IMPROVING THE SURGICAL TREATMENT OF PERITONEAL METASTASES OF COLORECTAL ORIGIN

MOLECULAR IMAGING, CLINICAL AND PHARMACOLOGICAL ASPECTS

Fortuné M.K. Elekonawo
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MOLECULAR IMAGING, CLINICAL AND PHARMACOLOGICAL ASPECTS

Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 12 november 2020
om 10.30 uur precies

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General introduction
GENERAL INTRODUCTION

The peritoneal cavity is the most extensive cavity of the human body. Its surface is lined by the peritoneum which consists of mesothelial cells. By producing serous fluid, the mesothelial cells enable low friction movement of the intra-abdominal organs. Primary malignancies of the mesothelium, such as the various forms of peritoneal mesothelioma, are rare. More frequently, malignant disease of the peritoneum is the result of metastasized colorectal, appendiceal, ovarian, pancreatic or gastric cancer. Malignant disease of the peritoneal surface can, even when treated according to current intensive treatment standards, result in severe morbidity or death. Several hypotheses on the etiology of peritoneal metastases (PM) exist. Cancer cells may be released into the peritoneal cavity during the natural disease progression, especially following perforation of the primary tumor. In case of metachronous disease, one of the most accepted hypothesis is that tumor cell release and peritoneal entrapment can be caused by surgical trauma after resection of the primary tumor [1].

In colon cancer, the peritoneum is the second most affected site of distant metastases and PM have an incidence of 10-15% [2, 3]. In general, colorectal cancer patients with PM have a dismal prognosis and median overall survival does not exceed 7 months after palliative treatment [4]. Even with modern systemic chemotherapy, median overall survival only reaches up to a maximum of 22 months [5]. In some patients, PM are present without hematogenic metastases and should be regarded and treated as locoregional disease. When disease is limited to the peritoneum, treatment with cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has the most potential for curation and is widely practiced throughout the world [6]. Five-year survival can reach up to 40% after complete cytoreduction [7]. However, CRS-HIPEC is an extensive surgical procedure and can result in morbidity and mortality rates of 16-52% and 0-5%, respectively [6].
GENERAL INTRODUCTION

CYTOREDUCTIVE SURGERY AND HYPERTHERMIC

Intraperitoneal chemotherapy
The CRS-HIPEC procedure consists of four phases. In the first phase, the abdomen is approached via a standard midline incision and the peritoneal cancer index (PCI)[8], a standardized measure for the amount of intraperitoneal disease, is scored. If the tumor burden is too extensive, the abdomen is closed and the procedure cancelled, since no survival benefit of partial surgical resection is to be expected [9]. When complete resection is deemed possible, the next phase is the actual cytoreductive surgery. Here, a specialized surgeon removes all tumor that is macroscopically detectable (figure 1A). Subsequently, hyperthermic intraperitoneal chemotherapy of approximately 42°C is administered and continuously circulated in the abdomen for 30-90 minutes, depending on the type of chemotherapy that is prescribed (figure 1B). Mitomycin C, irinotecan and oxaliplatin are common chemotherapeutic drugs that are used for abdominal perfusion [10]. Hyperthermia is supposed to increase antitumor immune responses and impair DNA replication and multiple DNA repair pathways [11, 12], additionally it can enhance the cytotoxicity of cytostatic drugs [13]. In this way cancer cells are sensitized to cytotoxic agents.

Figure 1A
Schematic representation of the open abdominal setup for cytoreductive surgery. (Red dots represent peritoneal metastases)
Figure 1B  Simplified schematic representation of the commonly used "open coliseum" technique for HIPEC perfusion. Note the in- and outflow catheters that are connected to a perfusion system with a fluid reservoir and heat exchanger. The instilled fluid is pumped through the abdomen and perfusion system in a closed loop, ensuring equal heat exchange during the perfusion time.
The last phase of the CRS-HIPEC procedure consists of restoration of the gastro-intestinal tract and closure of the abdominal wall.

In the Netherlands, the main prognostic aspects of the procedure, i.e. patient selection and cytoreduction, are standardized. In this way, outcomes of patients treated with CRS-HIPEC have drastically improved over the past two decades [14]. However, throughout centers performing CRS-HIPEC large methodological differences continue to exist in postoperative care on surgical wards and the administration of HIPEC. Moreover, concentrations of intraperitoneal chemotherapy differ significantly between centers and are infrequently monitored [15].

**Patient selection**

Patient selection is crucial for reducing the risk of severe complications after CRS-HIPEC [16]. Currently, patients are selected based on medical history, comorbidity, extent of disease and performance score. Pre-operative computed tomography (CT) fulfills a central role in staging and assessment of intra-abdominal disease to determine eligibility for surgery. When the PCI exceeds a threshold, CRS-HIPEC is not beneficial for survival and/or the quality of life of patients. Therefore, accurate preoperative assessment of disease is essential in preventing futile laparotomies. Unfortunately, the sensitivity of CT for small peritoneal lesions (<1 cm) is limited and reaches only 10-43% [17, 18]. Diagnostic laparoscopy has been introduced into the clinical workflow prior to CRS-HIPEC to identify patients with high PCI and it can prevent non-therapeutic laparotomies [19]. Since current imaging methods fail to accurately estimate the extent of peritoneal disease preoperatively, imaging methods that have a higher predictive value and accuracy are needed.

**Complete cytoreduction**

Patient selection is an important aspect of treatment that can influence prognosis of patients treated with CRS-HIPEC. Another main determinant of prognosis is reaching macroscopic complete cytoreduction (CC0) during surgery. Macroscopic residual disease drastically reduces survival after CRS-HIPEC [7]. Throughout the procedure, an experienced surgeon relies on a combination of the pre-operatively acquired images, visible and tactile information to identify and subsequently resect malignant tumors. Adhesions or fibrosis can mimic the appearance of PM and are frequently present in the abdomen of patients, especially in those who underwent previous abdominal surgery. Identification of such lesions as malignant may result in more and larger resections, thus creating more surgical trauma to the peritoneum and viscera than necessary.
CHAPTER 1

TUMOR-TARGETED IMAGE-GUIDED SURGERY FOR COLORECTAL CANCER

Since completeness of cytoreduction is a major prognostic factor, increasing the surgeon’s ability to find small tumors could ultimately prevent recurrent PM and increase survival. For this purpose, new techniques to image tumors pre- and intraoperatively, e.g. tumor-targeted imaging, are gaining more scientific interest. In tumor-targeted imaging, tumor-associated antigens expressed by tumor cells are used for targeting molecules. These include receptor ligands, peptides, monoclonal antibodies or antibody fragments. Carcinoembryonic antigen (CEA) is a tumor-associated antigen. This membrane anchored surface protein is overexpressed in more than 90-95% of all colorectal cancer cases [20, 21]. Therefore, CEA can be used as a potential target for tumor-targeted imaging in this type of cancer.

For single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging, a targeting molecule can be radiolabeled with a gamma- or positron emitting radionuclide. Because of their slow blood clearance, monoclonal antibodies are labeled with radionuclides with half-lives matching the biological half-life of the antibody. For PET: Zirconium-89 (⁸⁹Zr, t₁/₂ 3.2 days) and Iodine-124 (¹²⁴I, t₁/₂ 4.2 days) and for SPECT: Indium-111 (¹¹¹In, t₁/₂, 2.8 days) is commonly used. While radionuclides can be accurately quantified, real-time visualization and delineation of tumors by radionuclide detection in the operating theatre can be cumbersome. For real-time imaging and accurate tumor delineation, near infrared fluorescence (NIRF) imaging is more suitable. NIRF tumor-targeted imaging relies on fluorescent dyes that absorb light with wavelengths in the near infrared range (700-1000 nm) that are conjugated to the targeting molecule. The advantage of NIRF imaging is that the dyes are invisible to the human eye and, therefore, do not change the appearance of the surgical field. Moreover, the penetration depth of NIR light is higher than light in the visible light range and autofluorescence of tissues is lower in the NIR range [22]. The dyes are visualized using an external excitation source and detector, which are nowadays combined and integrated in commercially available fluorescence camera systems.

DUAL LABELING

Conjugation of a chelator for radionuclide labeling and fluorescent dye to the same targeting molecule results in a dual-labeled tracer enabling multimodal imaging. Combining the two modalities offers specific advantages. First, the radionuclide can be used for pre-operative detection and imaging (e.g. PET or SPECT, depending on the radionuclide) of the tracer. Second, during a surgical procedure, the radionuclide may be used to detect lesions even when they are covered by layers of other tissue. Finally, the fluorescent signal can be used for
real-time delineation of tumors where the tracer has accumulated. Pre-clinical assessment of multimodal anti-CEA antibodies indicates that tracers are detectable, that targeting is specific, and that they can be used for image-guided surgery [23, 24]. One of the tracers that is used in the studies described in this thesis is dual labeled labetuzumab, a humanized monoclonal antibody with high affinity for CEACAM5. CEACAM5 is an abundantly present member of the CEA family of surface proteins [25].

**PRETARGETING**

Labeled antibodies have slow pharmacokinetics, slow tumor accumulation and slow excretion rates [26]. This results in an extended residence time in the circulation which, in turn, requires relatively long waiting times to achieve optimal tumor-to-background ratios (multiple days). Smaller targeting molecules can offer specific advantages over IgG molecules, due to their faster pharmacokinetics and pharmacodynamics. For treatment of colorectal PM, another disadvantage of an antibody such as labetuzumab is that it is metabolized hepatically. Since colorectal PM can be present on the visceral peritoneum of the liver, the tumor-to-background ratio could be insufficient when tumors are located on the liver surface. Pretargeting is a technique that avoids hepatic clearance of the labeled tracer. In pretargeting, an unlabeled bispecific antibody, that serves as targeting molecule, is administered first (figure 2B). The bispecific antibody has high affinity for the target (i.e. CEA) and for a (dual)-labeled peptide that is administered subsequently (figure 2C). The renal clearance of this (dual)-labeled peptide is fast and results in minimal hepatic accumulation.
Figure 2 Simplified illustration explaining pretargeting. A: Cells that overexpress a target protein (e.g., CEA in colorectal cancer cells). B: After administration, the bispecific antibody (TF2) binds to its target (CEA) on the cell surface. C: After sufficient binding and clearance of circulating TF2, the radio- and fluorescently (green star) labeled hapten peptide RDC018 is administered. The green triangles represent the two histamine-succinyl-glycine (HSG) moieties of RDC018. D: The labeled hapten peptide is bound by the bispecific antibody, ensuring the presence of the hapten peptide on the cell surface.
TARGETED PHOTODYNAMIC THERAPY

While CRS-HIPEC is currently considered the best available treatment for intraperitoneal colorectal metastases, the (added) value of the hyperthermic chemotherapy above the cytoreductive surgery has become subject of debate [27, 28]. Targeted photodynamic therapy (tPDT) gained more interest over the last decade in oncology research. In tPDT a photosensitizer is used that is able to generate toxic (oxygen) radicals after excitement with the physical energy of near-infrared light (figure 3). When this photosensitizer is in close proximity of cells it can, through a series of photochemical reactions, induce local cell damage and death [29, 30]. Tumor targeting in tPDT is similar to targeted NIRF imaging but, instead of imaging, the goal is to create a local therapeutic effect. tPDT could for example be applied when complete resection of tumors is impossible due to involvement of vital structures such as nerves and blood vessels [31].

*Figure 3* Simplified illustration of the mechanism of action of tPDT. After an antibody (conjugated with a photosensitizer e.g. IRDye700DX) is administered, it binds to its target (e.g. CEA in colorectal cancer cells). Next, after irradiation with light near the maximum absorbance peak of the photosensitizer, the photosensitizer reaches an excited state. Before falling back to its ground state, it can cause the formation of reactive oxygen species (ROS) and other radicals which damage and disrupt the cell membrane and other organelles. This way, tPDT can induce various forms of cell death.
While the first clinical trials have been completed and others are ongoing, (dual-)labeled antibodies for image guided surgery, pretargeting and tPDT are always first tested in the preclinical setting [31-34]. Successful translation of preclinical research can be difficult. Hurdles include interspecies variability due to differences in absolute size of the tumors or different (intra) cellular processes. Translating findings from small murine subcutaneous tumors (millimeter to centimeter range) to larger human tumors (centimeter to decimeter range) can also be challenging. The physical properties of light in the near infrared range, e.g. scattering, absorption or the limited penetration depth, can be a limiting factor for successful translation due to these scale differences between animal model and the clinical situation. Genetic differences between laboratory animals and humans can restrict the use of syngeneic models, for example mice do not express human CEA. Therefore, investigating a CEA-targeted tracer in mice requires immunodeficient animals for successful CEA-positive tumor grafting. However, for therapeutic techniques such as tPDT, ideally the immune system is needed for investigating anti-tumor immune responses [35]. Bridging the gap between studies in laboratory animals and patients is therefore essential and one of the topics of this thesis.
THESIS OUTLINE

The aim of the studies described in this thesis was to explore the current clinical and pharmacological aspects of CRS-HIPEC and investigate the potential of novel molecular imaging techniques for pre-operative and intraoperative imaging.

PART 1 – CLINICAL AND PHARMACOLOGICAL ASPECTS

Learning from historical data is paramount for improving patient care. While the main aspects of the CRS-HIPEC procedure are highly standardized (inter)nationally, postoperative care on surgical wards is not. Several specific differences in postoperative care between two Dutch HIPEC centers allowed us to retrospectively analyze the impact of a series of parameters on recovery and morbidity of patients (chapter 2).

Unfortunately, patients are still diagnosed with local or systemic recurrence of disease after treatment with CRS-HIPEC. The incidence of recurrences and the treatment of these recurrences was studied in a historical multicenter cohort study (chapter 3).

Similar to postoperative care, methodological differences of HIPEC administration exist among centers performing CRS-HIPEC. Chapter 4 reviews the literature on pharmacological aspects of oxaliplatin-based HIPEC. This review highlights the variation that is present in current oxaliplatin-based HIPEC protocols and identifies several knowledge gaps. These findings formed the basis for studying the influence of variation in HIPEC concentration on survival and complications in three HIPEC centers (chapter 5).

One of the differences that was noticed in HIPEC protocols was post-instillation flushing of the abdomen with crystalloids. The main goal of the clinical trial described in chapter 6 was to determine the effect of post-instillation flushing with crystalloids on systemic and tissue exposure of patients treated with oxaliplatin-based HIPEC.
PART 2 – DUAL-LABELED ANTIBODIES FOR IMAGING AND TREATMENT

Since completeness of cytoreduction is a major prognostic factor, increasing the surgeon’s ability to discriminate between fibrosis, scar tissue or adhesions, and cancer could increase survival after treatment. As mentioned in the introduction, smaller targeting molecules can offer specific advantages over larger antibodies with slower pharmacokinetics and pharmacodynamics. In chapter 7 a multimodal pretargeting method is investigated using a bispecific anti CEA x HSG antibody combined with a small hapten peptide for targeting peritoneal metastases in a mouse model.

Sometimes complete resection is impossible during surgery. tPDT has potential to treat visible and invisible residual tumor cells after resection. In chapter 8 the potential of tPDT is investigated in vitro and in vivo. For this purpose, labetuzumab conjugated with a photosensitizer is used for treatment in a subcutaneous xenograft model of colorectal cancer.

Predicting the behavior of targeted agents in patients based on studies in mouse- or other laboratory animal-tumor models can be challenging. In Chapter 9 a method is described to use resected tumor specimens to gain insight into the potential of newly developed (and preclinically tested) multimodal tracers. Here dual-labeled labetuzumab is used in vitro to incubate freshly resected human peritoneal tumors in order to assess the tumor-targeting potential of the tracer.

After these preclinical and translational studies, a phase 1 clinical trial was initiated and discussed in chapter 10. Ten patients with colorectal PM were injected intravenously with dual-labeled labetuzumab one week prior to CRS-HIPEC and the safety and feasibility of the technique were evaluated. Finally, the subjects of the previous chapters are discussed in chapter 11 and it also provides future perspectives for the treatment of peritoneal metastases of colorectal origin.
REFERENCES

Comparison of two perioperative management protocols and their influence on postoperative recovery after cytoreductive surgery and HIPEC

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Digestive Surgery, July 2019, 36(5):394-401

*Equal contributions
CHAPTER 2

ABSTRACT

Background
Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS-HIPEC) is associated with considerable postoperative morbidity, including ileus and infectious complications. Perioperative care is believed to be an important factor for the development and treatment of postoperative morbidity.

Methods
Data on case matched patients from a retrospective database of two Dutch HIPEC centers was compared. Patient selection and procedures were identical in both hospitals although perioperative management items differ slightly. In centre B immediate total parenteral nutrition (TPN), suprapubic urine bladder catheter placement (SPCs) and selective decontamination of the digestive-tract (SDD) are standard care for CRS-HIPEC patients, in centre A they are not.

Results
From a total of 223 patients, 68 consecutive patients from centre B were compared to 68 matched patients from centre A. TPN was administered to 54.4% of patients in centre A because of prolonged ileus, whereas it was standard of care in centre B. 105 (77.2%) patients experienced postoperative complications including 17.6% who had a grade III-IV complication. The incidence of grade III-V complications was 18 (26.4%) in centre A and 8 (11.8%) in centre B (p=0.03). Median hospital stay was: 12 days (7-84) in A and 11(6-80) in centre B (p=0.546).

Conclusion
Gastrointestinal recovery after CRS-HIPEC seems to take longer as compared to other surgical procedures. Between the two centres a significant difference in severe complications was found whilst standard TPN, selective bowel decontamination and suprapublic bladder catheters were the only identified differences in perioperative care.
INTRODUCTION

Globally, colorectal cancer (CRC) is the third most common cancer in males and the second in females [1]. Advanced stage of disease is present in approximately 20% of patients at first presentation [2], including 4-7% presenting with synchronous peritoneal carcinomatosis (PC) [3]. Patients with advanced T and N stage, poor tumor differentiation grade, mucinous tumours, younger age and right sided localization of the primary tumor have a higher risk for PC [3-5]. PC of colorectal origin has always been associated with poor prognosis and early studies in which patients were treated with palliative chemotherapy, reported median survival ranging from 3.1 to 7.0 months [5,6]. Later studies indicate that modern chemotherapy regimens can increase survival up to a median of 12.5 months [7]. Complete cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have demonstrated to significantly increase survival in selected patients with PC of colorectal origin [8-11]. Postoperative recovery in these patients is slower and associated with more morbidity compared to other abdominal procedures [12].

In elective colorectal surgery, fast track recovery programs that adhere to the ‘enhanced recovery after surgery’ (ERAS) principles, [13,14] have been demonstrated to be safe and effective [15,16]. ERAS programs in colorectal surgery cause faster recovery and fewer complications [17,18]. While patients operated for conventional colorectal, liver or gastrointestinal tumors do benefit from its effectiveness, the distinctive post-operative course after CRS and HIPEC does not allow application of standard ERAS protocols. This is not surprising since the procedure is usually much longer and leads to extensive peritoneal trauma. Prolonged postoperative ileus (POI) frequently occurs after HIPEC. POI hinders patient recovery and increases postoperative morbidity, hospital costs, readmission rates and postoperative hospital stay (HS) [19-22]. Patients with PC of CRC treated with CRS and HIPEC in two centers between 2010 and 2015 were case-matched and analyzed retrospectively. Both hospitals share identical patient selection standards and operative procedures in accordance with the nationwide protocol. However, peri-operative patient management is different on three major items. These are the standard use of total parenteral nutrition (TPN), selective decontamination of the digestive tract (SDD) and suprapubic bladder catheters (SPCs) in one of the centres.

The aim of the current study was to gain insight in early gastrointestinal recovery and peri-operative morbidity in this specific group of patients and to evaluate the effect of two different perioperative care protocols on these outcomes.
CHAPTER 2

PATIENTS & METHODS

Patients
Complete details on consecutive patients with PC of colorectal origin treated with CRS and HIPEC in Radboudumc Nijmegen and Catharina Ziekenhuis Eindhoven between 2010 and 2015 were available for this study. Exclusion criteria were non-primary HIPEC procedures or other origins of disease, e.g. mesothelial origin. A total of 68 consecutive patients from Radboudumc Nijmegen were included and compared with 68 best matching patients selected from the 155 consecutive patients available from Catharina Ziekenhuis Eindhoven. Relevant patient-, tumor-, recovery- and treatment(procedure)-related data were collected in a retrospective database. Matching variables were: age at time of surgery, BMI, metachronous or synchronous peritoneal carcinomatosis and PCI. After matching, a total of 136 patients were analyzed. Data acquisition and analysis was approved by the local ethics committee.

HIPEC Centers
Both institutes are Dutch HIPEC referral centers located in the southern region of The Netherlands. Indications for treatment with CRS and HIPEC and the applied operative techniques are identical and based on national consensus. Perioperative care protocols for these patients are identical in both hospitals, except for 3 items: selective decontamination of the digestive tract, peroperative placement of suprapubic catheters and TPN started on the first day after operation are standard in centre B. In the other centre Foley type urethral catheters are the standard of care and TPN and SDD are not included in the standard care protocol. These differences provided the opportunity to evaluate gastrointestinal recovery and complications as related to standard TPN plus SDD and suprapubic urine bladder catheters versus on-demand TPN, no SDD and transurethral catheters.

Standard TPN protocol
The nutritional needs of all patients in centre B were calculated on an individual patient level to provide adequate TPN. A dietician used the revised Harris and Benedict equation to calculate base caloric demand [23]. To reach adequate caloric intake for surgically treated patients, a surplus of 30-50% was added to the outcome of the equation.

Antibiotic prophylaxis
Centre A administered an i.v. dose of 2000 mg cefazolin and 500 mg metronidazole 30 minutes prior to incision, adequate dosing of profylaxis was continued for the duration of the procedure. In centre B the same dose of metronidazole was administered 30 minutes prior to incision. On the day prior to the procedure patients received an intravenous dose of 2000 mg
Ceftriaxone at 10 pm. Infectious complications that required additional antibiotic treatment were treated according to local protocols.

**Selective decontamination of the digestive tract in centre B**

For SDD, additional to the prophylaxis mentioned above, 1000 mg Tobramycin/Colistin/Amphotericin-B oromucosal paste was applied 4 times per day, complemented by a 10 ml gastrointestinal solution until oral food intake was resumed. SDD was used according to local protocol and is considered a valuable addition of antibiotic treatment to reduce the risk of infections after colorectal surgery [24,25].

**Urine catheters**

In centre B suprapubic bladder catheters are routinely placed during the first stage of the CRS-HIPEC procedure.

**HIPEC and CRS**

All procedures were performed by a specialized surgical team with ample experience in HIPEC. Leading HIPEC surgeons from both teams have crossed over to the other centre to participate in CRS-HIPEC surgery. All surgeons were directly or indirectly trained by the Netherlands Cancer Institute surgical team and therefore adhere to identical surgical techniques and procedures. Surgeons from both centers operated cases together in both hospitals. Peritoneal tumor extensiveness was scored using the peritoneal cancer index (PCI) [26,27]. Peritonectomy procedures were performed according to principles previously described by Sugarbaker [26]. Standard total omentectomy was part of all cytoreduction procedures that were performed in both centres. The gastro-omental arcade, previously known as gastro-epiploic arcade, is often part of the omental resection specimen. In several cases the arcade was spared.

The aim of each procedure was to achieve complete macroscopic resection which was scored using the CC-score (CC0: complete macroscopic resection, CC1: residual tumor nodules <2.5mm, CC2: residual tumor nodules larger than 2.5 mm). All procedures were performed as open “coliseum” surgery. For HIPEC with Mitomycin C (MMC) a solution of 35 mg/m² (maximum 70 mg) in 0.9% NaCl was used to perfuse the abdomen at a temperature of 42–43 °C for 90 minutes. In patients perfused with Oxaliptatin (OX), systemic chemotherapy with leucovorin and 5-fluoro-uracil (20 mg/m² and 400 mg/m²) was administered prior to HIPEC. Oxaliptatin, 460 mg/m², in 5% dextrose 42-43 °C was used for 30 minutes perfusion of the peritoneal cavity at 42-43 °C. During the historic period from 2010 to 2015 of the study the treatment protocol was revised and changed from MMC to OX in 2014, concording with the national protocol update.
Postoperative recovery
The date of the HIPEC procedure was defined as day 0. Multiple characteristics were registered as indicators of postoperative gastro-intestinal status: relief of nasogastric tube (used for gastric drainage), first day of tolerance of oral nutrition (not being clear fluids), first stool, start- and termination of TPN. Relief of nasogastric tube was defined by the day on which the tube was removed, provided no replacement in the following days during hospitalization was necessary. Oral tolerance was considered to be present when a patient experienced no nausea or vomiting after oral intake other than water or transparent liquid. First stool was defined as the day of the first defecation or enterostomy production (other than the commonly observed serous or postoperative early production on day 0-1). Termination of TPN was defined by the last day a patient received any amount of intravenous nutrition.

Complications
Complications were scored using the Clavien Dindo complication classification [28]. Since TPN was part of the care protocol in centre B, TPN was excluded as complication criterion to score complications.

Statistical analysis
Statistical analyses were performed using the Statistical Package for Social Sciences, Version 22.0 (IBM Corp., Armonk, NY, USA). Comparisons of means and medians respectively were conducted with student’s T-test or Mann-Whitney-U test, depending on distribution. Categorical variables were cross-tabulated and significance was determined by a Chi-Square test or Fisher’s exact test according to sample size. Pearson correlation was used to identify correlated variables. Correlations were tested one-sided with a p value of 0.05. All other tests were performed two sided and a significance level of <0.05 was used to reject the null hypothesis. For all calculations, cases in which essential data missed were excluded from analysis.
RESULTS

A total of 223 patients treated in both hospitals between 2010 and 2015 with primary CRS and HIPEC for colorectal PC were included. All 68 eligible patients from centre B were included and compared with the 68 best matching patients from centre A. Table 1 summarizes general characteristics. After matching no significant differences could be demonstrated in patient and tumor characteristics of patients in both groups.

Complete cytoreduction (CC0) was achieved in 95.6% of treated patients. Procedure time was shorter in centre A than in B with 367 and 417 minutes, respectively (p<0.001). Patients treated in B had a higher estimated intraoperative loss of blood volume (p<0.001), this however did not result in a difference in postoperative blood haemoglobin levels. Mitomycin C and Oxaliplatin protocols were used in 115 patients (84.6%) and 21 patients (15.4%), respectively. All patients who were treated with Oxaliplatin had surgery in centre B. Total omentectomy including the gastro-omental arcade was performed in 25 patients (18.4%) in centre B. In 23 (16.9%) B patients the gastro-omental arcade was spared and in the remaining 88 patients of (64.7%) the arcade status was not unequivocally described. Time to removal of nasogastric tube and oral tolerance of food was not statistically different in these groups (p=0.933 and p=0.633).
### Table 1. Characteristics of patients, primary tumours and treatment procedures.

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<td><strong>Patient Characteristics</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td>Male 60 (44.1)</td>
<td>32 (47.1)</td>
<td>28 (41.2)</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>Female 76 (55.9)</td>
<td>36 (52.9)</td>
<td>40 (58.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, year, mean ± SD</strong></td>
<td>61.4 ± 10.7</td>
<td>61.6 ± 10.1</td>
<td>61.1 ± 11.4</td>
<td>0.749</td>
</tr>
<tr>
<td><strong>BMI, kg/m², median (range)</strong></td>
<td>24.4 (17.9-40.0)</td>
<td>24.6 (18-36)</td>
<td>24.3 (18-40)</td>
<td>0.969</td>
</tr>
<tr>
<td><strong>ASA</strong></td>
<td>I-II 127</td>
<td>66</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III-IV 9</td>
<td>2</td>
<td>7</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>Tumour Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PC1 presentation</strong></td>
<td>Synchronous 68 (50.0)</td>
<td>34 (50.0)</td>
<td>34 (50.0)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Metachronous 68 (50.0)</td>
<td>34 (50.0)</td>
<td>34 (50.0)</td>
<td></td>
</tr>
<tr>
<td><strong>PCI2, median (range)</strong></td>
<td>9.0 (0-24)</td>
<td>7.5 (2-21)</td>
<td>10 (0-24)</td>
<td>0.349</td>
</tr>
<tr>
<td><strong>Tumour location</strong></td>
<td>Appendix 3 (2.2)</td>
<td>0 (0)</td>
<td>3 (4.4)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Right colon 53 (39.0)</td>
<td>23 (33.8)</td>
<td>30 (44.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transverse 6 (4.4)</td>
<td>4 (5.9)</td>
<td>2 (2.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left colon 9 (6.6)</td>
<td>5 (7.4)</td>
<td>4 (5.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sigmoid 48 (35.3)</td>
<td>27 (39.7)</td>
<td>21 (30.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rectum 16 (11.8)</td>
<td>9 (13.2)</td>
<td>7 (10.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple 1 (0.7)</td>
<td>0 (0)</td>
<td>1 (1.5)</td>
<td>0.211</td>
</tr>
<tr>
<td><strong>pTstatus</strong></td>
<td>≤3 69 (50.7)</td>
<td>37 (54.4)</td>
<td>32 (47.1)</td>
<td>0.568</td>
</tr>
<tr>
<td></td>
<td>4 65 (47.8)</td>
<td>31 (45.6)</td>
<td>34 (50.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>x 2 (1.5)</td>
<td>0 (0)</td>
<td>2 (2.9)</td>
<td></td>
</tr>
<tr>
<td><strong>pNstatus</strong></td>
<td>N0 40 (29.4)</td>
<td>22 (32.3)</td>
<td>18 (26.5)</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>N1 41 (30.1)</td>
<td>18 (26.5)</td>
<td>23 (33.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N2 51 (37.5)</td>
<td>28 (41.2)</td>
<td>23 (33.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nx 10 (2.9)</td>
<td>0 (0)</td>
<td>4 (5.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Procedure and treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Completeness of cytoreduction</strong></td>
<td>CC0 133 (97.8)</td>
<td>66 (97.1)</td>
<td>67 (98.5)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>CCI 3 (2.2)</td>
<td>2 (2.9)</td>
<td>1 (1.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Procedure time, min, mean ± SD</strong></td>
<td>475 ± 126</td>
<td>367 ± 69</td>
<td>417 ± 162</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Blood Loss, mL, median (range)</strong></td>
<td>1500 (100-7500)</td>
<td>800 (100-4200)</td>
<td>1993 (150-7500)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Postoperative Hb difference</strong></td>
<td>-1.3 ± 1.1</td>
<td>-1.5 ± 1.0</td>
<td>-1.2 ± 1.3</td>
<td>0.104</td>
</tr>
<tr>
<td><strong>HIPEC</strong></td>
<td>Mitomycin C 115 (84.6)</td>
<td>67 (98.5)</td>
<td>48 (70.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Oxaliplatin 21 (15.4)</td>
<td>1 (1.5)</td>
<td>20 (29.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bowel anastomosis</strong></td>
<td>0 51 (37.5)</td>
<td>25 (36.8)</td>
<td>26 (38.2)</td>
<td>0.619</td>
</tr>
<tr>
<td></td>
<td>1 65 (47.8)</td>
<td>33 (48.5)</td>
<td>32 (47.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1 19 (14.0)</td>
<td>10 (7.4)</td>
<td>9 (13.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>1 (1.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Protective ostomy</strong></td>
<td>Yes 8 (5.8)</td>
<td>6 (8.8)</td>
<td>2 (2.9)</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>No 128 (94.2)</td>
<td>62 (91.2)</td>
<td>66 (97.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Peroperative serosal injury</strong></td>
<td>46 (33.8)</td>
<td>17 (25)</td>
<td>29 (42.6)</td>
<td>0.396</td>
</tr>
<tr>
<td><strong>Peroperative JJ-stent placement</strong></td>
<td>15 (11.0)</td>
<td>13 (19.1)</td>
<td>2 (2.9)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Days ICU</strong></td>
<td>2 (0-38)</td>
<td>2 (0-38)</td>
<td>2 (1-3)</td>
<td>0.471</td>
</tr>
<tr>
<td><strong>Hospital stay, days, median (range)</strong></td>
<td>11 (6-84)</td>
<td>12 (7-84)</td>
<td>11 (6-80)</td>
<td>0.546</td>
</tr>
</tbody>
</table>

1 Peritoneal cancer; 2 Peritoneal cancer index; 3 Intensive care unit
### Table 2. Complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>All Patients n = 136 (100%)</th>
<th>A n = 68 (50%)</th>
<th>B n = 68 (50%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Complication</td>
<td>105 (77.2)</td>
<td>48 (70.6)</td>
<td>57 (83.8)</td>
<td>0.066</td>
</tr>
<tr>
<td>Uncomplicated</td>
<td>31 (22.8)</td>
<td>20 (29.4)</td>
<td>11 (16.2)</td>
<td></td>
</tr>
<tr>
<td>Clavien Dindo score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17 (12.5)</td>
<td>8 (11.8)</td>
<td>9 (13.2)</td>
<td>0.028</td>
</tr>
<tr>
<td>II</td>
<td>62 (45.6)</td>
<td>22 (32.4)</td>
<td>40 (58.8)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15 (11.0)</td>
<td>10 (14.7)</td>
<td>5 (7.4)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>9 (6.6)</td>
<td>6 (8.8)</td>
<td>3 (4.4)</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>2 (1.5)</td>
<td>2 (2.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Any infection</td>
<td>42 (30.9)</td>
<td>29 (42.6)</td>
<td>13 (19.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Wound</td>
<td>24 (17.6)</td>
<td>17 (25.0)</td>
<td>7 (10.3)</td>
<td>0.024</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>11 (8.1)</td>
<td>9 (13.2)</td>
<td>2 (2.9)</td>
<td>0.028</td>
</tr>
<tr>
<td>Line</td>
<td>5 (3.7)</td>
<td>N</td>
<td>5 (7.4)</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>13 (9.6)</td>
<td>10 (14.7)</td>
<td>3 (4.4)</td>
<td>0.041</td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td>20 (14.7)</td>
<td>14 (20.6)</td>
<td>6 (8.8)</td>
<td>0.053</td>
</tr>
<tr>
<td>Other</td>
<td>8 (5.9)</td>
<td>1 (1.5)</td>
<td>7 (10.3)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

*Table 2* Complications per hospital. *N* = not scored or described.
Complications

A complicated post-operative course was observed in 105 (77.2%) of all patients and 24 (17.6%) had grade III-IV Clavien-Dindo complications (Table 2). Grade III-V complications were more commonly observed in A: n=18 (26.4%) versus n=8 (11.8%) in B (p=0.03). Most common grade III-IV complications were anastomotic leakages, fistulas and intra-abdominal abscesses.

Infectious complications were more prominent in centre A; 29 (42.6%) versus 13 (19.1%) in B (p=0.003), including a difference in the incidence of wound-, urinary tract-, other infections and the incidence of anastomotic leakage. In centre B, fewer urinary tract infections occurred: n=2 (2.9%) versus n=9 (13.2%) in centre A (p=0.028). In centre A 13 (19.1%) patients had a double J stent placed peroperatively. Of those patients, 5 (38.5%) developed an infection of the urinary tract. The numbers for the whole study were 15 and 5 respectively (33%). Median hospital stay was not statistically different between the centers: 12 days (7-84) in A and 11(6-80) in B (p=0.546).

A higher PCI was related with a longer HS (p=0.03). And a longer HS was moderately correlated with oral intolerance (r=0.632, p<0.001).

Standard TPN and TPN on demand

TPN was administered per protocol in 98.5% (one patient did not receive TPN due to line failure) of patients in centre B. It was judged indicated and given to 54.4% of centre A patients (Table 3). Duration of TPN was longer in A with a median of 10 (4-65) versus 8 (0-28) days postoperatively in B (p=0.03). Generally TPN was ended before discharge. The day of first oral tolerance and nasogastric tube removal was 1 day earlier in A (p=0.03 and p<0.001) but in A nasogastric tubes were reinserted more frequently: n=18 versus n=7 (p=0.018). First stool occurred on day 5 in both groups and abdominal wound drains were removed earlier in A, median day 3 versus day 7 in B (p<0.001).
DISCUSSION

CRS combined with HIPEC is a curative multimodality approach for peritoneal carcinomatosis with a relatively high rate of morbidity and delayed return of gastrointestinal functions compared to other abdominal surgical procedures. Postoperative complications and gastrointestinal recovery were carefully registered in two Dutch tertiary referral hospitals for this surgical technique. Despite central intravenous catheter related postoperative morbidity, a postoperative protocol in with standard SDD, TPN and SPCs showed a decreased incidence of severe postoperative morbidity. The incidence of delayed return of gastrointestinal function was remarkably high compared to what is described for other gastrointestinal surgical procedures [29-32]. To minimize selection bias and create a representative patient group all consecutive patients over 5 years from one centre were included and matched for all known important risk factors with patients from the larger cohort of the other centre. The difference in medication used for the perfusion between the centers differed between study groups. However, available studies comparing the results of mitomycin C with oxaliplatin in the setting of HIPEC have shown very similar results with respect to postoperative complications [33-35].

<table>
<thead>
<tr>
<th>Table 3. Gastrointestinal Status</th>
<th>All Patients n = 136 (100%)</th>
<th>A n = 68 (50%)</th>
<th>B n = 68 (50%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPNa n, (%)</td>
<td>104 (77)</td>
<td>37 (54.4)</td>
<td>67 (98.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPN start day</td>
<td>1 (0-23)</td>
<td>3.5 (0-23)</td>
<td>1 (0-3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPN stop day, median (range)</td>
<td>8 (0-65)</td>
<td>10 (4-65)</td>
<td>8 (2-28)</td>
<td>0.043</td>
</tr>
<tr>
<td>TPN Total days</td>
<td>7 (1-61)</td>
<td>5 (1-61)</td>
<td>7 (2-20)</td>
<td>0.243</td>
</tr>
<tr>
<td>NTb stop day, median (range)</td>
<td>3 (0-71)</td>
<td>3 (0-10)</td>
<td>4 (1-71)</td>
<td>0.002</td>
</tr>
<tr>
<td>NT reinsertion n, (%)</td>
<td>25 (18.4)</td>
<td>18 (26.5)</td>
<td>7 (10.3)</td>
<td>0.018</td>
</tr>
<tr>
<td>First stool day, median (range)</td>
<td>5 (1-12)</td>
<td>5 (1-11)</td>
<td>5 (1-12)</td>
<td>0.430</td>
</tr>
<tr>
<td>OTc day, median (range)</td>
<td>5 (1-76)</td>
<td>5 (1-17)</td>
<td>6 (2-76)</td>
<td>0.034</td>
</tr>
<tr>
<td>AWDd stop day, median (range)</td>
<td>5 (2-20)</td>
<td>3 (2-10)</td>
<td>7 (2-20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First stool &gt; day 4 (%)</td>
<td>68 (50) *</td>
<td>34 (50)</td>
<td>34 (50)</td>
<td>0.924</td>
</tr>
<tr>
<td>First OT &gt; day 4 (%)</td>
<td>76 (55.9) **</td>
<td>32 (47.1)</td>
<td>44 (64.7)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*Table 3 Parameters of Gastrointestinal status. aTotal parenteral nutrition, bNasogastric tube, cOral tolerance, dAbdominal wound drains. * 9 not described, **13 Not described.*
Complications and infections
Total complication rate was higher in centre B but severe complications were more common in centre A. Differences in perioperative care protocols might have an effect on type of complications and complication rates but the retrospective nature of this study prevents any direct association. There was a substantial difference between the centers considering the time abdominal drains were kept, but a recent meta-analysis showed that early drain removal after abdominal surgery is neither beneficial nor detrimental when related to infection rates [36]. In pelvic surgery a lower incidence of urinary tract infections was previously described with the use of SPCs over transurethral catheters [37]. More urinary tract infections were found in centre A where urethral catheters were standard of care. However, the incidence of double-J stents was also higher in this population. J-J stents were placed when indicated after distal ureteral reimplantations or reconstructions. In the total cohort 45% of patients with a urinary tract infection (n=11) had a double-J stent (n=5), suggesting a higher susceptibility for urinary tract infections in these patients. The effect of urethral- or suprapubic catheters in combination with double-J-stents remains unclear because the limited amount of patients with double J stents.

Gastrointestinal recovery
Gastrointestinal recovery seems to take more time in CRS and HIPEC procedures than in other fields of abdominal surgery [29-32]. Although we observed a relation between PCI and hospital stay in the total cohort, differences in postoperative recovery did not seem to correspond with differences in length of postoperative stay between the two centers. After major abdominal surgical procedures, patients may experience severe nausea and vomiting after oral ingestion of food for several days postoperatively. In those cases, patients are at risk of aspiration, dehydration or malnutrition. Relief of symptoms may be achieved by nasogastric tube placement and additional TPN for nutritional purposes. Reinsertion of nasogastric tubes was more often necessary in group A, which could be due to premature removal postoperatively.
Enteral nutrition is suggested to be the preferred option whenever adequately possible, since it is associated with fewer (septic) complications, reduced costs, lower incidence of ileus or anastomotic leakage and a shorter hospital stay [38-41].
Also, a better peri-operative nutritional status is associated with less complications in gastrointestinal surgery patients [42] and the patient with peritoneal carcinomatosis is often in suboptimal nutritional status even before surgery. TPN is standard of care in centre B, as opposed to the other centre where it is used when oral intake of food and calories was insufficient after several days. The number of days to oral tolerance and nasogastric tube relieve was smaller in centre A, which might be explained by the early enteral feeding
policy and a more aggressive policy in removing nasogastric tubes and enteral feeding. The difference in time to oral tolerance however, did not result in a difference in hospital stay. Theoretically a better nutritional status resulting from TPN could reduce the susceptibility for infections, although this remains unclear. The present study shows that TPN was still a necessity in over half of patients to ensure adequate nutrition intake whilst an early enteral feeding policy is supposed to result in an earlier oral tolerance for food. Ideally clinicians should be able to determine which specific patients would benefit from different feeding policies. However the exploratory setup of this study does not allow a fair comparison of TPN versus early enteral feeding.

**Conclusions**
The current exploratory study provides insight in duration of recovery of the gastrointestinal tract in CRS and HIPEC patients. Significantly less severe infectious complications were observed in a group of CRS and HIPEC patients treated with a care protocol which involved standard TPN, SDD and SPCs. Moreover, TPN was unavoidable in a large part of CRS and HIPEC patients with an early enteral feeding policy.
The study shows a significant difference in relevant early outcome of the treatment. The differences between care protocols might well explain these differences on theoretical grounds. The burden of these complications for patients and hospital finance demands prospective evaluation in a randomized, prospective multicenter study.
REFERENCES


Treatment of isolated peritoneal recurrences in patients with colorectal peritoneal metastases previously treated with cytoreductive surgery and HIPEC

Wijntje J. van Eden, Fortuné M.K. Elekonawo, Bas J. Starremans, Niels F.M. Kok, Andreas J.A. Bremers, Johannes H.W. de Wilt, Arend G.J. Aalbers

ABSTRACT

Background
Colorectal peritoneal carcinomatosis (PC) is preferably treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal intraperitoneal chemotherapy (HIPEC). Peritoneal recurrence of disease after treatment can occur without distant metastases, with a variety of treatment options. This study aimed to evaluate the management of isolated peritoneal recurrence after primary CRS-HIPEC.

Methods
In two tertiary referral centers all, patients who underwent CRS-HIPEC for colorectal PC between 2004 and 2015 and who developed isolated peritoneal recurrences were retrospectively evaluated. Location, treatment of peritoneal recurrences and curative or palliative treatment intent were reported. Univariable and multivariable Cox regression analysis and survival analyses were performed.

Results
Of 414 patients treated with CRS-HIPEC for colorectal PC, 106 patients (26%) developed isolated peritoneal recurrence. Forty-three patients (41%) were treated with curative intent and 63 patients (59%) with palliative intent. Median overall survival in the patients treated with curative intent was 24.7 months (interquartile range (IQR) 12.1-61.7) compared to 7.6 months (IQR 2.5-15.9) in those treated with palliative intent (P<0.001). In the patients treated with curative cytoreductive surgery (n = 17) and curative second CRS-HIPEC (n = 15) median overall survival was 51.7 months (IQR 14.4-NA) and 29.0 months (IQR 18.1-63.0), respectively (P=0.620). The latter group had a significant higher region count (median 1 vs 3; P<0.001). Postoperative complications and hospital stay did not significantly differ between first and second CRS-HIPEC.

Conclusion
After CRS-HIPEC for colorectal cancer, approximately one out of four patients will develop isolated peritoneal recurrences. A substantial amount of these patients can be safely treated with curative intent yielding long-term survival.
INTRODUCTION

Patients with colorectal peritoneal carcinomatosis (PC) are preferably treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). PC occurs in approximately 10% of all colorectal cancer patients and is associated with poor survival [1, 2]. Five-year overall survival rates are increasing up to 50% when selected patients have been treated with CRS-HIPEC although locoregional and distant recurrences are common [3-5]. Although nearly all patients who develop distant metastases in combination with peritoneal carcinomatosis are treated with systemic palliative treatment or best supportive care [6], patients with isolated peritoneal disease can be candidates for curative treatment. Recurrence is strongly associated with lymph node status, extent of peritoneal disease and completeness of cytoreduction [7, 8]. Isolated peritoneal recurrences may have different drivers than the aforementioned factors. Golse et al. described no correlation between the extent of peritoneal disease and isolated peritoneal recurrences [9]. Isolated peritoneal recurrence may still be treated with curative intent, either with complete cytoreduction or CRS-HIPEC in carefully selected patients [10, 11]. Limited data are available in the literature but second CRS-HIPEC procedures are reported as a potential option to curatively treat patients. Brouquet et al. described a 5-year overall survival of 72.5% in patients with isolated peritoneal recurrences who underwent a second CRS-HIPEC. The objective of this study was to evaluate the management of isolated peritoneal recurrence and the effect on survival after previous CRS-HIPEC in patients with colorectal PC.
METHODS

Patients
The current study was performed in two Dutch tertiary CRS-HIPEC centers, The Netherlands Cancer Institute (NCI) in Amsterdam and Radboud University Medical Centre (Radboudumc) in Nijmegen. The NCI started to perform CRS-HIPEC procedures in 1996. The Radboudumc started in 2010. Data was retrieved from prospectively maintained databases. Patients with colorectal peritoneal carcinomatosis who had been treated with CRS-HIPEC between January 2004 and December 2015 and were diagnosed with isolated peritoneal recurrence of disease during follow up were eligible for inclusion in the study. Patients were excluded if an R2b-resection was performed (remaining tumor nodules >2.5 mm after cytoreduction) or if distant metastases were present at time of peritoneal recurrence. Patients were usually followed for at least ten years or up to death. This study was performed in accordance with medical ethical institutional guidelines, and board approval was not considered necessary because of the study’s retrospective nature.

Surgical procedure
Details of the procedure used for CRS-HIPEC have been previously described [12]. Cytoreduction preceded hyperthermia and intraperitoneal chemotherapy. Until March 2014, Mitomycin C (MMC) 35 mg/m² diluted in Dianeal was used, which was heated to 42-43°C over a 90 min period. Thereafter, leucovorin and 5-fluorouracil (20 mg/m² and 400 mg/m², respectively) was administered intravenously and oxaliplatin 460 mg/m² diluted in Dianeal® (NCI) or 5%-dextrose (Radboudumc) heated to 42-43°C was inserted in the abdominal cavity during 30 minutes while the abdomen was still open. In almost all patients who underwent a second CRS-HIPEC, the chemotherapeutic agent used was different from the agent used at the first CRS-HIPEC. However, two patients experienced neurotoxicity from previously received intravenous oxaliplatin, and Mitomycin C was again preferred during the second CRS-HIPEC in these patients.

The extent of peritoneal disease was scored with the Dutch region count, which divided the abdomen into seven regions, and the Peritoneal Cancer Index (PCI) [13]. The completeness of cytoreduction was scored based on the size of remaining tumor nodules. In an R1-resection no visible macroscopic tumor nodules were seen; in an R2a-resection remaining tumor nodules < 2.5 mm were left behind; and in case of an R2b-resection tumor nodules > 2.5 mm were left behind in the abdomen after cytoreduction.

Postoperatively, patients were admitted to the intensive care unit. Every patient was pre-and postoperatively discussed in a multidisciplinary team meeting. Whether or not patients received perioperative or adjuvant chemotherapy was decided by the multidisciplinary team.
Follow-up and recurrences
During follow-up, details of recurrences were accurately recorded. The date of recurrence was reported, together with the modality which was used to diagnose peritoneal recurrence of disease, which was either radiological, surgical or based on clinical symptoms or elevated tumor markers. The presence of histopathological evidence for peritoneal recurrence was recorded, along with the treatment and date of treatment of isolated peritoneal recurrences. Treatment could have had curative or palliative intent, which was discussed in a multidisciplinary team meeting. CRS-HIPEC, cytoreductive surgery and radiotherapy were considered to be curative treatment options, whereas palliative cytoreductive surgery, palliative radiotherapy, systemic chemotherapy and supportive care were considered to be palliative treatment options. Based on the treatment intent, a curative and a palliative treatment group were created. Hospital records were consulted to extract the intent of the treatment in patients with isolated peritoneal recurrences or in case of incomplete data. Disease free survival (DFS) was defined as time in months from date of curative treatment for isolated peritoneal recurrence to date of peritoneal re-recurrence. The date of last follow-up or death was also recorded, and overall survival (OS) was defined as time in months between treatment for isolated peritoneal recurrence to date of last follow-up or death.

Statistical analysis
Statistical analyses were performed using IBM SPSS Statistics version 22 (IBM Corp, Armonk, NY). Categorical data were presented as numbers with percentages and Pearson’s Chi square test, linear by linear, and Fisher’s exact test were used as appropriate. Continuous data were presented as medians with their interquartile range (IQR) or with minimum and maximum values, and the Kruskal-Wallis, Mann-Whitney-U and Wilcoxon Signed Ranks test were used as appropriate. Using the Kaplan Meier method, survival analyses were performed, and the log-rank test was used to test for statistical differences between groups. Furthermore, using Cox regression univariable analyses were performed and variables with a p value <0.05 or clinically relevant variables were included in multivariable analysis. An interaction term was created for complication grade and treatment intent. Subgroup analyses were performed for patients treated with curative intent. A p value <0.05 was used to reject the null hypothesis. For the Cox models, proportionally assumption was checked and fulfilled.
RESULTS

Patients
Overall, 414 patients were treated with CRS-HIPEC for colorectal peritoneal carcinomatosis between January 2004 and December 2015; 106 these patients (25.6%) were diagnosed with isolated peritoneal recurrence of disease after CRS-HIPEC, of whom 43 patients (40.6%) were treated with curative intent and 63 patients (59.4%) underwent treatment with palliative intent (Figure 1). Details on the baseline characteristics of all patients with isolated peritoneal recurrence are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
<th>Curative Treatment n = 43</th>
<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
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<td></td>
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<td></td>
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<td>Intraperitoneal drug regimen</td>
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<td><strong>P value</strong></td>
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46
### Table 1. Continued

<table>
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<tr>
<th>Classification of SAE</th>
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<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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<table>
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<th>Hospital stay (days)</th>
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<th>%</th>
<th>P value</th>
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<td></td>
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<td>15 (12-23)</td>
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<th>%</th>
<th>P value</th>
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<td>pT-stage</td>
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<th>P value</th>
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<tr>
<td>2</td>
<td>13</td>
<td>30.2</td>
<td>20</td>
<td>31.7</td>
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<td>2.3</td>
<td>4</td>
<td>6.3</td>
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<table>
<thead>
<tr>
<th>Systemic metatases prior to CRS-HIPEC</th>
<th>Curative Treatment n = 43</th>
<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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<tbody>
<tr>
<td>Absent</td>
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<td>74.4</td>
<td>59</td>
<td>93.7</td>
<td>0.009</td>
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<th>Peritoneal carcinomatosis</th>
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<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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<tr>
<td>Synchronous</td>
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<td>51.2</td>
<td>39</td>
<td>61.9</td>
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<td>Metachronous</td>
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<td>48.8</td>
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<table>
<thead>
<tr>
<th>Location primary tumor</th>
<th>Curative Treatment n = 43</th>
<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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<tbody>
<tr>
<td>Appendix</td>
<td>4</td>
<td>9.3</td>
<td>6</td>
<td>9.5</td>
<td>0.273</td>
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<td>Colon</td>
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<td>74.4</td>
<td>53</td>
<td>84.1</td>
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<td>Rectum</td>
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<td>16.3</td>
<td>4</td>
<td>6.3</td>
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Table 1. Continued

<table>
<thead>
<tr>
<th></th>
<th>Curative Treatment n = 43</th>
<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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<td>Differentiation primary tumor</td>
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<td>62.8</td>
<td>31</td>
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<td>Poor</td>
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<td>20.9</td>
<td>26</td>
<td>41.3</td>
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<td>Unknown</td>
<td>7</td>
<td>16.3</td>
<td>6</td>
<td>9.5</td>
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<tr>
<td>Histology primary tumor</td>
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<td>29</td>
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<td>Signet ring cell carcinoma</td>
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<td>2</td>
<td>12</td>
<td>19.0</td>
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Table 1 Baseline characteristics. Results are presented as numbers with percentages and medians with minimum and maximum values (age) or interquartile range (hospital stay). Abbreviations. ASA, American Society of Anaesthesiologists; MMC, Mitomycin C; SAE, Serious Adverse Event; CTCAE, Common Toxicology Criteria of Adverse Events; CRS, cytoreductive surgery; HIPEC, hyperthermic intraperitoneal chemotherapy.
Figure 1 Patient selection and treatment characteristics. RTx radiotherapy, CTx systemic chemotherapy, SC supportive care, FU follow-up, CRS cytoreductive surgery, HIPEC hyperthermic intraperitoneal chemotherapy, pCRS palliative cytoreductive surgery, pCRS-HIPEC palliative cytoreductive surgery
Peritoneal recurrences
Details of the peritoneal recurrences are presented in Table 2. After diagnosis of isolated peritoneal recurrence, patients were treated after a median of 1.2 months (IQR 0.4-2.4). In the curative intent group, 42 patients (97.7%) were treated with CRS, whether combined with HIPEC or not (Figure 1). In 15 out of 16 CRS-HIPEC procedures, and 19 of 26 CRS procedures a R1-resection was achieved. Two patients treated with a R1-resection died due to complications related to the procedure. One patient (2.3%) was curatively treated with radiotherapy. During follow-up of the remaining 33 patients treated with curative intent, recurrence of disease was observed in 30 patients (90.9%), all of whom underwent treatment. Only three patients (9.1%) remained free of disease until the last date of follow-up. Details on the treatment strategies for the patients treated with palliative intent are also visualized in Figure 1.

<table>
<thead>
<tr>
<th>Table 2. Characteristics of isolated peritoneal recurrences</th>
<th>Curative Treatment n = 43</th>
<th>%</th>
<th>Palliative Treatment n = 63</th>
<th>%</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Time until recurrence*</td>
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<td>-</td>
<td>11.0 (6.3-15.2)</td>
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<td>&lt; 0.001</td>
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<td>Diagnostic method</td>
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<td>32</td>
<td>74.4</td>
<td>30</td>
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<td>Surgical</td>
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<td>7.9</td>
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<td>Clinical (symptoms)</td>
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<td>28</td>
<td>44.4</td>
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<tr>
<td>Recurrence histologically confirmed†</td>
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<td>&lt; 0.001</td>
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<tr>
<td>Yes</td>
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<td>88.4</td>
<td>31</td>
<td>49.2</td>
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<td>Extent of peritoneal recurrence∞</td>
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<td>Multifocal</td>
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<td>6.3</td>
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</table>

Table 2 Characteristics of peritoneal recurrences after initial CRS-HIPEC. Numbers are presented with percentages and medians with interquartile ranges.
* Time from first CRS-HIPEC to time of peritoneal recurrence.
† At time of diagnosis of isolated peritoneal recurrence.
∞ At imaging or surgical inspection.
Survival after treatment of isolated peritoneal recurrences

During follow up, the median time until recurrence was 13.7 months (8.3-19.6) in all patients, and 11.0 months (6.3-15.2) in patients treated with palliative intent compared with 17.7 months (3.8-26.0) in those treated with curative intent (p <0.001).

Median OS after treatment of isolated peritoneal recurrence was favorable in patients treated with curative intent compared to those treated with palliative intent; 24.7 months (IQR 12.1-61.7) versus 7.6 months (IQR 2.5-15.9) respectively (p <0.001) (Figure 2).

Patients treated with curative intent had 1-, 2- and 3-year survival rates after treatment of 74, 50 and 37%, respectively, while survival rates for patients treated with palliative intent were 36, 16 and 6% after 1-, 2- and 3-years, respectively.

In multivariable Cox regression analysis, complication grade 3-4 after first CRS-HIPEC (hazard ratio (HR) 7.02 (95% CI 2.51-19.62) compared with complication grade 0-2 (p <0.001), and treatment of peritoneal recurrence with palliative intent (HR 2.74 (95% CI 1.49-5.05) compared with treatment with curative intent; P=0.001) were associated with poorer OS (Table 3). There was a significant interaction between complication grade and treatment intent in multivariable analysis that was inserted in the model (HR 0.20 (95%CI 0.06-0.61); p =0.005) (Table 3).

Figure 2 Overall survival of patients since treatment of the first peritoneal recurrence with curative or palliative intent
<table>
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<tr>
<th>Table 3. Cox regression for OS</th>
<th>Univariable OS</th>
<th>Multivariable OS</th>
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<td>Median (IQR)</td>
<td>HR (95% CI)</td>
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<td></td>
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<td>Female</td>
<td>14.4 (5.8-44.5)</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>12.0 (7.0-29.3)</td>
<td>0.99 (0.61-1.61)</td>
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<tr>
<td>Present</td>
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<td>0.87 (0.57-1.33)</td>
</tr>
<tr>
<td><strong>Systemic metastases prior to CRS-HIPEC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>12.0 (5.0-24.7)</td>
<td>1</td>
</tr>
<tr>
<td>Present</td>
<td>31.7 (13.6-44.5)</td>
<td>0.45 (0.22-0.89)</td>
</tr>
<tr>
<td><strong>Systemic chemotherapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10.6 (6.2-21.9)</td>
<td>1</td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>5.8 (2.2-16.6)</td>
<td>1.51 (0.77-2.98)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>17.4 (6.3-39.4)</td>
<td>0.76 (0.45-1.28)</td>
</tr>
<tr>
<td>Perioperative</td>
<td>12.2 (3.0-27.2)</td>
<td>0.87 (0.40-1.89)</td>
</tr>
<tr>
<td><strong>Chemotherapy IP used</strong>*</td>
<td>Mitomycin C</td>
<td>13.6 (5.8-29.5)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>6.6 (1.3-12.9)</td>
<td>1.66 (0.76-3.61)</td>
</tr>
<tr>
<td><strong>Region Count</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2 regions</td>
<td>15.5 (5.8-51.7)</td>
<td>1</td>
</tr>
<tr>
<td>3-5 regions</td>
<td>12.2 (5.8-24.7)</td>
<td>1.48 (0.93-2.36)</td>
</tr>
<tr>
<td>6-7 regions</td>
<td>10.6 (3.7-24.0)</td>
<td>1.60 (0.73-3.51)</td>
</tr>
<tr>
<td><strong>Completeness of cytoreduction</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>13.4 (5.6-31.7)</td>
<td>1</td>
</tr>
<tr>
<td>R2a</td>
<td>10.6 (5.8-15.9)</td>
<td>1.68 (0.92-3.05)</td>
</tr>
<tr>
<td><strong>Complication grade</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTCAE 0-2</td>
<td>16.6 (5.8-39.4)</td>
<td>1</td>
</tr>
<tr>
<td>CTCAE 3-4</td>
<td>7.6 (3.8-12.0)</td>
<td>2.76 (1.58-4.20)</td>
</tr>
<tr>
<td><strong>pT-stage</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ pT3</td>
<td>15.8 (6.6-29.7)</td>
<td>1</td>
</tr>
<tr>
<td>pT4</td>
<td>10.3 (3.7-29.5)</td>
<td>1.14 (0.74-1.77)</td>
</tr>
<tr>
<td><strong>pN-stage</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>15.5 (6.6-36.3)</td>
<td>1</td>
</tr>
<tr>
<td>pN1</td>
<td>8.7 (3.0-29.3)</td>
<td>1.37 (0.80-2.34)</td>
</tr>
<tr>
<td>pN2</td>
<td>13.6 (4.1-27.2)</td>
<td>1.39 (0.80-2.44)</td>
</tr>
<tr>
<td><strong>Peritoneal carcinomatosis</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>10.6 (3.7-29.0)</td>
<td>1</td>
</tr>
<tr>
<td>Metachronous</td>
<td>13.4 (7.7-29.7)</td>
<td>0.78 (0.51-1.21)</td>
</tr>
<tr>
<td>Table 3. Continued</td>
<td>Univariable OS</td>
<td>Multivariable OS</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Location primary tumor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appendix</td>
<td>8.2 (6.9-22.3)</td>
<td>0.89 (0.43-1.86)</td>
</tr>
<tr>
<td>Colon</td>
<td>12.2 (5.0-29.3)</td>
<td>1</td>
</tr>
<tr>
<td>Rectum</td>
<td>19.3 (6.2-29.7)</td>
<td>0.81 (0.40-1.62)</td>
</tr>
<tr>
<td><strong>PA type primary tumor</strong></td>
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<td></td>
</tr>
<tr>
<td>AC</td>
<td>13.4 (3.3-39.4)</td>
<td>1</td>
</tr>
<tr>
<td>MC</td>
<td>15.8 (7.7-29.0)</td>
<td>0.98 (0.62-1.55)</td>
</tr>
<tr>
<td>SRCC</td>
<td>7.0 (3.0-12.6)</td>
<td>1.56 (0.81-3.00)</td>
</tr>
<tr>
<td><strong>Differentiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good/Moderate</td>
<td>15.8 (6.3-29.7)</td>
<td>1</td>
</tr>
<tr>
<td>Poor</td>
<td>7.7 (2.5-29.3)</td>
<td>1.34 (0.84-2.14)</td>
</tr>
<tr>
<td><strong>Treatment intent of recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curative</td>
<td>24.7 (12.2-61.7)</td>
<td>1</td>
</tr>
<tr>
<td>Palliative</td>
<td>7.6 (2.5-15.9)</td>
<td>2.68 (1.68-4.28)</td>
</tr>
<tr>
<td><strong>Extent recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>31.7 (12.0-71.2)</td>
<td>1</td>
</tr>
<tr>
<td>Multifocal</td>
<td>10.7 (3.7-22.3)</td>
<td>2.46 (1.36-4.44)</td>
</tr>
<tr>
<td><strong>Time until recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>8.7 (2.6-21.8)</td>
<td>1.38 (0.90-2.11)</td>
</tr>
<tr>
<td>≥ 1 year</td>
<td>15.8 (6.3-32.4)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Complication grade * Treatment intent</strong></td>
<td>NA</td>
<td>2.20 (1.30-3.71)</td>
</tr>
</tbody>
</table>
Table 3 Univariable and multivariable Cox regression analyses for overall survival in all patients (n=106) after treatment of isolated peritoneal recurrence.

*: Factors related to the first CRS-HIPEC.

Abbreviations. OS, overall survival; IQR, interquartile range; HR, Hazard ratio; CI, confidence interval; ASA, American Society of Anesthesiologists; IP, intraperitoneal; CTCAE, Common Toxicology Criteria of Adverse Events; AC, adenocarcinoma; MC, mucinous adenocarcinoma; SRCC, signet ring cell carcinoma.

Treatment with curative intent

In the 33 patients successfully treated with curative intent, time to second recurrence was 10.3 months (IQR 5.7-15.7). Median OS in these patients was 36.3 months (IQR 15.2-63.0) compared with 7.7 months (IQR 5.9-18.7) in patients who did not respond to intentionally curative treatment (p = 0.005).

Median OS in the 17 patients who underwent curative CRS was 51.7 months (IQR 14.4-NA), and 29.0 months (IQR 18.1-63.0) in the 15 patients who underwent curative CRS-HIPEC (p =0.620) (Supplementary Figure 1). In patients with recurrences limited to one or two regions, CRS only was the preferred treatment; these patients had a median region count of 1 (IQR 1-1) during surgery. Patients with multifocal peritoneal recurrences were significantly more often treated with CRS-HIPEC, and these patients had a median region count of 3 (IQR 1-5) (p <0.001).

Comparing the first and second CRS-HIPEC procedures, low- and high-grade postoperative complications did not significantly differ. Median hospital stay was 15 days (IQR 11-20) for the first CRS-HIPEC and 14 days (11-28) for the second CRS-HIPEC.
DISCUSSION

A substantial survival benefit was observed in patients who had isolated, recurrent peritoneal metastases who were treated with curative intent. Approximately 25% of all patients treated with CRS-HIPEC in our cohort recurred intraperitoneally only; in 41% of these patients a curative treatment option was considered. Although most of these patients face recurrence again, their average survival is prolonged.

Repeated CRS-HIPEC was feasible in some patients with isolated peritoneal recurrences in this study. This is concordant with several other studies [9-11, 14-16] and appears to be similar in our study. Complication rates and length of hospital stay for the first and second CRS-HIPEC procedure in this cohort were not statistically significantly different as opposed to the study of Golse et al. in which a higher complication rate for secondary CRS-HIPEC procedures was reported [9]. The only systematic review about repeat CRS (by Williams et al.) concluded that repeat CRS gives possible survival benefit in carefully selected patients [17]. Braam et al. evaluated the treatment options of all recurrences after CRS-HIPEC[18], and showed that patients with locoregional and/or systemic recurrences who were treated with curative intent showed significantly better survival than those who received palliative treatment, i.e., 43 months versus 12 months respectively [18]. The same conclusion could be drawn based on the current study, which is focused on a cohort with isolated peritoneal recurrence.

Ideally, patients eligible for locoregional treatment would be appropriately selected preoperatively to limit the burden of surgery to patients in which complete cytoreduction is feasible. Patients who were scheduled for surgery with curative intent but, in whom CRS was not feasible, clearly did not benefit from therapy and were exposed to surgical complications. In patients with primary colorectal PC, it is hard to preoperatively estimate the extent of disease and to select patients appropriately using computed tomography (CT) imaging only [19]. Laparoscopy is often used to get better insight into the peritoneal tumor burden. In recurrent peritoneal metastases laparoscopy is difficult, due to previous CRS-HIPEC, and it may be hard to distinguish scar tissue and limited peritoneal metastases on CT imaging. Magnetic Resonance Imaging (MRI), whether or not combined with Diffusion Weighted Imaging (DWI), may aid radiological diagnosis. Recently published studies from Dohan et al. and Michielsen et al. support the possible additional value of MRI in patients with peritoneal carcinomatosis [20, 21].
Predictive factors associated with a poor prognosis in patients with primary colorectal peritoneal carcinomatosis who underwent CRS-HIPEC include the extent of peritoneal disease, completeness of cytoreduction, postoperative complications and lymph node status of the primary tumor, which is previously described [7, 8]. Only the presence of postoperative complications after the first CRS-HIPEC was identified as a factor in multivariable analysis when isolated peritoneal recurrences were treated. These complications probably influenced the decision as to whether patients received curative or palliative treatment. Patients with high grade complications were more often treated with palliative intent. The lack of other associations in this cohort could imply that isolated peritoneal recurrences behave independently of the primary CRS-HIPEC characteristics, i.e. positive lymph nodes of the primary tumor. The exact mechanism of peritoneal carcinomatosis remains unclear; however, it is known that most tumor cells in the peritoneal cavity die, but a small fraction can survive and attach to the mesothelium [22]. The tumor microenvironment and cancer-associated fibroblasts (CAFS) present in the stroma play an important role in these attached cells, which contributes to tumor growth, invasion and progression [22, 23]. It could be that due to the biology of the tumor, these patients respond differently to intraperitoneal MMC or oxaliplatin, which was also one of the main reasons to preferably change the chemotherapeutic agent during the second CRS-HIPEC. Patients with PC, even those with relatively limited disease, clearly represent a heterogeneous group.

The retrospective design of this study causing selection bias is the most important limitation that precludes firm inferences. These patients have been selected among others to undergo primary CRS-HIPEC. Subsequently, they recurred intraperitoneally and some of them were selected for treatment with curative intent. However, it is clear that surgery or radiotherapy led to prolonged survival in some patients.

In conclusion, locoregional treatment of isolated peritoneal recurrences after CRS-HIPEC is feasible in approximately half of the patients and should be considered if distant metastases are absent at the time of diagnosis. When curative treatment is obtained, long-term survival can be achieved.
REFERENCES


Hyperthermic intraperitoneal chemotherapy with oxaliplatin for peritoneal carcinomatosis: a clinical pharmacological perspective on a surgical procedure


CHAPTER 4

ABSTRACT

Rationale
Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has become standard of care in the treatment of patients with peritoneal carcinomatosis of colorectal origin. The use of oxaliplatin for HIPEC has gained popularity. Although the HIPEC procedure is adopted throughout the world, major differences exist between treatment protocols regarding the carrier solution, perfusate volume, the use of an open or closed technique, duration of the perfusion and the application of additional flushing. These differences can influence pharmacokinetics and pharmacodynamics of oxaliplatin and may thereby impact efficacy and/or safety of the treatment. Clinicians should be aware of the clinical importance of pharmacology when performing HIPEC surgery.

Conclusion
This review adds new insights into the complex field of pharmacology of HIPEC and highlights an important worldwide problem: the lack of standardisation of the HIPEC procedure.
INTRODUCTION

Metastases to the peritoneal cavity, referred to as peritoneal carcinomatosis (PC), is a common phenomenon in distant metastatic colorectal cancer [3-5]. Patients with isolated metastasis to the peritoneum who are treated with palliative surgery alone or with 5-FU-based regimens have poor overall survival rates of approximately 6 months [6-8]. As a result of the introduction of cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC), median overall survival has significantly increased up to 63 months with a 5-year survival rate of approximately 40% [9]. CRS-HIPEC procedure has become the standard of care in the treatment of patients with PC of colorectal origin [10]. Adequate patient selection remains one of the main challenges since CRS-HIPEC is associated with approximately 1-3% mortality and significant morbidity in one third of patients [9]. The most important prognostic factors that have been identified to influence the outcome after CRS-HIPEC procedure are the extent of the peritoneal disease, the completeness of cytoreduction and the histological subtype of the primary tumour [11].

Different chemotherapeutic drugs can be administered in HIPEC for colorectal PC. Traditionally, mitomycin C (MMC) was the most commonly used drug, but since a few years oxaliplatin is more often used worldwide. Oxaliplatin is the cornerstone in the systemic treatment of patients with colorectal cancer. Results on survival in HIPEC series for CRC are comparable for MMC and oxaliplatin [12-19]. No statistically significant differences were demonstrated in survival and postoperative morbidity after HIPEC with MMC or oxaliplatin [20, 21]. Since a randomised phase III trial comparing MMC and oxaliplatin is lacking, there is no consensus on the intraperitoneal drug of choice. Based on time duration of the perfusion, 30 min for oxaliplatin vs. 90 min for MMC, oxaliplatin is the preferential drug in CRS-HIPEC procedure for colorectal PC in many centres.

The oxaliplatin dose used for HIPEC is 3.5 - 5.4 times the intravenous dose of an infusion at one time delivered to patients with metastatic colorectal cancer in the different treatment regimens [22]. Although CRS-HIPEC is widely applied as the standard treatment for PC of colorectal origin, the exact procedure of HIPEC differs between institutions and surgeons. There is no consensus on applied dose, duration, carrier solution, perfusate volume, perfusate concentration, the use of an open vs. a closed technique, or the usefulness of additional flushing with crystalloids at the end of the HIPEC procedure. These differences
can play an important role in the pharmacokinetics (PK) of oxaliplatin and thereby might influence efficacy and/or safety of the HIPEC procedure. This review provides an overview about pharmacokinetics and pharmacodynamics of oxaliplatin during HIPEC procedures and the implications for clinical practice. It is surprising that a high risk procedure as HIPEC with great impact on survival is not standardised yet.

**ANALYTICAL TECHNIQUES**

Oxaliplatin is highly reactive in blood and forms a variety of hydrolysed intermediates after intravenous infusion, including monochloro- dichloro- and diaquo-platinum species [23, 24]. Up to 17 platinum-containing derivatives have been observed in plasma ultra filtrate (UF) samples from patients [25]. These intermediates rapidly react with endogenous low-molecular-weight molecules such as glutathione, cysteine, and methionine, and high-molecular-weight compounds such as albumin, globulin and haemoglobin [23, 26, 27]. At the end of a two hour intravenous administration of oxaliplatin, approximately 40% of the administered platinum is bound to erythrocytes and approximately 33% is bound to plasma proteins [28]. The unbound platinum is generally considered as the pharmacologically active moiety [23, 24], although the relationship between free platinum and the pharmacological activity and toxicity is not as clear as for carboplatin [29]. Free platinum concentrations are a sum of active as well as inactive forms of free platinum. Some authors suggest that the parent drug oxaliplatin is the pharmacologically active moiety [26, 30, 31]. Analysis of the free fraction of the parent drug oxaliplatin has revealed a very short terminal half-life for intact oxaliplatin of only 14 minutes in blood [26].

Considering this, it is important to differentiate between the analytical techniques that are used for the detection of platinum derivatives when interpreting pharmacokinetic data. The majority of PK studies that have been performed with the HIPEC procedure, determined the platinum content by flameless atomic absorption spectrometry (AAS) [32-38], direct current plasma emission spectroscopy (DC-PES) [39], inductively coupled plasma optical emission spectrometry (ICP-OES) [40-42] or inductively coupled plasma mass spectrometry (ICP-MS) [43, 44]. These analytical techniques measure both the parent drug oxaliplatin and other active and inactive platinum containing complexes that are formed as a result of high reactivity of oxaliplatin in vivo. This might lead to overestimation of the concentration of active drug, because of non-specificity. Two studies have investigated the PK of intact oxaliplatin during HIPEC [37, 45]. Measurement of intact oxaliplatin and total platinum content can result in
large differences in PK parameter estimation, such as a oxaliplatin plasma clearance of 28.4 l h⁻¹m⁻² based on intact oxaliplatin [45] versus 6.68 l h⁻¹m⁻² based on AAS [35]. On the other hand, the extent of drug absorbed during HIPEC (36-60% [45] vs. 40-68% [35]) and the volume of distribution (0.294 l/kg [46] vs. 0.235 l/kg [35]) seem to be consistent. To date, it is unknown which analytical measurement (intact oxaliplatin or the mixture of platinum derivates) is the best surrogate marker to predict both toxicity and efficacy of oxaliplatin-based HIPEC. Future research should be performed to provide answers to this unresolved question.

CARRIER SOLUTIONS IN HIPEC

The ideal carrier solution for HIPEC with oxaliplatin should provide an equal distribution of the cytotoxic drug and heat, with minimal loss of volume during perfusion. This requires minimal transport of fluid and electrolytes from the peritoneal compartment to the plasma and stability of the drug in the carrier solution. Because oxaliplatin can react with chloride ions, causing degradation, dextrose 5% is often used as carrier solution in oxaliplatin-based HIPEC protocols [47]. A disadvantage of the use of dextrose 5% is the inability to maintain a high intraperitoneal fluid volume, due to rapid absorption [47]. High intraperitoneal volumes of dextrose 5% require high doses of insulin to prevent severe hyperglycaemia, causing major electrolyte disturbances during perfusion [48-51]. Other carrier solutions that have been investigated are hypertonic solutions, hypotonic solutions and isotonic high molecular weight solutions. The main disadvantage of hypertonic solutions is dilution of the drug due to fluid shift towards the peritoneal cavity [47]. Both in vitro and animal studies suggest that the use of hypotonic solutions can enhance platinum accumulation in tissue [52, 53]. Nevertheless, this effect could not be replicated in humans and because of an increased risk of haemorrhage and thrombocytopenia, the use of hypotonic solutions for HIPEC with oxaliplatin is discouraged [54]. Some centres advise the use of high molecular weight solutions such as icodextrin [42, 55]. The small differences that are found in rate and extent of oxaliplatin absorption between glucose 5% and icodextrin 4% are deemed clinically irrelevant [55]. Promising results have been seen with the use of the peritoneal dialysis fluids Physioneal 40 dextrose 2.27% solution [37] and Dianeal PD4 dextrose 1.36% solution [56, 57], showing minimal electrolyte and glycemic disturbances during HIPEC procedure. The cytotoxic properties of oxaliplatin in Physioneal 40 dextrose 2.27% solution remain unchanged during HIPEC procedure [37]. These findings support the use of perfusates containing lower concentrations of dextrose.

However, there is currently no consensus on the best type of carrier solution, showing the need for further research, as different carrier solutions may result in different outcomes.
DRUG PENETRATION IN TUMOUR TISSUE

The goal of intraperitoneal administration of oxaliplatin is to obtain high local concentrations and high penetration in tumour tissue to enhance efficacy. Although tissue penetration seems important, a relationship between tissue concentration and efficacy of the HIPEC procedure has not yet been described. Given the heterogeneity of colorectal carcinoma, the optimal tissue concentration might differ for each patient or even in different tumours within the same patient. Drug penetration is limited to only a few cell layers under the tumour surface [58]. This highlights the importance of complete cytoreduction to warrant optimal effect of intraperitoneal administered oxaliplatin. Elias et al. [32] showed that concentration of platinum in healthy peritoneum tissue exposed to oxaliplatin solution during HIPEC is a good reflection of platinum concentration in peritoneal tumour tissue. A recent study collected tissue samples after HIPEC with oxaliplatin and demonstrated no significant difference (p=0.38) between the platinum concentration in dry tissue of peritoneum and in subjacent fascia [37].

Table 1 provides an overview of human and animal studies in which platinum tissue concentration during HIPEC procedure with oxaliplatin were measured. Because of major differences in the procedure of HIPEC it is hard to compare the individual studies. Factors that have been identified to have an impact on tumour tissue platinum concentration are hyperthermia, pressure, dose, perfusate concentration, type of carrier solution and pre-treatment with 5-FU [32, 37-40, 54, 59, 60].

Effect of hyperthermia and pressure

The rationale for using hyperthermia in cancer treatment relates to tumour cells being more susceptible to heat than non-malignant cells. Hyperthermia impairs DNA replication and disturbs multiple DNA repair pathways, and thus sensitises cancer cells to cytotoxic agents leading to increased cell death [61]. Local heating of tumours also triggers multiple anti-tumour immune responses and facilitates increased trafficking of immune cells between tumours and draining lymph nodes [62].

Besides intrinsic anti-tumour activities, hyperthermia also enhances cytotoxicity of oxaliplatin [59, 63]. A study in rats showed that peritoneal tissue concentration of oxaliplatin significantly increased with higher temperature [59]. An animal study in a swine model using a semi open technique with constant oxaliplatin concentration showed that hyperthermia (42°C) increased tissue concentration of oxaliplatin in visceral peritoneum compared to normothermia. High pressure also increased oxaliplatin tissue concentration in both visceral
and parietal peritoneum compared to normal pressure [38]. High intra-abdominal pressure can be achieved using a HIPEC procedure with a closed abdominal wall [38, 64]. On the other hand, higher tissue concentrations and a homogenous distribution of oxaliplatin in perfusate can be achieved using the open technique [65, 66].

**Effect of dose and concentration**

A study in rats showed that tissue distribution is significantly increased by the use of higher doses of oxaliplatin. A six fold increase in tissue concentration was seen with a four fold increase of dose [59]. In a phase I clinical study an increase in tumour platinum concentration was found with every dose escalation step of 50 mg/m². Tumour platinum exposure increased with a factor 1.5 between the lowest and highest dose tested, which were 260 mg/m² and 460 mg/m² [32]. HIPEC with oxaliplatin perfused at a temperature of 40°C over a time period of two hours showed a factor 1.3 increase in tumour platinum exposure between a dose of 200mg/m² and 250mg/m². Nevertheless, the maximum tolerated dose for 2-h perfusion of oxaliplatin was 200mg/m² in that study [40].

Perfusate volume and perfusate concentration are important variables for pharmacokinetics during HIPEC procedure. Diffusion of oxaliplatin from perfusate to peritoneal tissue and blood is driven by a concentration gradient. A higher perfusate volume of 2.5 l/m² instead of 2 l/m² decreases platinum intraperitoneal concentration by 20% [32]. Plasma maximal concentration ($C_{\text{max}}$) and systemic exposure is similar for 410 mg/m² oxaliplatin in 2.5 l/m² and 310 mg/m² oxaliplatin in 2 l/m². This indicates that pharmacokinetics and tissue penetration of oxaliplatin are influenced more by the concentration in perfusate than by the total dose administered [32, 59]. Some publications describe the use of a standard perfusate volume of 2 l/m² with a fixed dose of 460mg/m². In this case all patients are treated with a fixed concentration of 230 mg/l oxaliplatin in perfusate at the beginning of the HIPEC procedure. Using perfusate volumes above 2 l/m² along with a fixed dose per m², might negatively influence efficacy because tumour exposure will be decreased. On the other hand, the use of fixed volumes of 2 l/m² can cause inadequate tissue contact time in patients with a relative large abdominal cavity. Some centres fill the abdominal cavity completely before the administration of oxaliplatin. This causes great variation in oxaliplatin concentrations and therefore is likely to influence tumour penetration and systemic absorption of oxaliplatin which may influence efficacy and/or safety of the treatment.
<table>
<thead>
<tr>
<th>Author/year</th>
<th>Subjects</th>
<th>Treatment strategy</th>
<th>Oxaliplatin dose (mg/m²)</th>
<th>Carrier solution</th>
<th>Duration, flow rate</th>
<th>Temperature (°C)</th>
<th>Flushing afterwards</th>
<th>Analytical method</th>
<th>Total platinum tissue concentration (ng/mg)</th>
</tr>
</thead>
<tbody>
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<td>Elias et al. / 2002 [32]</td>
<td>n = 5</td>
<td>Open Coliseum technique</td>
<td>260</td>
<td>5% dextrose, 2 L/m²</td>
<td>30 min (+ approximately 10 min required to reach high homogeneous temperature), 2 L/min</td>
<td>42 – 44</td>
<td>no</td>
<td>AAS</td>
<td>Tumor tissue: 228 μg/g dry tissue Normal peritoneum: 230 μg/g dry tissue</td>
</tr>
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<td>n = 3</td>
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<td>460</td>
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<td>n = 4</td>
<td>Open Coliseum technique</td>
<td>460</td>
<td>100 mosm/L dextrose, 2 L/m²</td>
<td>30 min (+ approximately 5 min required to reach high homogeneous temperature), 2 L/min</td>
<td>42 – 44</td>
<td>no</td>
<td>AAS</td>
<td>Tumor tissue: 480 ± 82 μg/g dry tissue Normal peritoneum: 396 ± 73 μg/g dry tissue</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Subjects</th>
<th>Treatment strategy</th>
<th>Oxaliplatin dose (mg/m²)</th>
<th>Carrier solution</th>
<th>Duration, flow rate</th>
<th>Temperature (°C)</th>
<th>Flushing afterwards</th>
<th>Analytical method</th>
<th>Total platinum tissue concentration (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elias et al. / 2002 [54]</td>
<td>n = 4</td>
<td></td>
<td>150 mosm/L dextrose, 2 L/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor tissue: 440 ± 108 μg/g dry tissue Normal peritoneum: 449 ± 83 μg/g dry tissue</td>
</tr>
<tr>
<td></td>
<td>n = 3</td>
<td></td>
<td>200 mosm/L dextrose, 2 L/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor tissue: 322 ± 76 μg/g dry tissue Normal peritoneum: 314 ± 101 μg/g dry tissue</td>
</tr>
<tr>
<td></td>
<td>n = 3 (+3)</td>
<td></td>
<td>300 mosm/L dextrose, 2 L/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor tissue: 299 ± 87 μg/g dry tissue Normal peritoneum: 339 ± 92 μg/g dry tissue</td>
</tr>
<tr>
<td>Stewart et al. / 2008 [40]</td>
<td>n = 12</td>
<td>Closed</td>
<td>200</td>
<td>5% dextrose, fixed volume of 3 L</td>
<td>120 min, 0.8-1 L/min</td>
<td>40.6 ± 0.5</td>
<td>Yes, with 3L of crystalloid</td>
<td>ICP-OES</td>
<td>Tumor tissue: 15.9 ± 12.0 μg/g Normal peritoneum: 17.7 ± 10.8 μg/g</td>
</tr>
<tr>
<td></td>
<td>n = 3</td>
<td></td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor tissue: 20.3 ± 9.7 μg/g Normal peritoneum: 26.3 ± 14.7 μg/g</td>
</tr>
<tr>
<td>Löffler et al. / 2017 [37]</td>
<td>n = 9</td>
<td>not mentioned</td>
<td>300</td>
<td>Physioneal 40 dextrose 2.27% w/v Clear-Flex peritoneal dialysis solution, 5-6L (different concentrations; 93.7 ± 12.2 [75.0-108.9] μg/ml)</td>
<td>30 min, ?</td>
<td>42</td>
<td>not mentioned</td>
<td>AAS</td>
<td>Normal peritoneum: 50 μg/g dry tissue (range 5-203 μg/g) Subjacent fascia: 70 μg/g dry tissue (range 0-103 μg/g)</td>
</tr>
<tr>
<td>Author/year</td>
<td>Subjects</td>
<td>Treatment strategy</td>
<td>Oxaliplatin dose (mg/m²)</td>
<td>Carrier solution</td>
<td>Duration, flow rate</td>
<td>Temperature (°C)</td>
<td>Flushing afterwards</td>
<td>Analytical method</td>
<td>Total platinum tissue concentration (ng/mg)</td>
</tr>
<tr>
<td>-------------</td>
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<td>--------------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td><strong>Animal studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Facy et al. / 2012 [38] (pigs) | n = 5 | Semi open | 150 mg/l | 5% dextrose, 4L | 30 min, ? | 38 | Yes, with 1L of 5% dextrose | AAS | Parietal peritoneum: 35.62 ± 8.81 μg/g  Visceral peritoneum: 5.48 ± 0.70 μg/g  
| | n = 5 | Semi open | 150 mg/l | 5% dextrose, 8L | 38 | Yes, with 1L of 5% dextrose | 38 | Parietal peritoneum: 53.61 ± 10.32 μg/g  Visceral peritoneum: 9.99 ± 1.66 μg/g  
| | n = 5 | Semi open | 150 mg/l | 5% dextrose, 8L | 38 | Yes, with 1L of 5% dextrose | 38 | Parietal peritoneum: 66.16 ± 13.03 μg/g  Visceral peritoneum: 10.39 ± 3.49 μg/g  
| | n = 5 | Closed | 920 | 5% dextrose, 15mL | 25 min, 16mL/min | 37 | Yes, with 20mL of 5% dextrose | HPLC with Phenomenex column and a precolumn of C18 | Normal peritoneum: 5.7 ± 0.4 μg/mL  
| Piché et al. / 2011 [59] (rats) | n = 5 | Closed | 920 | 5% dextrose, 15mL | 25 min, 16mL/min | 37 | Yes, with 20mL of 5% dextrose | HPLC with Phenomenex column and a precolumn of C18 | Normal peritoneum: 5.7 ± 0.4 μg/mL  
| | n = 7 | | | | | | | | Normal peritoneum: 6.5 ± 0.3 μg/mL  
| | n = 13 | | | | | | | | Normal peritoneum: 7.9 ± 0.6 μg/mL  
| | n = 6 | | | | | | | | Normal peritoneum: 2.4 ± 0.7 μg/mL  
| | n = 6 | | | | | | | | Normal peritoneum: 15.5 ± 6.6 μg/mL  
| | | | | | | | | |  

**Table 1.** Continued
<table>
<thead>
<tr>
<th>Author/year</th>
<th>Subjects</th>
<th>Treatment strategy</th>
<th>Oxaliplatin dose (mg/m²)</th>
<th>Carrier solution</th>
<th>Duration, flow rate</th>
<th>Temperature (˚C)</th>
<th>Flushing afterwards</th>
<th>Analytical method</th>
<th>Total platinum tissue concentration (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pestieau et al. / 2001 [39] (rats)</td>
<td>n = 5</td>
<td>Closed</td>
<td>15 mg/kg</td>
<td>5% dextrose, 150 mL</td>
<td>90min, 80 ml/min</td>
<td>35-37</td>
<td>n o mentioned</td>
<td>DC-PES</td>
<td>Colonic tissue: 25 ± 9 μg/mL</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40-42</td>
<td></td>
<td></td>
<td>Colonic tissue: 28 ± 6 μg/mL</td>
</tr>
</tbody>
</table>

Table 1. Overview of human and animal studies measuring total platinum tissue exposure during HIPEC procedure with oxaliplatin.

AAS = atomic absorption spectrometry  
ICP-OES = inductively coupled plasma optical emission spectrometry  
HPLC = High-performance liquid chromatography  
DC-PES = direct current plasma emission spectroscopy
Table 2. Pharmacokinetic studies of oxaliplatin in patients undergoing HIPEC procedure with oxaliplatin

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Subjects</th>
<th>Treatment strategy</th>
<th>Oxaliplatin dose (mg/m²)</th>
<th>Carrier solution</th>
<th>Duration, flow rate</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elias et al. / 2002 (30)</strong></td>
<td></td>
<td>Open Coliseum technique</td>
<td>260</td>
<td>5% dextrose, 2 L/m²</td>
<td>30 min (+ approximately 10 min required to reach high homogeneous temperature), 2 L/min</td>
<td>42 – 44</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>n = 3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 3+3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n = 3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Elias et al. / 2002 (52)</strong></td>
<td></td>
<td>Open Coliseum technique</td>
<td>460</td>
<td>100 mosm/L dextrose, 2 L/m²</td>
<td>30 min (+ approximately 5 min required to reach high homogeneous temperature), 2 L/min</td>
<td>42 - 44</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n = 3</td>
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<td></td>
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</tr>
<tr>
<td>n = 3 (+3)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Mahteme et al. / 2008 (43)</strong></td>
<td></td>
<td>Open Coliseum technique</td>
<td>427 ± 29</td>
<td>5% dextrose, 3.2 ± 0.7 L</td>
<td>30 min (+ time required to reach high homogeneous temperature), ?</td>
<td>41.5 - 43</td>
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<tr>
<td>n = 8</td>
<td></td>
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<td><strong>Ferron et al. / 2008 (33)</strong></td>
<td></td>
<td>Open Coliseum technique</td>
<td>360 (n=7) and 460 (n=17)</td>
<td>5% dextrose, 2 L/m²</td>
<td>30 min (+ approximately 8-10 min required to reach high homogeneous temperature) (12x) and 30min (12x), 2 L/min</td>
<td>42 – 43</td>
</tr>
<tr>
<td>n = 24</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Chalret du Rieu et al. / 2014 (34)</strong></td>
<td></td>
<td>Open Coliseum technique</td>
<td>360</td>
<td>5% dextrose, 2 L/m²</td>
<td>30 min (+ approximately 8-10 min required to reach high homogeneous temperature) (12x) and 30min (63x), 2 L/min</td>
<td>42 – 43</td>
</tr>
<tr>
<td>n = 58</td>
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<td>n = 17</td>
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</table>
### Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Flushing afterwards</th>
<th>Analytical method</th>
<th>Total platinum $t_{1/2}$ perfusate (min)</th>
<th>Total platinum $C_{\text{max}}$ (µg/ml)</th>
<th>AUC (µg/ml/h)</th>
<th>Ultrafilterable platinum $C_{\text{max}}$ (µg/ml)</th>
<th>AUC (µg/ml/h)</th>
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<tbody>
<tr>
<td>no</td>
<td>AAS</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
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<td>-</td>
<td>-</td>
<td>14</td>
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<td></td>
<td></td>
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<td>-</td>
<td>-</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.2</td>
<td>-</td>
<td>8.5</td>
<td>17</td>
</tr>
<tr>
<td>no</td>
<td>AAS</td>
<td>35</td>
<td>15.0 ± 2.3</td>
<td>92.3 ± 10.1</td>
<td>8.7 ± 1.7</td>
<td>16.7 ± 2.3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>13.6 ± 1.7</td>
<td>104.0 ± 12.1</td>
<td>9.1 ± 1.4</td>
<td>18.6 ± 2.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10.4 ± 0.9</td>
<td>72.3 ± 6.3</td>
<td>7.6 ± 1.2</td>
<td>15.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.0 ± 1.8</td>
<td>81.6 ± 7.2</td>
<td>7.8 ± 1.1</td>
<td>17.6 ± 1.9</td>
</tr>
<tr>
<td>yes, with an unknown amount of saline</td>
<td>LC with porous graphitic carbon (PGC) and post-column derivation with sodium diethyldithiocarbamate (DDTC) in a microwave field followed by photometric detection</td>
<td>29.5 [21.1-41.2] †</td>
<td>-</td>
<td>-</td>
<td>8.3 ± 1.8 †</td>
<td>26220 ± 4290 †</td>
</tr>
<tr>
<td>yes, with an unknown amount of saline</td>
<td>AAS</td>
<td>29 [18-42]</td>
<td>-</td>
<td>-</td>
<td>13.7 [8.0 – 20.0]</td>
<td></td>
</tr>
<tr>
<td>yes, with an unknown amount of saline</td>
<td>AAS</td>
<td>29.6 ± 6</td>
<td>-</td>
<td>-</td>
<td>16.1 ± 4.9</td>
<td>22.9 ± 4.7</td>
</tr>
</tbody>
</table>
Table 2. Pharmacokinetic studies of oxaliplatin in patients undergoing HIPEC procedure with oxaliplatin.
† unbound oxaliplatin parent drug
AAS = atomic absorption spectrometry
ICP-OES = inductively coupled plasma optical emission spectrometry
ICP-MS = inductively coupled plasma mass spectrometry
LC = Liquid chromatograph
### Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Flushing afterwards</th>
<th>Analytical method</th>
<th>Total platinum ( t_{1/2} ) perfusate (min)</th>
<th>C( _{\text{max}} ) (µg/ml)</th>
<th>AUC (µg/ml/h)</th>
<th>C( _{\text{max}} ) (µg/ml)</th>
<th>AUC (µg/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not mentioned</td>
<td>ICP-MS</td>
<td>-</td>
<td>-</td>
<td>138.1 ± 33.1 mg( \times ) min/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>yes, with 3L of crystalloid</td>
<td>ICP-OES</td>
<td>70.1 ± 23.8</td>
<td>2.2 ± 0.77</td>
<td>23.2 ± 11.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.3 ± 10.3</td>
<td>3.2 ± 0.6</td>
<td>31.1 ± 3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not mentioned</td>
<td>ICP-OES</td>
<td>132</td>
<td>2.56 ± 0.9</td>
<td>87.20 ± 123.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not mentioned</td>
<td>ICP-OES</td>
<td>76.8 ± 21</td>
<td>20.5 ± 4.3</td>
<td>192 ± 45.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.4 ± 29.4</td>
<td>22.3 ± 9.1</td>
<td>213 ± 72.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### PHARMACOKINETICS OF OXALIPLATIN DURING HIPEC

Table 2 provides an overview of PK studies of oxaliplatin in patients undergoing HIPEC procedure.

**Absorption of platinum from the peritoneal compartment**

In the peritoneal compartment the great majority of administered drug is present as unbound platinum, which is available for anti-tumour activity and transfer to the bloodstream. This indicates a low protein-platinum binding in perfusate solution. Although unbound platinum in perfusate has only been studied for intraperitoneal administration of cisplatin [67, 68], this may also apply to the administration of oxaliplatin. Given the high reactivity of oxaliplatin with serumproteins such as albumin, it can be speculated that extensive cytoreduction, bleeding, or severed tissue surfaces, will decrease the fraction of unbound platinum in perfusate. No significant association has been found between oxaliplatin systemic exposure and the extent of the surgery or the peritoneal cancer index (PCI) [36].
Oxaliplatin is rapidly absorbed from the peritoneal compartment with reported mean peritoneal half-lives \( (t_{1/2}) \) of 29 min [35, 36], 35min [54] and 40 min [32], indicating that approximately half of the dose is cleared from the peritoneal compartment during a 30-min HIPEC procedure. Some studies report much longer peritoneal \( t_{1/2} \) up to 2.2 hours [40-42]. The absorption rate constant of oxaliplatin from peritoneal perfusate to plasma is independent of the administered dose and shows low inter-patient variability (coefficient of variation of 22%) [35]. Furthermore, modifications in osmolarity of the carrier solution (with fixed oxaliplatin concentration) does not affect systemic oxaliplatin absorption [54]. A positive correlation has been found between the percentage of systemic absorbed platinum and the body mass index (BMI) of patients [45]. This might be due to a larger peritoneum surface area in patients with higher BMI. This could not be replicated in another study using closed HIPEC procedure [43]. Nevertheless, this study shows that body surface area (BSA) is a predictor of systemic exposure of total platinum. Patients with higher BSA show a lower plasma oxaliplatin Area Under the Concentration-time curve (AUC) over a 1-hour closed HIPEC procedure, possibly caused by lower drug concentrations in perfusate in these patients [43]. Peritoneal AUC and systemic AUC of oxaliplatin during HIPEC is not influenced by disease burden or the extent of peritonectomy, indicating that an intact peritoneum is not required to maintain the concentration differences between perfusate and plasma observed during HIPEC procedure [43, 69].

HIPEC performed with sodium bicarbonate containing carrier solution shows a fast decline of free platinum compounds in the perfusate with a recovery of only 50% at 5 minutes after the start of perfusion [37]. This fast decline of free platinum compounds in the perfusate might not only be explained by absorption from the peritoneal compartment, but also by reaction with solid tissues in the peritoneum and degradation in the perfusate solution, most probably caused by reaction with erythrocytes and other cell types or debris circulating in the perfusate. Only a small fraction of 10-15% of the parent drug oxaliplatin is consistently detectable during 30-min HIPEC with 300mg/m² oxaliplatin. This can be the result of rapid nonenzymatic transformation into reactive compounds in perfusate. Nevertheless, it was shown that bioactivity in perfusate was preserved during the whole HIPEC procedure of 30 minutes [37]. When dextrose 5% is used as carrier solution, the parent drug oxaliplatin is more stable in perfusate, with a degradation of only 5-10% at the end of the HIPEC procedure [45]. These great differences in recovery of the parent drug oxaliplatin could be explained by different carrier solution, given a higher reactivity with sodium bicarbonate containing carrier solutions [37]. Nevertheless, both studies use different analytical methods making it hard to adequately compare these results.
Systemic pharmacokinetics of oxaliplatin with HIPEC

One of the advantages of intraperitoneal administration of oxaliplatin is the use of high drug doses with relatively low systemic exposure. Inter-individual variability of central volume of distribution and plasma clearance is larger than for peritoneal volume of distribution and peritoneal clearance [35]. The $C_{\text{max}}$ of platinum in plasma is reached at the end of the HIPEC procedure [32, 54]. After evacuation of the oxaliplatin solution from the abdominal cavity, the plasma concentration of platinum rapidly drops. Some studies show a relative small systemic exposure (AUC) that is comparable to AUC values observed after a two hour intravenous infusion of oxaliplatin at a dose of 130 mg/m$^2$ [32]. Others found a two times higher systemic exposure of the parent drug oxaliplatin after HIPEC compared to intravenous infusion of 130 mg/m$^2$ oxaliplatin over 30min [45]. Systemic exposure of oxaliplatin increases with higher doses [32, 36]. A study in rats showed that higher perfusion temperature decreased systemic exposure of oxaliplatin [59] and decreased drug absorption in kidney tissue [39]. The reason for these findings is unknown, but might be associated with higher reactivity in peritoneum.

High systemic exposure of oxalipatin during HIPEC can lead to thrombocytopenia and neutropenia. The most frequently reported toxicities after HIPEC with oxaliplatin are haemoperitoneum (23%), neuropathy (19%), thrombocytopenia (13%) and ascites (4%) [36]. Although haemoperitoneum is a surgical postoperative complication, high systemic oxaliplatin exposure can increase the risk of haemoperitoneum. Neutropenia is rarely observed [36].

To date, only two studies investigated the PK of intact oxaliplatin during HIPEC [37, 45]. Huge differences were found between the plasma clearance of unbound intact oxaliplatin (28.4 l h$^{-1}$ m$^{-2}$) [45] and total unbound platinum (6.68 l h$^{-1}$ m$^{-2}$) [35]. The systemic exposure of unbound intact oxaliplatin is about four times lower as the systemic exposure of total unbound platinum. This can be explained by the fact that, with time, the amount of intact oxaliplatin will constitute a gradually decreasing fraction of total unbound platinum due to reactivity with endogenous compounds [45].

Additional Flushing

There is no consensus about the usefulness of flushing the abdominal cavity with crystalloids at the end of oxaliplatin administration. When flushing is performed, its purpose is to minimise systemic exposure of both patient and personnel as well as evacuate remaining debris and clots due to the surgery and resulting bleeding. However, HIPEC without flushing might increase tumour exposure because intraperitoneal tumour cells might be exposed to high concentrations of oxaliplatin for a longer time period. Currently, there is a lack of knowledge on the effect of additional flushing on oxaliplatin pharmacokinetics.
PHARMACODYNAMICS OF OXALIPLATIN DURING HIPEC

Few studies have been performed investigating pharmacodynamics of oxaliplatin during HIPEC [36, 41, 70, 71]. These studies use pharmacokinetic-pharmacodynamic (PK/PD) models to find associations between pharmacokinetic parameters and pharmacodynamic toxicities of the treatment. It has been demonstrated that HIPEC induced neutropenia largely depends on the duration of the HIPEC procedure and the oxaliplatin concentration in perfusate, which is related to systemic concentrations [41]. It is being predicted that each 400mg/L increase in initial oxaliplatin concentration causes 28% decrease in absolute neutrophil count (ANC) at day 7 [70]. Extension of the duration of HIPEC from 30 to 60 minutes is predicted to result in a 23% decrease in ANC at day 7 [70]. This is relevant because postoperative infectious complications can be expected within the first week after surgery. Main determinants for the duration and severity of HIPEC-induced thrombocytopenia are the initial oxaliplatin concentration and the duration of the HIPEC procedure [71].

The systemic exposure to oxaliplatin is associated with the severity of thrombocytopenia and the occurrence of haemoperitoneum [36]. An increase of approximately 20% in systemic oxaliplatin exposure resulted in a decrease of platelets and an increase in chance of developing haemoperitoneum. No associations were found between neither intraperitoneal nor systemic oxaliplatin exposure and the onset of ascites or neuropathy [36].

The pharmacokinetic studies that have been performed so far suggest that higher doses of oxaliplatin could be used for HIPEC procedure, without substantially increasing the risk of major haematological toxicity. Nevertheless, based on the data summarised above, this should be performed with great caution.

THE RATIONALE FOR ADMINISTRATION OF 5-FU PRIOR TO ADMINISTRATION OF OXALIPLATIN

The combination of intravenous administration of oxaliplatin with 5-fluorouracil-leucovorin significantly improves antitumour efficacy in patients with metastatic colorectal cancer [72]. There is a synergistic effect of oxaliplatin and 5-fluorouracil [73]. 5-fluorouracil cannot be mixed with oxaliplatin due to incompatibility. This is the reason for the clinical use of intraoperative intravenous administration of 5-fluorouracil and leucovorin in conjunction with intraperitoneal perioperative oxaliplatin [32]. Although the peritoneum and abdominal cavity have an impaired blood supply causing a limited efficacy of systemic therapy, it is demonstrated that intravenous administration 5-fluorouracil penetrates rapidly into heated tumour nodules during HIPEC [74]. The simultaneous administration of intravenous and
intraperitoneal chemotherapy creates an ideal situation for enhancement of cytotoxicity in the heated tumour nodules. A recent study in rats showed that intravenous administration of 5-fluorouracil enhances peritoneal absorption of oxaliplatin [60]. This highlights the importance of the administration of 5-fluorouracil in the management of PC of gastrointestinal origin.

**KNOWLEDGE GAPS**

Although several studies have investigated drug penetration in tumour tissue during HIPEC procedure, a relationship between tumour platinum exposure and clinical outcome is lacking. It can be assumed that tissue penetration is an important factor for optimal drug effect. Nevertheless, the optimal drug concentration to attain complete tumour cell death remains unclear and might differ for individual patients and tumours. Higher tumour exposures can be achieved with the use of higher perfusate concentrations and a longer duration of the procedure, but this will also cause increased systemic absorption which is related to toxicity. Future studies should be performed to investigate the relationship between tumour exposure and efficacy and systemic exposure and toxicity. This will help to find the most optimal HIPEC procedure for each patient.

**FUTURE PERSPECTIVES**

For patients with advanced PC who are not eligible for HIPEC with curative intent, a novel treatment has been recently introduced called pressurised intraperitoneal aerosol chemotherapy (PIPAC). This technique is minimally invasive and combines the advantages of local administration with pressurised vaporisation. Although no clear indication for PIPAC has been defined yet, the treatment was reported to be feasible, well tolerated and safe [75]. However, available data is limited by small sample sizes, heterogeneity and the lack of control groups.

New techniques, such as organoid technology, [76] create great opportunities for future HIPEC research. Organoids are three-dimensional (3D) stem cell cultures that self-organise into ex vivo ‘mini-organs’. Organoids generated from colorectal carcinomas can be used to test several cytotoxic agents, concentrations, durations, temperatures and frequencies, to optimise current intraperitoneal chemotherapy. The first study using this approach showed that oxaliplatin was the most efficient cytotoxic agent in patients with PC from colorectal
cancer origin [77]. Using organoids generated from colorectal carcinomas of individual patients will create opportunities to individualise HIPEC procedures. Future studies should investigate the opportunities of these individualised approaches, which can theoretically create the most optimal treatment with high tumour exposure and efficacy with acceptable systemic exposure and toxicity.

**CONCLUSIONS**

Currently, there is a wide variety in procedures and a lack of clinical data on PK in HIPEC. Several important factors can influence the pharmacokinetic profile of oxaliplatin in HIPEC procedures. The variety of analytical techniques and HIPEC procedures make it difficult to compare individual studies. Although HIPEC is nowadays widely accepted as effective curative treatment option, the exact procedure can differ between institutions. There is a need for standardisation of the first line HIPEC procedure with oxaliplatin in patients with PC of colorectal origin. Given the complexity of the procedure, there is a need for a multidisciplinary approach, combining the expertise of surgeons, medical oncologists, perfusionists, anaesthesiologists and pharmacists.
REFERENCES


Effect of intraperitoneal chemotherapy concentration on morbidity and survival


British Journal of Surgery Open, April 2020, 4(2):293-300

*Equal contributions
ABSTRACT

Background
Selected patients with colorectal peritoneal metastases (PM) are treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). Concentration of intraperitoneal chemotherapy reflects the administered dose and perfusate volume. The aim of this study was to calculate intraperitoneal chemotherapy concentration during HIPEC and see whether this was related to clinical outcomes.

Methods
An observational multicentre study included consecutive patients with colorectal PM who were treated with CRS-HIPEC between 2010 and 2018 at three Dutch centres. Data were retrieved from prospectively developed databases. Chemotherapy dose and total circulating volumes of carrier solution were used to calculate chemotherapy concentrations. Postoperative complications, disease-free and overall survival were correlated with intraoperative chemotherapy concentrations. Uni- and multivariable logistic regression, Cox regression and survival analyses were performed.

Results
Of 320 patients, 220 received intraperitoneal Mitomycin C (MMC) and 100 oxaliplatin (OXA). Median perfusate volume for HIPEC was 5.0L (range 0.7–10.0). Median intraperitoneal concentration was 13.3 mg/L (range 7.0–76.0) for MMC and 156.0 mg/L (range 91.9–377.6) in patients treated with OXA. Grade 3 or greater complications occurred in 23.4% (n=75). Median overall survival was 36.9 months (IQR 19.5–62.9). Intraperitoneal chemotherapy concentrations were not associated with postoperative complications or survival.

Conclusion
CRS-HIPEC was performed with a wide variation in intraperitoneal chemotherapy concentrations that were not associated with complications or survival.
INTRODUCTION

Selected patients with colorectal peritoneal metastases (CRPM) are currently offered cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). This results in improved median survival compared to systemic chemotherapy [1-3]. Successful treatment of CRPM with CRS-HIPEC depends on several factors, including optimal patient selection and completeness of cytoreductive surgery [4, 5]. The concentration of the active agent used for intraperitoneal chemotherapy may also be important. The most widely used intraperitoneal chemotherapeutic drugs are Mitomycin C (MMC), oxaliplatin (OXA) or irinotecan[6], any of which may be used with systemic therapies. Existing studies show major differences regarding the intraperitoneal temperature, duration and perfusate volume [7, 8]. Intraperitoneal chemotherapy dosage is usually based on body surface area (BSA). Carrier solution volume has, however, received little attention. In the Netherlands the carrier solution volume is not standardized. Volumes used reflect the remaining abdominal capacity after cytoreductive surgery. This itself is influenced by variations in tumour load, visceral resections and muscle tone of the abdominal wall. These variations inevitably result in different intraperitoneal chemotherapy concentrations in patients with similar BSAs receiving similar drug doses.

Complication rates between patients treated with intraperitoneal MMC or OXA have been shown to be similar [9], but the effects of higher concentrations of intraperitoneal chemotherapy have not been evaluated to see if there is a relationship with increased occurrence of adverse events or to see whether lower concentrations might be associated with worse survival. The aim of the current study was to measure final intraperitoneal chemotherapy concentrations during the HIPEC and to evaluate its impact on complications and survival.
CHAPTER 5

METHODS

Patients
This cohort study was performed in three tertiary institutes in the Netherlands, the Radboud University Medical Centre (Radboudumc), the Netherlands Cancer Institute (NCI) and University Medical Centre Groningen (UMCG). Consecutive patients with CRPM who underwent primary CRS-HIPEC between 2010 and 2018 were eligible. Patients with appendiceal neoplasms other than adenocarcinoma and secondary and/or third HIPEC-procedures were excluded. Prospectively developed databases of all patients treated with CRS-HIPEC were in place at all three centres. Preoperatively, all patients were discussed in a multidisciplinary team meeting, involving surgeons, medical oncologists, radiologists, gastroenterologists and pathologists. This study was performed in accordance with local medical ethical guidelines and collection of coded data was approved by the local medical ethical committee of the Radboudumc.

Data collection and outcomes
Patient and treatment characteristics, along with operative details, details on the HIPEC procedure, histology, post-operative complications, disease-free survival (DFS) and overall survival (OS) were recorded. Intraperitoneal chemotherapy concentration was calculated by dividing the administered chemotherapy dose by the total volume of the instilled carrier solution.

Postoperative complications were scored according to the National Cancer Institute’s CTCAE Common Terminology criteria of adverse events (v4.03) or Clavien-Dindo classification. DFS and OS were defined as the time from the date of operation to the date of disease recurrence or death, date of censoring, or end of follow-up. Patients were excluded if they had surgery less than 6 months before data analyses.

To assess the impact of intraperitoneal chemotherapy concentrations on secondary outcomes, patients were classified in three groups; for both MMC and OX the different groups were based on the lowest 25%, middle 50% and highest 25% intraperitoneal chemotherapy concentrations.

The Peritoneal Cancer Index (PCI) [10] and Dutch Region Count [11] were combined to create patient groups based on volume of disease categorized as limited, moderate or extensive peritoneal metastases. Patients with a PCI less than 7 or a region count between 0 and 2 were placed in the lowest category. The moderate group consists of patients with a PCI ranging from 7 to 20 or a region count of 3, 4 or 5. Lastly, patients with a PCI above 20 or region count of 6 or 7 were placed in the group with extensive peritoneal metastases.
Surgical procedure
During explorative laparotomy the extent of peritoneal disease was scored according to the Peritoneal Cancer Index (PCI) and/or the Dutch region count [10, 11]. Generally, when the PCI was ≤20 and/or region count ≤5 the surgeons pursued complete cytoreduction. Completeness of cytoreduction score or the R score was used: CC0/R1-resections represents no visible macroscopic tumour nodules after cytoreduction, CC1/R2a-resections tumour nodules ≤2.5mm, CC2/R2b-resections tumour nodules 2.5 mm – 25 mm and CC3 represents >25 mm tumour nodules [12].

After exploratory laparotomy and cytoreductive surgery, HIPEC was performed. As described in detail elsewhere [2]. The open ‘coliseum-technique’ was used to create a basin in the abdominal cavity. Two to four inflow catheters and two outflow catheters were used. The abdominal cavity was filled with a carrier solution (Dianeal® PD1.36 (Baxter B.V., Utrecht, The Netherlands) in the NCI, 5% dextrose (Baxter) in RUMC and UMCG for OXA and 0.9% sodium chloride for MMC) until all peritoneal surfaces were submerged. Chemotherapeutic drugs were added when the optimal temperature was steadily reached, as described below. Dose of MMC or OXA was based on Body Surface Area (BSA) with a maximum of 2m² for MMC. Patients received MMC or OXA dependent on institutional practice. In March 2014 RUMC and NCI switched standard MMC protocols to OXA.

For HIPEC with MMC, 35 mg/m² heated to 41-43 °C was administered for 90 minutes. Half of the total MMC-dose was given at the start of the HIPEC-procedure, another quarter 30 minutes after the start and the last quarter of the total dose given 60 minutes after the start. When OXA was used, prior to HIPEC, intravenous leucovorin 20 mg/m² was administered followed by 5-fluorouracil 400 mg/m². Thereafter, the carrier solution was heated to 43°C with OXA 460 mg/m² added and perfused for 30 minutes. All patients were admitted to the Intensive Care Unit postoperatively.

Follow-up
Bi-annual Computed Tomography (CT) of the chest and abdomen was performed in the first five years following CRS-HIPEC along with measurement of the serum tumour markers carcinoembryonic antigen, cancer antigen 125 and cancer antigen 19-9.

In the NCI, CT and serum tumour markers were performed annually after the first two years of bi-annual follow up. Recurrences and overall survival were registered.
Statistical analysis
Means and medians were analysed with Student’s T-test or Mann-Whitney U test depending on distribution. Categorical variables were cross-tabulated and significant differences identified using Fisher’s exact test or Chi-square as appropriate. Kaplan-Meier estimates of survival were calculated. Overall survival was compared between groups with different intraperitoneal chemotherapy concentrations, using log-rank test. All tests were performed two-sided and a significance level of <0.05 was considered statistically significant. Statistical analyses were performed with the Statistical Package for Social Sciences, Version 22.0 (IBM Corp., Armonk, NY, USA).
Multivariable Cox regression analyses were performed with variables that were significant in univariable analysis or considered clinically relevant (tumour differentiation, N stage, completeness of cytoreduction score, and extent of disease).

RESULTS
A total of 320 patients underwent CRS-HIPEC and were included. Of these, 220 received intraperitoneal MMC and 100 intraperitoneal OXA. Median follow-up was 22.4 months (range 0.1-122.6). Baseline characteristics of the two groups are described in Table 1.
BSA ranged from 1.3m² to 2.5 m² (median 1.9 m²). Median total dose of chemotherapy concentration was 66.9 mg (range 35.0-89.1) of MMC and 877.0 mg (range 572.4-1060.0) for OXA. Median carrier solution volume was 5.0L and ranged between 0.7L and 10.0L (Figure 1a). Median calculated intraperitoneal chemotherapy concentration was 13.3 mg/L (range 7.0 –76.0) for MMC and 156.0 mg/L (range 91.9 – 377.6) for OXA (Figure 1b and c).
Grade ≥ 3 complications occurred in 81 of 320 (25.3%) patients (Table 1). Median OS was 36.6 months. Patients treated with MMC had median OS of 36.9 (0.1 - 122.6) months, patients treated with OXA had median OS of 29.5 (0.6 - 42.8) months (p=0.516). Median DFS in patients treated with MMC DFS was 12.9 (0.1 - 107.4) months and 13.1 (0.6 - 42.8) months in the OXA group.
<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>All patients</th>
<th>MMC</th>
<th>OXA</th>
<th>p value</th>
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<td>OXA</td>
<td>p value</td>
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<td>-------------</td>
<td>------</td>
<td>------</td>
<td>---------</td>
</tr>
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<td>184 (57.5)</td>
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<td>SRCC(^1)</td>
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<tr>
<td>Extent of peritoneal metastasis</td>
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<td>Completeness of cytoreduction</td>
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<td>R1</td>
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Table 1 General patient characteristics. Numbers are presented as means and percentages in parentheses. Symbols: \(^1\) American Society of Anesthesiologists physical status classification system, \(^2\) Peritoneal metastasis, \(^3\) Signet ring cell carcinoma
Figure 1 Details of intra-abdominal volumes and body surface area, and calculated concentrations of mitomycin C and oxaliplatin.
a Scatterplot of intra-abdominal volume and body surface area (BSA). Note the variation in intra-abdominal volume at the same BSA.
b, c Box-and-whisker plots of calculated concentrations of mitomycin C and oxaliplatin. Median values, interquartile ranges and ranges (excluding outliers) are denoted by horizontal bars, boxes and error bars respectively.

Effects of concentration of intraperitoneal chemotherapy
Table 2 summarizes the different concentrations in the three patient groups and the association with grade 3 surgical complications. Complication rates and grades were not significantly different between patients who received low, mid or high concentrations of intraperitoneal chemotherapy in either group (p=0.492 MMC and p=0.575 OXA).
Figure 2 and 3 illustrate the OS and DFS of MMC and OXA per group (lower quartile, midrange and upper quartile). No significant association was observed between the three different groups of concentrations and OS or DFS.
Multivariable cox regression analysis identified pN-stage, extent of peritoneal metastases and completeness as independent prognostic factors for OS (Figure 3). The calculated circulating chemotherapy concentration during HIPEC was not associated with adverse effects on survival nor to the presence of disease recurrence.
Figure 3: a, c Overall (OS) and disease-free (DFS) survival in patients receiving mitomycin C (MMC) and b, d OS and DFS in patients receiving oxaliplatin. Hazard ratios are shown with 95 per cent confidence intervals. PM, peritoneal metastases.
Table 2. Postoperative complications and concentrations

<table>
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<td>Midrange</td>
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<td>(7-11.2 mg/L)</td>
<td>(11.2-15.4 mg/L)</td>
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<tr>
<td>45 (81.8)</td>
<td>81 (73.6)</td>
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<tr>
<td>10 (18.2)</td>
<td>29 (26.4)</td>
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<table>
<thead>
<tr>
<th>Grade 0-II</th>
<th>Grade III-V</th>
</tr>
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<tbody>
<tr>
<td>Lower quartile</td>
<td>Midrange</td>
</tr>
<tr>
<td>(91.9-131.1 mg/L)</td>
<td>(131.3-184.0 mg/L)</td>
</tr>
<tr>
<td>16 (64.0)</td>
<td>37 (75.5)</td>
</tr>
<tr>
<td>9 (36.0)</td>
<td>12 (24.5)</td>
</tr>
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</table>

*Table 2* Postoperative concentrations and their relation to the used calculated chemotherapy concentration during HIPEC divided in 3 strata. Note complication data of 1 patient in the MMC group was unknown.
DISCUSSION

This study showed wide variation of intraperitoneal carrier solution volumes in patients with colorectal peritoneal metastases who were treated with CRS-HIPEC in three tertiary Dutch centres. Variations in carrier solution volume resulted in different calculated intraperitoneal chemotherapy concentrations for both MMC and OXA. Calculated intraperitoneal chemotherapy concentrations varied 10- and 4-fold for MMC and OXA respectively. Postoperative complication rate, disease-free survival and overall survival were not affected by differences in chemotherapy concentrations, regardless of HIPEC chemotherapy type.

The recently completed PRODIGE7 trial [13] (clinicaltrials.gov: NCT00769405) which compared systemic chemotherapy combined with CRS-HIPEC (oxaliplatin) or systemic chemotherapy with CRS alone did not find a significant benefit of OXA based HIPEC over CRS alone [14-16]. In both groups the median survival was more than 40 months. The interpretation of results of the PRODIGE7 study remains difficult because the additional effect of systemic chemotherapy remains to be proven by the CAIRO6 study (clinicaltrials.gov NCT02758951). Other recent studies; the Dutch COLOPEC trial (NCT02231086)[17] and French PROPHYLOCHIP trial (NCT01226394)[18]) have investigated different aspects of HIPEC treatment but have still failed to resolve the relative contributions made by cytoreductive surgery, hyperthermia, intra-peritoneal chemotherapy and perioperative systemic therapy on survival.

The present study aimed to address the potential impact of intraperitoneal concentration of the chemotherapeutic agent. The findings suggest that the difference in concentrations of the chemotherapy agent within the limits identified plays a minor role in outcome after CRS-HIPEC.

Theoretically a low concentration might reduce the efficacy of the agent, but no relationship existed between concentration and patient survival. Nor was there any relationship between complications and different calculated chemotherapy concentrations. Several explanations are possible for the lack of association between concentration and survival. If the lowest chemotherapy concentration was above a threshold concentration required to inhibit cell proliferation and cell cycle progression in tumour cells within the given perfusion period, higher concentrations might not impact on survival. The intra-peritoneal chemotherapy as administered in these protocols might not have added benefit above the other components of CRS-HIPEC, as suggested by the PRODIGE7 trial. Variable intra-peritoneal volumes may also have had a confounding effect [19].

A recent study with patient derived organoids did, however, find that currently used concentrations might be insufficient for complete eradication of all malignant cells [20].
This merits further investigation as drug concentrations may be critical as new agents are introduced.

Worldwide treatment variation in CRS-HIPEC regimens is well recognized and standardization can improve outcomes [21]. The American Society of Peritoneal Malignancies has proposed a standardized MMC protocol based on consensus [8]. Despite a willingness to standardize, differences in protocols still exist as shown in two recent systematic reviews [6, 22]. In the Netherlands there is no standardization for the intraperitoneal carrier solution volume. The total carrier solution volume is based on the intraperitoneal volume whereas the total chemotherapeutic dose is based on BSA, inevitably resulting in wide variations in concentration.

Although drug concentration was not identified as a significant risk factor influencing survival or complications, optimization of chemotherapy concentrations might contribute to standardized treatments, particularly as new agents are introduced.
REFERENCES


Wide variation in tissue, systemic and drain fluid exposure after oxaliplatin-based HIPEC: results of the GUTOX study

Loek A.W. de Jong, Fortuné M.K. Elekonawo, Marie Lambert, Jan Marie de Gooyer, Henk M.W. Verheul, David M. Burger, Johannes H.W. de Wilt, Etienne Chatelut, Rob ter Heine, Philip R. de Reuver, Andreas J.A. Bremers, Nielka P. van Erp

Cancer Chemotherapy and Pharmacology, July 2020, 86(1):141-150
ABSTRACT

Background
In this exploratory study, the effect of postprocedural flushing with crystalloids after oxaliplatin-based hyperthermic intraperitoneal chemotherapy (HIPEC) on platinum concentrations in peritoneal tissue, blood and drain fluid was studied. Interpatient variability in oxaliplatin pharmacokinetics and the relation between platinum concentration in peritoneal fluid and platinum exposure in tissue and blood was explored.

Methods
Ten patients with peritoneal carcinomatosis of colorectal origin were treated with HIPEC including postprocedural flushing, followed by ten patients without flushing afterwards. Tissue, peritoneal fluid, blood and drain fluid samples were collected for measurement of total and ultrafiltered platinum concentrations.

Results
Peritoneal tissue concentration and systemic ultrafiltered platinum exposure showed large inter individual variability, ranging from 65 - 1640 µg/g dry weight and 10.5 - 28.0 µg*h/ml, respectively. No effect of flushing was found on geometric mean platinum concentration in peritoneal tissue (348 vs. 356 µg/g dry weight), blood (14.8 vs. 18.1 µg*h/ml) or drain fluid (day 1: 7.6 vs. 7.7 µg/ml; day 2: 1.7 vs. 1.9 µg/ml). The platinum concentration in peritoneal fluid at the start of HIPEC differed twofold between patients and was positively correlated with systemic exposure (p=.04) and peak plasma concentration (p=.04).

Conclusion
In this exploratory study no effect was found for postprocedural flushing on platinum concentrations in peritoneal tissue, blood or drain fluid. BSA-based HIPEC procedure leads to large interpatient variability in platinum exposure in all compartments. The study was registered at ClinicalTrials.gov on 7 December 2017 under registration number NCT03364907.
INTRODUCTION

Peritoneal metastasis of colorectal origin is identified in 5-10% of patients undergoing primary resection, and metachronous colorectal peritoneal metastasis occurs in 20-50% of patients during follow-up [1-3]. Despite the use of modern systemic chemotherapy regimens, patients with peritoneal metastasis of colorectal cancer have poor outcome with a median overall survival of 10-16 months [4,5]. Since the introduction of cytoreduction combined with hyperthermic intraperitoneal chemotherapy (HIPEC), median overall survival increased to 32 – 41 months [6-10].

The rationale for HIPEC is to obtain high local drug concentrations and high penetration in tumour tissue with relatively low systemic exposure. The response of tumour cells is dependent on drug concentration in peritoneal fluid. In organoids derived from colorectal peritoneal metastases a platinum concentration of 118 – 275 µg/ml in peritoneal fluid is required to eliminate 50% of tumour cells during 30-min HIPEC procedure [11]. Although these findings cannot be easily extrapolated to in vivo tumour nodules in patients, it provides insight in the importance of the drug concentration in peritoneal fluid. As diffusion is the most dominant mechanism to penetrate in tissue for low molecular weight drugs, such as cisplatin and mitomycin C, higher drug concentration in peritoneal fluid results in higher drug concentration in tumour tissue [12]. Unfortunately, the optimal tissue concentration that is required to eliminate peritoneal metastases is unknown. It seems reasonable to strive for the highest local tissue concentration while limiting systemic exposure to prevent toxicity to the patient and the treating personnel in the postoperative period. [13,14].

Although cytoreductive surgery (CRS) procedures are more or less standardised, large variations exist in HIPEC treatment modalities [15]. Important methodological variations include: technique (open ‘coliseum’ vs. closed abdomen), temperature, type and dose of the drug, exposure time, type and volume of carrier solution and whether or not the peritoneum is flushed with crystalloids at the end of HIPEC. It is pivotal to understand the effects of these different variations on pharmacokinetics and pharmacodynamics of the treatment. Postprocedural flushing is predominantly performed with the idea to minimise platinum concentration in blood and decrease platinum concentration in drain fluid after surgery, resulting in lower personnel exposure risk. On the other hand, it might decrease peritoneal tissue concentration and as such decrease efficacy of the treatment. If there is an effect of postprocedural flushing on platinum concentrations in tissue, blood or drain fluid, this may affect efficacy and safety of the treatment.
The primary goal of this exploratory study was to evaluate the effect of flushing with NaCl 0.9% on platinum concentration in peritoneal tissue, blood and drain fluid after oxaliplatin-based HIPEC. In addition, the inter individual variability in tissue, blood and drain fluid was explored and the relation between platinum concentration in peritoneal fluid at the start of HIPEC and platinum exposure in tissue and blood was investigated.

**MATERIALS AND METHODS**

**Patients**
Patients ≥ 18 years old with a diagnosis of preoperatively identified primary or recurrent peritoneal metastasis of colorectal origin who were planned for HIPEC treatment with oxaliplatin according to routine clinical care were eligible for study entry. Patients were sequentially allocated over both groups, meaning that 10 patients were enrolled in the flushing group, followed by 10 patients in the non-flushing group.

The GUTOX trial was approved by the institutional ethics committee Arnhem-Nijmegen (Nijmegen) and was compliant with the Declaration of Helsinki. All patients provided written informed consent before entering the study. The study was registered at ClinicalTrials.gov, NCT03364907.

**Study design**
The GUTOX study was an exploratory, single-center, prospective, pharmacokinetic cohort study. The study design is graphically displayed in figure 1. In the flushing group 10 patients were treated with HIPEC including flushing afterwards. Flushing consisted of rinsing the abdominal cavity with 0.9% (w/v) sodium chloride immediately after the intra-peritoneal chemotherapy was drained out of the abdominal cavity. In the non-flushing group, 10 patients underwent HIPEC without flushing afterwards. Surgical procedure was performed according to the local routine protocol for CRS-HIPEC procedure [16]. Oxaliplatin-based HIPEC was performed using the open coliseum technique at a dose of 460mg/m², at a target temperature of 42-43 °C for a total duration of 30 minutes. Dextrose 5% was used as carrier solution. The volume of dextrose 5% was dependent on the abdominal volume which differed between patients. A flow rate of 1.2 – 2 L/min was used to circulate the perfusate. At the end of the chemoperfusion the instillation solution was drained from the abdominal cavity.
Pharmacokinetic sampling and analytical assay
Pharmacokinetic sampling was performed as described in figure 1. At the end of HIPEC, a small peritoneal tissue sample (~1x2cm) from the dorsal side of the posterior rectus sheath was collected in patients of both groups. In the patients treated in the flushing group, a second peritoneal sample was collected immediately after flushing with crystalloids. Peritoneal tissue pre-treatment was performed according to an earlier described method [14]. After HIPEC, four drainage tubes were fixed to drainage bags in order to collect outflowing drain fluid during the postoperative period as described in figure 1. The total volume of drain fluid per bag was noted, starting the morning after HIPEC. Immediately after sampling the drain bags were changed. All samples were stored at -40 °C until analysis. Platinum concentrations were measured using flameless atomic absorption spectrometry according to a previously described method [17].

For non-compartmental pharmacokinetic analysis, Phoenix WinNonlin® version 8.1 (Certara USA Inc, Princeton, NJ) was used.
### Inclusion
Patients (n=20) with diagnosis of peritoneal carcinomatosis of colorectal origin who are planned for CRS-HIPEC treatment with oxaliplatin

<table>
<thead>
<tr>
<th>Flushing group (n=10)</th>
<th>Non-flushing group (n=10)</th>
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<tbody>
<tr>
<td>CRS-HIPEC with postprocedural flushing</td>
<td>CRS-HIPEC without postprocedural flushing</td>
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</table>

**Cytoreductive surgery**
- Determination of PCI-score
- Determination of CC-score

**HIPEC** (t=0)
- Peritoneal fluid samples (t=5, 15, 30 min)
- Blood samples (t=10, 20, 30 min)
- Peritoneal tissue sample (t=30 min)

**Postprocedural flushing with NaCl 0.9%**
- Peritoneal fluid sample (t=35 min)
- Peritoneal tissue sample (t=40 min)

**Postoperative care**
- Blood samples (t=¾, 1, 1½, 2, 4, 12, 24, 36, 72)
- Drain fluid samples (postoperative day 1 + 2)

**Figure 1** Study design. CRS = cytoreductive surgery, HIPEC = hyperthermic intraperitoneal chemotherapy, PCI-score = Peritoneal Cancer Index score, CC score = completeness of cytoreduction score. a HIPEC was performed with oxaliplatin 460mg/m² at 42-43 °C for a duration of 30 minutes.
Haematologic toxicity
The occurrence of haematologic toxicity was monitored for up to 14 days or until hospital discharge. Haematological lab monitoring took place as part of routine clinical care. Leukopenia, anaemia, and thrombocytopenia was graded according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0 [18].

Statistics
Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Pharmacokinetic parameters were described as geometric mean with range. Unpaired t-tests were used to test for differences in patient characteristics with the exception of sex and CC-score where a χ2-test was performed. A paired sample t-test was used on logarithmic transformed data to compare platinum tissue concentrations before and after postprocedural flushing in the flushing group. Unpaired t-tests were used on logarithmic transformed data to compare platinum tissue concentrations, systemic exposure and platinum in drain fluid between both groups. A Fisher’s exact test was used to compare haematologic toxicity between both groups. Spearman’s rank correlation tests were used to test for correlations between platinum concentration in peritoneal fluid, tissue exposure and unbound and total systemic exposure. p < 0.05 were considered as statistically significant.
RESULTS

Patients
Twenty patients were included in the GUTOX trial between March 2018 and June 2019. Patient characteristics are summarised in table 1. Despite of a non-randomised study design, patient characteristics were equally distributed.

<table>
<thead>
<tr>
<th>Table 1. Patients’ characteristics</th>
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<tr>
<td>Age (year)</td>
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<tr>
<td>Sex (M/F)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Body surface area (m²)</td>
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<tr>
<td>PCI score</td>
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<tr>
<td>CC score</td>
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<tr>
<td>CC-0</td>
</tr>
<tr>
<td>CC-1</td>
</tr>
<tr>
<td>Karnofsky score</td>
</tr>
<tr>
<td>Peritoneal metastasis (primary/recurrent)</td>
</tr>
<tr>
<td>eGFR using CKD-EPI (ml/min/1.73m²)</td>
</tr>
</tbody>
</table>

*Table 1 Patients’ characteristics. Data are presented as mean ± SD or as median (IQR). PCI-score = Peritoneal Cancer Index score, CC score = completeness of cytoreduction score, eGFR = estimated glomerular filtration rate, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration.*

Peritoneal tissue
The interpatient variability in platinum concentrations in peritoneal tissue was substantial, ranging from 65 – 1640 µg/g dry weight. In the patients of the flushing group no difference was found between the geometric mean [range] platinum concentrations in peritoneal tissue before and after postprocedural flushing (348 [66 – 1571] µg/g dry weight vs. 356 [65 – 1025] µg/g dry weight, respectively; p = 0.927). The platinum tissue concentrations pre- and post-flushing are graphically displayed in figure 2. The non-flushing group showed similar geometric mean [range] platinum concentrations in peritoneal tissue compared to the flushing group before postprocedural flushing (478 [202 – 1640] µg/g dry weight vs. 348 [66 – 1571] µg/g dry weight, respectively; p = 0.416).
Blood

Interpatient variability in systemic exposure of ultrafiltered platinum and total platinum was 30% and 25%, respectively. Systemic exposure for ultrafiltered platinum and total platinum, ranged from 10.5 – 28.0 µg*h/ml and 62.3 – 168.4 µg*h/ml, respectively. The patients of the flushing group showed lower geometric mean [range] Cmax for both ultrafiltered platinum and total platinum than the patients of the non-flushing group (Cmax total platinum: 6.3 [5.0 – 8.6] µg/ml vs. 8.0 [5.5 – 11.6] µg/ml, respectively; p = 0.024 and Cmax ultrafiltered platinum: 4.6 [3.5 – 5.7] µg/ml vs. 5.9 [3.4 – 8.7] µg/ml, respectively; p = 0.043). Geometric mean [range] systemic exposure of total platinum was lower in the flushing group compared to the non-flushing group (90.4 [62.3 – 105.8] µg*h/ml vs. 116.4 [79.5 – 168.4] µg*h/ml, respectively; p = 0.019). There was no difference in geometric mean [range] systemic exposure of ultrafiltered platinum between both groups (14.8 [10.5 – 20.2] µg*h/ml vs. 18.1 [10.8 – 28.0] µg*h/ml, respectively; p = 0.141). Platinum pharmacokinetics of unbound and total platinum in plasma during HIPEC and the first 4 hours post-HIPEC are shown in figure 3.
Drain fluid

The geometric mean [range] of platinum cleared via drainage during the first two days after HIPEC did not differ between both groups (4.5 [2.6 – 7.7] mg vs. 6.3 [3.0 – 11.8] mg, respectively; p = 0.054). No differences were found between both groups in geometric mean [range] platinum concentration in drain fluid on day 1 post-HIPEC (7.6 [2.8 – 21.1] µg/ml vs. 7.7 [3.8 – 14.6] µg/ml, respectively; p = 0.953) or day 2 post-HIPEC (1.7 [0.7 – 5.4] µg/ml vs. 1.9 [1.4 – 3.1] µg/ml, respectively; p = 0.523). The platinum cleared via drainage appeared to be only a minor part (approximately 0.6%) of the totally administered dose.
Peritoneal fluid

Platinum concentration at start of HIPEC showed substantial interpatient variability, ranging from 122 - 246 µg/ml. On average, the total volume of peritoneal fluid, platinum concentration at start and total exposure over 0 - 30 minutes in peritoneal fluid did not differ between the two groups. The peritoneal fluid concentration-time curve during HIPEC is shown in figure 4. All the platinum in peritoneal fluid was unbound platinum. During the 30-minute oxaliplatin perfusion, total platinum concentration in peritoneal fluid decreased from 180 (± 39) µg/ml to 129 (± 26) µg/ml in the flushing group and from 191 (± 24) µg/ml to 133 (± 23) µg/ml in the non-flushing group, reflecting a decrease of approximately 28% and 30%, respectively. Postprocedural flushing decreased platinum concentration to negligible concentrations of 8 µg/ml, which is ~4% of the concentration at start.

Figure 4 Peritoneal fluid concentration-time curve during HIPEC. The start concentration at timepoint 0 is a theoretical concentration calculated using the administered dose and the total peritoneal fluid administered. Data are presented as mean ± SD, Pt = Platinum, UF Pt = ultrafiltered platinum.
Correlations
Spearman’s correlation tests showed that ultrafiltered platinum concentration in peritoneal fluid at the start of HIPEC was positively correlated with both total systemic exposure (p=.04) and peak plasma concentration (p=.04) and negatively correlated with perfusate volume (p=.01). The peak ultrafiltered platinum plasma concentration was negatively correlated with perfusate volume (p=.04). No correlations were found between platinum concentration in peritoneal fluid at the start of HIPEC and tissue exposure.
Pharmacologic parameters are summarised in table 2. Correlations between the described variables are shown in a scatterplot in figure 5.

Figure 5 Statistical significant correlations. Scatterplots with Spearman’s correlation coefficient (R) and Sig(2-tailed) p-value (p). Pt concentration in peritoneal fluid at start of HIPEC is positively correlated with both AUC_{0-72h} Pt UF (A) and C_{max} UF Pt (B) in plasma and negatively correlated with perfusate volume (C). C_{max} UF Pt in plasma is negatively correlated with perfusate volume (D). Pt = Platinum, UF Pt = ultrafiltered platinum, AUC_{0-72h} = Area under the curve from 0 to 72 hours, C_{max} = peak plasma concentration.
Table 2. Pharmacologic parameters. Data are presented as mean ± SD or as geometric mean [range]. P values < 0.05 are considered statistically significant and are flagged with one asterisk (*). Based on paired T-test. Pt = Platinum, AUC_{0-0.5h} = Area under the concentration-time curve from 0 to 0.5 hours, C_{max} = peak plasma concentration, AUC_{0-72h} = Area under the concentration-time curve from 0 to 72 hours, AUC_{0-inf} = Area under the concentration-time curve from 0 to infinite time, UF Pt = ultrafiltered platinum.

<table>
<thead>
<tr>
<th></th>
<th>Flushing (n = 10)</th>
<th>Non-flushing (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General information</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin dose (mg)</td>
<td>877±83</td>
<td>902±124</td>
<td>0.603</td>
</tr>
<tr>
<td>Intravenous fluid</td>
<td>1193±568</td>
<td>1114±592</td>
<td>0.764</td>
</tr>
<tr>
<td>administered between 1 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior to HIPEC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraabdominal fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt concentration in tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-flushing</td>
<td>348 [66–1571]</td>
<td>478 [202–1640]</td>
<td>0.416</td>
</tr>
<tr>
<td>(µg/g dry weight)</td>
<td></td>
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<td></td>
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<tr>
<td>Pt concentration in tissue</td>
<td></td>
<td></td>
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<tr>
<td>post-flushing</td>
<td>356 [65–1025]</td>
<td>–</td>
<td>0.927*</td>
</tr>
<tr>
<td>(µg/g dry weight)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
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<tr>
<td>C_{max} total Pt (µg/ml)</td>
<td>6.3 [5.0–8.6]</td>
<td>8.0 [5.5–11.6]</td>
<td>0.024*</td>
</tr>
<tr>
<td>AUC_{0-72h} total Pt</td>
<td>90.4 [62.3–105.8]</td>
<td>116.4 [79.5–168.4]</td>
<td>0.019*</td>
</tr>
<tr>
<td>(µg*h/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{max} UF Pt (µg/ml)</td>
<td>137.2 [77.2–209.2]</td>
<td>169.8 [117.6–285.2]</td>
<td>0.110</td>
</tr>
<tr>
<td>AUC_{0-72h} UF Pt (µg*h/ml)</td>
<td>4.6 [3.5–5.7]</td>
<td>5.9 [3.4–8.7]</td>
<td>0.043*</td>
</tr>
<tr>
<td>AUC_{0-inf} UF Pt (µg*h/ml)</td>
<td>14.8 [10.5–20.2]</td>
<td>18.1 [10.8–28.0]</td>
<td>0.141</td>
</tr>
<tr>
<td><strong>Drain fluid</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Volume produced during</td>
<td>1142 [460–2280]</td>
<td>1312 [650–3025]</td>
<td>0.524</td>
</tr>
<tr>
<td>first 2 days (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt cleared during first</td>
<td>4.5 [2.6–7.7]</td>
<td>6.3 [3.0–11.8]</td>
<td>0.054</td>
</tr>
<tr>
<td>2 days after HIPEC (mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt concentration on day</td>
<td>7.6 [2.8–21.1]</td>
<td>7.7 [3.8–14.6]</td>
<td>0.953</td>
</tr>
<tr>
<td>1 after HIPEC (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt concentration on day</td>
<td>1.7 [0.7–5.4]</td>
<td>1.9 [1.4–3.1]</td>
<td>0.523</td>
</tr>
<tr>
<td>2 after HIPEC (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peritoneal fluid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of perfusate (ml)</td>
<td>5077±1163</td>
<td>4755±756</td>
<td>0.474</td>
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<tr>
<td>Pt concentration at the</td>
<td>180±39</td>
<td>191±24</td>
<td>0.450</td>
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<tr>
<td>start of HIPEC (µg/ml)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pt concentration at the</td>
<td>129±26</td>
<td>133±23</td>
<td>0.734</td>
</tr>
<tr>
<td>end of HIPEC (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt concentration after</td>
<td>8±8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>flushing (µg/ml)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AUC_{0-0.5h} peritoneal</td>
<td>73.1 [48.6–109.6]</td>
<td>75.9 [63.7–100.4]</td>
<td>0.664</td>
</tr>
<tr>
<td>fluid (µg*h/ml)</td>
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</tbody>
</table>
Haematologic toxicity
No difference was found in haematological toxicity between both groups (table 3). None of the patients developed leukopenia. Any grade anaemia occurred in 90% of all patients (100% of the patients in the flushing group vs. 80% in the non-flushing group). Four patients in the flushing group and one patient in the non-flushing group experienced grade 3 anaemia. The median [range] time to nadir anaemia appeared 5 [1-10] days post-HIPEC in the flushing group and 3 [1-12] days post-HIPEC in the non-flushing group. Grade 1 thrombocytopenia occurred in 30% of the patients in both treatment groups. Median [range] time to nadir thrombocytes was 2 [1-4] days post-HIPEC.

<table>
<thead>
<tr>
<th>Table 3. Haematologic toxicity</th>
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<tbody>
<tr>
<td>Flushing (n = 10)</td>
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<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Leukopenia</td>
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<tr>
<td>Any</td>
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<tr>
<td>Severe (grade 3 and 4)</td>
</tr>
<tr>
<td>Anaemia</td>
</tr>
<tr>
<td>Any</td>
</tr>
<tr>
<td>Severe (grade 3 and 4)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Any</td>
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<tr>
<td>Severe (grade 3 and 4)</td>
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</table>

Table 3 Haematologic toxicity between the two patient groups
DISCUSSION

Substantial interpatient variability was demonstrated for platinum concentrations in both peritoneal tissue, blood and drain fluid during and after HIPEC procedure. Assuming that platinum exposure correlates with efficacy and safety of the treatment, the observed interpatient pharmacokinetic variability might affect treatment outcome and should be reduced. It is important to know which variations in HIPEC procedure contribute to high variability in platinum exposure. The GUTOX study evaluates the effect of postprocedural flushing on pharmacokinetics of oxaliplatin. In this exploratory study no significant effect of flushing after HIPEC on platinum concentration in peritoneal tissue, blood or drain fluid was detected. Although a yet unexplained difference in systemic exposure between patients who were and were not flushed after the HIPEC procedure was observed.

Since platinum concentrations were prospectively collected in multiple compartments, including peritoneal fluid, peritoneal tissue, blood and drain fluid, this study provides insights in the further understanding of oxaliplatin distribution during HIPEC procedure. High platinum concentration in microscopic remnants of tumour after cytoreduction is important for efficacy of HIPEC [19,11]. Platinum tissue concentrations in healthy peritoneal tissue instead of tumour tissue were measured because tumour nodules need to be resected prior to HIPEC to give the patient probably most benefit from the procedure. This is a feasible approach since Elias et al. showed in earlier studies that platinum tissue concentration after HIPEC is similar between tumour- and healthy peritoneal tissue [14].

The analytical method used to quantify platinum in this study was not validated for measurement of platinum in peritoneal tissue and drain fluid. Nevertheless, it is unlikely that matrix will influence the analysis using the technique of atomic absorption spectrometry since with this technique the total sample is burned at high temperature to vaporise and atomise elements including platinum that is being analysed. Patients were not randomised over both treatment groups but were sequentially allocated to the flushing- and non-flushing group. The local HIPEC protocol that was used for routine clinical care at the beginning of the study included postprocedural flushing. After the inclusion of the first ten patients in this study, the local HIPEC protocol was changed to HIPEC without postprocedural flushing. This indicates that all HIPEC patients, regardless of whether they participated in this study, were treated conform the prevailing local HIPEC protocol. Although no randomisation was performed, the patient characteristics were well balanced between both treatment groups. Additionally, no stratification was performed for factors that may influence platinum concentration in tissue, e.g. volume of perfusate and peritoneal fluid concentration, since these factors cannot be determined before the procedure.
Interpatient variability in platinum concentration in dried peritoneal tissue was high, which is in line with earlier findings [12]. The median tissue concentrations found in this study match with the results of Elias et al. who found a peritoneal platinum tissue exposure of 392 µg/g dry weight, using a similar HIPEC method [14]. HIPEC performed with a lower dose of 300 mg/m² resulted in a notable lower peritoneal tissue concentration of only 50 µg/g dry weight [range 5 – 203 µg/g dry weight] [20]. In the GUTOX study a correlation between platinum concentration in peritoneal fluid and peritoneal tissue could not be demonstrated. However, others demonstrated that higher platinum concentrations in peritoneal fluid resulted in higher tissue exposure [12]. Oxaliplatin was dosed based on BSA which resulted in a large range of platinum concentrations in peritoneal fluid of 122 - 246 µg/ml. From a pharmacological point of view the use of a fixed drug concentration should be preferred when performing HIPEC. Concentration-based HIPEC should be incorporated in global standardisation of HIPEC protocols, which is unfortunately still not the case. Nevertheless, even when HIPEC is performed with a fixed oxaliplatin concentration the interpatient variety is still high [12]. This suggests that in addition to peritoneal platinum concentrations, other factors will influence tissue exposure and thereby efficacy of the treatment.

The peak plasma concentration of total and ultrafiltered platinum was reached at the end of HIPEC and rapidly dropped after removing oxaliplatin from the abdominal cavity. The higher peak concentration and exposure over time for platinum in the non-flushing group was caused by a difference in absorption of platinum in the first 30 minutes of HIPEC, as can be seen in figure 3. The observed difference cannot be explained by an effect of flushing, because the procedure for both groups did not differ during the first 30 minutes of the HIPEC procedure. The observed difference could neither be explained by differences in renal function, platinum concentration in peritoneal fluid, absolute dose, extent of surgery nor the amount of intravenous fluid administered around HIPEC procedure. Therefore the observed difference is yet unexplained and needs further attention.

Interpatient variability in systemic exposure is considered moderate and is comparable with between-patient variability of 33% after intravenous administration [21], while others report lower interpatient variability for total platinum and ultrafiltered platinum of 12% and 4-15%, respectively [22]. The unbound platinum concentration is generally considered as the pharmacologically active moiety [21,23]. The peak plasma concentration of ultrafiltered platinum observed in the GUTOX study (flushing: 4.6 and non-flushing: 5.9 µg/ml) after intraperitoneal administration of oxaliplatin in a dose of 460 mg/m² was higher than the peak plasma concentration of ultrafiltered
platinum observed after a 2-h intravenous infusion of oxaliplatin at a dose of 130 mg/m² (1.21 µg/ml) [21]. This suggests a faster systemic absorption, which seems pharmacological plausible when administering a higher dose over a shorter time period. More important, the average total exposure over time for ultrafiltered platinum observed in the GUTOX study (15.5 and 18.8 µg*h/ml) is higher than the total systemic exposure for ultrafiltered platinum after a single 2-h infusion of oxaliplatin at 130 mg/m² (11.9 µg*h/ml) [21]. Nevertheless, no oxaliplatin induced haematologic toxicity was identified in this study. Anaemia is a very common complication in the immediate post-operative period being present in up to 90% of patients after major surgery [24]. Usually leukocytes and platelets reach their nadir within 7-14 days after chemotherapy. Erythrocytes live for approximately 120 days [25] and therefore will not reach a nadir for several weeks after treatment. In this study nadir anaemia was observed after a median time of 3-5 days post-HIPEC. Therefore the anaemia observed in this study should be contributed to the operative procedure and is unlikely related to oxaliplatin.

The most important contamination sources during postoperative care of the patients is the personnel exposure to urine and drain fluid from a HIPEC-patient. The concentration in drain fluid on day two was about five times lower compared to day one. The platinum concentration in drain fluid on both days did not differ between the groups. However, a trend towards a difference in the absolute amount of platinum cleared via drain fluid was found. This can be explained by a higher total volume of produced drain fluid in the non-flushing group. The risk for personnel exposure is considered to be related with the platinum concentration and not with the absolute amount in drain fluid [26]. These results are in line with earlier findings reporting ranges in platinum concentrations in drain fluid of 0.6 – 13.2 µg/ml on day 1 post-HIPEC and 0.2 - 3.2 µg/ml on day 2 post-HIPEC [27]. This data underlines the importance of safety precautions when handling drain fluid of HIPEC patients. Postprocedural flushing after HIPEC does not seem to reduce the risk for personnel exposure.
On average no difference was found between total volume of perfusate and platinum concentration in peritoneal fluid at start of HIPEC between both groups. Although it is important to notice that the platinum concentration in peritoneal fluid differed up to twofold between individual patients (122 vs. 246 µg/ml), which might effect antitumour activity at the peritoneal level. The decline of free platinum in peritoneal fluid is mainly the result of absorption from the peritoneal compartment towards peritoneal tissue and the systemic compartment. Reactions with erythrocytes and other cell types or debris in perfusate are unlikely to occur. All platinum in peritoneal fluid consisted of free unbound platinum. A decrease of approximately 30% in total platinum concentration in peritoneal fluid was found which is consistent with literature reporting decreases of 30 - 50% [28,12].

In this exploratory study no effect was found for postprocedural flushing after oxaliplatin-based HIPEC on platinum concentrations in peritoneal tissue, blood or drain fluid. A detrimental effect of flushing on efficacy or safety of the treatment seems unlikely and, therefore, the use of postprocedural flushing should be debated to simplify the HIPEC procedure. This study showed that BSA-based HIPEC procedure leads to large interpatient variability in platinum exposure in all compartments. Assuming that exposure correlates with treatment outcome, the observed sources of variability in platinum exposure needs to be further investigated.
REFERENCES


A pretargeted multimodal approach for image-guided resection in a xenograft model of colorectal cancer

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European Journal of Nuclear Medicine and Molecular Imaging: Research, September 2019, 9(1):86
ABSTRACT

Background
Image-guided surgery may improve surgical outcome for colorectal cancer patients. Here, we evaluated the feasibility of a pretargeting strategy for multimodal imaging in colorectal cancer using an anti-carcinoembryonic antigen (CEA) x anti-histamine-succinyl-glycine (HSG) bispecific antibody (TF2) in conjunction with the dual-labeled diHSG peptide (RDC018), using both a fluorophore for near-infrared fluorescence imaging and a chelator for radiolabeling.

Methods
Nude mice with subcutaneous CEA-expressing LS174T human colonic tumors and CEA-negative control tumors were injected with TF2. After 16 hours, different doses of $^{111}$In-labeled IMP-288 (non-fluorescent) or its fluorescent derivative RDC018 were administered to compare biodistributions. MicroSPECT/CT and near infrared fluorescence imaging were performed 2 and 24 hours after injection. Next, the biodistribution of the dual-labeled humanized anti-CEA IgG antibody $[^{111}\text{In}]$In-DTPA-MN-14-IRDye800CW (direct targeting) was compared with the biodistribution of $^{111}$In-RDC018 in mice with TF2-pretargeted tumors, using fluorescence imaging and gamma counting. Lastly, mice with intraperitoneal LS174T tumors underwent near-infrared fluorescence image-guided resection combined with pre- and post-resection microSPECT/CT imaging.

Results
$^{111}$In-RDC018 showed specific tumor targeting in pretargeted CEA-positive tumors (21.9 ± 4.5 and 10.0 ± 4.7 % injected activity per gram (mean ± SD %IA/g), at 2 and 24 hours p.i., respectively) and a biodistribution similar to $^{111}$In-IMP288. Both fluorescence and microSPECT/CT images confirmed preferential tumor accumulation. At post mortem dissection, intraperitoneal tumors were successfully identified and removed using pretargeting with TF2 and $^{111}$In-RDC018.

Conclusion
A pretargeted approach for multimodal image-guided resection of colorectal cancer in a preclinical xenograft model is feasible, enables preoperative SPECT/CT and might facilitate intraoperative fluorescence imaging.
INTRODUCTION

Colorectal cancer is the third most common cause of cancer deaths in the Western world [1]. In different stages of disease, surgery is a crucial part of the (curative) treatment of patients and complete resection of malignant tissue remains one of the main prognostic factors [2]. Surgical outcome may be improved by better pre- and intraoperative imaging tools to aid the surgeon in patient selection, tumor detection and radical resection. Intraoperative fluorescence imaging has shown potential to increase specificity and sensitivity of resections [3]. A combined approach to improve both pre- and intraoperative tumor detection using tumor-targeted multimodal imaging may be advantageous to achieve the best surgical outcome.

Overexpression of carcinoembryonic antigen (CEA) is present in 90-95% colorectal cancers [4, 5]. This biomarker may be targeted by the high-affinity monoclonal antibody MN-14. MN-14 is a humanized IgG directed against the carcinoembryonic antigen-related cell adhesion molecule 5 [6]. In a previous study, MN-14 labeled with Indium-111 ($^{111}$In) and conjugated to IRDye800CW ($[^{111}\text{In}]\text{In-DTPA-MN-14-IRDye800CW}$) was shown to specifically accumulate in CEA-expressing tumor xenografts and enabled radio- and fluorescence-guided surgery of colorectal tumor nodules [7]. However, due to the slow blood clearance of antibodies, high tumor-to-background signals can only be achieved at several days after injection.

An alternative approach to deliver radioactive, fluorescent or other agents to tumors for imaging and therapy is via a pretargeting strategy [8]. In this multistep approach, first a bispecific antibody (bsAb) is administered that specifically targets the tumor. Subsequently, a hapten, carrying a diagnostic or therapeutic load, is administered that binds to the bsAb. This hapten is a relatively small molecule with rapid renal clearance. Both high specificity and high tumor-to-background ratios may be achieved using this strategy, as it combines the specific targeting properties of antibodies with the favorable pharmacokinetics and clearance of small molecules [9-14].

The current study investigates the use of a tumor-specific multimodal pretargeting strategy. For this purpose, we apply the trivalent bispecific anti-CEA x anti-histamine-succinyl-glycine (HSG) antibody TF2 in combination with the multimodal di-HSG hapten-peptide RDC018, an IMP-288 derivative (Figure S1). RDC018 contains both a fluorophore and a chelator for $^{111}$In labeling, thus enabling both radionuclide and fluorescence imaging, whereas IMP-288 only harbors a chelator and no fluorophore. Here, we evaluate the feasibility of this multimodal pretargeting approach in a colorectal cancer model of peritoneal metastasis.
METHODS

Pretargeting molecules TF2 and RDC018
The bsAb TF2 contains two CEACAM5 and one HSG-binding sites [13] and was produced using the Dock-and-Lock method as described previously [15]. A schematic representation of the pretargeting agents is provided by Schoffelen et al.[13]. RDC018 is a peptide-hapten derived from IMP-288, a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-conjugated d-Tyr-d-Lys-d-Glu-d-Lys tetrapeptide, in which both lysine residues are substituted with a HSG moiety via their ε-amino group (Figure S1) [16]. In addition to the DOTA chelate for radiolabeling, RDC018 is C-terminally conjugated with the fluorescent DyLight™ 800 NHS ester. TF2 [15] and RDC018 were kind gifts from Immunomedics Inc.

Radiolabeling
All labeling procedures were performed under metal-free conditions. Briefly, $^{111}$InCl$_3$ (Mallinckrodt Medical BV/Curium, Petten, the Netherlands) was added to IMP-288 or RDC018 in two volumes of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH5.5. After 20 minutes of incubation at 95°C, 50 mM ethylenediaminetetraacetic acid (EDTA) was added to the labeling reaction to a final concentration of 1 mM EDTA to chelate unincorporated $^{111}$In. Lastly Tween80 (Sigma-Aldrich, Saint Louis, MO, USA) was added to the labeling product in a final concentration of 0.01%. The labeling efficiency was determined by instant thin-layer chromatography on Varian silicagel strips (ITLC-SG; Agilent Technologies, Amstelveen, The Netherlands) using 0.1 mM ammonium acetate (NH$_4$Ac) buffer with 0.1 M EDTA (pH 5.5) as the mobile phase. If labeling efficiency was below 95%, labeled products were purified using solid phase extraction on an HLB cartridge (Waters Chromatography B.V., Etten-Leur, The Netherlands) with 100% EtOH as mobile phase. Final radiochemical purity was >95% for all compounds.

The antibody MN-14 was conjugated to IRDye800CW (fluorophore:antibody substitution ratio 1.4) and diethylenetriaminepentaacetic acid (DTPA) which was labeled with $^{111}$InCl$_3$ at a specific activity of 0.78 MBq/µg, as previously described [7].
**Cell culture**

CEA-expressing human colon adenocarcinoma cells LS174T and CEA-negative human renal cell carcinoma cells SK-RC-52 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were tested for mycoplasma negativity. Cells were cultured in sterile conditions using RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum and 2 mM L-glutamine, without antibiotic additive. Cells were cultured in T150 tissue culture flasks in a humidified incubator with an atmosphere of 95% air and 5% carbon dioxide at 37°C. All cells were harvested with trypsin/EDTA.

**Xenograft Mouse Models**

All animal experiments were approved by the Institutional Animal Welfare Committee of the Radboud University Medical Center and were conducted in accordance to the guidelines of the Revised Dutch Act on Animal Experimentation (2014).

Female BALB/cAnNRj-Foxn1nu/nu nude mice (7 to 9 weeks-old, 18-22 g body weight; Janvier), housed in individually ventilated cages (5 mice per cage) under non-sterile standard conditions with free access to standard animal chow and water, were adapted to laboratory conditions for 1 week before experimental use. For the biodistribution and microSPECT/CT experiments, mice were subcutaneously inoculated with $2 \times 10^6$ LS174T cells (left flank) and $2 \times 10^6$ SK-RC-52 cells (right flank) both suspended in 200 µL RPMI-1640 medium. For the image-guided resection experiment, intraperitoneal tumor growth was induced by an intraperitoneal injection of $3 \times 10^5$ LS174T cells suspended in 200 µL RPMI-1640 medium. Tail vein injections were performed for intravenous administration of antibodies and peptides.

**Biodistribution studies**

**Biodistribution of $^{111}$In-IMP-288 versus $^{111}$In-RDC018**

In the first experiment the biodistributions of $^{111}$In-IMP-288 and $^{111}$In-RDC018 were compared. Three different dose levels (0, 0.8 and 8 nmol) of TF2 or controls were tested with a 1:20 TF2:HSG-peptide ratio of each HSG-peptide in two subsets of 30 mice (5 mice per group). Mice at the zero dose level received the same amount of HSG-peptide (0.4 nmol) as mice at the highest dose level. Seventeen days after tumor inoculation 200 µL TF2 in PBS-0.5% BSA or PBS-0.5% BSA was injected intravenously. Sixteen hours following TF2 administration the radiolabeled HSG peptide ($^{111}$In-IMP-288, 9.5 MBq.µg$^{-1}$ or $^{111}$In-RDC018, 5.8 MBq.µg$^{-1}$) was injected. Mice were euthanized by CO$_2$/O$_2$ asphyxiation and the biodistribution of $^{111}$In-IMP-288 and $^{111}$In-RDC018 was determined 2 or 24 hours after peptide injection. For this purpose, tissues of interest (tumor, muscle, lung, spleen, kidney, liver, pancreas, stomach and duodenum) were dissected and weighed after which activity was measured in a shielded 3”-well-type γ-counter (Perkin-Elmer, Boston, MA, USA). Blood samples were obtained.
by heart puncture. For calculation of the uptake of activity in each tissue as a fraction of the injected activity, three aliquots of the injection dose were counted in the y-counter simultaneously.

**Biodistribution of $^{111}$In-RDC018 versus dual-labeled MN-14**

In the second experiment the biodistribution profile of $^{111}$In-RDC018 in the pretargeted approach (TF2 - RDC018) was compared to the dual-labeled humanized monoclonal antibody MN-14 (reference compound) using the IVIS Lumina fluorescence camera (Xenogen VivoVision IVIS Lumina II, Caliper Life Sciences, Waltham, MA, USA) and MicroSPECT/CT (U-SPECT II; MILabs, Utrecht, The Netherlands). TF2 (1.4 nmol) and $[^{111}$In$]$In-DTPA-MN-14-IRdye800CW (32.2µg, 0.78 MBq.µg$^{-1}$) were injected intravenously 17 days following subcutaneous tumor cell inoculation in two groups of 5 mice. Radiolabeled RDC018 (126 MBq.µg$^{-1}$, 0.18 µg per mouse, 22 MBq per mouse) was administered 16 hours following TF2 injection. Mice which received TF2 and $^{111}$In-RDC018 were imaged at two time points (2 and 24 hours post administration of the radiolabeled peptide). The reference group (n = 5) was imaged 24 and 48 hours after dual-labeled MN-14 injection.

**MicroSPECT/CT and near-infrared fluorescence (NIRF) imaging**

Mice with one CEA-positive and one CEA-negative tumor were scanned on a small-animal microSPECT/CT scanner with a 1.0-mm diameter pinhole collimator tube (acquisition time, 2x15 minutes) in prone position, followed by a CT scan (spatial resolution, 160 µm; 65 kV; 612 µA) for anatomical reference. MicroSPECT/CT scans were reconstructed with MILabs reconstruction software, which uses an ordered-subset expectation maximalization algorithm. NIRF images were acquired on the IVIS fluorescence imaging system (acquisition time, 5 minutes; binning, medium; Fstop, 2; excitation, 745 nm; excitation autofluorescence, 675 nm; emission, ICG; lamp level, high; FOV, D).

**Image-guided (post mortem) resection**

After the biodistribution experiments, an image-guided resection experiment was performed. Intraperitoneal tumors were induced in 3 groups of mice and after three weeks the targeting agents were administered. In the first group, 6 nmol of TF2 was administered to 5 mice and 16 hours following TF2 injection, 0.3 nmol of radiolabeled RDC018 was administered. Two hours following $^{111}$In-RDC018 injection, mice were imaged with microSPECT/CT and NIRF imaging. Hereafter image-guided resection using the IVIS fluorescence imaging system was performed. To confirm complete resection, additional optical imaging and SPECT/CT
images were acquired. Next, animals were dissected to determine the biodistribution of the radiolabeled peptide as described above. The two remaining groups of mice served as controls. In two mice, peritoneal tumors grew faster than expected and reached a humane endpoint before start of the experiment. These mice were therefore euthanized prior to injection of the control compounds. As a result, each control group consisted of 4 mice. In the first group, (positive control) dual-labeled MN-14 [7] was administered to 4 mice with intraperitoneal LS174T tumors. Resection and analysis were performed 3 days after dual-labeled MN-14 injection. As negative control, we used 4 mice with tumors pretargeted with the trivalent anti-CD20 bsAb TF4 [17] in combination with 111In-RDC018 with the same dosing and timing as the TF2 group.

**Statistical analysis**
Statistical analyses were performed using GraphPad Prism software (version 5.03; GraphPad Software). Student’s T test was performed on the biodistribution studies IMP288 vs RDC018 (tumor, blood and kidney), corrected for multiple testing (Bonferroni). A p-value <0.05 was used to reject the null hypothesis. Data are presented as mean and standard deviation.

**RESULTS**

**Biodistribution**
To gain more insight into differences in the in vivo behavior between 111In-RDC018 and 111In-IMP-288, mice with s.c. tumors received different dose levels of TF2 and hapten-peptide. The biodistribution of 111In-RDC018 revealed high and target-specific uptake in CEA-expressing TF2-pretargeted tumors after 2 and 24 hours (22.0 ± 4.5 %IA/g and 10.0 ± 4.7 %IA/g, respectively). In contrast, uptake of 111In-RDC018 remained low in CEA-negative tumors (6.0 ± 1.8 %IA/g and 0.9 ± 0.4 %IA/g, respectively) as well as in other healthy tissues. Compared to the reference compound 111In-IMP-288, 111In-RDC018 showed similar tumor-specific uptake in CEA-positive TF2-pretargeted tumors (Figure 1: 8 nmol TF2, Figure S2: 0 nmol and 0.8 nmol TF2). However, in CEA-negative tumors, uptake of 111In-RDC018 was significantly higher than the uptake of 111In-DOTA-IMP-288 (0.13 ± 0 %IA/g and 0.07 ± 0 %IA/g p<0.001). The same observation was made for tracer accumulation in kidney and blood, which was significantly higher for 111In-RDC018 compared to 111In-IMP-288 after 2 and 24 hours (both p<0.001). For 0.8 nmol TF2, tumor uptake of pretargeted LS174T tumors was significantly higher compared to the negative control tumors for both timepoints. In more detail, for 0.8 nmol TF2 and 2 hours after peptide injection, the uptake in CEA-positive LS174T tumors was 22.0 ± 4.5 %IA/g and 6.0 ± 1.8 %IA/g in CEA-negative SK-RC-52 tumors. (p<0.001). This difference remained 24
hours after peptide injection (10.0 ± 4.7 %IA/g and 0.9 ± 0.4 %IA/g (p<0.001), respectively). We did not observe a statistically significant difference in uptake between $^{111}$In-RDC018 and $^{111}$In-IMP-288 for both time points. For 8.0 nmol TF2 we observed similar results (Figure 1). Two hours after peptide injection, uptake was 9.9 ± 0.4 %IA/g for LS174T and 3.8 ± 0.6 %IA/g for SK-Rc-52 cells (p<0.001). This difference remained 24 hours after peptide injection (10.2 ± 1.9 %IA/g and 1.6 ± 0.3 %IA/g (p<0.001), respectively). Two hours after peptide injection, $^{111}$In-RDC018 uptake in LS174T tumors was higher than $^{111}$In-IMP-288: 9.9 ± 0.4 %IA/g and 5.3 ± 1.8 %IA/g (p=0.0135). After 24 hours, however, the uptake of $^{111}$In-RDC018 and $^{111}$In-IMP-288 was not statistically significantly different (10.2 ± 1.9 and 6.6 ± 2.9 (p=0.2713).

To compare the in vivo behavior of $^{111}$In-RDC018 in TF2-pretargeted tumors to dual labeled MN-14 (direct targeting), the biodistribution patterns of both approaches were characterized. Both tracers were shown to specifically target the CEA-expressing tumors which was confirmed by microSPECT/CT and NIRF imaging (Figure 2). Also, the excretion routes of $^{111}$In-RDC018 (via the kidneys) and $^{111}$In-MN-14-IRDye800CW (via the liver) was clearly illustrated (Figure 2).

Figure 1 Biodistribution profiles of $^{111}$In-RDC018 and $^{111}$In-IMP-288 at 2 h and 24 h p.i. after pretargeting with 8 nmol TF2 in BALB/c nude mice, showing specific tumor uptake in the s.c. CEA-expressing LS174T tumor and pronounced renal uptake.
**Figure 2** Near-infrared fluorescence (left) and microSPECT/CT (right) images of mice bearing s.c. CEA-expressing tumors (left flank), acquired using the TF2 - $^{111}$In-RDC018 pretargeting strategy (A) or the dual labeled hMN-14 direct targeting strategy (B). Both series were acquired 24 h post injection. Note the uptake in the tumor, liver and kidneys depending on tracer type.
Fluorescence imaging
In addition to the quantitative biodistribution studies based on the radiosignal, we assessed tumor accumulation and distribution of the tracer by NIRF imaging. The imaging results show a similar distribution pattern compared to the quantitative biodistributions (Figure 3), demonstrating that our pretargeted approach can be used for NIRF imaging of CEA-positive tumors, which is essential for reliable image-guided surgery.

![Figure 3 Near-infrared-fluorescence image 24 h p.i. of $^{111}$In-RDC018 in a mouse bearing a s.c. CEA-expressing LS174T tumor (left flank) and a s.c. CEA-negative SK-RC-52 tumor (right flank), confirming the specific tumor targeting in the CEA-expressing tumor and renal clearance (black arrows) of $^{111}$In-RDC018.]

Image-guided (post mortem) resection
Finally, we assessed the feasibility for pretargeted image-guided surgery in a more clinically relevant setting in mice with intraperitoneal LS174T tumors undergoing resection. RDC018 was labeled at a specific activity of 20.1 MBq/µg. The microSPECT/CT images clearly identified intraperitoneal tumors (Figure 4). Subsequently, NIRF imaging was able to identify these tumors and serve as guidance during (post mortem) resection. Finally, post-resection microSPECT/CT and NIRF imaging confirmed complete resection of tumor tissue (Figure 4).
Figure 4 Near-infrared fluorescence images (B and C) and microSPECT/CT images (A and D) of a mouse with a CEA-expressing intraperitoneal LS174T tumor (arrow) 2 h after administration of $^{111}$In-RDC018. Pre-resection, the tumor can be clearly localized using microSPECT/CT (A, white arrow) and near-infrared fluorescence imaging (B, white arrow). Subsequently, after euthanization, the tumor was resected with fluorescence image-guidance (C, white arrow). Finally, a post-resection microSPECT/CT was acquired (D) confirming the complete resection of the tumor nodule. The radiosignal of the renal clearance of $^{111}$In-RDC018 can also be observed in (A) and (D).
DISCUSSION

In the present study we show that pretargeted multimodal image-guided resection is feasible in a model for peritoneal metastasis. $^{111}$In-RDC018 accumulation in the tumor is specific and RDC018 clears via the kidneys in TF2 pretargeted intraperitoneally xenografted tumors. These characteristics enabled ‘preoperative’ microSPECT- and ‘intraoperative’ NIRF imaging for successful image-guided post mortem resection and confirmation of complete tumor resection by postoperative SPECT.

For the pretargeting strategy to work, the bispecific antibody should not show fast internalization. TF2 internalizes only minimally [18]. Furthermore, Schmidt et al. showed that CEA is a slowly and minimally internalizing antigen [19]. RDC018 is an IMP-288 derived hapten-peptide conjugated with a fluorescent moiety. To evaluate the effect of the fluorophore of RDC018 on the in vivo behavior of the molecule, the biodistribution was compared to that of IMP-288. Our results illustrate that $^{111}$In-RDC018 and $^{111}$In-IMP-288 have, apart from uptake in the CEA-negative tumor and kidneys, similar distribution patterns after TF2 pretargeting (Figure 1). However, there is more nonspecific uptake of $^{111}$In-RDC018 across all included organs. These findings suggest that the fluorophore does not relevantly compromise the parental molecule’s binding properties and in vivo behavior. We did, however, find a higher uptake of $^{111}$In-RDC018 in the CEA-negative SK-RC-52 tumors (Figure 1), which indicates more nonspecific uptake of the tracer, possibly due to different molecular characteristics of the dye (e.g. lipophilicity and charge) and/or the enhanced permeability and retention effect due to the longer blood retention time [20]. An earlier study indicated that high dye:antibody conjugation ratio’s (>2) can significantly change biodistributions of antibody-dye conjugates [21]. Herefore, the smaller molecule $^{111}$In-RDC018 contains a single fluorescent moiety and MN-14 was conjugated at a final dye:antibody ratio of 1.4. Further evaluation of chemical differences was beyond the scope of this study. The uptake of $^{111}$In-RDC018 in CEA-positive tumors, however, was large enough to provide sufficient tumor specific uptake for imaging purposes (Figure 2,3,4). Earlier studies already showed that a non-CEA specific pretargeting IgG combined with a radiolabeled hapten resulted in very low tumor uptake [18]. Kidney uptake was higher for $^{111}$In-RDC018 than for $^{111}$In-IMP-288, indicating enhanced tubular reabsorption of the dual-labeled peptide in the kidneys, most probably caused by charge of the fluorescent moiety.

Sharkey et al. and Lütje et al. demonstrated a pretargeted approach for radioimmunotherapy and image-guided surgery in a model of prostate cancer [10, 17]. Similar to their studies, we found high specific tumor uptake, and higher kidney uptake when we injected different
doses of the bispecific antibody TF2 and the $^{111}$In-labeled diHSG hapten-peptide RDC018. High renal uptake of $^{111}$In-RDC018 as a result of renal clearance might impair imaging and image guidance in the vicinity of the kidneys and bladder. This would, however, not reduce its potential in colorectal cancer, since renal involvement or dissemination is rare [22]. On the contrary, renal clearance may be advantageous for tracers targeting colorectal cancer. For example, the dual-labeled humanized antibody MN-14 is mainly cleared via the hepatobiliary route, which results in a relatively high fluorescence- and radionuclide signal in the liver [7]. Therefore, intraoperative detection of liver metastasis or peritoneal metastasis present on the visceral peritoneum of the liver may remain particularly challenging with direct targeting strategies using antibodies. Figure 1 indicates higher liver uptake for $^{111}$In-RDC018 than $^{111}$In-IMP-288. The tumor-to-liver ratio however is still greater than 5. In addition, the more favorable pharmacokinetics of smaller molecules involved in pretargeting may result in higher tumor-to-background ratios at early time points after injection and might render them more suitable for theranostic purposes compared to antibody-based strategies [8].

Several challenges remain before successful clinical translation of this type of pretargeting strategies, including optimizing dosing and timing, for example the protein dose of the antibody, dose and activity of the hapten, time interval between antibody and hapten administration, and interval between hapten administration and imaging or surgery. Despite these challenges, several clinical trials that show the safety and feasibility of TF2 bsAb and hapten pretargeting strategies have been concluded [13, 23]. Recently, Liu published a more quantitative description of the pretargeting concept which contributes to overcoming the challenges of clinical translation [24]. For fluorescence image-guided surgery, also translation of the preclinical setup to the clinical setup at the operating rooms might be challenging.

For colorectal cancer patients, the cornerstone in surgical treatment is complete and radical resection. A surgeon’s ability to distinguish benign from malignant tissue can be hampered by adhesions, fibrous or scar tissue, which may be present due to earlier intra-abdominal procedures or disease. Reliable assessment of tumor burden can therefore sometimes only be performed during the surgical procedure [25]. Imaging techniques might aid in improving patient selection and the surgeon’s ability to distinguish between benign and malignant [3]. Recently, CEA-based NIRF image-guided surgery was shown to be safe and feasible in a clinical trial with pancreatic cancer patients [26]. The same tracer was used in a trial in colorectal cancer patients with recurrent disease or peritoneal metastasis [27]. These results indicate that CEA-targeted NIRF image-guided surgery may aid the surgeon in clinical decision-making during surgery; however, the specificity found in these trials was 62%. Fluorescence has a limited penetration depth and the occurrence of false-negative tumor lesions in the former trials was
mainly caused by overlying blood or tissue. Adding a radiotracer for targeted multimodality imaging can serve a multipurpose goal [14, 28]. It can serve as a preoperative detection tool (e.g., SPECT or PET) of primary tumor and/or (peritoneal) metastases [13]. Additionally, intraoperative detection of lesions using a gamma probe could be applied for deeper lesions and can potentially increase specificity and sensitivity. Furthermore, intraoperative fluorescence imaging may identify tumor lesions and might even be used as postoperative evaluation tool for complete removal. Another advantage of adding a radiotracer to the fluorescent targeting molecules is the ability to reliably quantify the amount of tracer present in surgical specimens. Standardization in quantification of fluorescent imaging is gaining more interest [29], and could benefit from quantitative techniques using radiotracers.

CONCLUSIONS

Our findings elucidate a potential role of pretargeting strategies in the search of optimum vehicles for image-guided surgery and theranostic approaches in modern treatment of colorectal cancer. In the current study, resection of pretargeted tumors with radio- and fluorescence guidance in a colorectal cancer model was shown to be feasible. A limitation of our approach is the post mortem dissection. Therefore, the next step is optimization of this strategy before clinical translation in the future.
REFERENCES

Carcinoembryonic antigen-targeted photodynamic therapy in colorectal cancer models

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ABSTRACT

Background
In colorectal cancer, survival of patients is drastically reduced when complete resection is hampered by involvement of critical structures. Targeted photodynamic therapy (tPDT) is a local and targeted therapy which could play a role in eradicating residual tumor cells after incomplete resection. Since carcinoembryonic antigen (CEA; CEACAM5) is abundantly overexpressed in colorectal cancer, it is a potential target for tPDT of colorectal cancer.

Methods
To address the potential of CEA-targeted PDT, we compared colorectal cancer cell lines with different CEA-expression levels (SW-48, SW-480, SW-620, SW-1222, WiDr, HT-29, DLD-1, LS174T, and LoVo) under identical experimental conditions. We evaluated the susceptibility to tPDT by varying radiant exposure and concentration of our antibody conjugate (DTPA-hMN-14-IRDye700DX). Finally, we assessed the efficacy of tPDT in vivo in 18 mice (BALB/cAnNRj-Foxn1nu/nu) with subcutaneously xenografted LoVo tumors.

Results
In vitro, the treatment effect of tPDT varied per cell line and was dependent on both radiant exposure and antibody concentration. Under standardized conditions (94.5 J/cm² and 0.5 µg/µL antibody conjugate concentration), the effect of tPDT was higher in cells with higher CEA availability: SW-1222, LS174T, LoVo, and SW-48 (22.8%, 52.8%, 49.9% and 51.9% reduction of viable cells, respectively) compared to cells with lower CEA availability. Compared to control groups (light or antibody conjugate only), tumor growth rate was reduced in mice with s.c. LoVo tumors receiving tPDT.

Conclusion
Our findings suggest cells (and tumors) have different levels of susceptibility for tPDT even though they all express CEA. Furthermore, tPDT can effectively reduce tumor growth in vivo.
INTRODUCTION

Surgery is a cornerstone in curative treatment of colorectal cancer; however, incomplete resection can drastically reduce survival after surgery [1, 2]. Radical resection of tumors might be hampered by involvement of critical structures including large vessels, nerves or visceral organs. In advanced-stage cancers, adjuvant local or systemic therapies can improve survival after (incomplete) resection. Survival of systemically-treated patients with advanced colorectal cancer has improved with modern systemic therapies [3], but the maximum dose of systemic therapy is limited by toxicity and side effects [4].

Photodynamic therapy (PDT) could play a role in overcoming the limitations of incomplete radical resection and toxicity from systemic therapy. PDT has gained a role in treatment in different fields of oncology nowadays [5-8]. It can be applied as standalone treatment modality, although it might also serve as adjuvant treatment to surgery after incomplete resection [9].

The principle of PDT is based on combining three non-toxic components: oxygen, light and a photosensitizer. The photosensitizer is excited with the physical energy of non-ionizing light (of a specific wavelength) which, through a series of photochemical reactions, results in formation of highly reactive oxygen species (ROS)[10, 11]. In turn, ROS may induce local cell apoptosis and necrosis, and/or cause microvascular damage. Furthermore, a change in photosensitizer structure and hydrophilicity has been proposed to contribute to cell damage [12]. Preferably, accumulation of the photosensitizer should be tumor-specific to prevent extensive damage to the normal tissue surrounding the tumor and to increase the intratumoral dose. Therefore, a tumor-targeted PDT approach has been developed [13]. In targeted photodynamic therapy (tPDT), a tissue of interest is selectively localized using a targeting vehicle conjugated to a photosensitizer. When the photosensitizer-vehicle conjugate has accumulated in targeted tissue, light of a specific wavelength is administered locally, making this therapy highly specific. Carcinoembryonic antigen (CEA) is a membrane-anchored glycoprotein and is overexpressed in 90-95% of colorectal cancer cases. Therefore, CEA can be used for primary targeting of colorectal carcinomas. hMN-14 (labetuzumab) is an IgG directed against the carcinoembryonic antigen-related cell adhesion molecule 5 with high affinity [14]. However, different tumors express different amounts of CEA. Therefore, in clinical practice CEA-targeted PDT might...
only be useful in tumors with sufficient CEA expression, when complete tumor resection is hampered by the presence of critical structures that are to be preserved. Here, we investigate whether the effect of tumor-targeted PDT is influenced by the availability of CEA on the cell surface of tumor cells with different CEA expression levels. Our multimodal conjugate (DTPA-hMN-14-700DX) consists of the humanized anti-CEA antibody, hMN-14 (labetuzumab), the photosensitizer IRDye700DX, and the chelator diethylenetriaminepentaacetic acid (DTPA). DTPA allows radiolabeling with $^{111}$In and subsequent in vivo tumor detection and precise quantification of the antibody conjugate accumulation. After the in vitro experiments on different colorectal cancer cell lines, the therapeutic effect of tPDT was evaluated in vivo in a xenograft mouse model.

**MATERIALS AND METHODS**

**Cell culture**
CEA-expressing human colon adenocarcinoma (primary or metastatic) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). SW-1222 cells were obtained from Sigma-Aldrich (Saint Louis, MO, USA). LS174T, SW-620, SW-480, SW-48, DLD-1 and HT-29 were cultured in RPMI-1640 (Gibco, Dun Laoghaire, Ireland) supplemented with 10% fetal bovine serum (FBS) and 2 mM L-Glutamine. WiDr and SW-1222 were cultured in DMEM high glucose (Gibco) supplemented with 10% FBS. LoVo was cultured in Ham’s F-12 Nutrient mix GLUTAMAX (Gibco) supplemented with 10% FBS. No antibiotic additives were used. All cells tested negative for Mycoplasma. Cells were cultured in tissue culture flasks in a humidified incubator at 37°C in an atmosphere of 95% air and 5% CO$_2$. Cells were harvested with 0.05% trypsin-EDTA (ethylenediaminetetraacetic acid).

**DTPA-hMN-14-IRDye700DX conjugation**

hMN-14 (labetuzumab) was kindly provided by Immunomedics, Inc., Morris Plains, NJ, USA. It was conjugated with IRDye700DX-NHS (LI-COR, Lincoln, NE, USA) and SCN-Bz-diethylenetriaminepentaacetic acid (DTPA) (Macrocyclics, Plano, TX, USA) in two steps. First, hMN-14 was conjugated with IRDye700DX-NHS in 0.1 M NaHCO$_3$, pH 8.5, with a 10-fold molar excess of IRDye700DX-NHS. Next, the reaction mixture was incubated for 1 hour at room temperature on an orbital shaker and protected from light. Second, SCN-Bz-DTPA in 0.1 M NaHCO$_3$, pH 9.5 was added to the reaction mixture in a 10-fold molar excess. After another hour of incubation on the orbital shaker in the dark, the mixture was dialyzed in a Slide-A-Lyzer (10 kDa cutoff; Thermo Fisher Scientific, Waltham, MA, USA) against phosphate-
buffered saline (PBS) containing 2 g/L Chelex® 100 Resin (Bio-Rad Laboratories, Inc.; Hercules, CA, USA). The final concentration of the conjugate was determined spectrophotometrically at 280 nm (Ultrospec 2000 spectrophotometer; Pharmacia Biotech), correcting for the absorption of IRDye700DX at that wavelength (3%, according to the manufacturer’s protocol). The molar substitution ratio of IRDye700DX was determined spectrophotometrically at 648 nm and reached 4.5.

**Radiolabeling of the hMN-14 conjugate**

Briefly, $^{[111}\text{In}]\text{InCl}_3$ (Curium, Petten, The Netherlands) was added to DTPA-hMN-14-IRDye700DX in three volumes of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 5.5. After 30 minutes of incubation at room temperature, 50 mM EDTA was added to the labeling reaction to a final concentration of 5 mM to chelate unincorporated $^{[111}\text{In}]\text{InCl}_3$. Labeling efficiency was determined by instant thin-layer chromatography on Varian silicagel strips (ITLC-SG; Agilent Technologies, Amstelveen, The Netherlands) using 0.1 mM ammonium acetate ($\text{NH}_4\text{Ac}$) buffer with 0.1 M EDTA, pH 5.5 as the mobile phase and labeling efficiency reached >95%.

For the in vitro binding assay, DTPA-hMN-14-IRDye700DX was radiolabeled with 0.5 MBq/µg of $^{[111}\text{In}]\text{InCl}_3$. For the biodistribution studies, in two mice with s.c. LoVo tumors, DTPA-hMN-14-IRDye700DX was radiolabeled with 0.23 MBq/µg of $^{[111}\text{In}]\text{InCl}_3$.

**In vitro binding assay**

The radiolabeled conjugate was diluted to contain 2.4 kBq per µL. To count the amount of activity, we used a shielded 3”-well-type $\gamma$-counter (Perkin-Elmer, Boston, MA, USA). Cells were counted and placed in culture medium supplemented with 0.5% Bovine Serum Albumin (BSA; Sigma-Aldrich Chemie N.V., Zwijndrecht, Netherlands) (Binding buffer; BB) at a concentration of 1∙10^7 cells per ml. Next, 4∙10^6 cells were pipetted in a 1.5 mL eppendorf tube (2 tubes per cell line; experiment performed in triplicate). We added 240 kBq of our radiolabeled antibody conjugate to cells in each tube. An excess of unlabeled DTPA-hMN-14-IRDye700DX (1.9 µg) was added to the last three tubes of each cell lines to determine the amount of nonspecific binding of the conjugate.

Cells were incubated at 37 °C with 5% CO₂ for 4 hours. Following incubation, cells were centrifuged at 805 g for 5 min, the supernatant was removed and the remaining activity in the tubes was measured in a $\gamma$-counter. A 100-µL standard, representing 100% activity, was measured in triplicate simultaneously. The antibody binding in the presence of an excess unlabeled antibody was subtracted from the antibody conjugate binding for each cell line. This results in a measure for the specific binding of our antibody conjugate, expressed as percentage of the total amount of added antibody conjugate.
PDT device
We used a standardized 690nm (SMBB690D-1100-02) high-output LED (Marubeni America Corporation; Santa Clara, CA, USA) device for illumination of targets [15]. All experiments were performed with the device set to its maximum power output of 200 mW/cm². To adjust light fluency rate, we varied the distance between light source and target. The bottom of the in vitro setup was transparent to prevent excessive heat generation and light reflection from the surface beneath the treated cells.

In vitro photodynamic therapy
To gain insight into the effects of tPDT and CEA availability, LoVo, LS174T, DLD-1 and HT-29 cells were treated with different antibody conjugate doses and light doses (Figure 1). Next, to directly compare the therapeutic effect on the 9 cell lines, all cells were treated with the same light and antibody-conjugate dose. Briefly, $2 \times 10^5$ cells per well were plated in 2 wells of a 24-wells plate (experiment performed in triplicate) one day prior to treatment. On the day of treatment, 0.5 μg/μL DTPA-hMN-14-700DX was added in 500 μL of BB. In this experiment tPDT was performed with a radiant exposure of 94.5 J/cm².

For both in vitro experiments, after harvesting, cells were plated in transparent 24-wells plates (Corning Inc.; Corning, NY, USA) ($1.5-2.0 \times 10^5$ cells per well) and allowed to adhere overnight. After cells were adherent, they were incubated with the antibody-conjugate in BB for 4 hours. Following incubation, BB was removed and cells were washed once with phosphate buffered saline to remove unbound antibody. Next, fresh medium was added and cells were exposed to specific radiant exposures. Cells rested for 1 hour after light exposure. Next, cells were washed to remove cell debris. Hereafter, cell viability was assessed using the cell titer glo® (Promega Corporation; Madison, WI, USA) luminescent cell viability assay. Incubation in demineralized water was used as positive control for 100% cell death.
All animal experiments were approved by the Dutch Central Committee for Animal Experiments, local protocols were approved by the Institutional Animal Welfare Committee of the Radboud University Medical Center and were conducted in accordance to the guidelines of the Revised Dutch Act on Animal Experimentation (2014).

Twenty male BALB/cAnNRj-Foxn1nu/nu nude mice (7 to 9 weeks-old, 18-22 g body weight; Janvier labs; Le Genest-Saint-Isle, France) were housed in individually ventilated cages (5 mice per cage) under standard non-sterile conditions. Mice had free access to standard animal chow and water. Animals were adapted to laboratory conditions for one week before experimental use. Subcutaneous (s.c.) tumors were induced by s.c. injection of $5\times10^6$ freshly harvested LoVo cells. Tumors grew in all mice that were injected s.c.

*Figure 1* In vitro cell viability (tPDT effect) on LoVo, LS174T, DLD-1, and HT-29 cells treated with different doses of light and antibody conjugate concentration. Statistically significant differences ($p<0.05$) are indicated with horizontal bars.
In vivo targeted photodynamic therapy

When average tumor size reached 45 mm³ and after stratification based on tumor size, 18 mice were randomly allocated into three experimental groups (treatment tPDT, PBS + 0.5% BSA with light exposure, antibody-conjugate without light exposure), 6 mice per group, mice were injected with 30 µg of unlabeled DTPA-hMN-14-IRDye700DX or PBS + 0.5% BSA via a 200-µl tail vein injection.

Tumors of mice in one control group (n=6) and the treatment group (n=6) were selectively exposed to 300 J/cm² of near infrared (NIR) light under inhalation anesthesia (2.5% isoflurane mixed with 100% O₂ (1 L/min)). All mice, including non-irradiated controls, were anesthetized for 12 minutes. The liver and other organs were protected from exposure to the NIR light by covering those areas with a gauze and aluminum foil. Treatment efficacy was determined based on tumor growth. Tumor diameters were measured in three dimensions by a blinded observer using a caliper three times per week. Tumor volume was calculated as the volume of an ellipsoid: \( \frac{4}{3} \pi r_1 \cdot r_2 \cdot r_3 \). Herein, \( r \) was calculated by dividing the tumor length, width, or height by two. Mice were euthanized by O₂/CO₂ asphyxiation when tumor volume exceeded more than 1,000 mm³. One mouse in the treatment group was excluded from the analyses, since we failed to irradiate its tumor with light (the aluminum foil shifted and covered the tumor).

Biodistribution

Two mice were used to determine the biodistribution of \(^{111}\text{In}-\text{labeled DTPA-hMN-14-IRDye700DX}\) (figure S2) ex vivo. Twenty-four hours after injection of the tracer, mice were euthanized and tissues of interest (tumor, muscle, lung, spleen, kidney, liver, pancreas, stomach, and duodenum) were dissected and weighed after which activity was measured in the \( \gamma \)-counter. Blood samples were obtained by cardiac puncture. For calculation of the uptake of activity in each tissue as a fraction of the injected activity, three aliquots of the injection dose were counted in the \( \gamma \)-counter simultaneously.
Statistical Analyses
Statistical analyses were performed using GraphPad Prism version 5.03 (GraphPad Software, Inc.; San Diego, CA, USA) and Statistical Package for Social Sciences, Version 22.0 (IBM Corp.; Armonk, NY, USA). A two-way ANOVA with Bonferroni correction for multiple testing was performed to analyze the effects of radiant exposure and antibody dose in the in vitro experiment with 9 different colorectal cancer cell lines. Furthermore, a one-way ANOVA with Dunnet correction for multiple testing was performed to compare the control condition without antibody and light (absolute control) with the control condition with light (internal control). For the in vivo experiment, a one-way ANOVA with Bonferroni correction for multiple testing was performed. A p-value <0.05 was used to reject the null hypothesis. Data are presented as mean and standard deviation.

RESULTS
First, we assessed the binding capacity of $^{111}$In-DTPA-hMN-14-IRdye700DX to cell lines with different CEA expression levels. This capacity can be considered as a surrogate value for accessible CEA epitopes on the cell surface. Figure S1 illustrates that the cells used in this study can be roughly classified into two groups: low and high CEA accessibility.

To gain insight into the effects of tPDT, we treated 2 cell lines with low CEA availability and 2 cell lines with high CEA availability under different conditions, varying both the antibody conjugate concentration and radiant exposure (light dose).

LS174T and LoVo cells (both high CEA availability) showed a light dose dependent increase of therapeutic efficacy of tPDT, regardless of antibody conjugate concentration (Figure 1). At the highest light dose (314.5 J/cm$^2$), the effect of tPDT on LoVo was higher at 3 µg/mL compared to 1 µg/mL antibody conjugate concentration (27.8% vs 15.1% viable cells, p = 0.002). At lower light doses, we did not observe an additional effect of increasing the antibody conjugate dose. Similar to LoVo, in LS174T the effect of tPDT increased with increasing light dose (Figure 1). So, in the cell lines with higher CEA availability, we observed a clear effect of increasing light dose and antibody conjugate dose on the effect of tPDT. In contrast, in cell lines with lower CEA availability (DLD-1 and HT-29), we did not observe this variation in susceptibility for tPDT. Decreased cell viability due to tPDT in DLD-1 was only observed at the highest dose level of light (p <0.001) and was not dependent on the antibody conjugate concentration used. In HT-29 cells (low to moderate CEA availability), we did not find an effect of tPDT at 1 µg/mL of antibody conjugate. At 3 µg/mL, however, there was an effect of tPDT at the two highest light doses (94.4 and 314.5 J/cm$^2$). From these experiments, we concluded that the effect of
antibody conjugate dose and light dose on the efficacy of tPDT seem to be dependent on the CEA availability in tumor cells.
Subsequently, we performed a separate experiment to investigate the relationship between CEA availability and tPDT effects. To explore this relationship, we included 9 cell lines with varying CEA expression levels. Figure 2 summarizes our findings on tPDT effect and its relation to amount of antibody conjugate binding of the different cell lines. No treatment effect was observed in cells with less than 5% specific binding of the antibody conjugate, which represents low CEA availability (HT-29, SW620, SW480, WiDr, and DLD-1). SW-1222 (5.5% specific binding) showed a moderate effect of tPDT after treatment (78.2% viable cells). LS174T, LoVo, and SW48 (7.7, 11.7, and 30.6% specific binding, respectively) were more sensitive to treatment with tPDT (47.2%, 50.1%, and 48.1% viable cells after treatment, respectively), suggesting that a minimum level of antibody conjugate binding to the cells is required for effective treatment with tPDT.
Subsequently, we evaluated the ability of tPDT to reduce in vivo tumor progression in a xenograft mouse model. Before evaluating the treatment, we performed an ex vivo biodistribution of $^{111}$In-labeled DTPA-hMN-14-IRDye700DX. We found 13.7 ± 3.8 %ID/g of our tracer in tumor, 8.8 ± 0.5 %ID/g in spleen and 29.4 ± 2.6 %ID/g in liver tissue (Figure S2). In mice treated with tPDT, we generally observed slower tumor growth than the control groups (n=17, p>0.05). The tumor of one mouse in the treatment group, however, showed a more aggressive growth pattern after treatment (mouse 1, Figure 3). This was possibly caused by incomplete tumor irradiation during tPDT, but because this was not reported during treatment of this mouse, this dataset was not rejected from the analyses.
Figure 2 Relation between the tPDT effect (amount of remaining viable cells 1 hour after treatment) and antibody conjugate binding (expressed as percentage of the total amount of added antibody conjugate) for the nine different cell lines. Note that in general more binding (more accessible/targetable CEA sites) leads to a greater effect of tPDT (fewer viable cells).
Figure 3 In vivo tumor growth progression after targeted PDT treatment (day 0) and control conditions followed up three times per week. The two control groups: DTPA-hMN-14-IRDye700DX only group (without light exposure) in blue and the light-only group in red. Only 5 mouse were treated due to a technical incident during the treatment of the 6th mouse. In general, mice in the tPDT (treatment) group show a delayed tumor growth pattern. One of the treated mice had a more aggressive growth pattern.
DISCUSSION

The current study demonstrates that in vitro tumor cell lines with different levels of CEA targetability have varying susceptibility to tPDT. Furthermore, the amount of $^{111}$In-DTPA-hMN-14-IRDye700DX binding to these cells seems to play a crucial role in the efficacy of tPDT. Our findings suggest that only cell lines that can be targeted with higher amounts of antibody-conjugate can be treated effectively with tPDT. In vivo, we observed a trend towards tumor growth delay in xenografted tumors of tPDT-treated mice.

In the tested cell lines, higher light dose increased the efficacy of tPDT. However, in DLD-1 and LS174T cells, the maximum light dose led to a statistically significant decrease in cell viability even without the presence of the antibody conjugate (Figure 1). This suggests that the energy provided by the light source may have been excessive in this condition. Hyperthermia can lead to decreased cell survival in (monolayer) cells and is likely to be responsible for the decrease in cell viability of the 0 $\mu$g/mL control condition of LS174T and DLD-1 [16, 17].

In this study, we included a large series of CEA-expressing cell lines in a direct comparison. To compare the tPDT effect in all cell lines under identical conditions, we performed a single experiment with fixed conditions: 0.5 $\mu$g/µL $^{111}$In-DTPA-hMN-14-IRDye700DX and total radiant exposure 94.5 J/cm². Higher radiant exposures could have an additional thermal effect on several cell lines (see above). Since we chose 94.5 J/cm², the observed effects depicted in Figure 2 are considered to be only tPDT-mediated, but may be an underestimation of the maximum tPDT effect that could be achieved. More specific binding of $^{111}$In-DTPA-hMN-14-IRDye700DX resulted in a larger treatment efficacy of tPDT in vitro (Figure 2). These findings are consistent with previous studies that showed an increased efficacy of tPDT when there is more photosensitizer available in the tumor [13].

Increasing the number of photosensitizer moieties per antibody molecule (substitution ratio) might further increase the amount of photosensitizer in tumors. However, as we use a random conjugation method, excessive conjugation of the photosensitizer to an antibody might affect the antibody binding affinity. Additionally, when conjugating high amounts of a photosensitizer or in general a fluorescent dye to an antibody, changes in chemical properties (lipophilicity and net charge) may lead to faster blood clearance, less tumor uptake, and more accumulation in liver and spleen [18-20]. Findings from these studies are in line with the findings of the biodistribution performed in the current study. Random conjugation strategies can, therefore, be considered a limitation regarding the preparation of an antibody-photosensitizer conjugate. Our results also suggest that tPDT efficacy is dependent on a combination of radiant exposure and amount of photosensitizer that is present on the target.

In addition, different coping strategies of tumor cells to tPDT-induced damage may affect
treatment efficacy, but this was not taken into account in the current study.

In contrast to in vitro tPDT, in vivo tPDT can be particularly challenging due to the multifactorial nature of the treatment and treatment effect. In the current study, we tested the efficacy of tPDT with subcutaneous LoVo tumors in BALB/c nude mice. LoVo cells were chosen as a representative because the antibody-conjugate binding to the cells was the most representative of the 3 cell lines with the highest binding percentage (SW-48, LoVo and LS174T) (Figure S1). We observed a steady increase in tumor size in the control groups during 6 weeks. The treated mice showed a slower tumor growth pattern in general (Figure 3). The delay in tumor growth was, however, not statistically different compared to the control groups. This was caused by one treated mouse that showed an accelerated tumor growth pattern. When cells survive after (incomplete) tPDT, cell cycle progression might be stimulated, resulting in increased proliferation, and invasive and metastatic growth [21]. This can particularly occur when treatment is not adequate. Furthermore, in a single tumor, heterogeneity in CEA expression may exist, which could be a limitation in adequate tumor targeting [22, 23]. Earlier studies indicate that host immunity can also play a crucial role in achieving a successful effect of (targeted) PDT [10, 24]. However, due to the use of an immunodeficient mouse model, the effects of immune system involvement could not be assessed in the current study. Moreover, fractionated and repeated tPDT might enhance the tPDT effect, as observed earlier by Mitsunaga and colleagues [25].

During the past years, targeted PDT approaches have gained more clinical and scientific interest. In the clinical situation, tPDT may mainly have a role as adjuvant therapy to surgery [9]. The main advantage of tPDT over other adjuvant therapies is the ability to selectively apply therapy to local areas and tissues of interest, thereby minimizing side effects. When combined with NIR fluorescence- or nuclear imaging, its potential for clinical use as theranostic approach is highlighted even further [26, 27]. Here, we focused on tPDT in a colorectal cancer model using a humanized monoclonal antibody directed against CEA (CEACAM5). Since other primary epithelial malignancies also express varying levels of CEA, CEA-targeted PDT could potentially be used in treatment of other malignancies as well. These include carcinomas of the gall bladder, urinary bladder, stomach, pancreas, ovary, endometrium, and lung [28]. Other conjugates of the same antibody have already been used in (clinical) studies investigating fluorescence- and radioguided surgery, radioimmunotherapy, and antibody-drug conjugates [29-35].
Our findings are in line with work by Shirasu et al., who showed phototoxic and dose-dependent effects of CEA-targeted PDT, with their anti-CEA antibody C2-45 conjugated with IRDye700DX [36]. Our radiolabeled antibody conjugate, $^{111}$In-DTPA-hMN-14-IRDye700DX, allows precise quantification of the tracer both in vivo and in vitro, and may enable SPECT or PET imaging prior to surgery. During surgery, when tumors are covered by overlying tissue, the radiolabel could guide the surgeon to the area of interest, using a gamma probe prior to resection of tumors [29, 37]. Subsequently, tumor margins may be visualized using near-infrared fluorescence imaging using the same radiolabeled antibody conjugate. Lastly, the photosensitizer can be irradiated to treat residual tumor that was excluded from excision [9].

**Conclusions**

Our findings suggest cells (and tumors) have different levels of susceptibility for tPDT even though they all express CEA. Furthermore, tPDT can effectively reduce tumor growth in vivo. In the current study, we treated whole subcutaneous xenografted tumors, whereas in the clinical situation one would optimally use tPDT after resection to treat (microscopically) small amounts of residual tumor cells. Thus, our findings could even be an underestimation of clinically-achievable results with tPDT.
REFERENCES


Ex vivo assessment of tumor-targeting fluorescent tracers for image-guided surgery

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Cancers, April 2020, 12(4):987
ABSTRACT

Background
Image-guided surgery can aid in achieving complete tumor resection. The development and assessment of tumor-targeted imaging probes for near-infrared fluorescence image-guided surgery relies mainly on preclinical models, but the translation to clinical use remains challenging. In the current study we introduce and evaluate the application of a dual-labelled tumor-targeting antibody for ex vivo incubation of freshly resected human tumor specimens, and assessed the tumor-to-adjacent tissue ratio of the detectable signals.

Methods
Immediately after surgical resection, peritoneal tumors of colorectal origin were placed in cold medium. Subsequently, tumors were incubated with $^{111}$In-DOTA-hMN-14-IRDye800CW, an anti-carcinoembryonic antigen (CEA) antibody with a fluorescent and radioactive label. Tumors were then washed, fixed, and analyzed for the presence and location of tumor cells, CEA expression, fluorescence, and radioactivity. Twenty-six of 29 tumor samples obtained from 10 patients contained malignant cells.

Results
Overall, fluorescence intensity was higher in tumor areas compared to adjacent non-tumor tissue parts ($p < 0.001$). The average fluorescence tumor-to-background ratio was $11.8 \pm 9.1:1$. A similar ratio was found in the autoradiographic analyses. Incubation with a non-specific control antibody confirmed that tumor targeting of our tracer was CEA-specific.

Conclusion
Our results demonstrate the feasibility of this tracer for multimodal image-guided surgery. Furthermore, this ex vivo incubation method may help to bridge the gap between preclinical research and clinical application of new agents for radioactive, near infrared fluorescence or multimodal imaging studies.
INTRODUCTION

Successful surgical treatment of many cancers relies on complete tumor resection; incomplete resection increases the odds of recurrence [1–7]. Especially in late-stage cancers, complete resection of tumors and accurate detection of their metastases remains challenging. Intraoperative imaging techniques, such as real-time near-infrared fluorescence imaging, might help to overcome these challenges [8]. Currently, several clinical trials in patients with various types of cancer are ongoing, and others have demonstrated that fluorescent imaging probes can be used for intraoperative or postoperative evaluation of tumor margins and the presence of cancer [9,10]. Tumor-specific fluorescent or multimodal tracers might help to increase the surgeons’ ability to discriminate between healthy and malignant tissue, and several clinical trials in different cancers evaluated their use [8].

There are several prerequisites for successful tumor-targeted fluorescent image-guided surgery. Obviously, the amount of tracer accumulating in the tumor should surpass the detection limit of the fluorescence imaging camera system. Second, the fluorescent tracer should reach sufficiently high tumor-to-background tissue ratios (TBR) in vivo to discriminate between tumor and adjacent normal tissue.

Newly developed fluorescent tracers for use in surgical oncology are initially tested in vitro for their tumor-targeting potential, using library-derived cancer cell lines that are cultured under standardized laboratory conditions. Subsequent steps may include small animals, (patient-derived) organoid models, or the ex vivo perfusion of organs. Animal models can provide information on biodistribution, clearance and toxicity. However, the ability of preclinical results to predict clinical practice is limited [11]. For fluorescent tracers this applies to the TBR found in patients.

Therefore, we present a method to assess the TBR for predicting the clinical feasibility of a fluorescently labeled tracer for image-guided surgery. In short, we incubated freshly-resected peritoneal tumors of patients with colorectal peritoneal carcinomatosis with a multimodal anti-carcinoembryonic antigen (CEA) antibody ($^{111}$In-DOTA-hMN-14-IRDye800CW), because CEA is overexpressed in colorectal cancer and can be used as a highly specific target for fluorescently-labeled tracers [12,13]. The feasibility of this multimodal antibody for fluorescence image-guided surgery and detection of metastasis has already been demonstrated in mice with human tumor xenografts [14,15]. The aim of this study was to determine the tumor to adjacent tissue contrast after ex vivo tissue incubation with $^{111}$In-DOTA-hMN-14-IRDye800CW to assess the feasibility of using this tracer for fluorescence image-guided surgery in cancer patients.
CHAPTER 9

MATERIALS AND METHODS

Ethical Approval
All experiments have been carried out according to the principles of the Declaration of Helsinki. The study protocol and collection of tissue were approved by the research ethics committee of the region Arnhem-Nijmegen (CMO regio Arnhem-Nijmegen) on 01-05-2017 (ethic code: 1004-2017).

Patients and Patient Tissue Samples
We collected tissue from 11 patients with peritoneal metastases of colorectal origin who underwent cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). According to the approved protocol, only surgical resection specimens of tumors that did not need standard pathological evaluation were collected (i.e. tissue surplus). Patient data was anonymized for members of the research team that did not have a direct treatment-relation with the patients. Therefore, no informed consent was required as approved by the ethics committee. One patient was excluded because the processing time (resection to incubation) was too long (> 2 hours), which led to tissue of inferior viable quality.

Antibody Preparation
hMN-14 (labetuzumab) was kindly provided by Immunomedics, Inc. (Morris Plains, NJ, USA). hMN-14 is a humanized IgG directed against carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) with high affinity [34]. We used a human isotype IgG (hlgG) as a negative control: human IgG-UNLB (SouthernBiotech, Birmingham, AL, USA). Prior to conjugation, stock hlgG was dialyzed in a Slide-A-Lyzer (10 kDa cutoff; Thermo Fisher Scientific, Waltham, MA, USA) against phosphate-buffered saline (PBS). For the current experiments, hMN-14 and hlgG were conjugated with IRDye® 800CW-NHS (LI-COR, Lincoln, NE, USA) and p-SCN-Bn-DOTA (Macrocyclics, Plano, TX, USA) in two steps. First, hMN-14 (9.6 mg/ml) and hlgG (0.63 mg/mL) were conjugated with IRDye800CW-NHS in 0.1 M NaHCO₃, pH 8.5, with a 3-fold molar excess of IRDye800CW-NHS. Next, the reaction mixture was incubated for 1 hour at room temperature on an orbital shaker and protected from light. Second, DOTA-NHS in 0.1 M NaHCO₃, pH 9.5, was added to the reaction mixture in a 10-(hMN-14) and 20-fold (hlgG) molar excess. After another hour of incubation on the orbital shaker in the dark, the mixture was dialyzed in a Slide-A-Lyzer (10 kDa cutoff; Thermo Fisher Scientific, Waltham, MA, USA) against 0.25 ammonium acetate (NH₄Ac), pH 5.5, containing 2 g/L Chelex® 100 Resin (Bio-Rad Laboratories, Inc.; Hercules, CA, USA). The final concentration of the conjugates was determined spectrophotometrically using the Infinite 200® Pro (Tecan group Ltd, Männedorf,
Switzerland) measuring at 280 nm, correcting for the absorption of IRDye800CW. The substitution ratio of the dye reached 1.09 for hMN-14 and 0.88 for hlgG.

Radiolabeling of the Antibody Conjugates
Briefly, [\[^{111}\text{In}\]InCl\(_3\)] (Curium, Petten, The Netherlands) was added to dual-conjugated hlgG or hMN-14 in two volumes of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 5.5. After 45 minutes of incubation at 40 °C, 50 mM ethylenediaminetetraacetic acid (EDTA) was added to the labeling reaction in a final concentration of 5 mM to chelate unincorporated \([^{111}\text{In}]\text{InCl}_3\). Labeling efficiency was determined by instant thin-layer chromatography (ITLC) on Varian silicagel strips (ITLC-SG; Agilent Technologies, Amstelveen, The Netherlands), using 0.1 mM ammonium acetate (NH\(_4\)Ac) buffer with 0.1 M EDTA, pH 5.5, as the mobile phase. Antibody-conjugates were purified by gel filtration on a PD-10 column and the radiochemical purity of the final dual-labeled conjugates reached >95%, as determined by ITLC.

In-Vitro Antibody Testing
To confirm that the hMN-14 antibody-conjugate was immunoreactive for CEA, and the non-specific control hlgG conjugate was not, the two antibody preparations were tested in an in vitro binding assay. DOTA-hlgG-IRDye800CW and DOTA-hMN-14-IRDye800CW were radiolabeled with 0.04 MBq/µg of \([^{111}\text{In}]\text{InCl}_3\).

Briefly, the radiolabeled conjugates were diluted to contain 400 Bq / µL. To determine the amount of activity, we used a shielded 3”-well-type γ-counter (Perkin-Elmer, Boston, MA, USA). Mycoplasma-negative, human CEA-positive colon adenocarcinoma LS174T cells (ATCC, Manassas, VA, USA) were cultured according the supplier’s instructions, without antibiotic additive. Cells were counted and placed in RPMI 1640 medium (ThermoFisher Scientific, Waltham, MA, USA) supplemented with 0.5% bovine serum albumin (BSA; Sigma-Aldrich Chemie N.V., Zwijndrecht, The Netherlands) (Binding buffer; BB). Next, cells were pipetted in 12 1.5 mL Eppendorf tubes (1.2⋅10^6 cells per tube). We added 40 kBq of the respective radiolabeled antibody conjugate (hMN-14 or hlgG) to cells in each tube (6 tubes with hMN-14; 6 with hlgG). An excess of unlabeled DOTA-hMN-14-IRDye800CW (> 2.0 µg) was added to 3 tubes of each antibody conjugate to determine the amount of nonspecific binding of the conjugate. Cells were incubated at 37 °C with 5% CO\(_2\) for 4 hours. Following incubation, cells were centrifuged at 800 x g for 5 min, the supernatant was removed and the remaining activity in the tubes was measured in the γ-counter. A 100-µL standard, representing 100% activity, was measured in triplicate simultaneously.

Analyses of the fluorescence signal of the antibody conjugate were performed as described by Rijpkema et al. [15].
**Patient Tissue Incubation and Handling**

Immediately after resection, specimens were placed in 4–8 °C low glucose Dulbecco’s Modified Eagle Medium (DMEM) (ThermoFisher Scientific, Waltham, MA, USA), supplemented with penicillin-streptomycin (Gibco™, ThermoFisher Scientific, Waltham, MA, USA) (10000 U/mL penicillin and 10000 µg/mL streptomycin), diluted in DMEM to a final concentration of 100 U/mL (Figure 1A). Within 1 hour after resection, adipose tissue parts were discarded and, to promote tissue penetration of the antibody-conjugate, several larger (>3 mm) individual tumors were divided into 2 or more similar parts by sharp dissection (max. 2 x 2 mm). Subsequently, tumors were placed in DMEM supplemented with 100 U/mL penicillin-streptomycin and 0.1% BSA, and 4 µg·ml⁻¹, indium-111-labeled antibody conjugate. The incubation concentration was similar to the blood concentration that is to be expected directly following intravenous injection of approximately 20 mg antibody-conjugate in a patient with 5L of blood. Clinical image-guided surgery studies with tumor-targeting antibody conjugates used similar doses [23,24]. Tumors were incubated in 5–10 mL of incubation medium on an orbital shaker at 37°C in an atmosphere containing 5% CO₂ (Figure 1B). Incubation was performed overnight (mean incubation time: 20 hours ± 1 hour). Following incubation, tumors were washed in at least 4.5 L of continuously moving and serially refreshed PBS supplemented with 0.1% BSA at 4–8 °C for a minimum of 4 hours (Figure 1C).

Specimens were fixed in 4% formaldehyde and embedded in paraffin. After embedding, 4 µm histological sections were cut to a maximum of 150 µm from the surface. Sections were mounted on immunohistochemical microscope glass slides. Four serial slides were used for fluorescence imaging, macroautoradiography, H&E staining, and immunohistochemistry (IHC) (Figure 1D). The involved pathologist (LB) microscopically evaluated on H&E slides if the tissue was suitable for histopathological analyses after the incubation process. IHC was performed to evaluate carcinoembryonic antigen (CEA), cytokeratin 20 (CK20), and homeobox protein 2 (CDX-2). The slide designated for H&E staining was first imaged on a closed field fluorescence imaging system (Odyssey CLx; LI-COR Biosciences, Lincoln, NE, USA). Next, the same slide was placed on a photostimulable phosphor plate. After 10 days, we imaged the plate using photo-stimulated luminescence on a phosphor imager (Typhoon FLA 7000 phosphor imager, GE Healthcare, Hoevelaken, The Netherlands). Thereafter, standard H&E staining was performed according to local protocol.

We included two patients for the hIgG antibody-conjugate control. In both patients, the tumors were cut into 2 equal halves so that the same tumor could be incubated with both antibody conjugates (¹¹¹In-labeled DOTA-hMN-14-IRDye800CW and ¹¹¹In-labeled DOTA-hlgG-IRDye800CW).
**Figure 1** Method of ex vivo incubation of surgical tissue samples with $^{111}$In-DOTA-hMN-14-IRDye800CW. (A) surgical resection of tumor specimen and tissue preparation. (B) Overnight incubation with $^{111}$In-DOTA-hMN-14-IRDye800CW at 37°C on orbital shaker with antibiotic additives. (C) 4 hours washing of unbound antibody-conjugate at 4°C. (D) Formalin-fixation, paraffin embedding, and mounting of serial slices on glass slides for (immuno)histochemical analyses.
**Immunohistochemistry**

Slides were deparaffinized with xylene, rehydrated in ethanol and rinsed in distilled water according to standard local protocol. Heat-induced antigen retrieval was performed in EDTA solution (pH 9.0). Endogenous peroxidase activity was blocked with 3% H₂O₂ in 10 mM PBS for 10 minutes at room temperature. Subsequently, tissue sections were washed with 10 mM PBS and stained with primary antibodies (CEA: Mouse monoclonal; Clone COL-1; MS-613-P; Neomarkers Fremont, CA, U.S.A. CK20: Rabbit Monoclonal; Clone E19-1; VWRKILM2133-C1; ImmunoLogic/VWR International B.V., Amsterdam, The Netherlands. CDX2: Rabbit monoclonal; Clone EPR2764Y; 235R-16; Cell Marque/Millipore Sigma, Darmstadt, Germany.) Next, sections were incubated with biotin free Poly-HRP-anti mouse/rabbit IgG (ImmunoLogic/ VWR International B.V., Amsterdam, The Netherlands) in EnVision™ FLEX Wash Buffer (Dako Denmark A/S, Glostrup, Denmark) (1:1) at room temperature for 30 minutes. Antibody binding was visualized using the EnVision™ FLEX Working Solution (Dako A/S) at room temperature for 10 minutes. All sections were counterstained with hematoxylin for 5 seconds before dehydration in ethanol and cover slipping.

**Image Analyses**

An experienced board-certified gastrointestinal pathologist (LB) reviewed the H&E, CEA, CK20 and CDX2 slides of all resected specimens and verified the location and presence of tumor cells. Based on H&E, regions of interest (ROI) (tumor and adjacent normal) were drawn in the corresponding fluorescent images using Image Studio Lite version 5.2.5 (LI-COR Biosciences, Lincoln, NE, USA). Fluorescence intensity was calculated as provided by the program: Mean fluorescence intensity per pixel (fluorescence intensity), which is an arbitrary unit. Background intensity (zero signal) was determined in an area in close proximity to the analyzed tissue and was automatically subtracted from the ROIs using background subtraction in Image Studio Lite. The total tumor fluorescence intensity was divided by the total area of the tumor ROI. The total normal intensity was divided by the total area of the normal ROI. The same principle, including automatic background subtraction, was adapted for analysis of the images acquired with photo stimulated luminescence. Here we used Aida Image Analyzer version 4.21 (Elysia-Raytest, Angleur, Belgium), which calculates a mean pixel intensity.
Statistical Analyses
Statistical analyses were performed with the Statistical Package for Social Sciences, Version 22.0 (IBM Corp., Armonk, NY, USA). All tests were performed two-sided and a significance level of <0.05 was considered to be statistically significant. To test for difference in tumor and normal fluorescence and autoradiography intensities, we performed a Mann-Whitney U test across all samples or control antibody-conjugate-incubated samples. For analyses of patients who received systemic therapy versus patients who did not, we also performed a Mann-Whitney U test, which divided the cases by history of systemic therapy.

RESULTS
We collected 29 peritoneal deposits from 10 colorectal cancer patients (5 male, 5 female). Mean age was 64.8 ± 10.7 years. Four patients received adjuvant systemic therapy for their primary tumor prior to CRS-HIPEC. Six patients were diagnosed with a peritoneally metastasized adenocarcinoma and in three patients the peritoneal metastases originated from a mucinous adenocarcinoma. In one patient, the metastases originated from signet-ring-cell carcinoma.

After incubation, tissue remained microscopically viable as was determined microscopically on H&E slides by the involved pathologist. Twenty-six tumors contained malignant cells, and depending on size and quality, multiple or single series of slides were produced from these tumors. This resulted in 46 series of slides that were analyzed. The other 3 tumors did not contain viable tumor cells, but mainly consisted of fibrosis or stroma. Median fluorescence intensity was 453.4 (IQR: 257.2–919.0) in tumors, whereas in adjacent tissue the median fluorescence intensity was 48.7 (IQR: 28.8–79.0) (\( P < 0.001 \)). Overall, fluorescence intensity was higher in tumorous areas compared to adjacent non-tumor tissue parts (Figure 2). Mean fluorescence intensity in tumor tissue did not differ between patients with or without a history of systemic therapy (\( P = 0.912 \)). Median intensity of the autoradiography for tumor tissue was 5.0 \( \cdot 10^6 \) (IQR: 2.4 \( \cdot 10^6 \)–9.2 \( \cdot 10^6 \)), while the median autoradiography intensity in non-tumor tissue was 9.9 \( \cdot 10^5 \) (IQR: 2.5 \( \cdot 10^5 \)–2.4 \( \cdot 10^6 \)) (\( P < 0.001 \)). TBRs for the fluorescence and radiosignal in each patient is shown in Figure S1. An example of tumor and normal tissue ROI is provided in Figure 3.

Tumors of two patients were incubated with dual-labeled hMN-14 (\(^{111}\)In-DOTA-hMN-14-IRDye800CW) in parallel with dual-labeled hIgG as control (Figure 2; last 2 patients). Median tumor fluorescence intensity of hIgG treated samples was 4.9 (IQR 2.7–8.5), which was similar to the fluorescence intensity of normal tissue in the same samples: 4.9 (IQR 3.6–13.3,
Similarly, the median intensity of the autoradiography was $5.6 \times 10^5$ (IQR: $4.5 \times 10^5$–$7.5 \times 10^5$) for tumor tissue and $4.4 \times 10^5$ (IQR: $3.8 \times 10^5$–$7.5 \times 10^5$) for non-tumorous tissue ($P = 0.465$). Furthermore, in the in vitro binding assay (Figure S2), dual-labeled hMN-14 showed higher binding to LS147T cells than the non-specific hlgG conjugate ($P < 0.001$). Additional blocking with an excess of unlabeled antibody led to a significant reduction in binding ($P < 0.001$), indicating specific binding of $^{111}$In-DOTA-hMN-14-IRDye800CW to CEA (Figure S2).

![Figure 2 Mean fluorescence intensity (arbitrary units) per pixel for tumor (blue dots) and normal tissue (black diamonds) in individual tumors. Each blue circle represents an included tumor. Vertical dashed lines separate patients. Note the higher fluorescence signal in all tumors compared to surrounding normal tissue ($P < 0.001$). The control condition (incubation with the non-specific antibody-conjugate DOTA-hlgG-IRDye800CW) shows no significant difference between tumor and normal tissue tracer accumulation (red circles and black open diamond; last two patients).]
Figure 3 Example of an ROI for tumor (orange line) and surrounding tissue (pink line), as drawn on the H&E stained slide (A). B: Consecutive slide with immunohistochemical CEA staining. C: fluorescence flatbed image of the same slide as A. D: autoradiography image of the same slide as A.
DISCUSSION

We observed high tumor-to-surrounding tissue ratios of our dual anti-CEA tracer $^{111}$In-DOTA-hMN-14-IRDye800CW after ex vivo incubation of freshly-resected colorectal peritoneal metastases. Together with earlier results on biodistribution and tumor accumulation, these results indicate that it is feasible to use this tracer for fluorescence image-guided surgery in patients with colorectal peritoneal metastases. This way, ex vivo incubation of surgical samples contributes to bridging the gap between preclinical studies and clinical application of novel tracers for fluorescence and multimodal image-guided surgery.

Fluorescent and radiolabeled bimodal imaging probes may serve a versatile role before, during and after image-guided surgery. This includes accurate tracer quantification for pharmacokinetic purposes, preoperative radionuclide imaging, real-time intraoperative radiodetection, real-time near-infrared fluorescent imaging, and qualitative and quantitative ex vivo analysis of resection specimens, as has been demonstrated in several translational studies for multiple diseases [15–18]. Furthermore, its feasibility has been demonstrated in recent clinical trials [19,20], and several clinical trials are currently ongoing [8]. Ex vivo incubation of patient tissue specimens with antibodies has previously been performed in different applications [21,22]. In the present study, we applied this approach to assess the TBR of multimodal antibody conjugates to be used for image-guided surgery. The involved pathologist assessed all included tumor specimens after incubation and they remained viable based on microscopic H&E assessment. However, the incubated tissue could undergo molecular changes that may not be visible on H&E stainings. These were not assessed in the current study.

Since clinical translation of preclinical results is usually challenging, we incubated freshly-resected human tumor specimens with the multimodal tracer in-vitro. We chose an incubation concentration similar to the blood concentration that is to be expected following intravenous injection of approximately 20 mg antibody-conjugate in a patient. This dose is similar to doses used in clinical image-guided surgery studies with tumor-targeting antibody conjugates [23,24]. We evaluated the potential of our tracer using colorectal peritoneal metastases from a group of patients receiving surgical treatment. On average, we found an almost ten-fold higher microscopic fluorescent signal in CEA-positive tumor areas compared with adjacent non-tumorous areas in all samples after incubation with dual-labelled hMN-14. Moreover, the fluorescent signal in cancer cells was at least 5-fold higher than the signal in adjacent normal tissue in all resected tumor specimens (Figure 2), indicating sufficient tumor-to-background contrast in all patients. We found a similar contrast in the autoradiographic analyses. Recent
clinical studies describe TBRs between 2 and 5 after intravenous injection of fluorescently or dual-labeled tumor-targeting antibodies [24–27]. Methodological differences in analyses or tumor type might explain the difference in TBR between literature and our findings. Moreover, the main limitation of ex vivo incubation of surgical specimens is that it does not represent the clinical administration route (intravenous; circulation) and the (slow) clearance in the clinical situation. Consequently, tracer accumulation is not limited by extravasation or plasma clearance. Instead, it relies on passive diffusion of the antibody conjugate into tissue and release of unbound tracer into the washing buffer during the washing step.

We noticed less penetration to CEA-positive areas when we analyzed deeper than 100 µm in incubated specimens. Longer incubation time or a smaller tumor-targeting molecule might result in deeper tissue penetration. However, in general during surgery tumor edges and margins are of more interest than tumor cores. Therefore, analyzing only the superficial layers after ex vivo incubation might be sufficient to assess the feasibility of a tracer for image-guided surgery (this may be different for therapeutic applications).

A key factor determining the TBR of tumor-targeting tracers is the presence and availability of the biomarker that the tracer targets. All evaluated tumor specimens were found positive for CEA. In vivo, membrane-bound CEA can shed into the interstitial compartment and circulation [28]. As a result, our tracer could bind to soluble serum CEA after shedding. An earlier study showed that the targeting sensitivity of MN-14 is not affected by complexation with plasma CEA, and that complexation reduces at increasing doses [29]. More recently, Boogerd et al. used an anti-CEA tracer in a clinical study with 26 patients, and observed that only a minor fraction (3%) of the administered dose was lost by binding to circulating CEA [26]. Due to the nature of our ex vivo incubation assay, investigation of CEA present in the circulation is not possible. However, we did observe that our tracer accumulated in CEA-positive mucin (supplementary Figure 3b). Primary mucinous adenocarcinomas are more often associated with positive resection margins [30]. Since viable tumor cells may reside in or between pools of mucin, fluorescence imaging might also be of added value during surgery of this subtype of colorectal cancer. In contrast to mucin, necrotic areas showed less uptake of our tracer compared to viable tumor cells (Figure S3), indicating high specificity of our tracer for CEA-expressing tumor cells.

Another method to more closely mimic the clinical situation is ex vivo perfusion of organs or tumors. There, the biological architecture, including blood vessels, remains largely preserved [31]. Unfortunately, ex vivo perfusion studies are limited by the need of afferent arteries that can be cannulated and connected to a perfusion system. When tumors are small and vascular
architecture does not allow for such connection, ex vivo incubation can be an alternative method. This way, tissue integrity remains intact even when a (macro)vascular structure is missing. Recent advances also indicate that patient-derived organoids can be used to predict patient-specific drug responses [32]. While developments in coculturing the tumor immune-micro environment are promising [33], creating similar stroma compositions including the presence of necrosis, immune cells or other non-malignant components of the tumor remains challenging in tumor models. Therefore, the method presented in this study offers specific advantages for translational evaluation of tracers for imaging, especially when tumors of interest are small. Furthermore, it may contribute to bridging the gap between translational research and clinical application of new agents for multimodal or near-infrared fluorescence imaging studies. We showed that high TBRs can be found after ex vivo tissue incubation with dual-labeled hMN-14. These promising results would support further clinical studies using this tracer in patients with peritoneal metastases of colorectal origin.
REFERENCES


Multimodal image-guided surgery of colorectal peritoneal carcinomatosis: a phase 1 clinical trial


Adapted from: Nederlands Tijdschrift voor Oncologie, 2018, 15:226-30

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CHAPTER 10

ABSTRACT

Background
The best available treatment for colorectal peritoneal metastases is cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). Accurate preoperative estimation of tumor burden and intraoperative detection of tumor nodules remains difficult. The current study evaluates the safety and feasibility of preoperative SPECT/CT imaging and fluorescence image-guided surgery using two doses of a dual-labeled anti carcinoembryonic antigen (CEA) antibody in patients who undergo CRS-HIPEC for colorectal peritoneal carcinomatosis.

Methods
Five or six days prior to the CRS-HIPEC procedure, two groups of five patients each, received a single dose (2- or 10 mg) of the radio- and fluorescently labeled anti-CEA antibody Labetuzumab. One or two days prior to the operation, preoperative SPECT/CT imaging of the thoracoabdominal region was performed in all patients. During the CRS-HIPEC procedure, the signal originating from the dual-labeled antibody was used for intraoperative gamma detection and real-time fluorescence imaging for in vivo and ex vivo analyses.

Results
Administration of dual-labeled Labetuzumab was safe and no injection related adverse events were observed. No SPECT or intra-operative signal was detected in the 2 mg protein dose group. Preoperative SPECT/CT images with 10 mg visualized larger extraperitoneal metastases, but no peritoneal metastases. Intraoperative fluorescence imaging was feasible and enabled detection and delineation of colorectal peritoneal metastases during surgery in the 10 mg protein dose group which provided a more pronounced contrast between tumor and surrounding background tissue than the 2 mg dose group.

Conclusion
Labetuzumab is a safe and promising dual-labeled anti-CEA antibody for fluorescence image-guided surgery in patients who undergo CRS-HIPEC for colorectal peritoneal carcinomatosis.
INTRODUCTION

Colorectal cancer is one of the most commonly diagnosed cancers worldwide [1]. Synchronous peritoneal metastases (PM) are present in 3-13% of patients with colorectal cancer [2, 3]. Metachronous PM are found during follow-up in up to 19% of patients, depending on tumor location, stage and histological subtype [4]. According to international standards, treatment of PM consists of complete cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). While this treatment drastically improves survival of selected patients with colorectal PM compared to systemic chemotherapy regimens, successful treatment remains dependent on several critical factors, such as adequate patient selection and achieving complete cytoreduction during surgery [5-7]. Another critical patient-related factor for long-term outcome is the peritoneal cancer index (PCI) [8]. The PCI represents the amount and distribution of tumor present in the abdomen and is one of the main prognostic factors of CRS-HIPEC [7]. It is assessed by an experienced surgeon scoring the various peritoneal deposits in the exposed abdomen. The score is based upon the size and number of metastases that are present and can reach a maximum of 39. When the PCI is above 20, the tumor burden appears to be too high for successful treatment and in those patients cytoreduction is usually not performed and the procedure is of no benefit to the patient. Futile laparotomy rates of 5-15% have been reported in recent studies from large CRS-HIPEC centers [9-12]. Diagnostic laparoscopy (DLS) may be used as method to score the PCI prior to CRS-HIPEC. However, it may be limited by the presence of adhesions or the rigid tubing of the laparoscope [13]. Current preoperative imaging methods such as computed tomography (CT) and \(^{18}\)F-FDG-PET/CT still fail to accurately estimate the PCI [14]. Therefore, more accurate imaging methods are needed for patients with PM who appear eligible for CRS-HIPEC.

After preoperative evaluation of patients, the surgical challenge is to achieve a complete cytoreduction. Cytoreduction may be hampered by involvement of crucial anatomical structures such as vessels or large parts of the small bowel. In addition, discrimination between adhesions or fibrosis and tumor lesions may be difficult since surgeons can rely on visual and tactile information only. CRS-HIPEC is often the second or third abdominal procedure and as a result patients regularly present with both intraabdominal adhesions and peritoneal tumors. Improving the surgeons’ ability to discriminate between tumors and adhesions or fibrous tissue might increase the number of patients that receive complete cytoreduction.

Near infrared fluorescence (NIRF) image-guided surgery (IGS) using specific tumor-targeting tracers could help surgeons to more accurately discriminate between tumor and nonmalignant tissues [15]. Radionuclides, depending on their mode of decay, can be used for single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging. This could enable sensitive preoperative imaging for estimation of the PCI.
Combining radionuclides and NIRF on the same targeting molecule can offer specific advantages. First, the radionuclide can be used for preoperative imaging. Next, the NIRF signal can guide the surgeon during surgery and radionuclides may even be used for intraoperative detection of deeper seeded lesions [16]. After resection, the surgical bed can be assessed with NIRF or radionuclide detection to identify residual lesions and resection margins. Finally, the fluorescent signal may be used for ex vivo analysis of resection margins of freshly resected tissue. Therefore, the combination of radionuclides and NIRF offers a potential synergy in preoperative imaging and intraoperative imaging or tumor detection [16].

Carcinoembryonic antigen (CEA) is overexpressed on the surface of colorectal cancer cells in more than 90% of all cases [17, 18]. Therefore, it can serve as a target for imaging tracers labeled with a radionuclide and fluorescence moiety for radiodetection and real-time intraoperative fluorescent imaging, respectively. The dual-labeled anti-CEA antibody \([^{111}\text{In}]\) In-DOTA-Labetuzumab-IRDye800CW has been tested for these purposes in vitro, in vivo and ex vivo with promising results [19-21]. Here, we evaluate the safety and initial feasibility of preoperative SPECT imaging and fluorescence IGS using \([^{111}\text{In}]\)In-DOTA-Labetuzumab-800CW in patients who undergo CRS-HIPEC for colorectal peritoneal carcinomatosis. This study is part of a larger phase I/II clinical trial.

**METHODS**

**Patients**

The current study was approved by the local ethical committee (CMO region Arnhem-Nijmegen) and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients. Patients were eligible if they were scheduled for CRS-HIPEC for colorectal PM and were over 18 years old. Patients with a serum CEA of > 500 ng/ml were excluded from participation.

**\([^{111}\text{In}]\)In-DOTA-Labetuzumab-IRDye800CW preparation**

Labetuzumab was kindly provided by Immunomedics Inc. (Morris Plains, NJ, USA). \([^{111}\text{In}]\) In-DOTA-Labetuzumab-IRDye800CW was produced under metal free conditions and in compliance with Good Manufacturing Practice (GMP) in the radiopharmacy facilities of the Radboudumc. The manufacturing process is similar to the preparation of Girentuximab-DOTA-IRDye800CW 5 mg·ml\(^{-1}\) which was described earlier [16]. In short, Labetuzumab 5 mg·ml\(^{-1}\) was incubated with a 2-2.5-fold molar excess (to reach to the preferred substitution ratio of 0.5-2.0) of IRDye800CW-NHS ester (LI-COR biosciences, Lincoln, NE, USA) followed by incubation with a 25-fold molar excess (preferred substitution ratio 0.5-3.0) of the chelator
DOTA-NHS (Macrocyclics, Dallas, TX, USA) in a 1.25 M NaHCO₃ buffer, pH 8.5. Hereafter, excess IRDye800CW-NHS ester and DOTA-NHS ester were removed by dialysis against 0.25 M ammoniumacetate, pH 5.5 containing Chelex 100 resin (Bio-Rad Laboratories Inc., Hercules, CA, USA) for 3 days to ensure metal free conditions. After purification, the product was sterile filtered and aliquoted. DOTA-Labetuzumab-IRDye800CW was stored at 4 °C in the dark until use. To confirm the tumor-specific binding of the dual-labeled antibody conjugate, the immunoreactive fraction of [¹¹¹In]In-DOTA-Labetuzumab-IRDye800CW was determined as described by Lindmo et al. [22]. Before each intravenous administration, the integrity of the dual-labeled antibody conjugate and radiochemical purity after radiolabeling was determined by high-performance liquid chromatography analysis.

Before injection, 2- or 10 mg DOTA-Labetuzumab-IRDye800CW was labeled with ¹¹¹In. In short, unlabeled DOTA-Labetuzumab-IRDye800CW was incubated with 100-120 MBq [¹¹¹In]InCl₃ (Curium, Petten, The Netherlands) at 45 °C for 30 min in the dark. 100 MBq of the final product was diluted in 0.9% NaCl to a final volume of 10 mL and drawn up in a syringe shortly before injection.

**Safety and intravenous dual-labeled Labetuzumab injection**

Five or six days prior to surgery, patients received a single intravenous dose of [¹¹¹In]In-DOTA-Labetuzumab-IRDye800CW. Patients were monitored for adverse events until 3 hours after injection and blood samples were drawn for safety analyses. Blood was drawn before injection, 3 hours after injection, before the SPECT/CT and before the operation. In blood, hematologic markers for liver and renal function were analyzed. Temperature was measured before, and 30 and 180 minutes after injection. Blood pressure was measured at the same timepoints as temperature. Treatment-related adverse events were defined as any adverse event associated with the study procedure but not necessarily related to the study intervention (i.e. [¹¹¹In]In-DOTA-Labetuzumab-IRDye800CW administration) for up to 10 days after surgery, using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03).

**SPECT/CT imaging**

One or two days prior to surgery, whole body anterior and posterior SPECT/CT images of the thorax and abdomen were acquired with a dual-head Symbia T16 Truepoint SPECT/CT scanner (Siemens Healthcare, The Hague, The Netherlands). After acquisition of a low dose non-contrast enhanced CT, scintigraphic imaging was performed with the following settings: non-circular, angle 0-180°, 64 views per detector, 19 seconds per view with medium energy all-purpose parallel-hole collimators. Accumulation of [¹¹¹In]In-DOTA-Labetuzumab-IRDye800CW was scored by a nuclear medicine physician.
Image-guided surgery and CRS-HIPEC

Surgery was performed by a specialized surgical CRS-HIPEC team and the procedure has been described in detail previously [23]. After initial opening of the abdomen, inspection and adhesiolysis, the surgeon scored the PCI as usual. Hereafter, the PCI was re-scored using a fluorescence imaging system (QMI Spectrum NIR fluorescence camera, Quest Medical Imaging, Middenmeer, the Netherlands). Subsequently, surgery continued as normal and researchers scored all resected tissue specimens ex vivo for the presence of fluorescence and radiosignal using the fluorescence imaging system and the gamma probe (Europrobe 3.2, SOE 3216-7, Eurorad SA, Eckbolsheim, France), respectively. Once the surgeon completed cytoreduction and preceding the HIPEC, the abdomen was inspected using the fluorescence camera and the gamma probe once more. Any additional lesions that were found were re-assessed by the surgeon after which he/she decided if this lesion needed additional resection.

Tissue processing

Surgical resection specimens underwent standard pathological processing for analysis of presence of tumor including H&E staining. To enable 1-on-1 comparisons between intraoperative observations, ex vivo observations, and microscopic analyses, were reviewed during the first steps of pathological processing (cassette allocations after formalin fixation). For research purposes, the presence or absence of tumor was confirmed with additional H&E, CEA, CK20 and CDX2 in all relevant tissues. Additional H&E staining allowed fluorescence detection in identical slides. Therefore, all research sections were scanned for the presence of fluorescence signal (800 nm) on the Odyssey CLx imaging system (LI-COR Biosciences, Lincoln, NE, USA) before H&E staining.

Tissue and image analyses

An experienced board-certified gastrointestinal pathologist reviewed the H&E, CEA, CK20 and CDX2 stained sections of all resected specimens and verified the location and presence of tumor cells.

Based on H&E, regions of interest (ROIs) (tumor and adjacent normal tissue) were drawn in the corresponding fluorescence images using Image Studio Lite version 5.2.5 (LI-COR Biosciences, Lincoln, NE, USA). Fluorescence intensity was calculated as provided by the program and expressed as mean fluorescence intensity per pixel (fluorescence intensity), which is an arbitrary unit. Background intensity (zero signal) was determined in an area in close proximity to the analyzed tissue and was automatically subtracted from the tumor and normal tissue ROIs using background subtraction in Image Studio Lite. The total fluorescence intensity was divided by the total area of the ROI, both for tumor and adjacent normal tissue ROIs.
**Statistical analyses**
This study describes 2 groups of the dose escalation part of a larger clinical study. The primary endpoint of the study is safety (number of injection related (serious) adverse events). Secondary parameters included the optimal dose after dose escalation in 3 groups of patients. More details are described in the original study protocol (NCT03699332).

For the current study, statistical analyses considering tumor-to-background ratios were performed using Prism 5.03, GraphPad Software, San Diego, CA, USA. The mean tumor-to-background ratio of both patient groups was compared with a two-sided T-test and if $p < 0.05$ the null hypothesis was rejected.

**RESULTS**

**Patients**
Five patients were intravenously injected with 2 mg and another five patients with 10 mg 100 MBq $^{111}$In-DOTA-Labetuzumab-IRDye800CW. Table 1 summarizes basic patient characteristics. Mean hospital stay was 12 days after surgery.
Table 1. Patient characteristics

Table 1 Patient characteristics. *: Procedure cancelled due to extraperitoneal metastases/irresectable disease. !: CC2 cytoreduction, PCI not scored due to discovery of retroperitoneal disease and cancellation of the procedure. CAIRO6: Preoperative chemotherapy regimen according to the study protocol of the CAIRO6 clinical trial [24]. 3,10: Patients received adjuvant systemic therapy for their primary tumor (prior to CRS-HIPEC). 6: Palliative chemotherapy after which the disease was considered resectable and the treatment intent switched to curative. 9: Neoadjuvant to increase chances of complete resection of the primary tumor. LAMN: Low-grade appendiceal mucinous neoplasm, MA: Mucinous adenocarcinoma.
Safety analysis

No infusion-related (serious) adverse events (S)AE were observed. No adverse events were observed. Temperature, blood pressure and hematologic markers for liver and renal function remained stable in the first hours after injection, at time of the SPECT/CT and on the day of surgery. Complications after CRS-HIPEC or possible adverse events are provided in table 2. Three patients experienced no complications or possible adverse events.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Protein dose</th>
<th>Days*</th>
<th>Adverse event</th>
<th>Intervention</th>
<th>Related</th>
<th>CTCAE</th>
<th>SAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 mg</td>
<td>11</td>
<td>Fever (without focus)</td>
<td>Antibiotics</td>
<td>No</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>2 mg</td>
<td>28</td>
<td>Delayed gastric emptying/ gastroparesis</td>
<td>Yes (enteral tube)</td>
<td>No</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>2 mg</td>
<td>16</td>
<td>Seroma</td>
<td>Yes (drainage)</td>
<td>No</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>2 mg</td>
<td>24</td>
<td>Elevated liver enzymes (transient)</td>
<td>No</td>
<td>Unlikely</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>2 mg</td>
<td>15</td>
<td>Intraabdominal hemorrhage (arterial)</td>
<td>Yes (radiological coiling)</td>
<td>No</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>2 mg</td>
<td>20</td>
<td>Anastomotic leakage</td>
<td>Yes, relaparotomy</td>
<td>No</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>10 mg</td>
<td>10</td>
<td>Chest pain</td>
<td>none</td>
<td>No</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>10 mg</td>
<td>7</td>
<td>Paralytic ileus</td>
<td>Yes (TPN)</td>
<td>No</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>10 mg</td>
<td>11</td>
<td>Abdominal discomfort and fever</td>
<td>Yes (antibiotics)</td>
<td>Unlikely</td>
<td>1</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2. Adverse events. *: After injection, CTCAE grade: common terminology criteria for adverse events grade (v4.03), SAE: Serious adverse event.
Preoperative SPECT/CT imaging
In none of the patients, peritoneal lesions could be visualized using SPECT/CT imaging. In two patients, preoperative SPECT/CT imaging detected extra-abdominal nodal disease. These lesions were all larger than 1 cm. In patient #7, accumulation of $[^{111}\text{In}]$In-DOTA-Labetuzumab-IRDye800CW was present in bilateral para-aortocaval nodes and in the left supraclavicular region (figure 1). In patient #9 accumulation of $[^{111}\text{In}]$In-DOTA-Labetuzumab-IRDye800CW was present in unilateral para-aortal nodes.

![Figure 1 SPECT/CT images of patient #7. Arrows indicate increased signal in areas of interest. Green arrows: supraclavicular lymph node(s). Blue arrows: para-aortocaval lymph nodes. Red arrow: increased uptake in the liver. A: Anterior-posterior maximum intensity projection (MIP) of the thoraco-abdominal region. B: Transversal SPECT section of the supraclavicular region. B*: Corresponding fused image of CT and SPECT images. C: Transversal SPECT section of the mid-abdominal region. C*: Corresponding fused image of CT and SPECT images.](image)
After histopathological frozen section analyses, the para-aortocaval nodes were found positive for adenocarcinoma. Figure 2 illustrates the intra-abdominal view on these lymph nodes during the procedure. The supraclavicular node(s) were not sampled, since it had no clinical consequences.

![Figure 2](image)

Figure 2 Tumor-positive retroperitoneal lymph nodes visualized with near-infrared fluorescence. White light image (A), overlay image (B), image of the fluorescence signal (C).

**Intraoperative multimodal imaging and back-table analyses**

The majority of lesions were identified by fluorescence imaging during surgery. In patients who received 2 mg of the dual-labeled antibody, back-table analyses in the operating theatre revealed that 17 out of 28 (61%) of malignant lesions could be detected with fluorescence imaging (table 3). In the 10 mg group, 16 out 17 (95%) of malignant lesions were found positive for fluorescence (table 3). We found 4 false positive lesions originating from 3 patients in the 10 mg group. The first 3 individual lesions were histologically identified as a granulocytic inflammatory process with necrosis, fibrotic inflammation and local colitis. The last lesion contained heavy cauterization defects which did not allow for further histological determination. An example of intraoperative NIR fluorescence imaging is provided in figure 3. The gamma probe was used to confirm the presence of the tracer in vivo and for ex vivo back-table confirmation of the presence of the tracer.
Figure 3 peritoneal tumor deposits in Douglas’ pouch of patient #7 (10 mg). White light image (A), overlay image (B), image of the fluorescence signal (C). Note that these lesions were not clearly visible on preoperative SPECT/CT imaging (figure 1).

Table 3. Fluorescence signal found

<table>
<thead>
<tr>
<th></th>
<th>2 mg Malignant</th>
<th>Benign</th>
<th>10 mg Malignant</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence +</td>
<td>17</td>
<td>0</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Fluorescence-</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 3 Fluorescence signal found in malignant and benign lesions in both dose groups using intraoperative and back-table imaging in the operating theatre.

Microscopic ex vivo tissue analysis

The back-table analyses were confirmed with subsequent microscopic analyses. Microscopic analyses of the tumor-to-background ratios in the two patient groups showed a higher mean tumor-to-background ratio in the 10 mg group than in the 2 mg group (4.1 ± 0.8 vs 1.7 ± 0.3; \( p = 0.04 \)). Figure 4 provides a typical image of an ex vivo examination of patient tissue.
Figure 4  Ex vivo examination of resected tissue. A: peritoneal deposit; white light with fluorescence overlay. B: fluorescence image. C: H&E section of part of the tissue in A and B. D: CEA-immunohistochemical staining of a subsequent slide of C. E: fluorescence image of the same slide as C. Note the presence of fluorescence signal in the CEA-positive areas and in hepatocytes (orange circle).
DISCUSSION

This study describes the feasibility and safety of dual-labeled Labetuzumab for pre- and intraoperative imaging in patients with colorectal peritoneal metastases who receive CRS-HIPEC treatment. No infusion-related (S)AE were observed after dual-labeled Labetuzumab injection in ten patients. With the used doses, preoperative imaging of intraperitoneal metastases with SPECT/CT proved impossible. Yet, preoperative SPECT/CT did reveal extraperitoneal disease in two patients. Intraoperative NIRF imaging of dual-labeled Labetuzumab was feasible in the 10mg dose group, which provided a more pronounced contrast between tumor and surrounding background tissue than after administration of 2 mg antibody conjugate, resulting in detection of 95% of the lesions with NIRF imaging. These observations are supported by quantitative ex vivo tumor-to-background analyses. Our findings indicate that multimodal imaging using $^{111}$In-DOTA-Labetuzumab-IRDye800CW is safe and feasible in patients who undergo CRS-HIPEC for colorectal peritoneal carcinomatosis. In patients with colorectal PM, preoperative injection of $^{111}$In-labeled DOTA-Labetuzumab-IRDye800CW was well tolerated, which was in line with previous experience and phase I trials with (radio)labeled Labetuzumab for other clinical indications [25-28]. Postoperative hospital stay of patients, a surrogate marker for postoperative recovery, was similar to what to historical data from our center [29]. These results indicate that preoperative injection and imaging of $^{111}$In-labeled DOTA-Labetuzumab-IRDye800CW in patients with PM who undergo CRS-HIPEC combined with intraoperative imaging is safe.

Preoperative SPECT/CT was not able to identify individual small peritoneal metastases. This could be a result of insufficient tracer accumulation in the small peritoneal tumors. Therefore, in this study preoperative scoring of the PCI was not possible with SPECT/CT. Figure 1 however, illustrates that larger lesions such as lymph node metastases can be visualized with this technique, which is important because extraperitoneal or systemic disease spread is not treated with CRS-HIPEC. If such metastases are found in combination with PM, the treatment intent shifts from curative (CRS-HIPEC) to palliative (systemic therapy or best supportive care) and a futile laparotomy or CRS may be avoided. Therefore, identification of extraperitoneal disease with preoperative imaging can be of great additional value for the treatment plan of patients. Similarly to CT, SPECT/CT at the doses used in the current study, seems to lack sensitivity and specificity for accurate estimation of the PCI and especially for detection of small lesions [30-32]. Recent results of a prospective study using diffusion weighted magnetic resonance imaging for scoring the PCI are promising [33].

NIRF was able to identify individual metastases during surgery (figure 3). Table 3 shows that more false negatives were found when 2 mg of dual-labeled Labetuzumab was administered compared to 10 mg. The higher dose resulted in 4 false positive lesions in 3 patients.
Histopathological analyses showed that these lesions consisted of inflammatory processes. One lesion showed excessive expression of CEA, which could explain the increased uptake of our tracer and presence of fluorescence signal in that lesion. The uptake in the other lesions could be a result of the enhanced permeability and retention effect that can be pronounced in inflamed tissue [34].

The high expression of CEA in more than 90% of all colorectal cancers and their metastases renders it suitable as target for targeted imaging of colorectal cancer. In vivo, we observed that the amount of fluorophores that accumulated in peritoneal tumors after injection of 2 mg dual-labeled Labetuzumab was below the detection limit of the imaging system. Increasing the number of fluorophores on the tumor surface increases contrast. However, further increasement of the dose can also result in an increase in background signal and thus deteriorate the tumor-to-background contrast. Another way to increase the number of fluorescent moieties is conjugating more IRDye800CW groups to one antibody molecule. In turn however, this may deteriorate the antibody’s binding capacity and alter the physiological clearance rate [35]. Therefore, careful dose escalation remains important before further adjustments to the antibody or clinical implementation.

Multimodal imaging offers specific advantages over imaging with a single modality. The radionuclide, e.g. indium-111, enables preoperative SPECT/CT imaging and intraoperative gamma detection. Additionally, it can be used for quantitative pharmacological analyses [16]. NIRF-imaging enables real-time intraoperative feedback of the location of the tracer and the NIRF signal can facilitate ex vivo analyses of resected tissue. The advantage of combining the two modalities on one molecule over co-injection of two separate antibodies is that the detected signals originate from the same targeting molecule. Especially when cancer tissue is covered by fibrous or other nonmalignant tissue, this allows for an intraoperative detection approach in two steps. Since radiosignals have a high tissue penetration, they can first be used to detect the presence of cancer cells. After careful dissection of the overlaying tissue, the fluorescent signal may be utilized to identify and delineate the tumor of interest. Furthermore, while quantification of real-time fluorescence remains challenging, another advantage of a dual-labeled antibody includes the possibility for accurate quantification of the tracer.
Ultimately, in vivo and ex vivo analyses of freshly resected tissue might expose positive resection margins during surgery. Recent studies showed that this is feasible using fluorescently-labeled antibodies in the surgical setting [36-39]. Back-table tissue analyses could enable the surgeon to make corrections to the resection margin in the same session. Not only could this be a faster method than the classical histopathological approach, i.e. frozen section analysis, it also provides direct 3-dimensional insight in the relationship of resected tissue and surgical field. Even after an initial correction, tissue can again be evaluated with ex vivo imaging until the desired margin has been reached. Furthermore, this ex vivo tissue diagnosis method can more reliably be standardized than in vivo fluorescence imaging since imaging distance, background and settings can be more easily set on fixed values. Therefore it can be a great addition to in vivo fluorescence imaging [40].

**Conclusion**

This study shows that CEA-targeted multimodal imaging can safely be used in patients who undergo CRS-HIPEC treatment. Preoperative SPECT/CT enables identification of extraperitoneal disease, while intraoperative fluorescence imaging enables detection and delineation of colorectal peritoneal metastases during surgery.

**Acknowledgements**

We thank M. Hekman for her early drafts of the study protocol. We also thank D. Bos for her assistance in the lab and operating theatre. The authors would like to thank our colleagues from the radiopharmacy laboratory (“Hotlab”) for preparation of the dual-labeled antibody prior to each injection. We also would like to thank M. de Bakker for writing the SPECT/CT protocol. Lastly, we thank all involved technicians, surgeons, surgical nurses and the rest of the surgical team for their help during the procedures.
REFERENCES


General discussion and future perspectives
GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Even after more than two decades of international experience with CRS-HIPEC, successful treatment of peritoneal metastases remains a challenge. Adequate patient selection and experience with the surgical technique of the procedure are important factors for outcome. Many centers throughout the world have adopted different HIPEC perioperative care protocols to treat patients with colorectal peritoneal metastases (PM). Improving the surgical treatment of patients with colorectal PM starts with exploring the effects or impact of these different protocols, in order to provide the best guidelines for optimal treatment.

The main prognostic factors of CRS-HIPEC outcome are peritoneal cancer index (PCI), reaching complete cytoreduction, postoperative complications and natural behavior of the various tumor subtypes [1]. Treatment of patients with PM should therefore focus on optimal patient selection (including preoperative assessment of the PCI and tumor biology), increasing the probability of reaching complete cytoreduction and reducing postoperative complications.

The studies described in this thesis focused on exploring the current clinical and pharmacological aspects of CRS-HIPEC and investigating the potential of novel molecular imaging techniques for preoperative and intraoperative imaging and treatment.

IMPROVING PATIENT SELECTION

A major issue in the treatment of patients with PM is the chance of encountering excessive or irresectable tumor after opening of the abdomen. This scenario results in a futile laparotomy and is considered an unsuccessful treatment. The extent of tumor spread can be scored via various scoring systems. Internationally the most commonly used system is the PCI. A less invasive method for PCI-scoring is diagnostic laparoscopy (DLS). DLS may prevent non-therapeutic laparotomies and has been widely implemented in the standard path of care of patients with colorectal PM [2]. However, accurate scoring of the PCI remains difficult due to physical limits of the rigid laparoscope tubing and regular presence of intraabdominal adhesions during DLS. Because of these limitations, other strategies to improve preoperative imaging are being explored [3].

Currently, patient selection for CRS-HIPEC is performed based on clinical characteristics and findings from imaging modalities such as computed tomography (CT). However, the sensitivity of CT for detection of PM is limited and accurate preoperative assessment of the PCI remains difficult [4-6]. 18-fluorodeoxyglucose-positron emission tomography ([18F]FDG-PET) combined with CT seems to be of limited additional value for detecting and scoring intraperitoneal disease when compared to the standard abdominal CT-scan [7]. Therefore, it
is not considered part of the standard clinical workflow of patients who undergo CRS-HIPEC. The limited added value of $^{18}$F-FDG-PET/CT for accurate detection of peritoneal metastases, especially in mucinous adenocarcinomas, is most probably caused by the metabolic basis of the technique which results in relatively low uptake in small lesions and not by the resolution of the technique (millimeter range)[8]. Another form of PET is immuno-PET in which a positron emitting nuclide is coupled to a targeting molecule such as an antibody. It has potential to non-invasively and accurately reveal tumor antigen expression, immune checkpoints or inflammatory processes [9]. A commonly used positron emitting radionuclide for immuno-PET is Zirconium-89 ($^{89}$Zr). Under identical conditions, the spatial resolution and image quality of $^{89}$Zr-based immuno-PET is in similar range as $^{18}$F-FDG-PET/CT [10]. Immuno-PET imaging, using a tumor-specific antibody labeled with $^{89}$Zr, could overcome the low uptake issues of $^{18}$F-FDG in small (mucinous) peritoneal tumors. However, combining a long circulating antibody with a high energy gamma emitting radionuclide such as $^{89}$Zr could be troublesome for intraoperative use due to higher radiation exposure for the surgical team and other medical personnel. Due to these safety considerations, single photon emission computed tomography (SPECT), which relies on (lower energy) gamma-emitting radionuclides, might be more suitable for this purpose. We used dual-labeled labetuzumab to pre- and intraoperatively image peritoneal tumors using the gamma-emitting radionuclide indium-111 ($^{111}$In) in patients with colorectal PM who were treated with CRS-HIPEC. Preliminary results suggest that preoperative $^{111}$In-SPECT/CT imaging with dual-labeled labetuzumab fails to accurately assess the different intraperitoneal deposits [11]. This could be a result of the limited spatial resolution of the technique combined with low levels of activity that accumulated in peritoneal tumors. However, since $^{111}$In enables intraoperative gamma detection, the conjugation of this radionuclide to the dual-labeled tracer still has additional value. Moreover, a higher antibody dose could also improve the uptake and contrast for SPECT/CT imaging but was not tested in the described study.

Another promising non-invasive method for assessment of intra- and extraperitoneal disease is diffusion-weighted magnetic resonance imaging (DW-MRI) [12]. DW-MRI is often included in routine MRI investigations, requires no exogenous contrast agents and has extensive clinical applications [13]. In short, together with the corresponding apparent diffusion coefficient maps, it shows the effect of diffusion of water molecules within the cellular microenvironment [14]. For tumor imaging, DW-MRI relies on the fact that in tissues with increased cellularity, e.g. tumors, water diffusion is usually more restricted. A recent meta-analysis found that (DW)MRI had the highest sensitivity (91%; CI 84-96%) for detection of peritoneal metastases when compared to CT and $^{18}$F-FDG-PET/CT [4]. Other recent studies also found that DW-MRI significantly outperforms CT [12, 15]. Limitations of DWI-MRI include bowel movement
artefacts and the relatively long scanning time compared with CT. Despite these limitations, DW-MRI has potential for non-invasive preoperative PCI scoring and a prospective randomized multicenter clinical trial investigating this potential is currently ongoing in the Netherlands (DISCO trial – clinicaltrials.gov: NCT04231175).

**PERSONALIZATION OF PATIENT SELECTION**

Clinical patient characteristics such as age, sex, comorbidity and tumor type provide essential information for treatment planning, eligibility for surgery, and type of chemotherapy. These characteristics are important but do not reveal information regarding molecular properties and corresponding potential tumor biology which is important for personalized treatment. For example, KRAS/BRAF mutation status and deficiencies in the mismatch repair (MMR) system are predictive biomarkers for outcome in colorectal cancer. In more detail KRAS and BRAF are both members of the potentially oncogenic RAS-RAF-MAPK pathway, and have been used for optimization and prediction of treatment with epidermal growth factor receptor (EGFR) blockers. The EGFR lies upstream from this pathway which is involved in cell proliferation, growth, apoptosis resistance, survival, invasion and migration of cells [16, 17]. Metastatic colorectal cancers with KRAS-mutations are resistant to anti-EGFR treatment and BRAF-mutations are indicative for poor prognosis [18, 19]. Also for patients with PM of colorectal origin who are treated with CRS-HIPEC, BRAF mutation marks a poor prognosis [20]. However, in these patients KRAS mutation does not seem to have an effect on survival. Moreover, MMR repair status can predict the clinical benefit of immune checkpoint blockade with anti-PD-1 antibodies [21].

Including molecular tumor characteristics such as KRAS, BRAF and MMR status in tailoring treatment could potentially improve selection and outcome of CRS-HIPEC patients. Next generation sequencing (NGS), where new individual mutations in relevant genes may be identified can potentially even reveal more prognostic markers. Current limitations of NGS include the limited analytic sensitivity, limitations in databases and knowledge of the meaning of certain mutations [22]. Especially in the case of peritoneal metastases, where patient numbers are limited, more research is needed to provide insight for large scale clinical implementation of (personalized) NGS.
IMPROVING CYTOREDUCTIVE SURGERY

Improving the surgeon’s ability to detect and remove peritoneal metastases could ultimately improve survival of patients. Multimodal tumor-targeted image-guided surgery is a promising method to further improve cytoreductive surgery, especially to obtain complete removal of all macroscopically detectable tumor deposits (CC0). In the literature, clinicians and researchers have reported that the use of different targets for antibody-based tumor-targeting molecules is safe and useful [11, 23-25].

Image-guided surgery relies on adequate contrast between tumor and surrounding tissue. Further conceptual refinement of antibody-based image-guided surgery can improve contrast for imaging. The slow plasma clearance of antibodies (a few days) limits the in vivo tumor-to-normal tissue uptake ratio at early timepoints after intravenous administration. Furthermore, hepatic clearance of large molecules such as antibodies can result in insufficient contrast for colorectal liver- or peritoneal metastases present on the visceral peritoneum of the liver. In pretargeting, fast renal clearance of the labeled hapten peptide resulted in decreased signal in the liver [26]. Since pretargeting relies on the use of a bispecific antibody combined with a labeled peptide, which are administered at independent timepoints and doses, it requires optimization of more variables than the classic antibody-based approach. Before reaching optimal conditions for clinical use, several dose finding- and timepoint optimization studies have to be performed. Variables to consider are time interval between administration of the antibody and the hapten peptide, antibody dose, hapten peptide dose, and finally the time interval between the administration of hapten peptide and actual imaging. Due to the complexity of this process, successful clinical translation of pretargeting remains difficult.

Translation to the clinical situation

While multimodal-image guided surgery is a promising method to further improve (cytoreductive) surgery, there is a need for translational methods to bridge the gap between the clinical and preclinical situation. Several techniques such as cell cultures and patient-derived organoids can be useful to investigate the in vivo targeting potential of new tracer molecules [27]. Similarly to patient-derived organoids, patient-derived whole tumors or tumor tissue may contribute to successful clinical translation of novel tracers [28]. Another promising method is ex vivo incubation of patient-derived tumor samples. Here, freshly resected tumor samples are directly incubated with the tracer of interest, with this sophisticated technique tumor architecture and integrity remain intact. This way it provides estimation of feasibility for in vivo application. Ex vivo incubation is a feasible method for bridging the gap between translational research and clinical application of new agents for multimodal or near infrared fluorescence imaging or studies [29].
Centers throughout the world, including our center, are investigating different methods for image-guided surgery during the CRS-HIPEC procedure. These (smaller) separate studies provide essential information about feasibility and safety. The next step should be initiating large multicenter phase 3 studies to reveal if these techniques can improve the surgical results and provide a survival benefit in this specific patient group.

**IMPROVING HIPEC**

**Role of systemic therapy**

There is a lack of consensus regarding the role and the optimal protocol of HIPEC. Large heterogeneity in protocols, drugs, carrier solutions and in methods of administration (open, semi-open, closed) continues to exist. Methodological differences in the administration and monitoring of oxaliplatin-based HIPEC were identified in one of the studies of this thesis which highlights the need for a multidisciplinary approach towards standardization of the procedure [30]. On the other hand, the recent ‘negative’ results of the French PRODIGE7 trial, which did not find a difference in (disease free) survival in patients treated with oxaliplatin-based CRS-HIPEC or CRS alone, reopened the discussion amongst specialists treating PM [31]. However, the results of the PRODIGE7 should be interpreted with caution due to the limitations in study design and methodology [32, 33]. The investigators estimated that median overall survival in their experimental arm (CRS-HIPEC) would reach up to 48 months compared to 30 months in the control arm (CRS). This seems an overestimation of the additional effect of HIPEC which is aimed at increasing local control of the disease. Previous trials for systemic adjuvant therapy in the metastatic and non-metastatic setting of colorectal cancer have been designed with more conservative estimations of survival benefits [34, 35]. Another limitation of the study is that patients in the study received many cycles of neoadjuvant systemic chemotherapy and patients were not included if they did not receive at least 6 months of systemic treatment. As a result of this, patients with PM who responded to systemic therapy were preselected from those who did not. This introduced bias in the actual effect of HIPEC since a large number of patients who would normally receive CRS-HIPEC was excluded from the study. Currently, the CAIRO6 trial investigates the effect of perioperative systemic chemotherapy combined with CRS-HIPEC [36]. Since all patients will be treated with CRS-HIPEC, this trial will not demonstrate whether HIPEC is essential after a complete CRS. However, this study will demonstrate whether preoperative systemic therapy is of benefit for patients with colorectal PM and the results of this trial are eagerly awaited by the international CRS-HIPEC community.
Intraperitoneal chemotherapy concentration

Analogous to intravenous chemotherapy dosing schemes, the dose of chemotherapy administered during HIPEC is calculated on body surface area (BSA). While this is common practice and important for drug-safety, this method might be misplaced in the case of HIPEC. First of all, plasma concentrations of platinum-based cytostatic drugs after abdominal or peritoneal administration are lower than after intravenous or oral administration. Therefore, administered HIPEC drug doses are higher than intravenous doses. For the amount of drug needed for HIPEC, the intra-abdominal volume might be a more important parameter than BSA because patients with similar BSA may have different intra-abdominal volumes. Unlike the protocol for HIPEC in mesothelioma as established by the group of Deraco [37], working with a standard intraperitoneal concentration of drugs, BSA-based doses of cytostatic drugs for HIPEC in CRC are often dissolved in a volume of carrier solution that fills the abdomen rather than a fixed volume or concentration. We investigated the variation in intraperitoneal chemotherapy in three CRS-HIPEC centers by assessing the BSA, intraperitoneal volume and chemotherapy dose that patients received. BSA-based dosing of cytostatic drugs resulted in a marked interpatient variation in absolute chemotherapy concentrations during oxaliplatin-based HIPEC [38]. The study also demonstrated that variations in chemotherapy concentration did not influence overall survival or the incidence of complications. In a recent clinical trial, we prospectively measured the oxaliplatin concentrations in plasma, perfusion fluid and abdominal drain fluid in twenty patients. Substantial interpatient variability was demonstrated for platinum concentrations in peritoneal tissue (range 65 – 1640 µg/g dry weight), blood and drain fluid during and after HIPEC procedure. Furthermore, no significant effect of flushing with crystalloids after HIPEC on platinum concentration in peritoneal tissue, blood or drain fluid was found [39].

Targeted photodynamic therapy

Similarly to HIPEC, targeted photodynamic therapy (tPDT) for colorectal peritoneal metastases could have a possible role in treating microscopic residual disease, since it has potential to specifically reduce tumor growth [40]. Reactive oxygen species formed by PDT can physically damage cells and induce apoptosis and other mechanisms of cell death by disrupting crucial cellular processes or destroying the integrity of the cell membrane. Additionally, tPDT may directly damage anti-apoptotic factors and drug efflux pumps that are involved in classical drug resistance [41]. Since chemo resistant disease sometimes relies on these pumps and factors, this could be one of the reasons why tPDT might be effective in chemo resistant disease [42]. While dermatologists and oral-maxillofacial surgeons are familiar with (non-targeted) PDT for treatment of non-melanoma skin cancers, other skin conditions and head and neck cancer, the use of (t)PDT in other fields of oncology is still in its infancy [43]. While
there is ample experience with topical application of PDT tracers, tumors that do not allow topical administration require intravenous administration of the photosensitizer coupled to a targeting molecule. A great advantage of tPDT is that intravenous administration allows more precise targeting of the tracer, since it is not applied to adjacent healthy tissue. Most recent tPDT studies use intravenous photosensitizers, in contrast to topically applied dermatological PDT agents [44-47]. This results in clearance of a significant part of the tracer before it reaches the tumor. Furthermore, the efficacy of antibody based tPDT might be dependent on the antigen expression on target cells [40]. Because of these limitations the technique could be optimized further. Other promising targeting techniques such as delivery platforms that are triggered by the lower pH of the tumor environment might overcome these limitations [48, 49]. Another important factor for tPDT is the light source. The complexity, size and folds of the peritoneum require an illumination source which can be conveniently aimed at the desired anatomical location with the correct distance. Due to the precision of lasers, a laser-based system might be more optimal than less focused light emitting diodes.

Since the immune response is an essential part of tPDT, the treatment should be further optimized in humans for new applications [50]. Therefore, the potential of using tPDT in conjunction with- or instead of (CRS)HIPEC should be further explored, especially in the case of recurrent disease where the chance of encountering chemo-naïve cells is theoretically larger.

**Improving perioperative care and outcome**

Standardization of treatment protocols can improve outcome of patients treated for peritoneal malignancies [51]. This could be a result of reaching multidisciplinary consensus on the optimal treatment protocols for specific patients based on the literature [52]. The main surgical aspects of the CRS-HIPEC procedure are standardized but perioperative care is not. Nutritional, physical and psychological support and optimization of patients undergoing complex surgery has numerous benefits on clinical outcome for patients undergoing complex surgery [53, 54]. A recent French pilot study indicates that these findings are also applicable to patients who undergo CRS-HIPEC [55]. Therefore, these prehabilitation programs should be further explored in the near future.

In turn, enhanced recovery after surgery (ERAS) programs have proven their safety and efficacy in reducing complications, mortality and hospital stay after colorectal surgery [56, 57]. ERAS programs for CRS-HIPEC are feasible and may be associated with a reduction in use of narcotics and total fluid, length of hospital stay, and complication rates [58-61]. However, implementation of ERAS programs remains difficult due to the multidisciplinary nature and the number of variables to consider [62]. Moreover, recovery and complications might be
affected by type of perioperative care that is provided [63]. These findings highlight the importance of perioperative care for improving patient outcome. Therefore, future studies should focus on finding the optimal ERAS program for patients with colorectal PM treated with CRS-HIPEC.

CRS-HIPEC is a local treatment and the ultimate goal is removal of all peritoneal disease to prevent local recurrence intraabdominally. Unfortunately, in up to 26% of patients treated with CRS-HIPEC, recurrent peritoneal metastases are found during follow-up [64] and median 5 year overall survival does not exceed 50% [65]. Locoregional treatment of isolated peritoneal recurrences after CRS-HIPEC can safely lead to increased overall survival of carefully selected patients [64]. However, still a majority of patients that are diagnosed with isolated recurrent peritoneal disease are treated with palliative intent (67%) [64]. Optimizing the HIPEC procedure could reduce the incidence of recurrence and, thereby, increase the overall survival of the total patient group.

Prehabilitation and ERAS programs for CRS-HIPEC, HIPEC protocols and treatment of recurrences after CRS-HIPEC share the same general problem that relatively little decisive and robust clinical trials have been performed. The last decades, a vast increase in observational data on the treatment of colorectal peritoneal metastasis has become available. These results are valuable and form the fundament for further research. The international scientific community interested in peritoneal surface malignancy, should therefore focus on initiating well designed multicenter studies. These studies should include identification of new molecular prognostic factors for patient selection, HIPEC protocols for improved outcome, ERAS programs for a reduction in postoperative complications, and improvement of image-guided surgery. Furthermore, more (inter)national collaboration can increase the size and robustness of the trials that are initiated.
CHAPTER 11

CONCLUSION

The treatment of patients with PM of colorectal origin has shown successful developments in the last two decades. The ultimate goal of all topics covered in this thesis is improving the outcome of patients after CRS-HIPEC. While the findings from studies in this thesis contribute to scientific progress of CRS-HIPEC research, the search for optimal treatment continues. In the future, the clinical workup of patients with colorectal PM should contain the most accurate non-invasive diagnostic modalities. Furthermore, a personalized approach to identify tumor characteristics contributes to selecting the most ideal treatment. This could ultimately include (a combination of) multimodal image-guided cytoreductive surgery with enhanced recovery in combination with more personalized intraperitoneal and systemic treatment. Furthermore, targeted alternatives such as targeted photodynamic therapy need to be further explored in addition to the currently used treatment standards.
REFERENCES

17. McCubrey, J.A., et al., Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT...


49. Voskuil, F.J., et al., Image-guided surgery for tumor agnostic detection of solid


64. van Eden, W.J., et al., Treatment of Isolated Peritoneal Recurrences in Patients with Colorectal Peritoneal Metastases Previously Treated with Cytoreductive

12.1

Summary
SUMMARY

The best available treatment for patients with colorectal peritoneal metastases (PM) is cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). This treatment is effective in well selected patients and several predictive factors for successful treatment are available. These include the amount of PM present in the abdomen, complete macroscopic cytoreduction of all malignant peritoneal lesions, and the occurrence of major postoperative complications.

To further improve and expand the knowledge on the treatment of peritoneal metastases that is currently available, the current clinical and pharmacological aspects of CRS-HIPEC and the potential of novel molecular imaging techniques for pre- and intraoperative imaging were investigated in this thesis.

Part 1 includes five chapters on the clinical and pharmacological aspects of CRS-HIPEC. In chapter 2 the effect of specific differences in postoperative care including the standard use of total parenteral nutrition (TPN), suprapubic catheters (SPCs) and selective bowel decontamination (SBD) were retrospectively compared between two centers with otherwise similar treatment protocols. One hundred thirty-six matched patients (68 in each center) were included and 105 patients (77.2%) experienced postoperative complications. The incidence of severe complications (clavien-dindo grade III-V) was significantly higher in the center without standard use of TPN, SPCs and SBD: 26.4% versus 11.8%. No differences in postoperative hospital length of stay were found. These results suggest that the incidence of severe postoperative complications may be improved by specific postoperative care elements.

In chapter 3 the historical treatment of 414 patients with PM in two centers is described. After initial treatment with CRS-HIPEC, 106 patients (26%) were diagnosed with isolated peritoneal recurrence of disease. Forty-three of these patients (41%) were treated with curative intent, whereas the rest was treated with palliative intent. After treatment with curative intent, median survival was 24.7 (IQR 12.1-61.7) months, whereas survival after palliative treatment was only 7.6 (IQR 2.5-15.9) months. Fifteen patients underwent a second CRS-HIPEC procedure (14.1%), their median overall survival reached 29.0 (IQR 18.1-63.0) months. These findings indicate that a substantial number of patients with recurrent disease after CRS-HIPEC can be safely treated with curative intent yielding long-term survival.

Methodological differences in the administration of HIPEC continue to exist between centers that perform CRS-HIPEC. These include differences regarding the carrier solution, perfusate volume, use of an open or closed technique, duration of the perfusion and application of
additional flushing following chemoperfusion. In chapter 4 the literature is reviewed on these subjects in oxaliplatin-based HIPEC and identified a wide variety in the methods with respect to administration of HIPEC. This underlines the need for standardization of the HIPEC procedure with oxaliplatin in patients with colorectal peritoneal metastases. Usually, the chemotherapy dose during HIPEC is based on body surface area (BSA), as is usual in systemic treatment. The calculated dose is then dissolved in the available abdominal volume of the patient during HIPEC. However, BSA and intraperitoneal volume are not well correlated. This results in different drug concentrations in patients. In chapter 5 the influence of the variation that is present in HIPEC drug concentration is described on complications and survival by retrospectively analyzing databases of three CRS-HIPEC centers. Intraperitoneal chemotherapy concentrations ranged from 7 to 76 mg/L for mitomycin C and from 92 to 378 mg/L for oxaliplatin. However, no correlation between drug concentration and postoperative complications or survival was found.

In chapter 6 a clinical trial to determine the effect of post-HIPEC flushing with crystalloids on systemic and tissue exposure of patients treated with oxaliplatin-based HIPEC is described. Two groups of ten CRS-HIPEC patients were included, one group with post-instillation flushing and one without. Large inter-individual variability in platinum exposure were found in peritoneal tissue (65-1640 µg/g dry weight) and ultrafiltered plasma (10.5 – 28.0 µg*h/ml). No effect of flushing was found on median platinum concentration in peritoneal tissue, blood or drain fluid in the first hours and days following the procedure. The platinum concentration in the instillation fluid at the start of HIPEC differed twofold between patients and was positively correlated with systemic exposure and peak plasma concentration. The findings from this clinical trial confirm findings from chapter 5 that BSA-based HIPEC leads to large interpatient variability in platinum exposure in all compartments.

Part 2 of this thesis focused on the use of tumor-targeting antibodies for imaging and treatment of colorectal PM.

In chapter 7 the use of a bispecific anti-CEA x HSG antibody (TF2) combined with a small dual-labeled hapten peptide (RDC018) for image-guided resection of colorectal tumors in a mouse model is described. Mice with subcutaneous human CEA-positive LS174T and CEA-negative tumors were injected with the bispecific anti-CEA x HSG antibody and different doses of the small dual-labeled peptide. We analyzed the biodistribution of the tracer and performed post-mortem image-guided resection of intraperitoneal tumors. RDC018 showed specific tumor targeting after pretargeting with the bispecific antibody TF2. During post-mortem resection, intraperitoneal tumors were successfully identified and removed. These results show the
feasibility of image-guided resection after pretargeting of peritoneal colorectal cancer lesions in a mouse model.

Surgery combined with HIPEC remains the main treatment for colorectal PM. Targeted photodynamic therapy (tPDT), a new and developing technique, could be considered for the treatment of colorectal PM as an alternative or complementary treatment for HIPEC. In chapter 8 the treatment effect of tPDT was evaluated in vitro in 9 different CEA-expressing colorectal cell lines under identical experimental conditions. Furthermore, the effect of tPDT on tumor growth was evaluated in a mouse model with subcutaneous tumors. tPDT effect was greater in cells with higher CEA-availability. In vivo, tumor growth rate was reduced in mice treated with tPDT. These findings indicated that tumors have different levels of susceptibility for tPDT, largely as a result of different CEA-expression and availability levels. Furthermore, the results indicate that tPDT can effectively reduce tumor growth progression in vivo.

The goal of the study described in chapter 9 was to contribute to tackling one of the challenges in research concerning tumor-targeted approaches: adequately translating findings from in vitro or animal studies to the clinical situation. We describe a method to use resected tumor specimens to gain insight into the potential of newly developed multimodal tracers. Twenty-nine freshly resected peritoneal tumors of 10 colorectal cancer patients with PM were incubated with the dual-labeled anti-CEA antibody labetuzumab. In tumors with malignant cells, overall fluorescence was higher than in adjacent non-malignant tissue. The average fluorescence tumor-to-background ratio was 11.8 ± 9.1 : 1. These findings suggest the feasibility of the use of this tracer for multimodal image-guided surgery in humans.

In chapter 10 the results of a phase 1 clinical trial on image-guided surgery with dual-labeled labetuzumab of colorectal PM is presented. Ten patients with colorectal PM were intravenously injected with dual-labeled labetuzumab one week prior to CRS-HIPEC. Five patients received 2 mg of the dual-labeled antibody, the other 5 received 10 mg. Four or five days after injection, single photon emission computed tomography combined with computed tomography (SPECT/CT) scanning was performed to image the administered tracer. On the day of surgery, after opening the abdomen, before cytoreduction, gamma probe detection and fluorescence imaging was used for identifying peritoneal metastases. The safety and feasibility of the technique were evaluated. No infusion-related (serious) adverse events (S)AE were observed after dual-labeled labetuzumab injection and hospital stay remained similar to normal. While we were not able to identify individual peritoneal metastases with SPECT/CT,
it enabled us to visualize larger extraperitoneal disease. Intraoperative fluorescence imaging enabled us to identify peritoneal metastases, however contrast in the low dose group was less that in the highest dose group. Injection of dual-labeled labetuzumab was safe and pre-operative SPECT/CT imaging was feasible in patients with colorectal peritoneal metastases that underwent CRS-HIPEC treatment.

Overall, the broad content of this thesis contributes to improving the surgical treatment of peritoneal metastases of colorectal origin and can form a fundament for further research.
12.2 Samenvatting
SAMENVATTING

De best beschikbare behandeling voor patiënten met buikvliesuitzaaiingen van dikkedarmkanker (BU) is cytoreductieve chirurgie (CRS) gecombineerd met verwarmde chemotherapie spoeling in de buik (HIPEC). Dit is een grote en ingrijpende operatie. Met cytoreductieve chirurgie bedoelen we het zo compleet mogelijk weghalen van alle zichtbare kanker. De behandeling met CRS-HIPEC is alleen effectief wanneer patiënten zorgvuldig geselecteerd zijn. Er zijn enkele voorspellende factoren die aangeven of een patiënt een goede en reële kans heeft om succesvol behandeld te worden met CRS-HIPEC. Enkele van deze factoren zijn: de hoeveelheid van tumor aanwezig in de buik, of het lukt om al deze tumoren te verwijderen en het optreden van ernstige complicaties na de ingreep.

Om de kennis over de behandeling van buikvliesuitzaaiingen die momenteel beschikbaar is uit te breiden, zijn in dit proefschrift de huidige klinische en farmacologische aspecten van CRS-HIPEC onderzocht. Ook onderzochten we of nieuwe methoden om tumoren beter zichtbaar te maken vóór en tijdens de operatie nuttig en veilig zijn.

Deel 1 bevat vijf hoofdstukken over de klinische en farmacologische aspecten van CRS-HIPEC. In hoofdstuk 2 wordt het effect van bepaalde verschillen in de postoperatieve behandeling op het optreden van complicaties onderzocht. Honderdzesendertig patiënten (68 uit elk centrum) zijn geïncludeerd. Bij 105 patiënten (77.2%) traden complicaties op. Het voorkomen van ernstige complicaties was hoger in het centrum waar totaal parenterale voeding, suprapubische katheters en selectieve darm decontaminatie niet standaard werden toegepast: 26.4% tegenover 11.8%. Er werden geen verschillen gevonden in de lengte van het postoperatief verblijf in het ziekenhuis. Deze resultaten suggereren dat het optreden van ernstige postoperatieve complicaties kan worden verbeterd door het toepassen van specifieke postoperatieve zorgmethoden.

In hoofdstuk 3 is de behandeling van 414 patiënten met BU in twee centra beschreven. Na eerste een eerste behandeling met CRS-HIPEC voor BU, werden er in 106 patiënten (26%) opnieuw BU gevonden. Drieënveertig van deze patiënten (41%) kregen een nieuwe behandeling gericht op het genezen van de ziekte. De rest werd behandeld om de ziekte en symptomen te remmen, maar niet om te genezen. Na behandeling gericht op het genezen van de ziekte overleefde mensen gemiddeld zo’n 2 jaar. Na behandeling die gericht was om de ziekte en symptomen te remmen was dat slechts 7.6 maanden. Vijftien patiënten ondergingen een tweede CRS-HIPEC procedure (14.1%), hun overleving was gemiddeld 29 maanden. Deze bevindingen tonen aan dat een substantieel gedeelte van patiënten met terugkerende BU veilig en succesvol behandeld kan worden met eventueel een tweede CRS-HIPEC procedure.
Tussen verschillende behandelcentra bestaan er methodologische verschillen in de manier van toediening van de verwarmde chemotherapie in de buik (HIPEC). Dit zijn verschillen in onder andere: Oplosvloeistof voor de chemotherapie, spoelen met open of gesloten buik, de duur van de daadwerkelijke spoeling (perfusieduur) en het toepassen van eventueel naspoelen met zout na de chemotherapie spoeling. In hoofdstuk 4 is de literatuur samengevat en bediscussieerd over bovengenoemde punten. Er werd een grote variatie gevonden in de manieren waarop HIPEC wordt toegepast. Dit onderstreept het belang van standaardisering van de procedure, om zo de beste methode voor de individuele patiënt te kunnen waarborgen. Normaal gesproken wordt de dosering van HIPEC gebaseerd op het totaal lichaamsoppervlak van de patiënt (BSA), net als dat bij systemische chemotherapie gebeurt. De berekende dosis is dan opgelost in het volume dat in de buik van de patiënt past tijdens de HIPEC spoeling. Het probleem is echter dat BSA en het buikvolume van de patiënt niet goed met elkaar gecorreleerd zijn. Dit zorgt ervoor dat vergelijkbare patiënten soms een andere concentratie chemotherapie krijgen. In hoofdstuk 5 wordt de invloed van de variatie in chemotherapie concentratie op complicaties en overleving beschreven door historische data te analyseren die beschikbaar was in 3 behandelcentra. De chemotherapieconcentratie in de buik liep uiteen van 7 milligram per liter tot 76 milligram per liter voor het middel Mitomycine C, en 92 tot 378 milligram per liter voor het middel Oxaliplatine. Er werd geen correlatie gevonden tussen chemotherapie concentratie en overleving of complicaties.

In hoofdstuk 6 wordt een klinisch onderzoek beschreven dat het effect van het naspoelen met zout na HIPEC op de systemische en weefselbloodstelling van patiënten onderzocht. Twee groepen van tien CRS-HIPEC patiënten zijn hiervoor geïncludeerd. In één van de groepen werd er nagespoeld met zout, in de andere niet. We vonden een groot verschil in platinum (werkzame stof in Oxaliplatine) bloodstelling tussen de patiënten. We vonden geen effect van spoelen met zout op de concentratie platinum in weefsel, bloed of drainvloeistof in de eerste uren en dagen na de CRS-HIPEC procedure. Er zat een tweevoudig verschil in de platinum concentratie in de spoelvloeistof bij aanvang van de HIPEC spoeling. Ook bleek dat deze concentratie gerelateerd was aan de piek-concentratie in het bloed. Deze bevindingen bevestigen de resultaten uit hoofdstuk 5 waarin we concludeerden dat BSA-gebaseerde dosering leidt tot een grote variatie in de blootstelling aan chemotherapie in weefsel en bloed.

Het tweede deel van dit proefschrift was gericht op het gebruik van tumor-specifieke antilichamen voor beeldvorming en behandeling van buikvliezuitzaaiingen van dikke darmkanker.
In hoofdstuk 7 wordt het gebruik van een bispecifiek antilichaam (TF2) gecombineerd met een kleiner eiwit (RDC018) voor resectie met behulp van beeldvorming in een muis model beschreven. Muizen met onderhuidse darmkankertumoren werden geïnjecteerd met TF2 en verschillende doses van RDC018. Na het offeren van de muizen werd de verdeling van RDC018 in het lichaam van de muis geanalyseerd. Het was mogelijk om met deze methode tumoren zichtbaar te maken door middel van het fluorescentie en radioactieve signaal. Deze resultaten laten de haalbaarheid van de methode zien in een muis model.

Chirurgie gecombineerd met HIPEC is de beste behandeling voor BU die er op dit moment is. Gerichte fotodynamische therapie (tPDT) is een nieuwe en zich nog ontwikkelende techniek die gebruikt maakt van licht en een lichtgevoelige stof. Wanneer deze twee gecombineerd worden, wordt er een dodelijk milieu voor de kankercel gecreëerd. tPDT kan overwogen worden voor de (aanvullende) behandeling van BU van dikkedarmkanker. In hoofdstuk 8 wordt de behandeling met tPDT geëvalueerd in 9 verschillende dikkedarmkanker cellijnen. Tevens wordt het effect van tPDT op tumor groei geëvalueerd in een muis model met onderhuidse tumoren. Het effect van tPDT was groter in cellen met meer expressie van tumoreiwit (CEA) op hun oppervlak. De groei van onderhuidse tumoren werd geremd met het toepassen van tPDT. Deze bevindingen geven aan dat tumoren verschillende gevoeligheden hebben voor de behandeling met tPDT als gevolg van (onder andere) het verschil in expressie van tumoreiwit. Ook geven de bevindingen aan dat tPDT effectief kan zijn in het remmen van tumorgroei in een muis model.

Het doel van de studie die beschreven is in hoofdstuk 9 was om bij te dragen aan een probleem dat bij veel onderzoeken naar antilichamen of andere targeting-moleculen of medicijnen speelt: tussen het gebruik van cellen, muizen en de mens zit een groot verschil. In dit hoofdstuk beschrijven we een methode om net verwijderde tumoren te gebruiken om inzicht te krijgen in de effectiviteit en mogelijkheid om met een targeting-molecuul die tumoren zichtbaar te maken met behulp van fluorescentie en/of radioactiviteit. Aan negenentwintig tumoren van 10 patiënten werd in het laboratorium een fluorescent en radioactief antilichaam toegediend. We zagen dat in tumoren met kanker cellen er meer fluorescentie aanwezig was en dat er in normale cellen veel minder fluorescentie aanwezig was. Dit betekent dat we op deze manier een antilichaam of ander molecuul kunnen testen voordat het toegediend wordt aan de mens.
In hoofdstuk 10 worden de resultaten van een eerste fase klinisch onderzoek beschreven. Hier keken we naar de toepassing van een fluorescent en radioactief antilichaam dat we toediende aan 10 patiënten met BU. Vijf patiënten kregen 2 milligram van het antilichaam, de andere 5 patiënten kregen 10 milligram toegediend. Vervolgens werd er bijgehouden of er bijwerkingen optraden en dit was niet het geval. Vier of vijf dagen na de injectie werd er een SPECT/CT-scan gemaakt van deze patiënten. Tevens werd er tijdens de CRS-HIPEC operatie gekeken met een fluorescentie camera en radioactiviteit detector of we de tumoren zichtbaar konden maken en konden vinden. Met de scan werden geen individuele BU gevonden, wel werden grote lymfekliermetastasen gevonden. Vooral in de 10 milligram groep konden we tijdens de operatie de tumoren duidelijk zichtbaar maken met fluorescentie. Concluderend draagt de brede inhoud van dit proefschrift bij aan het verbeteren van de behandeling van patiënten met buikvliesuitzaaiingen van dikkedarmkanker en kan een verdere basis vormen voor toekomstig onderzoek.
Appendices
Figure S1 Chapter 3 OS of 33 patients treated with curative intent CRS or HIPEC.
Supplementary figures chapter 7: Pretargeting

*Figure S1 chapter 7* Structural formulas of IMP-288 and RDC018. Blue: DOTA chelate. Red: DyLight™ 800
Figure S2 chapter 7 Biodistribution profiles of $^{111}$In-RDC018 and $^{111}$In-IMP-288 at 2 h and 24 h p.i. after pretargeting with 0 and 0.8 nmol TF2 in BALB/c nude mice with s.c. tumors.
Supplementary figures chapter 8: Photodynamic therapy

Figure S1 chapter 8 the CEA binding availability of the cell lines used in this study. Note the (arbitrary) classification in high (>5% specific binding) and low (<5% specific binding) binding of DTPA-hMN-14-IRDye700DX.

Figure S2 chapter 8 Ex vivo biodistribution of two mice with subcutaneous LoVo tumors, one day after $^{111}$In-labeled DTPA-hMN-14-IRDye700DX injection.
Supplementary figures chapter 9

Figure S1 chapter 9 Tumor-to-background ratio for fluorescence and autoradiography of tissue incubated with $^{111}$In-DOTA-hMN14-IRDye800CW per patient. Tissue of patient 9 and 10 was split and also incubated with the control antibody conjugate, $^{111}$In-DOTA-hIgG-IRDye800CW. The autoradiography of patient 6 is missing due to failure of the radiolabeling.

Figure S2 chapter 9 Binding assay with CEA-positive LS174T cells using dual-labeled hMN-14 and dualabeled hIgG (control antibody).
Figure S3a chapter 9 Tumor with a necrotic core (other patient than depicted in figure 3b). A. H&E staining and zoom, B. CEA immunohistochemical staining, C. Flatbed fluorescence scan image, D. Autoradiography. Note the tracer uptake in the viable tumor border (yellow arrows) and the necrotic tumor core (green arrows).
Figure S3b. Figure 9. Tumor with mucin pools (other patient than depicted in Figure 3a). A. H&E staining and zoom. B. CEA immunohistochemical staining. C. Flatbed fluorescence scan image. D. Autoradiography. Note the tracer accumulation in the mucin pools (green arrows).
DATA MANAGEMENT PLAN

The primary and secondary data obtained during my PhD at the Radboud university medical center (Radboudumc) have been captured and stored on the local archive of the department of Radiology, Nuclear Medicine and Anatomy of the Radboudumc. All human studies were approved by the appropriate ethics committee. Data is stored anonymized and coded, uncoding can be performed by the third party trial bureau of the department of Radiology, Nuclear medicine and anatomy from the Radboudumc. Anonymized data from chapter 2, 3 and 5 is stored on the departments’ digital archive accessible by dr. M. Rijpkema or other senior researchers from the department. Data from chapter 4 and 6 is stored securely by the 1st author of the papers on the local Radboudumc server of his department. Mouse studies described in Chapter 7 and 8 were approved by the Central Animal Laboratory and the Animal Ethics Board of the associated universities and is stored in the same digital archive as chapter 2, 3 and 5. Data and methodology from chapter 9 was stored on the department’s Labguru server and accessible by the associated senior staff members. Data from chapter 10 is stored on the local department’s server and is currently under supervision by drs. JM de Gooyer and dr. Rijpkema. Published data generated or analyzed in this thesis are part of published articles and its additional files are available from the associated (corresponding) or last authors upon reasonable request. The data will be saved for 15 years after publication of the individual studies. Using these patient data in future research is only possible after a renewed permission by the patient as recorded in the informed consent.
LIST OF PUBLICATIONS

van Eden WJ, Elekonawo FMK, Starremans BJ, Kok NFM, Bremers AJA, de Wilt JHW, Aalbers AGJ.

Elekonawo FMK, de Gooyer JM, Boerman OC, Bremers AJA, Rijpkema M, de Wilt J.H.W.

Elekonawo FMK, Lütje S, Franssen GM, Bos DL, Goldenberg DM, Boerman OC, Rijpkema M.
A pretargeted multimodal approach for image-guided resection in a xenograft model of colorectal cancer. EJNMMI Res. 2019 Sep 4;9(1):86.

De Jong LAW, Elekonawo FMK, de Reuver PR, Bremers AJA, de Wilt JHW, Jansman FGA, Ter Heine R, van Erp NP.

Elekonawo FMK, Bos DL, Goldenberg DM, Boerman OC, Rijpkema M.

Elekonawo FMK, van der Meeren MMD, Simkens GA, de Wilt JHW, de Hingh IH, Bremers AJA

Elekonawo FMK, van Eden WJ, van der Plas WY, Ewalds RSG, de Jong LAW, Bremers AJA, Hemmer PHJ, Kok NFM, Kruijff S, Aalbers AGJ, de Reuver PR.
Effect of intraperitoneal chemotherapy concentration on morbidity and survival. BJS Open. 2020 Apr;4(2):293-300.
Elekonawo FMK, Starremans B, Laurens ST, Bremers AJA, de Wilt JHW, Heijmen L, de Geus-Oei LF.

Elekonawo FMK, de Gooyer JM, Bos DL, Goldenberg DM, Boerman OC, Brosens LAA, Bremers AJA, de Wilt JHW, Rijpkema M.

de Jong LAW, Elekonawo FMK, Lambert M, de Gooyer JM, Verheul HMW, Burger DM, de Wilt JHW, Chatelut E, Ter Heine R, de Reuver PR, Bremers AJA, van Erp NP.

de Gooyer JM, Elekonawo FMK, Bos DL, van der Post RS, Pèlegin A, Framery B, Cailler F, Vahrmeijer AL, de Wilt JHW, Rijpkema M.
### PHD PORTFOLIO

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**Department:** Radiology, nuclear medicine & Anatomy  
**Graduate School:** Radboud Institute for Health Sciences  
**PhD period:** 01-03-2016 – 01-03-2020  
**Promotor(s):** Prof. JHW de Wilt, Prof. OC Boerman  
**Co-promotor(s):** Dr. MJP Rijpkema, Dr. AJA Bremers

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<td>2.0</td>
</tr>
<tr>
<td>- Research Meeting Pathology (Weekly attendance and regular presentations)</td>
<td>2017-2020</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>c) Symposia &amp; congresses</strong></td>
<td>Year(s)</td>
<td>ECTS</td>
</tr>
<tr>
<td>- Radboud Frontiers</td>
<td>2016-2019</td>
<td>1.0</td>
</tr>
<tr>
<td>- PhD retreat (multiple posters presented)</td>
<td>2016-2019</td>
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<tr>
<td>- Radboud Science Day</td>
<td>2016-2019</td>
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<td>- NvH Chirurgendagen (oral presentation)</td>
<td>2016-2019</td>
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<tr>
<td>- Fluorescence Theranostics symposium</td>
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<tr>
<td>- 10th international congress on peritoneal surface malignancies (Washington DC; poster presenter)</td>
<td>2016</td>
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<tr>
<td>- 11th international congress on peritoneal surface malignancies (Paris; poster presenter)</td>
<td>2018</td>
<td>1.25</td>
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<tr>
<td>- Academy colloquium Precision surgery by tumor targeted molecular imaging (Amsterdam)</td>
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<tr>
<td>- European association of nuclear medicine (oral presentation)</td>
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<td>- ESMI annual meeting EMIM (Glasgow) (oral presentation)</td>
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<tr>
<td>- Nederlands congres voor peritoneale oncologie</td>
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<tr>
<td>- European image guided surgery congress</td>
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<tr>
<td><strong>d) Other</strong></td>
<td>Year(s)</td>
<td>ECTS</td>
</tr>
<tr>
<td>- Organizing committee research meeting department</td>
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<tr>
<td>- Organizing Department end of the year research meeting</td>
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<tr>
<td>TEACHING ACTIVITIES</td>
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<tr>
<td><strong>e)</strong></td>
<td>Lecturing</td>
<td>Explaining my project to various students “Meet your PhD”</td>
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<td>-</td>
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<tr>
<td><strong>f)</strong></td>
<td>Supervision of internships / other</td>
<td></td>
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<td>-</td>
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<td><strong>TOTAL</strong></td>
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ABOUT THE AUTHOR

DANKWOORD

Ja! Daar is ‘ie dan... Het heuse dankwoord, een van de meest gelezen delen van dit proefschrift.

Wat begon met een interesse voor onderzoek in traumatologie heeft zich ontplooid tot een proefschrift in een andere hoek van de heelkundige zorg. Via mijn studiementor van destijds (dr. Bremers) ben ik begonnen met wat onderzoek naast het reguliere curriculum. Dit werd al snel opgeschaald naar een wetenschappelijke stage, en heeft uiteindelijk geleid tot een promotietraject dat bijna naadloos aansloot op de kennis die ik tot op dat moment verkregen had. Dit proefschrift was nooit tot stand gekomen zonder hulp van velen, ook mensen die ik niet bij naam noem, maar wel hun steen hebben bijgedragen wil ik uiteraard bedanken ook voor hun bijdrage.

Als eerste wil ik mijn dank uitspreken naar de patiënten die meegewerkt hebben aan de klinische onderzoeken die beschreven zijn in dit proefschrift. Een deel van jullie is inmiddels overleden. Jullie waren, ondanks de ziekte en het zware behandeltraject, bereid om een bijdrage te leveren aan kennis die in potentie toekomstige patiënten kan helpen.

Dr. Rijpkema, beste Mark, als dagelijkse supervisor stond de deur altijd bij je open. Ik waardeer de vrijheid die je mij hebt gegeven in het werk als onderzoeker. In onze meetings bracht je altijd weer een kritische noot of bedacht je dat het goed zou zijn als er tóch nog een extra experiment gedaan moest worden (of juist niet)... Soms ben ik daar in meegegaan, soms ook niet en dat was allemaal geen probleem erg fijn!

Prof. dr. de Wilt, beste Hans, onze besprekingen hadden geen standaard moment, soms spraken we elkaar een maand niet, soms meerdere malen per maand. Op de poli of OK, in de auto of op kantoor over muziek, werk of random anekdotes alles was goed, zolang het werk maar af kwam. Dat is gelukt en dankzij jouw begeleidende rol als promotor heb ik dit proefschrift goede inhoud kunnen geven.

Dr. Bremers, beste André, wat begon als wetenschappelijke interesse evolueerde zich in een promotie. Dankzij jou ben ik gemotiveerd geraakt om het onderzoek uit dit proefschrift te gaan doen. Bijzondere patiëntengroep, bijzondere operatie en bijzonder veel vraagstukken die nog op te lossen zijn. Je kwam vaak met ideeën of hypothesen, soms te veel om allemaal te onderzoeken. Bedankt voor deze eindeloze inspiratie.
Dr. Boerman, beste Otto, je hebt geholpen mijn traject op te starten. Helaas besloot je al vrij snel na mijn komst te vertrekken als professor bij de nucleaire geneeskunde. Gelukkig lag dat niet aan mij! Desalniettemin veel dank voor alle kritische feedback die ik van je heb mogen ontvangen op papers, abstracts en posters.

Veel dank aan de leden van de manuscriptcommissie bestaande uit de professoren Mulders, de Geus-Oei en Meijerink voor het beoordelen van mijn proefschrift.

Desirée, de eerste échte collega, iemand waarmee je dagelijks samen werk verricht. Je hebt me wegwijs gemaakt op het lab. Vele experimenten waren niets geworden zonder jou. Je had altijd creatieve ideeën om voor mij soms ingewikkeld lijkende problemen in het lab op te lossen. We hebben veel gelachen en nooit gehuild, dank voor deze geweldige jaren!

Dr. Hekman, beste Marlène, de eerste dagen was het allemaal aftasten, ik kreeg te horen: ‘je gaat doen wat Marlène heeft gedaan, alleen dan bij colorectaal’. Uiteindelijk gebruikte we inderdaad het zelfde concept bij verschillende ziektebeelden. Bedankt dat je me de 1e twee jaar op sleeptouw hebt genomen en wegwijs hebt gemaakt op de afdeling. Naast dat serieuze was het ook altijd gezellig om met jou en de rest van kamer 3 te borrelen of feesten.

Jan Marie, kerel, partner in crime, dynamic duo, hoe je het ook wil noemen... We hebben een mooie tijd samen gehad. Soms liep het soepel, soms wat stroever, maar nooit echte problemen gehad. Dank voor de momenten waarop we hebben gelachen en ook samen hebben gebaald. Zonder jou was mijn promotie toch wel wat minder avontuurlijk geweest. Zet hem nog op met jouw laatste loodjes!

Natuurlijk wil ik ook iedereen die met mij op kantoor heeft gezeten op kamer 3 bedanken. In het bijzonder Tessa, Charlotte, Tom, Daphne L, Daphne (M)D, Peter W, Fokko, Yvonne, Gerwin, Massis, Robin, Joey, Maike, Sarah, Bastiaan, Esther, Melline, Estelle, Maria, Marti en Lieke. Samen hebben we gelachen, gehuild, geborreld, gereisd, en noem maar op... We maakten we het leven als PhD-student voor elkaar dragelijk door de relaxte sfeer die er altijd hing.

Dr. de Reuver, Philip, alhoewel je niet direct betrokken was in mijn promotieteam toch bedankt voor je bijdrage, eindeloos enthousiasme en betrokkenheid bij mijn onderzoek.

Loek, het was top om samen onderzoek te doen, jij als ziekenhuisapotheker hebt een andere blik op het onderzoek dat we deden en we vulden elkaar goed aan. Dat heeft geleid tot een aantal mooie publicaties die in de nabije toekomst hopelijk ook tot jouw promotie gaan leiden!

Natuurlijk wil ik alle analisten van de NucMed hartelijk bedanken voor hun bijdrage aan mijn proefschrift. Ook op het lab waren jullie altijd bereid om te helpen en mijn vragen te beantwoorden. Buiten het lab dronken we gezellig wat drankjes. Dankjewel Cathelijne, Janneke, Milou en Gerben.

Veel dank ook voor de collega’s van het trialbureau, in het bijzonder Michel en Judith. Zonder jullie ondersteunende rol zou het allemaal veel stroever gegaan zijn. Team-Hotlab bedankt voor jullie bijdrage en flexibiliteit. Maichel, Danny, Natascha en Sandra zonder jullie was een groot deel van dit proefschrift niet tot stand gekomen... Bedankt voor alle bereidingen. Tevens natuurlijk ook dank aan de collega's van de apotheek, waaronder Katja, voor alle inzet die jullie geleverd hebben om het halffabrikaat labetuzumab klinisch approved te krijgen!

Veel dank gaat uit naar Antoi en Steffie van de klinisch fysica. Antoi bedankt voor je praktische hulp rondom het meten van activiteit en gebruik van de gamma-counter. Steffie ik wil je bedanken voor de ondersteuning die je bood bij alle berekeningen en regels rondom het klinisch gebruik van radiopharmaca.

De rest van de senioren bedankt voor de mooie tijd en hulp die geboden werd wanneer dat gevraagd werd. Soms was een luisterend oor genoeg om stappen te zetten. Bedankt Peter, Sandra, Frank, Martin, René en Sanne.
Alle radiodiagnostisch laboranten bedankt voor jullie eindeloos geduld, gezelligheid en flexibiliteit. In het bijzonder wil ik degene waarmee ik het meeste heb gewerkt bedanken: Jur, Martin, Eddy, Maarten en Sandra.

Ook bedank ik graag de nucleair geneeskundigen die regelmatig onze scans bekeken. Erik en Marcel, bedankt voor jullie enthousiasme en inhoudelijke bijdrage aan dit proefschrift.

Researchgroep Prof. dr. Nagtegaal, in het bijzonder Shannon, Carlijn W, Femke, Valentina, Tessa, Carlijn en Iris bedankt dat jullie mij ook betrokken bij jullie researchgroep. Ik heb veel geleerd van het onderzoek dat jullie doen!

Floor en Ingrid bedankt voor jullie geduld en behulpzaamheid op de uitsnijkamer.

Ik wil alle collega’s van het CDL bedanken, in het bijzonder Bianca, Kitty, Wilma en Iris. Jullie passie voor dieren zorgt ervoor dat wij als onderzoekers gedegen proefdieronderzoek kunnen doen. Zonder jullie bestond een groot deel van dit proefschrift niet. Veel dank voor jullie kennis en hulp bij de dierexperimenten!

Ik wil ook alle OK-assistenten van het HIPEC-OK team bedanken voor jullie flexibiliteit en bereidheid om te helpen met het uitvoeren relatief ingewikkeld onderzoek op OK. Hetzelfde geldt voor de perfusionisten en medewerkers van de anesthesie.


Vrienden uit Bergen (en Sambeek), Gijs, Jaap, Joep, Collin en Nikolai ondanks dat ieder zijn eigen weg gegaan is hebben we nog steeds goed contact. Dank voor de ontspanning die we samen regelmatig vinden, al is het op een hutje op de hei of gewoon bij iemand op de slaapbank, het is altijd gezellig.

Sjakies a.k.a. Groepje 1! Nnaniki, Maud, Janne, Niekje, Lienieke, Inge en Myrdhin, bedankt voor alle leuke momenten die we samen hebben gehad en nog gaan hebben.
Jasper en Jaron, AM trio, thanks voor alle AM avonden en andere gezelligheid. Ik hoop dat we dat nog vaak kunnen blijven doen om zo wat afleiding te vinden in het werk van alle dag.

Martijn, jammer dat je verhuisd bent naar de hoofdstad, anders konden we nog vaker elkaar de oren van de kop lullen. Thanks voor alle goede gesprekken die we hadden en hebben.

Stef, vriend van het 1e uur, dankjewel voor je bijdrage als paranimf! Maar ook bedankt voor de steun en advies dat je geeft als dat nodig is.

Victoire, jij ook bedankt voor je bijdrage als paranimf en als grafisch ontwerper van dit proefschrift. Kleine broer waarmee ik altijd kan lachen, discussiëren of van gedachten kan wisselen. Fijn om je erbij te hebben en bedankt voor alle goede gesprekken die we hebben gehad over de jaren.

Eveil, andere kleine broer. Bedankt voor de tijden dat we tot laat hebben gelachen en meezongen met bollywood films. Dat zorgde pas voor ontspanning.

Joujou en Suze bedankt voor de steun en het vertrouwen dat jullie in mij hebben. Zonder jullie waren de moeilijke momenten van onderzoek doen nog moeilijker omdat ik dan niemand had om advies te vragen. Jullie hebben mij op de wereld gezet en opgevoed en hebben mij geleerd om door te zetten. Dank jullie!

Lieve Jolijn, ik weet dat ik nooit genoeg vertelde over wat voor onderzoek ik nou precies deed. Het liefst hoorde je alle details. Die kun je nu rustig nalezen in dit proefschrift! Dankjewel voor al je steun over de afgelopen jaren! Tevens bedankt voor de mooie foto die op de achterkant van dit proefschrift staat (southern carmine bee-eater/zuidelijke karmijnrode bijeneter/ *Merops nubicoides*). Het is heel fijn om met jou samen te leven, te reizen en te lachen. Dank je voor je eindeloze geduld en ik hoop dat we nog vele mooie dingen kunnen meemaken samen.