Final Study Report

Study Title: INTRAOPERATIVE INTRAPERITONEAL CHEMOPERFUSION TO TREAT PERITONEAL MINIMAL RESIDUAL DISEASE IN STAGE III OVARIAN CANCER: A RANDOMIZED PHASE II TRIAL

EudraCT number: 2015-000418-23

Study protocol code: AGO/2015/002

ClinicalTrial.gov identifier: NCT02567253

Sponsor: UZ Ghent

National Coordinator/ Coordinating Investigator: prof. Wim Ceelen

Funder: Vlaamse Liga tegen kanker (VLK)

Date of report: *27/07/2022*

Name and signature Sponsor:

Date signature Sponsor: .....

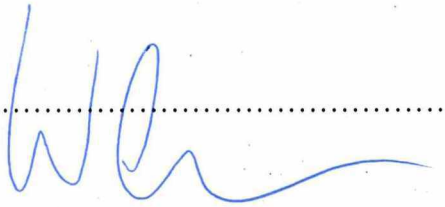


Table of content

1. Introduction

With more than 20.000 new cases annually in the United States and more than 65.000 new cases annually in Europe, ovarian cancer (OC) represents the second most common gynecological malignancy and main cause of death.[1–3] In comparison to other common solid cancers, OC is often diagnosed in an advanced stage, because of a lack of specific symptoms at earlier stages. Consequently, almost 70% of patients are diagnosed with stage IIIC OC, of which the majority (~90%) are of epithelial origin. Despite the fact that OC is usually widespread throughout the peritoneal cavity at the time of diagnosis, the disease generally remains confined to the peritoneal cavity.[4] Although significant research efforts have been made over the last 3 decades and despite the frequency and potential morbidity of peritoneal recurrence in advanced OC, only a modest improvement in treatment, prevention or survival has been achieved. This is explained by the fact that even after successful initial treatment, most patients (~80%) will eventually develop recurrent peritoneal disease, which can only arise from peritoneal minimal residual disease (pMRD), left after primary cytoreductive surgery (CRS).[3,5,6] Recently, the addition of intraoperative intraperitoneal chemoperfusion (IPEC) to the combination of CRS and systemic chemotherapy has been demonstrated to be beneficial, as OC usually remains confined to the peritoneal cavity.[6] The epidemiology and risk factors for PC in OC are well established. While the incidence of OC is low before the menopause, it rises afterwards with a median age at the time of diagnosis of 60-63 years. The lifetime risk of OC is approximately 1 in 70 in developed countries, but there are women with a much higher risk. The most important risk factor is a strong family history of breast or ovarian cancer, although a genetic predisposition (mostly germline mutations in tumor suppressor genes BRCA1 and/or BRCA2) is only present in only 10-15% of patients. For women with a BRCA1 or BRCA2 mutation, the risk of an OC is approximately 39-46% and 12-20%, respectively. Other factors like null parity, late menopause, early menarche and alcohol lead also to an increased risk, while breast feeding, oral contraceptives, pregnancy and tubal ligation are associated with a reduced risk of OC. [7,8]

1.1. Current first-line treatment of Stage III ovarian cancer

1.1.1 Cytoreductive (debulking) surgery

Most patients with ovarian cancer are diagnosed with advanced stage disease (stage III or IV) and when untreated, the outlook of these patients is poor, with a long-term survival (>10 years) of approximately 10-30% for women older than 65 years.(9, 10) Currently, complete debulking surgery (cytoreductive surgery, CRS) combined with systemic carboplatin/paclitaxel-based chemotherapy is the standard of care for primary OC.[7] The goal of CRS is to remove all macroscopically visible disease, since this has been shown to be associated with significantly increased overall survival (OS) and progression free survival (PFS) in patients with advanced OC.[4,9,10]

1.1.2 Neo-adjuvant chemotherapy

When tumor dissemination is too extensive and optimal primary CRS impossible, neo-adjuvant chemotherapy has been proposed in order to reduce tumor load and reduce postoperative complications. Vergote and colleagues compared the effect of neo-adjuvant chemotherapy followed by interval CRS with primary CRS followed by platinum-based chemotherapy, in patients with stage IIIC or IV OC. This study showed a higher rate of post-operative adverse effects in the group treated with primary CRS followed by chemotherapy. Residual tumor nodules of one cm or less were found in 41,6% of patients treated with primary CRS and in 80.6% of patients treated with interval CRS. Additionally, the hazard ratio (HR) for death was 0.98 (90% confidence interval [CI], 0.84 to 1.13; P=0.01 for non-

inferiority) and the HR for progressive disease was 1,01 (90% CI, 0.89 to 1.15), when comparing the group treated with interval CRS with the group treated with primary CRS. In conclusion, this study demonstrates first that neo-adjuvant therapy followed by interval CRS was not inferior to primary CRS followed by adjuvant chemotherapy and second that performing maximal cytoreduction remains the strongest independent variable for overall survival, whether administered at primary or at interval CRS.

1.1.3 Intraperitoneal chemotherapy

Since OC rarely spreads systemically, intraperitoneal (IP) chemotherapy might offer a benefit, since its pharmacokinetic advantage allows to achieve higher concentrations into the peritoneal cavity, as compared to i.v. therapy.[7] Several large randomized trials have demonstrated a statistically significant survival benefit associated with IP-platinum based chemotherapy as a first-line treatment after primary CRS (Table 1). [3,9] Kyrgiou et al. showed in a meta-analysis of multiple treatments, that the best survival in women with OC is obtained with a combination of IP administration of platinum and a taxane. They calculated that a platinum and taxane combination with IP administration resulted in a 55% relative risk reduction (95% confidence interval [CI] = 39% to 67%) for mortality as compared with nonintraperitoneal monotherapy.[11] Another meta-analysis by Hennessy et al., confirmed a benefit for catheter based IP chemotherapy in OS in patients with small (< 1cm) or no residual disease after surgery. Nevertheless, adjuvant IP chemotherapy through a catheter is currently not universally accepted as a standard treatment, mainly because of the potential for locoregional toxicity, port or catheter malfunction, and infection.[4,8]

Table 1. Randomized controlled trials of IP-platinum based chemotherapy as a first-line treatment after CRS, in women with primary stage III OC

Author	Year	N eligible patients	Setting	OS (months)	PFS (months)
Alberts[12]	1996	546	IV cyclophosphamide (600mg/m2) + IP cisplatin (100mg/m2) *OR* IV cyclophosphamide (600mg/m2) + cisplatin (100mg/m2)	IP arm: 49 95% CI [42-56] IV arm: 41 95% CI [34-47]	-
Markman[13]	2001	462	IV carboplatin (AUC9) + paclitaxel (135mg/m2) + IP cisplatin (100mg/m2) *OR* IV paclitaxel (135mg/m2) + cisplatin (75mg/m2)	IP arm: 63 IV arm: 52 RR 0.81 P=0.05 by log-rank test One-tail	IP arm: 28 IV arm: 22 RR 0.78 P=0.01 by log-rank test One-tail

Armstrong[14]	2003	415	IV paclitaxel (135mg/m2) + IP paclitaxel (60mg/m2) + cisplatin (100mg/m2) *OR* IV paclitaxel (135mg/m2) + cisplatin (75mg/m2)	IP arm:65.6 IV arm: 49.7 P=0.03 by log-rank test	IP arm:23.8 IV arm:18.3 P=0.05 by log-rank test

IV intravenous, IP intraperitoneal, OS overall survival, PFS progression free survival, CI 95% confidence interval, RR relative risk

1.1.4 Intraperitoneal Cisplatin

Cisplatin (cis-diamminedichloroplatinum [II]) is among the most frequently used chemotherapeutic agents for the treatment of solid tumors and has a molecular weight of approximately 300 and a high AUCi.p./AUCplasma ratio ranging from 12-22. [1,6,15,16] It executes its cytotoxic activity via formation of intra- and interstrand crosslinks in DNA, whereby the two major reaction products are guanine-guanine (Pt-[GG]) and adenine-guanine (Pt-[AG]) intrastrand crosslinks. These crosslinks results in inhibitory effects on DNA replication and transcription, which subsequently trigger apoptosis.[17] Recently, Bianga and colleagues demonstrated with laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) that cisplatin (75mg/m2, 42°C, 90min) penetrates deeper into tumor tissues (3-5mm), while oxaliplatin is mostly found at the periphery of the tumors. Therefore, cisplatin could provide a higher tumor drug concentration in pMRD.[8,18] The efficacy of cisplatin is enhanced by the addition of hyperthermia (thermal sensitization). Raaphorst et al. showed that a combination of mild hyperthermia (40-41°C) and low doses of cisplatin is a more effective treatment, due to the inhibition of DNA damage repair by hyperthermia. This synergistic effect persists at least for 2 hours. Other studies have confirmed that hyperthermia is a good sensitizer for cisplatin treatment.[19,20]

1.2 Hyperthermic intraperitoneal chemoperfusion (HIPEC)

HIPEC was first described in an animal model in 1974 by Euler [21].The first clinical application of combined cytoreduction and HIPEC was reported in 1980 by Spratt and co-workers, who treated a 35 years-old man, suffering from pseudomyxoma peritonei (PMP) with extensive surgery followed by IP chemoperfusion of thiotepa under hyperthermic conditions using a delivery system consisting of a heat exchanger and pump [22]. After the procedure, the drains were left in place and 5 days later another HIPEC procedure with methotrexate was performed. In that publication, the authors stressed the importance of removing free floating cancer cells by the microfilters in the perfusion circuit. The advantage of intraoperative chemoperfusion, as opposed to adjuvant, is the possibility to achieve optimal chemotherapy exposure of all peritoneal surfaces at risk. The use of hyperthermia is based on several observations. First, hyperthermia is selectively cytotoxic for malignant cells.[23] Second, the cytotoxicity of several chemotherapeutic agents (e.g. cisplatin) is enhanced by hyperthermia via different mechanisms like: increased cell membrane permeability, increased production of free oxygen radicals, increased DNA alkylation and increased activity at low pH. [9,10,19,20] Third, hyperthermia enhances tissue perfusion and oxygenation, and may improve drug penetration. Los et al. demonstrated a significant increase in peritoneal tumor Pt concentrations when IP cisplatin therapy was combined with regional hyperthermia (41.5 °C) in a rat colon cancer model.[24]

Although prospective, randomized controlled trials have demonstrated a significant benefit of CRS followed by HIPEC in colon cancer with PC and high-grade gastric cancer, up until recently there was only one prospective, randomized trial published up to now of HIPEC for PC in OC patients by Spiliotis et al. [6,25,26] On the other hand a number of non-randomized small trials have been published. Table 2 summarizes the most important trials presenting survival data in patients with primary advanced or recurrent OC treated with CRS and HIPEC.[3]

These results were summarized in two systematic reviews. Bijelic and colleagues found a median OS ranging from 22-54 months, a median disease free survival (DFS) ranging from 10-26 months, a significant morbidity of 5-36% and a median mortality of 3%. [10] Chua et al. found a similar median OS ranged from 22-64 months, a median PFS varied from 10-57 months, similar rates of severe perioperative morbidity ranged from 0-40% and mortality rates varied from 0-10% and are associated with treatment.[27]

Recently, Spiliotis et al.compared the effect of CRS followed by HIPEC and systemic chemotherapy with CRS followed by systemic chemotherapy in women with recurrent EOC, after initial debulking and systemic chemotherapy. Noteworthy, in the HIPEC group there was no difference in mean survival between patients with platinum-sensitive versus platinum-resistant disease (26.6 vs. 26.8 months, p=0,287). This was in contrast to the non-HIPEC group, where there was a statistically significant difference in mean survival between platinum-sensitive versus platinum-resistant disease (15.2 vs. 10.2 months, p<0.002).[26] At this time, several other prospective, randomized controlled trials were initiated to compare outcomes of CRS (interval or secondary) with or without HIPEC and are currently recruiting participants (Table 3).

Table 2. Results of trials presenting survival data in patients with primary advanced or recurrent OC treated with cytoreductive surgery followed by hyperthermic intraperitoneal chemoperfusion.

Author	Year	N	Disease type	CR (%)	Mortality (%)	Morbidity (%)	OS (months)	DFS/PFS (months)
Zanon[28]	2004	30	Recurrent or advanced primary	-	3.3	16.7	28.1	17.1
Reichman[29]	2005	13	Recurrent or advanced primary	38	None	-	-	15.4
Rufian[30]	2006	33	Recurrent or advanced primary	52	None	36	48a	-
Raspaglesi[31]	2006	40	Persistent recurrent	82	None	5	414a	23.9a

Cotte[32]	2007	81	Chemoresistent recurrent	55	2.5	136	28.4	19.2
Helm[33]	2007	18	Persistent recurrent	61	5.5	17	31	10
Di Giorgio[34]	2008	47	Advanced primary or recurrent	59	4.2	21.3	24	20
Fagotti[35]	2009	25	Recurrent	92	None	28	-	10
Tentes[36]	2010	29	Recurrent	58.6	3.4	21.4	-	-
Deraco[37]	2011	26	Primary advanced	65.2	3.8	15.2	60.7	15.2
Bakrin[38]	2012	246	Recurrent/ persistent	92.2	0.37	11.6	48.9	-
Ceelen[3]	2012	42	Recurrent	50	None	21	37	13
Bakrin[38]	2013	566	Recurrent or advanced primary	74.9	0.8	31.3	Recurrent: 45.7 Advanced: 354	-
Cascales[39]	2013	91	Recurrent or advanced primary	80.2	None	27	-	-

CR macroscopically complete (CC-0) resection, mortality 30-day or in-hospital mortality, morbidity major morbidity rate, OS median overall survival, DFS median disease-free survival, PFS median progression-free survival

Generally, the quality of available evidence is low, but it is reasonable to assume that in selected patients in whom complete or optimal CRS can be achieved, HIPEC could be a feasible addition to the standard treatment of systemic chemotherapy and surgery, with potential benefits and significant prolongation of survival in patients suffering from primary or recurrent OC.

2. Objectives of the study

The aim of this randomized, single-blinded phase II trial is to study the effect of cytoreductive surgery (CRS) and efficacy of cisplatin-based intraoperative intraperitoneal chemoperfusion (IPEC) in patients with primary or recurrent serous epithelial ovarian cancer (OC), in order to treat peritoneal minimal residual disease (pMRD). Additionally, we aim to study the pharmacodynamics of IP drug delivery, the value of different doses of cisplatin and hyperthermic administration.

Endpoints

2.1 □ Primary:

o Tissue penetration distance of cisplatin in peritoneal tumor tissue nodules

2.2 □ Secondary:

o Postoperative morbidity and mortality (within 30 days after debulking)

o Quality of Life (before and after debulking + 3, 6 and 12months after debulking)

o Pharmacokinetics of cisplatin in peritoneal perfusate and plasma samples

o Pharmacodynamics: Pt DNA adduct formation

o Overall survival, disease free survival, peritoneal recurrence free survival (24 months after debulking)

o Effects of (H)IPEC on peritoneal cytology

o Translational research: analyzing gene expression of selected biomarkers for Pt sensitivity, stromal density and composition of tumor slices.

3. Investigational Medicinal Product

3.1 Intraperitoneal chemoperfusion

The open (coliseum) technique is used as per standard of care, and a semi-closed circuit created consisting of inflow- and outflow drains, temperature probes, heat exchanger, and a peristaltic pump. When optimal cytoreduction is achieved, patients will be randomized to normothermic (37°C) or hyperthermic (41°C) chemoperfusion immediately before installation of the IPC system, using sealed envelopes. Chemoperfusion duration is 90 minutes, starting from the addition of drug to the circuit.

□ *Drug regimen, administration way and composition*

Cisplatin is used in a dose of 100 mg/m2 or 75mg/m2, dissolved in a balanced peritoneal dialysis solution (Physioneal™ 1.36, Baxter). The volume of the perfusate is calculated as 2 liters/m2 body surface area (BSA).

A systemic sodium-thiosulfate (Na2S2O3) solution is administered IV 20 minutes before start of the chemoperfusion (4g/m2, dissolved in 150ml NaCl 0,9%) and during 6 hours after completion of the chemoperfusion (12g/m2 dissolved in 1000ml NaCl 0,9%), in order to prevent nephrotoxicity.

An adjusted hydration and urinary workup have to be carried out during the first 24hours following infusion. Administration of cisplatin has been associated with aberrant concentrations of the serum

electrolytes (Mg, K, Na, Ca), including a symptomatic hypomagnesaemia. Hence, it is recommended to check these serum concentrations during and after each treatment.

 Producer

Hospira Benelux BVBA. Noorderplaats 9, 2000 Antwerpen.

 Distributor

Cisplatin Hospira 50 mg/50 ml Onco-Tain : BE 197486.

Cisplatin Hospira 100 mg/100 ml Onco-Tain : BE 197495.

 Packaging

Solution for injection. Cisplatin Hospira 50 mg/50 ml Onco-Tain: 1 glass vial containing 50 mg cisplatin/50 ml. Cisplatin Hospira 100 mg/100 ml Onco-Tain: 1 glass vial containing 100 mg cisplatin/100 ml. The following particulars will be added to the original vial, but will not obscure the original labelling: 'For clinical trial use only', study reference code, study site, study participant and investigator's name

 Storage conditions

Storage beneath 25°C and protect against light.

In-use stability: from a microbiological point of view, unless dilution has taken place in controlled and validated aseptic conditions, the product should be used immediately. If not used immediately, storage times and conditions for use are the responsibility of the user.

The resulting solution should not be stored in the refrigerator, due to precipitation.

 Known side effects of the medication

- Nephrotoxicity:

o Kidney failure

o Hyperuricaemia

- Ototoxicity

- Neurotoxicity:

o Peripheral neurotoxicity

- Hematological toxicity:

o Myelosuppression

o Leucopenia

o Thrombocytopenia

o Anaemia

- Gastro-intestinal toxicity:

o Emetogenesis

o Diarrhea

o Nausea

- Liver and bile disease:

o Increased transaminases: serum glutamate-oxaloacetate transaminase (SGOT)

4. Study Protocol Summary

4.1 Inclusion criteria

 Tumor type

o Biopsy proven serous epithelial ovarian carcinoma or peritoneal carcinoma

 Primary or recurrent disease

 Extent of disease

o Positive retroperitoneal lymph nodes and /or microscopic metastasis beyond the pelvis (FIGO stage III)

o Stage IV with unilateral pleural fluid allowed

o Complete or nearly complete macroscopic cytoreduction at the time of surgery (CC-0 or CC-1) deemed possible based on imaging, laparoscopy, or both

 Second-line patients; platinum sensitive

 Age over 18 years

 No major cardiac or respiratory disease

 Absent or limited (<500ml) clinical ascites

 Adequate performance status (Karnofsky index > 70%)

 Adequate mental faculty, allowing to understand the proposed treatment protocol and provide informed consent

 Expected life expectancy more than 6 months

 Laboratory data

o Serum creatinine 1.5 mg/dl or a calculated GFR (CKD-EPI) ≥ 60 mL/min/1.73 m2

o Serum total bilirubin 1.5 mg/dl, except for known Gilbert's disease

o Platelet count > 100.000/µl

o Hemoglobin > 9g/dl

o Neutrophil granulocytes > 1.500/ml

o International Normalized Ratio (INR) 2

 Absence of alcohol and/or drug abuse

 No other concurrent malignant disease

 No inclusion in other clinical trials interfering with the study protocol

 No concurrent chronic systemic immune or hormone therapy, except neoadjuvant chemotherapy

 Absence of any severe organ insufficiency

 No pregnancy or breast feeding

 Written informed consent

4.2 Exclusion criteria

 Severe or uncontrolled cardiac insufficiency, including recent (< 6 months) occurrence of myocardial infarction, the presence of congestive cardiac insufficiency, of symptomatic angor in spite of optimal medical care, of cardiac arrhythmia requiring medical treatment presenting insufficient rhythm control, or uncontrolled arterial hypertension

 Pregnancy or breast feeding

 Platinum resistant (relapse > 12 months after completion of Pt containing therapy) or refractory disease

 Active bacterial, viral or fungal infection

 Active gastro-duodenal ulcer

 Parenchymal liver disease (any stage cirrhosis)

 Uncontrolled diabetes mellitus

 Severe obstructive or restrictive respiratory insufficiency

 Psychiatric pathology capable of affecting comprehension and judgment faculty

 Tumor in the presence of obstruction

 Evidence of extra-abdominal disease (with the exception of unilateral malignant pleural effusion) or extensive liver metastasis Peritoneal cancer index (sPCI) ≥ 25

4.3 Primary endpoint

- Tissue penetration distance of cisplatin in peritoneal tumor tissue nodules

4.4 Secondary endpoints

- Postoperative morbidity and mortality (within 30 days after debulking)
- Quality of Life (before and after debulking + 3, 6 and 12months after debulking)
- Pharmacokinetics of cisplatin in peritoneal perfusate and plasma samples
- Pharmacodynamics: Pt DNA adduct formation
- Overall survival, disease free survival, peritoneal recurrence free survival (24 months after debulking)
- Effects of (H)IPEC on peritoneal cytology

- Translational research: analyzing gene expression of selected biomarkers for Pt sensitivity, stromal density and composition of tumor slices.

4.5 Procedures

- Patient selection and staging

Staging is performed as per standard of care, but should minimally include CT scan of the chest and abdomen and CA125 measurement.

Other imaging techniques (DWI-MRI, 18F-FDG-PET-CT) and diagnostic laparoscopy are optional.

- Neoadjuvant therapy

Neoadjuvant therapy is performed as the current standard of care (carboplatin and paclitaxel). The duration and treatment schedule are decided by the treating oncologist. Ideally, 3 or 4 courses are administered before surgery and IPC. A minimal waiting period of two or three weeks should be respected for weekly and three-weekly scheduled chemotherapy, respectively, between the last dose of chemotherapy and the date of surgery.

- Surgery
    - Assessment and confirmation of optimal resectability

The extent of the disease is reported and scored as the simplified peritoneal cancer index (sPCI).

    - Cytoreductive surgery

A combination of organ resections and peritonectomies is performed aiming to achieve optimal (macroscopically complete) resection. Two tumor nodules resected during surgery will be kept for analysis and serves as controls, and two other parietal peritoneal index nodules will be left during chemoperfusion, and are resected for analysis after the IPC.

    - Intraperitoneal chemoperfusion

The open (coliseum) technique is used as per standard of care, and a semi-closed circuit created consisting of inflow- and outflow drains, temperature probes, heat exchanger, and a peristaltic pump. When optimal cytoreduction is achieved, patients will be randomized to normothermic (37°C) or hyperthermic (41°C) chemoperfusion immediately before installation of the IPC system, using sealed envelopes. Chemoperfusion duration is 90 minutes, starting from the addition of drug to the circuit.

- Postoperative care and follow-up

Postoperative care and follow up are performed as per current standard treatment and protocols. The Dindo-Clavien classification will be used to score post-operative complications until 3 months after surgery and (H)IPEC. Participants of the study will be asked to fill in a cancer-specific (C30) and disease-specific (OV28) questionnaire, on specific time points (before and after debulking and after 3, 6 , 12, 18 and 24 months) to analyze the quality of life. In appendix you can find the EORTC questionnaires and manuals.

4.6 Randomisation and blinding

This study is a randomized, single-blinded phase II trial. The primary objective is to study the penetration of cisplatin in tumor tissue nodules, which are resected after CRS and (H)IPEC, in patients with stage III ovarian cancer. Patients will be randomized after CRS to the normothermic (37°C) or hyperthermic (41°C) treatment and within this two groups, there will be another randomization in terms of dose of cisplatin (75mg/m2 or 100mg/m2). Randomization will be performed by drawing sealed envelopes and this will be done by the perfusionist. There will be two papers in these envelopes: one paper with the patient code, and one paper with the treatment. Analyses of the primary endpoint (Pt Penetration) and of the pharmacokinetics of the total Pt concentration was done by independent blinded analysts.

5. Study analysis

5.1 Power calculation

A sample size of 48 patients (N=12/group) was calculated to detect an effect size of 10%, with a significance level of 0.05 ($\alpha = 0.05$) and of power of 0.8 ($\beta = 0.20$). A total of 60 patients will be included in order to account for the estimated drop-out rate of 25% post randomization. Patients will be followed-up to 24 months postoperatively. An interim analysis will be made and used to estimate the power to detect significant differences in tumor penetration between the normo- and hyperthermic cohort and will be used to derive the final sample size of the study.

5.2 Analysis of endpoints

5.2.1 Primary endpoint

The H0 hypothesis is that hyperthermia (41°C) doesn't enhance the Pt penetration in tumor tissue nodules. On the contrary, the Ha hypothesis is that hyperthermia does enhance the Pt penetration in tumor tissue nodules. Differences between the groups will be analyzed with a t-test.

Data on penetration was generated by utilizing Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) on tumor samples harvested as per the study protocol. This analysis was performed at the department of analytical chemistry (PI: Prof. Frank Vanhaecke).

5.2.2 Secondary endpoints

1. Overall survival, disease free survival and time to peritoneal recurrence was calculated using the Kaplan Meier product limit method, and differences were assessed with the Logrank and Cox proportional hazards analyses. Survival was measured from the date of surgery to progression or death.

2. Three months postoperative morbidity and mortality will be analyzed using effect methods (95% confidence interval), following classification using the Dindo-Clavien and Comprehensive Complication Index (CCI) classification methods.

3. Differences between groups will be analyzed with Chi square, Fisher exact, t-test and U-test if appropriate.

4. Pharmacokinetic analyses of intact and total Pt in blood, serum and perfusate were performed using UHPLC MS/MS at the department of Pharmaceutical Sciences (PI:Prof. Katrien Remaut) and Analytical Chemistry (PI: Prof. Frank Vanhaecke). A PK/PD model was established using nonlinear mixed-effects modelling (NONMEM) Pharmacokinetics were analyzed using PKsolver, an Excel plugin.

5. Pharmacodynamic effects (DNA Adduct formation) were estimated by IHC on frozen cryosections of harvested peritoneal tissues (cfr protocol). These analyses were carried out at the University Hospital of Essen, dept of pathology (PI: Prof. Dr. Thomale), as previously published. Analyses of semi-quantitative results were performed using a Chi-squared test.

6.Transcriptomic data were analyzed on frozen and preserved resection tissues, both pre- and post chemoperfusion. Following optimal cytoreduction, two parietal peritoneal index nodules were left during chemoperfusion, and resected for analysis after perfusion. Resected samples were kept in RNALater preservative medium following surgery, and frozen to -80 degrees. All samples were shipped to BGI technologies, Hong Kong, where RNA extraction and RNA-Seq (DNBSeq) was performed. Reads were aligned to GRCh38 using STAR and genes quantified using featureCounts. Differential gene expression was performed using DESeq2 followed by geneset enrichment analyses. Immune cell deconvolution was performed using CIBERSORT.

After RNA extraction and sequencing carried out by a commercial partner (BGI, Taiwan, China), GSEA and DE analyses were performed at the lab of computational cancer genomics and tumor evolution, UGent (PI: Prof. Dr. Jimmy van Den Eynden).

All calculations and plotting were performed with IBM SPSS®, version 24, and Graphpad Prism v8.

6. Independent Ethics Committee and Competent Authority

OVERVIEW APPROVED DOCUMENTS

Initial submission:	**Approval EC:**	**Approval FAMHP:**
- Protocol v2 dd. 13/03/2015 - ICF v1 dd. 18/12/2015 (NL) - Questionnaires EORTC QLQ-C30 (v3) and EORTC QLQ-OV28 - CRF v1 dd. 25/03/2015	18DEC2015	09JUL2015
Amendment 1:	**Approval EC:**	**Approval FAMHP:**
- Protocol v3 dd. 20/06/2017	10OCT2017	16OCT2017

- ICF v2 dd. 14/06/2017 (NL)
- ICF v2 dd. 28/08/2017 (FR)
- CRF v2 dd. 28/06/2017

Amendment 2: - Extension of study duration until 30SEP2018	**Approval EC:** 19FEB2018	**Approval FAMHP:** NA
Amendment 3: - Protocol v4 dd. 11/04/2018	**Approval EC:** 09MAY2018	**Approval FAMHP:** 30APR2018
Amendment 4: - Extension of study duration until 30SEP2019	**Approval EC:** 26SEP2018	**Approval FAMHP:** NA
Amendment 5: - Extension of study duration until 31DEC2019	**Approval EC:** 19FEB2019	**Approval FAMHP:** NA

7. Results

7.1 Subject enrollment and demographics

From 23/09/2016 to 2/09/2019, 54 patients were included in total. 50 Patients were included at the main study site, UZ Ghent, and an additional 4 patients were included in CHU Liege (local investigator Dr. Joseph Weerts). Follow-up of the final patient at UZ Ghent was concluded at 02/09/2021, and at CHU liège at 12/07/2020.

Full follow-up of two years or up until death was reached for 50/54 patients. All patients underwent the treatment as proposed by the protocol.

Groups were balanced for age and other factors (Table 4):

Table 4. Patient characteristics

		Groups				
Characteristics		Low dose / normo (N=16)	Low dose / Hyper (N= 13)	High dose / normo (N=12-	High dose /hyper (N=13)	Total (N=54)
Age (Mean (SD)) (yr)		59,88 (7,19)	61,15 (4,83)	61,42 (12,30)	64,08 (7,90)	61,54 (8,24)
BMI (kg/m2) (Mean (SD))		26,28 (5,10)	23,79 (3,71)	25,75 (5,61)	27,16 (6,51)	25,78 (5,30)
CCI (Mean (SD))		7,87 (0,96)	7,85 (0,56)	8,33 (1,50)	8 (0,82)	8 (0,99)
CA125 (U/mL) (Mean (SD))		224,76 (393,84)	68,18 (89,42)	288,81 (664,13)	278,14 (431,38)	212,48 (433,72)
PCI (Mean (SD))		10,85 (6,44)	8,83 (4,09)	12,91 (8,02)	10,92 (5,96)	10,83 (6,21)
Recurrent disease - no. (%)	Yes	13 (81,25)	8 (62)	10 (83,33)	10 (76,92)	41 (75,93)
	No	3 (18,75)	5 (38,46)	2 (16,67)	3 (23,08)	13 (24,07)
Completeness of cytoreduction -no.	CC-0	7	7	4	5	23
	CC-1	7	5	7	8	27
	CC-2	1	0	0	0	1
	CC-3	0	1	0	0	1

7.2 Study specific results

7.2.1 Primary endpoint: tissue penetration

Cisplation penetration dynamics

We visualized platinum concentrations in post-treatment tissue samples utilizing LA ICP MS in a semi-quantitative manner. After microscopic screening of eligible samples based on presence of tumoral cells and peritoneal architecture, we selected 24 samples for analysis. We

analysed post-treatment Pt195 distributions in these tissues using LA-ICP-MS imaging, using a Analyte G2 LA unit (Teledyne Photon Machines, Bozeman, MT, USA) . Tissue regions including tumor and tumoral stroma, and histology were annotated on consecutive H&E sections.

Post-treatment platinum distribution in tumor tissues was heterogeneous, with a clear gradient of uptake with high levels of intensity at the treated peritoneal membrane diminishing towards deeper layers of the submesothelial stroma. To characterize the penetration depth in this heterogenous context, we established a model in which tissue sections were split up in zones of 200µm thickness. After post-processing to remove artifacts and zones of no signal (such as tears in the tissue or large vascular structures), Pt195 signal intensity was averaged to acquire the mean signal intensity per layer. This mean intensity was then normalized to allow for direct comparison between samples. While in some cases, a linear gradient of penetration could be found, most samples demonstrated a very heterogenous pattern, with some samples demonstrating an increase in signal in deeper layers (figure 1). No clear differences were found between dose or temperature groups. After analysis of HE-stained sections in comparison to the Pt maps, "dark zones" of Pt signal were found to overlap with presence of tumoral cells, and were the main factor influencing total Pt uptake in tissues.

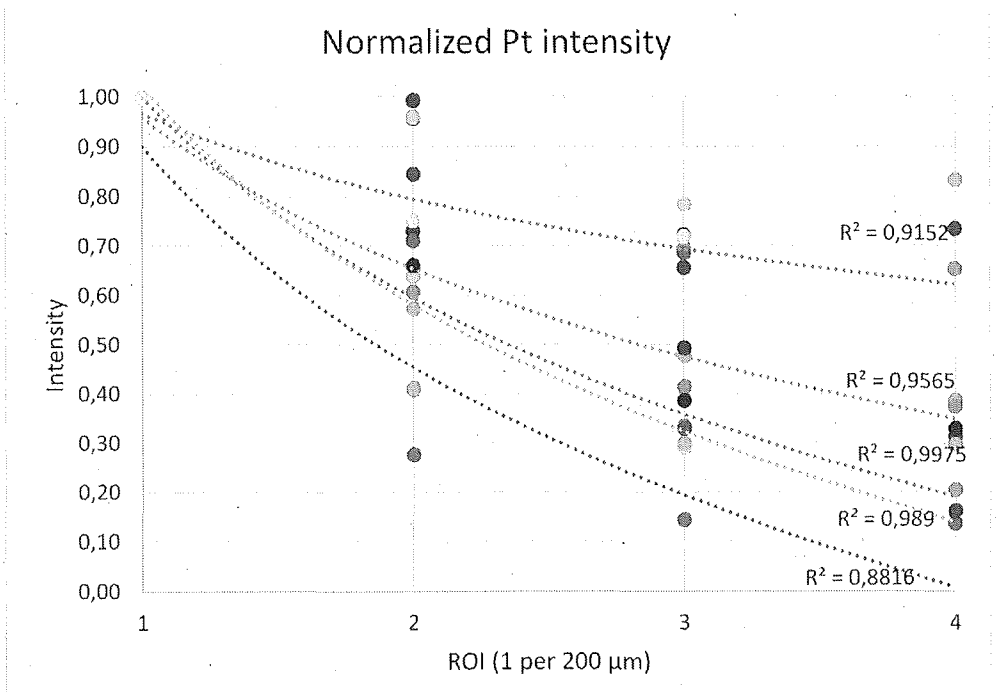


Figure 1. Pt intensity averaged and normalized per layer

Cisplatin uptake in tumoral cells is limited

Pt signal was decidedly lower in tumoral cells, when compared to their surrounding stroma (figure 2). To analyze this effect, we annotated all zones of neoplastic cells, and compared the averaged signal in neoplastic cells to their surrounding stroma. Platinum signal in malignant cells was consistently lower than in the surrounding tumoral stroma and ranged from 83% to 24%, with a mean ratio of platinum signal intensity between stromal and cancer cells of 2,4 (SD 0,887).

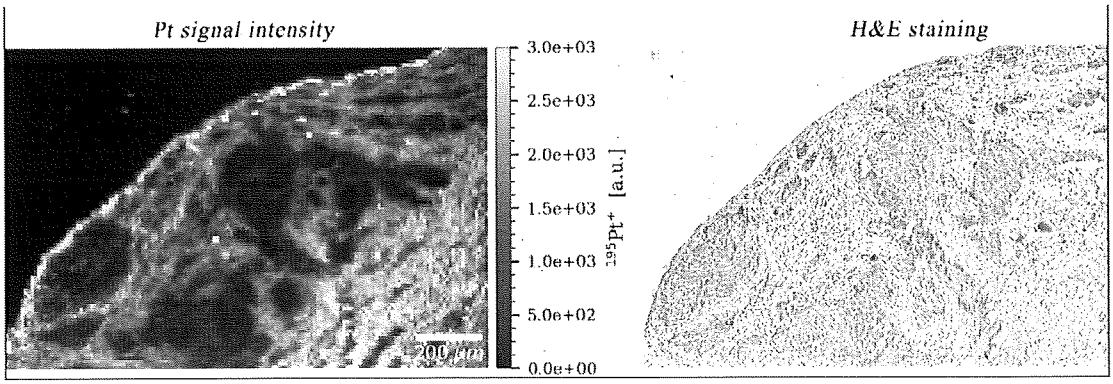


Figure 2. Consecutive sections of platinum heatmaps and HE-stained samples demonstrate limited uptake of platinum in tumoral cells.

Hyperthermia does not influence cisplatin uptake ratio

When comparing temperature and dose groups, no differences were found in cisplatin uptake in neoplastic cells (figure 3).

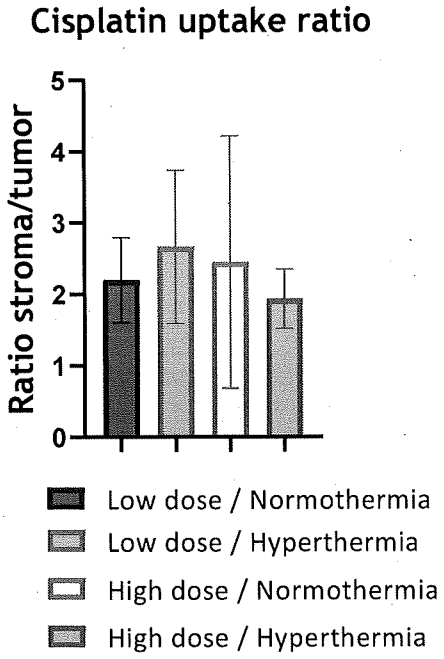


Figure 3. Bar chart indicating the mean ratio of stromal and tumoral Pt intensity per group.

8.2.2. Secondary endpoints

Morbidity

As described above, the highest dose was amended to 100 mg/m^2 due to higher than expected renal toxicity, with 4 out of 13 patients treated with 120mg/m^2 developing clavien-dindo grade III or IV acute kidney injury. Following dose reduction, no further reports of AKI were received.

Following surgery , the mean length of stay in the ICU was 2.76 (+- 1.79) days. The mean number of nights hospital stay total was 18.28 (+-12.21) days. Both outcomes were similar across all four groups, with no significant differences.

Hyperthermia did not influence morbidity. Complication rate as measured by the mean Comprehensive complication index (CCI) was similar between the normothermic and hyperthermic chemoperfusion groups (respectively 37.1 versus 28.5, P=0.20). Furthermore, reoperation rate was not negatively impacted by perfusion temperature (6/28 patients in the normothermia group vs 2/26 in the hyperthermia group, respectively). The most common indications for reoperation were leakage (4 patients) and compartment syndrome requiring limb fasciotomies (2 patients.)

Similarly, readmission rate was similar regardless of perfusion temperature, but was significantly influenced by chemotherapy regimen, with 2/29 patients needing to be readmitted within 30 days post-discharge at 75mg/m^2, compared to 1/12 at 100mg/m^2 and 5/12 patients at 120mg/m^2 (p= 0.014).

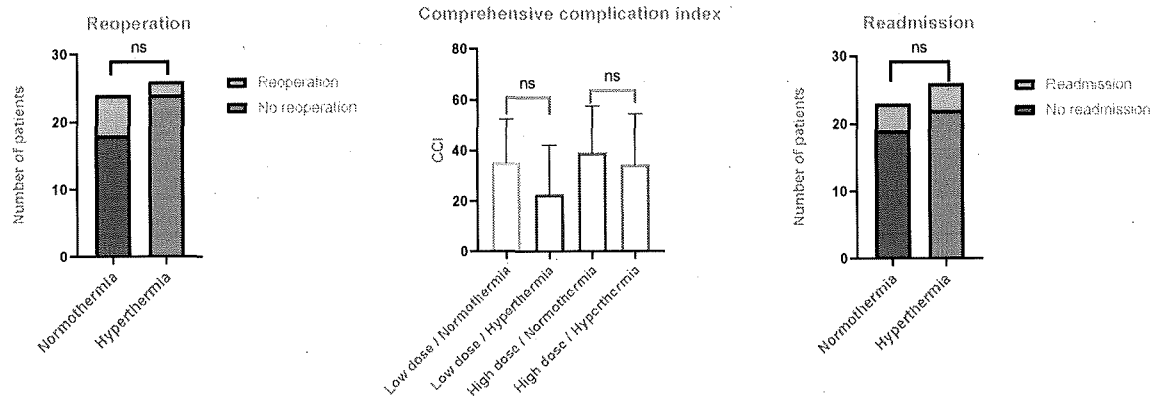


Figure 4. Bar charts of reoperation, CCI and readmission rates demonstrate no significant effects of hyperthermia on morbidity

Survival

50 out of 54 patients were followed up for two years post-surgery. In an intention-to-treat analysis, one-year survival of 90.9% and a two-year survival ratio of 75% were reached. Median Overall Survival was not reached. During follow-up, 27 patients recurred or died, leading to a RFS of 13.45 months. Hyperthermia and dose level did not affect OS and RFS significantly.

The most common extraperitoneal sites of recurrence included: liver metastases (5) and retroperitoneal adenopathies (5).

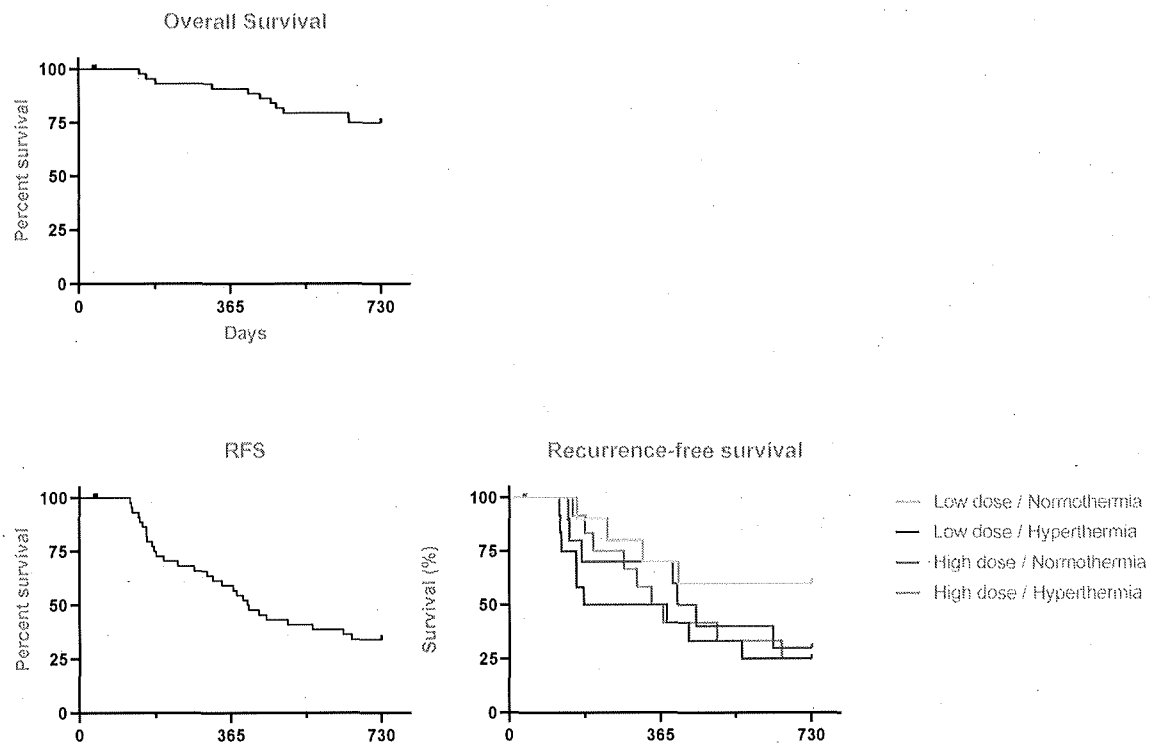


Figure 5. Kaplan Meier plots of overall and recurrence-free survival

Self-reported quality of life outcomes

Both Cancer- and Disease specific quality of life were surveyed by the EORTC QLQ-C30 and OV28 questionnaires. Patients were surveyed 3 weeks before operation, 6 weeks after and 3, 6, 12, 18 and 24 months after surgery and chemoperfusion.

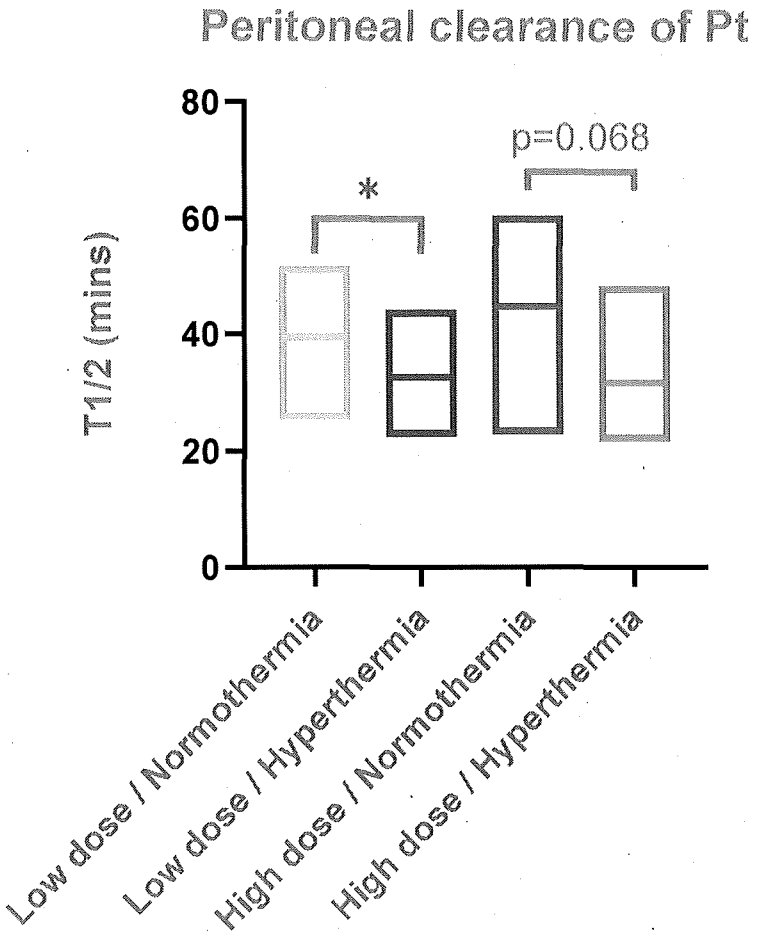
34 / 54 Patients responded to their baseline survey. At 12 months, a response rate for patients currently alive of 77% was reached. Further detailed analysis is ONGOING.

Pharmacokinetics

Hyperthermia influences peritoneal clearance

The maximal concentration of drug in the peritoneal perfusate was measured at the start of perfusion, and additional measurements were made at 45 and 90 minutes of perfusion. Area under the curve was analyzed following 90 minutes, and was similar between groups at both low and high dose (P=0.63 and 0.52, respectively). Mean T1/2 in perfusate was significantly lowered by hyperthermia in the low dose group (39.3 min vs 33 min, P=0.041), and to a lesser extent in the high dose group (42.8 min vs 33.4 min, P=0.068) (Figure 6).

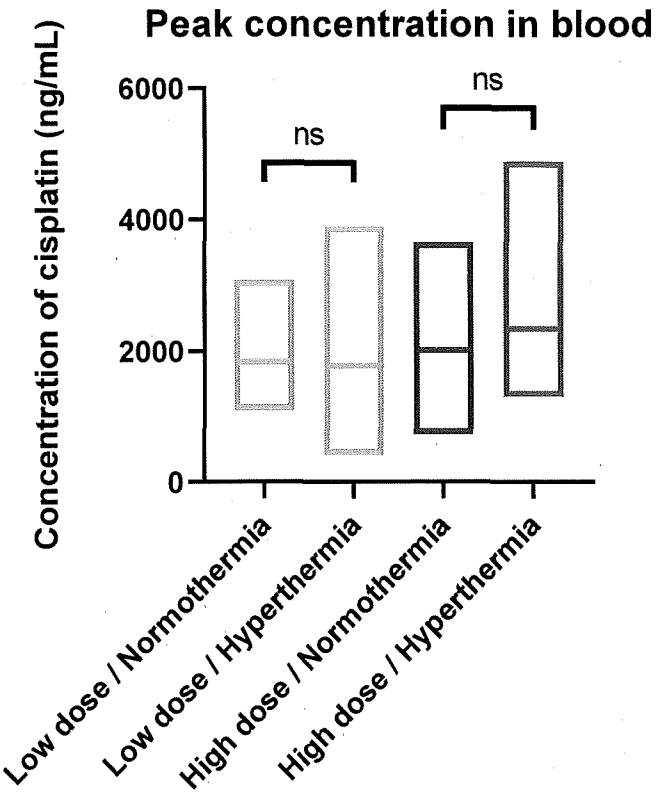
Figure 6. Boxplot of peritoneal clearance of intact Platinum demonstrating increased clearance in hyperthermic conditions



Plasma pharmacokinetics

The mean maximal concentration in plasma was 2100 ng/mL (+-1080 ng/mL), and was not influenced by hyperthermia (figure 7). Peak concentrations were reached at 24,50 minutes of perfusion (+- 15,65 mins). Mean AUC was 193218ng*minutes/mL (+-92117) AUC ratio of perfusate/plasma was similar in all groups (9.6 , P=0.26) .

Figure 7. Boxplots of peak concentrations in plasma demonstrating no effect of hyperthermia



Transcriptomic analysis RNAseq

We analyzed the transcriptome of 37 post-HIPEC samples utilizing bulk RNA-seq. We performed differential gene expression analysis to detail the effects of both dose and hyperthermia, in addition to immune cell deconvolution and survival analysis.

We analyzed post-perfusion samples from 37 patients. In accordance with previous *in vitro* data, hyperthermia induced a clear heat shock response when compared to normothermic chemoperfusion, exemplified by significant upregulation of *HSPB1*, also known as *Hsp27*. Additionally an upregulation of genes associated with poor prognosis in several cancers such as *FXR1*, *HIST1H2BK*, *CKS2* and *FKBP4* was observed. Pathways upregulated by hyperthermia included those linked to cisplatin response such as the E2F pathway and the G2M/DNA Damage checkpoints. Relatedly, we observed upregulation of both the mitotic spindle pathway and MTORC1 signaling, both known to be associated with cisplatin resistance. Immune cell deconvolution indicated a temperature-specific depletion of CD8+ and CD4+ memory cells, with no dose effect. Other cell types were not significantly affected.

8. Safety

During the study, a total of 76 SAEs occurred at UZ Ghent. Monitor confirms that all SAE forms are filed at site in a separate binder. The filing location was documented in a Note To File in ISF section 6.

The reporting of all SAEs to the EC, and FAMHP if applicable, was checked by the monitor. For SAE n° 15, the submission of the first follow-up report (15FU1) to FAMHP could not be found by the monitor. Therefore, the report was submitted to FAMHP on 19APR2022 by the monitor. For SAE n° 18 and 19, the submission of the initial report to the EC could not be found by the monitor. Therefore, the report was submitted to the EC on 19APR2022 by the monitor.

The monitor noticed that SAE n° 27, 48 and 73 were still ongoing and a follow-up report was missing. The SC confirmed that the SAEs were reported as 'ongoing' by mistake. SC corrected the initial reports of SAE n° 48 and 73 and the first follow-up report of SAE n° 27. A scan was taken by the monitor for filing purposes.

All SAEs are now closed. An overview of all SAEs and the reporting of it is given below.

SAE n°	Subject n°	Outcome	SUSAR?	Reported to EC	Reported to FAMHP
1	1	Resolved	no	25/05/2016	NA
2	6	Resolved	YES	3/02/2017	3/02/2017
3	5	Resolved	no	3/02/2017	NA
4	5	Resolved	YES	3/02/2017	3/02/2017
5	7	Ongoing	no	3/02/2017	NA
5FU1	7	Resolved	no	22/02/2019	NA
6	3	Resolved	YES	3/02/2017	3/02/2017
7	2	Resolved	YES	3/02/2017	3/02/2017
8	2	Resolved	YES	3/02/2017	3/02/2017
9	2	Resolved	YES	3/02/2017	3/02/2017
10	1	Ongoing	no	8/02/2017	8/02/2017
10FU1	1	Resolved	no	22/02/2019	NA
11	4	Resolved	YES	8/02/2017	8/02/2017
12	11	Resolved	no	27/02/2017	NA
13	15	Resolved	no	27/02/2017	NA
14	14	Resolved	YES	13/03/2017	13/03/2017
14FU1	14	Resolved	no	25/04/2017	NA
15	16	Ongoing	YES	13/03/2017	13/03/2017
15FU1	16	Resolved	YES	22/02/2019	19/04/2022
16	10	Resolved	no	24/03/2017	NA
17	15	Resolved	no	24/03/2017	NA
18	13	Resolved	no	19/04/2022	NA
19	13	Resolved	no	19/04/2022	NA
20	14	Resolved	no	25/04/2017	NA
21	10	Resolved	no	25/04/2017	NA
22	16	Resolved	YES	25/04/2017	25/04/2017
23	1	Resolved	no	8/05/2017	NA
24	21	Ongoing	no	8/05/2017	NA
24FU1	21	Resolved	no	29/05/2017	NA
25	16	Ongoing	no	11/05/2017	NA
25FU1	16	Resolved	no	22/02/2019	NA
26	20	Ongoing	no	17/05/2017	NA
26FU1	20	Resolved	no	22/02/2019	NA
27	24	Ongoing	YES	29/05/2017	29/05/2017
27FU1	24	Resolved	no	22/02/2019	NA

28	16	Resolved	no	30/05/2017	NA
29	18	Resolved	no	31/05/2017	NA
30	12	Resolved	no	31/05/2017	NA
31	1	Resolved	no	31/05/2017	NA
32	12	Resolved	no	9/06/2017	NA
33	25	Resolved	YES	7/08/2017	7/08/2017
34	22	Resolved	no	11/08/2017	NA
35	25	Resolved	no	11/08/2017	NA
36	25	Resolved	no	22/09/2017	NA
37	23	Ongoing	no	25/09/2017	NA
37FU1	23	Resolved	no	22/02/2019	NA
38	6	Death	no	25/09/2017	NA
39	8	Death	no	28/09/2017	NA
40	22	Resolved	no	24/10/2017	NA
41	25	Resolved	YES	24/10/2017	24/10/2017
42	21	Ongoing	no	23/11/2017	NA
42FU1	21	Resolved	no	22/02/2019	NA
43	13	Resolved	no	28/11/2017	NA
44	15	Ongoing	no	28/11/2017	NA
44FU1	15	Resolved	no	22/02/2019	NA
45	2	Resolved	no	28/11/2017	NA
46	13	Resolved	no	27/12/2017	NA
46FU1	13	Resolved	no	27/12/2017	NA
46FU2	13	Resolved	no	27/12/2017	NA
46FU3	13	Resolved	no	27/12/2017	NA
46FU4	13	Resolved	no	11/01/2018	NA
46FU5	13	Resolved	no	11/01/2018	NA
46FU6	13	Resolved	no	11/01/2018	NA
46FU7*	-	-	-	-	-
46FU8	13	Resolved	no	22/03/2018	NA
46FU9	13	Resolved	no	22/03/2018	NA
46FU10	13	Resolved	no	16/04/2018	NA
46FU11	13	Resolved	no	17/05/2018	NA
47	25	Resolved	no	3/01/2018	NA
48	12	Resolved	no	11/01/2018	NA
49	12	Resolved	no	23/01/2018	NA
50	11	Resolved	no	23/01/2018	NA
51	2	Resolved	no	2/02/2018	NA
52	11	Resolved	no	22/03/2018	NA
53	23	Resolved	no	22/03/2018	NA
54	12	Resolved	no	22/03/2018	NA
54FU1	12	Resolved	no	22/03/2018	NA
55	24	Resolved	no	22/03/2018	NA
56	5	Resolved	no	22/03/2018	NA
57	29	Resolved	no	22/03/2018	NA
57FU1	29	Resolved	no	22/03/2018	NA
57FU2	29	Resolved	no	16/04/2018	NA
58	32	Resolved	no	16/04/2018	NA
59	12	Death	no	16/04/2018	NA
60	16	Resolved	no	16/04/2018	NA
60FU1	16	Resolved	no	16/04/2018	NA
61	29	Resolved	no	16/04/2018	NA
62	2	Resolved	no	16/04/2018	NA
63	26	Resolved	no	17/05/2018	NA
64	19	Resolved	no	17/05/2018	NA
65	16	Resolved	no	17/05/2018	NA
66	13	Death	no	25/05/2022	NA
67	18	Resolved	no	25/05/2022	NA
68	24	Resolved	no	25/05/2022	NA
69	24	Resolved	no	25/05/2022	NA

70	24	Resolved	no	25/05/2022	NA
71	11	Death	no	31/05/2018	NA
72	30	Resolved	no	7/02/2019	NA
73	33	Resolved	no	15/02/2019	NA
74	35	Resolved	no	15/02/2019	NA
75	38	Resolved	no	15/02/2019	NA
76	41	Resolved	no	15/02/2019	NA

*46FU7: The 7th follow-up report of SAE n°46 does not exist as this number was skipped by mistake. A Note To File was created by the monitor to clarify this.
Non-serious Adverse Events are directly documented on the paper CRF. A Note To File was present to clarify this.

9. Protocol deviations

During the first monitoring visit, it was already noted that non-approved versions of the ICFs were used for 9 subjects. A detailed overview of the non-approved ICFs and the actions taken is given below:

For subject 1, non-approved ICF version dd. 10/02/2015 was used. For subjects 2, 3, 5, 6, 7, 8 and 9, non-approved ICF version NCT02567253 was used. For subject 4, a non-approved French ICF version (without footer) was used. All subjects were contacted by phone to sign the correct ICF version. This was documented in a Note To File for each subject. All subjects, except subject 4 who was lost to follow-up, were re-consented with ICF v1 dd. 28/10/2015.

For subject 5 and 9, Note To Files were created to explain the inconsistencies in dates of the signed ICF version NCT02567253 (see Investigator AI 4 of previous monitoring report).

For subject 7, monitor noticed that the Sub-Investigator signed the ICF before the subject. The SC added an explanation on the Note To File concerning the re-consent of this subject.

For subject 41, monitor noticed that the correct ICF v2 dd. 14/06/2017 (NL) was filed, although the signature page of ICF v1 dd. 28/10/2015 was added and signed. During the Close-out Visit, a clarification was added by the PI to confirm the subject was consented with the correct ICF version and to confirm he agreed to include the subject.

For subject 45, monitor noticed that only ICF v4 dd. 08/01/2018 (side study) was present. SC explained that the original ICF for this study got lost. During the Close-out Visit, a clarification was added by the SC and signed off by the PI.

Retraining was done by the monitor.

The study duration was approved by the EC until 31DEC2019 (see Amendment 5). The Last Subject Last Visit took place on 25AUG2021, which exceeds the approved duration of the study. Monitor discussed this with the CI and explained that an amendment should be submitted to the EC if the study will take longer than expected. The CI explained that the PM was out for a long period and confirmed that they are now actively following this up for ongoing studies. This was also documented on the Protocol Deviation Log.

10. Discussion and overall conclusions

In this study we report results of the first randomized clinical trial investigating differences between normothermic chemoperfusion (NIPEC) and hyperthermic chemoperfusion (HIPEC) in ovarian cancer. While preclinical and translational information might point towards an added benefit of hyperthermia when utilized with cisplatin [40,41], conflicting data exist [42], and limited pharmacokinetic and dynamic data is available in the context of a clinical trial.

We noted in our cohort that the addition of cisplatin HIPEC to cytoreductive surgery is feasible and safe at dose levels not exceeding 100 mg/m^2, and leads to a favorable pharmacokinetic profile as evidenced by a high $AUC_{peritoneum/plasma}$ ratio.

Compared with normothermic chemoperfusion, hyperthermia did not affect surgical morbidity. Furthermore, hyperthermia enhances peritoneal cisplatin absorption, without significant effects on plasma drug exposure. While these data might point towards an additional benefit, with no demonstrated risks in our cohort, care should be taken in generalizing this data. The primary endpoint of this study was platinum tissue penetration depth. We noted a marked heterogeneity in platinum tissue distribution, both in normothermic or hyperthermic temperatures. The main driver in this heterogeneity was demonstrated to be the presence of tumoral implants. Interestingly, tumoral implants demonstrated limited uptake in cancer cells of ovarian metastases, compared to their microenvironment, raising questions about the validity of penetration depth as a marker for pharmacodynamic effect. While the clinical efficacy of cisplatin HIPEC in the context of peritoneal metastases of ovarian cancer becomes more clear following the results of the OVHIPEC-1 trial [43], limited uptake in the target cells might prove to be a bottleneck in increasing the efficacy of this treatment, and was not alleviated by hyperthermia. Further study into mechanisms influencing intratumoral uptake, such as tumor interstitial fluid pressure (TIFP), could be critical in enhancing effectiveness by enhancing intratumoral drug concentrations.

To summarize, our data demonstrate that the addition of hyperthermia to cisplatin chemoperfusion is associated with a pharmacokinetic benefit with no added toxicity in our cohort, while demonstrating no clear survival benefit .

11. References

1. Grosso, G. *et al.* Intraperitoneal chemotherapy in advanced epithelial ovarian cancer: a survey. *Arch Gynecol Obstet* **290**, 425–434 (2014).
2. Siegel, R., Ma, J., Zou, Z. & Jemal, A. Cancer statistics, 2014. *CA Cancer J Clin* **64**, 9–29 (2014).
3. Ceelen, W. P. Cytoreduction and hyperthermic intraperitoneal chemoperfusion in women with heavily pretreated recurrent ovarian cancer. *Ann Surg Oncol* **19**, 2352–9 (2012).

4. Ledermann, J. A. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **24 Suppl 6**, 24–32 (2013).
5. Heintz, A. P. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet* **95 Suppl 1**, 161–92 (2006).
6. de Bree, E. & Helm, C. W. Hyperthermic intraperitoneal chemotherapy in ovarian cancer: rationale and clinical data. *Expert Rev Anticancer Ther* **12**, 895–911 (2012).
7. Halkia, E., Spiliotis, J. & Sugarbaker, P. Diagnosis and management of peritoneal metastases from ovarian cancer. *Gastroenterol Res Pract* **2012**, 541842 (2012).
8. Hennessy, B. T., Coleman, R. L. & Markman, M. Ovarian cancer. *Lancet* **374**, 1371–1382 (2009).
9. Ceelen, W. P., Hesse, U., de Hemptinne, B. & Pattyn, P. Hyperthermic intraperitoneal chemoperfusion in the treatment of locally advanced intra-abdominal cancer. *Br J Surg* **87**, 1006–1015 (2000).
10. Bijelic, L., Jonson, A. & Sugarbaker, P. H. Systematic review of cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy for treatment of peritoneal carcinomatosis in primary and recurrent ovarian cancer. *Ann Oncol* **18**, 1943–1950 (2007).
11. Kyrgiou, M., Salanti, G., Pavlidis, N., Paraskevaidis, E. & Ioannidis, J. P. A. Survival benefits with diverse chemotherapy regimens for ovarian cancer: meta-analysis of multiple treatments. *J Natl Cancer Inst* **98**, 1655–1663 (2006).
12. Alberts, D. S. *et al.* Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. *N Engl J Med* **335**, 1950–1955 (1996).
13. Markman, M. *et al.* Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and

intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. *J Clin Oncol* **19**, 1001–1007 (2001).

14. Armstrong, D. K. *et al.* Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* **354**, 34–43 (2006).

15. Wang, D. & Lippard, S. J. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* **4**, 307–320 (2005).

16. Pavlov, M. J. *et al.* Cytoreductive surgery and modified heated intraoperative intraperitoneal chemotherapy (HIPEC) for advanced and recurrent ovarian cancer -- 12-year single center experience. *Eur J Surg Oncol* **35**, 1186–1191 (2009).

17. Liedert, B., Pluim, D., Schellens, J. & Thomale, J. Adduct-specific monoclonal antibodies for the measurement of cisplatin-induced DNA lesions in individual cell nuclei. *Nucleic Acids Res* **34**, e47 (2006).

18. Bianga, J. *et al.* Complementarity of MALDI and LA ICP mass spectrometry for platinum anticancer imaging in human tumor. *Metallomics* **6**, 1382–1386 (2014).

19. Oršolić, N. & Car, N. Quercetin and hyperthermia modulate cisplatin-induced DNA damage in tumor and normal tissues in vivo. *Tumour Biol* **35**, 6445–6454 (2014).

20. Raaphorst, G. P. & Yang, D. P. The evaluation of thermal cisplatin sensitization in normal and XP human cells using mild hyperthermia at 40 and 41 degrees C. *Anticancer Res* **25**, 2649–2653 (2005).

21. Euler, J. *et al.* [Hyperthermic peritoneal perfusion in ascites tumours in rats (author's transl)]. *Wien Klin Wochenschr* **86**, 220–225 (1974).

22. Spratt, J. S., Adcock, R. A., Muskovin, M., Sherrill, W. & McKeown, J. Clinical delivery system for intraperitoneal hyperthermic chemotherapy. *Cancer Res* **40**, 256–260 (1980).

23. Hildebrandt, B. *et al.* The cellular and molecular basis of hyperthermia. *Crit Rev Oncol Hematol* **43**, 33–56 (2002).

24. Los, G. *et al.* Optimisation of intraperitoneal cisplatin therapy with regional hyperthermia in rats. *Eur J Cancer* **27**, 472–477 (1991).

25. Vj, V. *et al.* Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **21**, (2003).

26. Spiliotis, J. *et al.* Cytoreductive surgery and HIPEC in recurrent epithelial ovarian cancer: a prospective randomized phase III study. *Ann Surg Oncol* **22**, 1570–1575 (2015).

27. Chua, T. C. *et al.* Intraoperative hyperthermic intraperitoneal chemotherapy after cytoreductive surgery in ovarian cancer peritoneal carcinomatosis: systematic review of current results. *J Cancer Res Clin Oncol* **135**, 1637–1645 (2009).

28. Zanon, C. *et al.* Cytoreductive surgery and intraperitoneal chemohyperthermia for recurrent peritoneal carcinomatosis from ovarian cancer. *World J Surg* **28**, 1040–1045 (2004).

29. Reichman, T. W. *et al.* Cytoreductive surgery and intraoperative hyperthermic chemoperfusion for advanced ovarian carcinoma. *J Surg Oncol* **90**, 51–56; discussion 56-58 (2005).

30. Rufián, S. *et al.* Radical surgery-peritonectomy and intraoperative intraperitoneal chemotherapy for the treatment of peritoneal carcinomatosis in recurrent or primary ovarian cancer. *J Surg Oncol* **94**, 316–324 (2006).

31. Raspagliesi, F. *et al.* Cytoreduction combined with intraperitoneal hyperthermic perfusion chemotherapy in advanced/recurrent ovarian cancer patients: The experience of National Cancer Institute of Milan. *Eur J Surg Oncol* **32**, 671–675 (2006).

32. Cotte, E. *et al.* Cytoreductive surgery and intraperitoneal chemo-hyperthermia for chemo-resistant and recurrent advanced epithelial ovarian cancer: prospective study of 81 patients. *World J Surg* **31**, 1813–1820 (2007).

33. Helm, C. W. *et al.* Cytoreduction and intraperitoneal heated chemotherapy for the treatment of endometrial carcinoma recurrent within the peritoneal cavity. *Int J Gynecol Cancer* **17**, 204–209 (2007).

34. Di Giorgio, A. *et al.* Cytoreductive surgery (peritonectomy procedures) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) in the treatment of diffuse peritoneal carcinomatosis from ovarian cancer. *Cancer* **113**, 315–325 (2008).

35. Fagotti, A. *et al.* Secondary cytoreduction plus oxaliplatin-based HIPEC in platinum-sensitive recurrent ovarian cancer patients: a pilot study. *Gynecol Oncol* **113**, 335–340 (2009).

36. Tentes, A.-A. K. *et al.* Cytoreductive surgery and perioperative intraperitoneal chemotherapy in recurrent ovarian cancer. *Tumori* **96**, 411–416 (2010).

37. Deraco, M. *et al.* Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy as upfront therapy for advanced epithelial ovarian cancer: multi-institutional phase-II trial. *Gynecol Oncol* **122**, 215–220 (2011).

38. Bakrin, N. *et al.* Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) for persistent and recurrent advanced ovarian carcinoma: a multicenter, prospective study of 246 patients. *Ann Surg Oncol* **19**, 4052–4058 (2012).

39. Cascales Campos, P., Gil, J. & Parrilla, P. Morbidity and mortality outcomes of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with primary and recurrent advanced ovarian cancer. *Eur J Surg Oncol* **40**, 970–975 (2014).

40. Schaaf, L. *et al.* Hyperthermia Synergizes with Chemotherapy by Inhibiting PARP1-Dependent DNA Replication Arrest. *Cancer Res* **76**, 2868–2875 (2016).

41. Gabano, E., Colangelo, D., Ghezzi, A. R. & Osella, D. The influence of temperature on antiproliferative effects, cellular uptake and DNA platination of the clinically employed Pt(II)-drugs. *J Inorg Biochem* **102**, 629–635 (2008).

42. Cesna, V. *et al.* Narrow line between benefit and harm: Additivity of hyperthermia to cisplatin cytotoxicity in different gastrointestinal cancer cells. *World J Gastroenterol* **24**, 1072–1083 (2018).

43. van Driel, W. J. *et al.* Hyperthermic Intraperitoneal Chemotherapy in Ovarian Cancer. *New England Journal of Medicine* **378**, 230–240 (2018).