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# Adjuvant dendritic cell-based immunotherapy after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in patients with malignant peritoneal mesothelioma: a phase II clinical trial

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## ABSTRACT

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

**Methods** This open-label, single-center, phase II clinical trial, performed in the Erasmus MC Cancer Institute Rotterdam, the Netherlands, included patients with epithelioid MPM. 4–6 weeks before CRS-HIPEC leukapheresis was performed. 8–10 weeks after surgery, DCBI was administered three times biweekly. Feasibility was defined as administration of at least three adjuvant vaccinations in 75% of patients. Comprehensive immune cell profiling was performed on peripheral blood samples prior to and during treatment.

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

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

**Trial registration number** NTR7060; Dutch Trial Register (NTR).

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) improves survival outcomes of patients with malignant peritoneal mesothelioma (MPM), but recurrence rates are high. Dendritic cell-based immunotherapy (DCBI) showed promising results in patients with pleural mesothelioma and murine MPM models.

### WHAT THIS STUDY ADDS

⇒ The MESOPEC trial showed that DCBI as adjuvant treatment after CRS-HIPEC in patients with MPM was feasible and safe. DCBI showed promising survival outcomes, had an immune modulatory effect on lymphoid cells, and induced memory T-cell activation.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These data provide rationale for future research to investigate the effect of DCBI on survival outcomes, identify possible combination treatment strategies to optimize the effect of DCBI to improve the prognosis of patients with MPM.

## INTRODUCTION

Malignant peritoneal mesothelioma (MPM) is an aggressive malignancy. Due to its non-specific symptoms, such as abdominal pain,

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

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

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

## METHODS

### Study design

The MESOPEC trial was an open-label, single arm, single center phase II clinical trial, conducted in the Erasmus MC Cancer Institute, Rotterdam, the Netherlands. The MESOPEC study protocol, as well as a detailed description of MesoPher production, have been published earlier.[7 11] This study was conducted in accordance with the Declaration of Helsinki.

### Eligibility criteria

Patients diagnosed with epithelioid MPM and an indication for CRS-HIPEC, were screened to participate in the study. Patients undergoing palliative resections with HIPEC, in case of symptomatic tumor lesions and/or ascites, were also eligible to participate in the trial. Eligibility for CRS-HIPEC was based on multiple factors. Patients had to have a WHO-Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, the disease had to be confined to the abdominal cavity, and the expected survival had to be at least 6 months. Peritoneal Cancer Index (PCI) above 17 was considered a contraindication for CRS-HIPEC, when the Ki67 index was higher than 9%. When the Ki67 index was below 10%, PCI was not considered in evaluating CRS-HIPEC eligibility. If feasible, PCI and feasibility of cytoreduction were determined up front by diagnostic laparoscopy.

### Study procedures

A timeline of study procedures is given in figure 1. A leukapheresis procedure was performed to obtain autologous monocytes for dendritic cell (DC) vaccination production 4–6 weeks prior to CRS-HIPEC (a detailed description of the production of DC vaccination is provided in the online supplemental data). CRS-HIPEC was performed following the standard of care. The patients received DCBI in three biweekly vaccinations at the outpatient clinic, 8–10 weeks after surgery, followed by a booster after 3 and 6 months. One-third of the MesoPher dose was injected intradermally and two-thirds were administered intravenously. Prior to every vaccination, peripheral blood samples cells were obtained.

### Safety evaluation

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

### Objectives

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

### Surgical outcomes

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

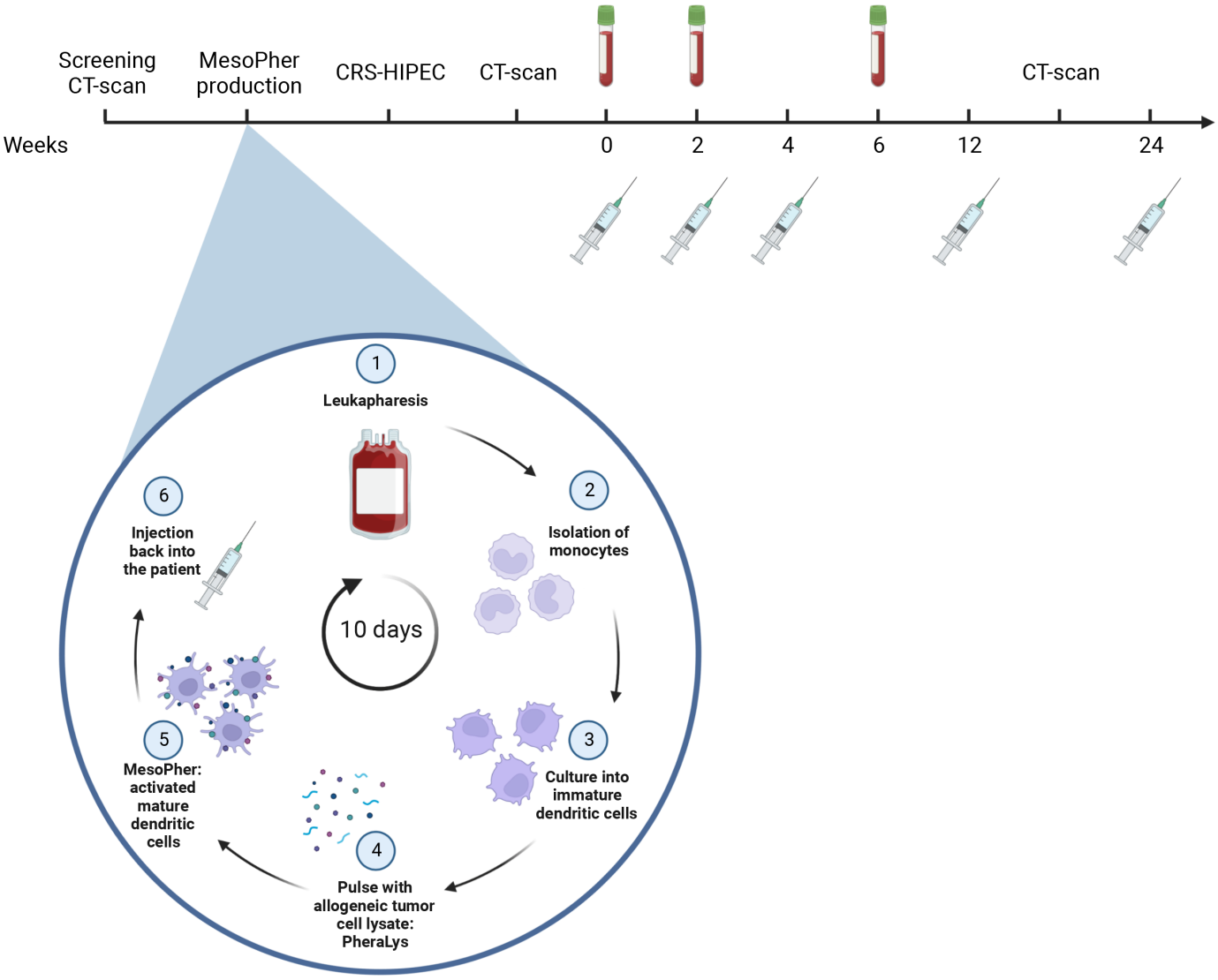


**Figure 1** Illustration of the treatment regimen of patients with malignant peritoneal mesothelioma treated with MesoPher dendritic cell vaccination (indicated by the syringes) after cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). Blood was drawn before every vaccination.

unit management due to single organ dysfunction (4a) or multiorgan dysfunction (4b); and CD grade 5 denoting any complication resulting in the death of a patient.

### Follow-up and clinical response evaluation

Further treatment and follow-up were performed according to standard protocol. Approximately 6 weeks after CRS-HIPEC, a CT-scan was performed to act as a baseline measurement. After this, CT-scans were made every 6 months during the first 3 years of follow-up. In postoperative years 4 and 5, CT-scans were performed once a year. Recurrence of disease was defined as measurable disease on imaging or as recurrent disease found by laparotomy or laparoscopy. Progression-free survival (PFS) was defined as the time interval between CRS-HIPEC and recurrence of disease for patients with a complete cytoreduction or progression of disease for patients with an incomplete cytoreduction. Patients who had no progression at the time of database lock were censored at the date of the last follow-up visit. OS was defined as the time interval between CRS-HIPEC and the date of death, or date of last follow-up visit in censored cases.

### Immune cell profiling

Flow cytometry staining was performed on cryopreserved peripheral blood mononuclear cells at baseline (before start of vaccination) and on treatment time points (two weeks after the first vaccination and two weeks after the third vaccination, figure 1). Three panels were designed to characterize the T cells (co-stimulatory, co-inhibitory, cytokine) and one panel for the DC-myeloid fraction. An automated, computational pipeline, based on the one described by Quintelier *et al* was developed to analyze the data (figure 2A).[12] First, margin events were removed, compensation, transformation, quality control, manual pregating and normalization were performed. Next, FlowSOM was used to cluster the data of the T-cell panels and the data of the DC-myeloid panel based on

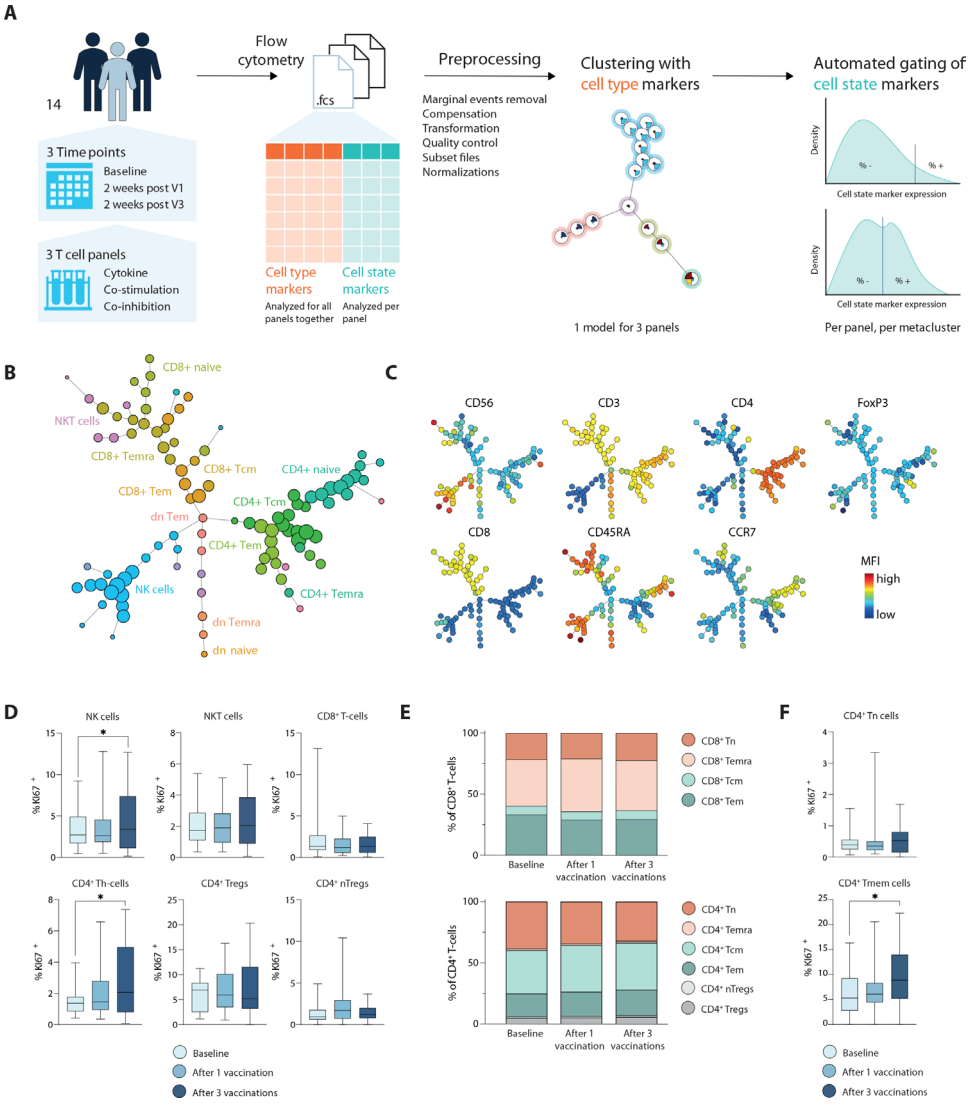


**Figure 2** Treatment induced changes in the abundance and proliferation of CD4+ Th-cells. (A) Pipeline for the FlowSOM unsupervised clustering analysis of the T-cell panels. (B–C) Identification of different lymphocyte clusters (B) by cell type marker expression (C) from the FlowSOM algorithm. (D)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

the cell type markers.[13] These clusterings were evaluated by mapping a manual gating of a subset of the data (online supplemental figure 1) and as a result, cell subtype abundances were obtained. Additionally, thresholds were computed for all cell state markers to obtain the complete immune profiles. Detailed descriptions of the flow cytometry and computational analysis are provided in the online supplemental data.

## Statistics

The sample size was calculated by: $N = z^2_{0.975} \cdot \frac{sens(1-sens)}{w^2 \cdot prev}$, assuming a sensitivity of diagnosing grade 3 toxicity of 99% and a prevalence of grade 3 toxicity in the study population of 2.5%. Total width of the CI is 0.20 (0.10 below and 0.10 above). Confidence level of the interval is 95% and α=0.05. The sample size that was necessary at least to obtain 95% CIs with a width of 20% for a prevalence of 2.5% was 19 patients. Rounded, this is a total of 20 patients. Feasibility was defined as administration of at least three adjuvant vaccinations in 75% of patients (ie, 15 patients). Kaplan-Meier survival analysis was used to estimate the median PFS and OS. Wilcoxon signed-rank tests (non-parametric, paired data) and Student's t test (parametric, paired data) were used to determine the statistical significance. Statistical analyses were executed using GraphPad Prism software (GraphPad Software, San Diego, California, USA). Continuous variables were shown as median with the range of values or

IQR. Categorical variables were presented as counts with percentages. P values of 0.05 and below were considered statistically significant.

## RESULTS

### Baseline characteristics

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

### Perioperative characteristics

Table 1 provides the perioperative characteristics for patients who underwent CRS-HIPEC (n=16). In 14 patients, the HIPEC regimen consisted of cisplatin and doxorubicin. In two patients, mitomycin-C (MMC) was used. For one patient this was because of induction therapy with carboplatin and possible resistance to cisplatin HIPEC. For the other patient MMC was used to minimize the risk of complications due to a perioperative expectancy of incomplete cytoreduction. In 10 patients complete cytoreduction was performed, resulting in a completeness of cytoreduction (CC-) score of 0 (n=7) or 1 (n=3). In six patients, complete cytoreduction was not feasible and palliative resections (CC-score of 3) and HIPEC were performed. Most common complications after CRS-HIPEC were pneumonia (n=4, 25%) and chylous leakage (n=5, 44%). Severe complications (ie, Clavien Dindo grade 3b) were reported in two patients (12.5%). One patient was diagnosed with an ileus and intra-abdominal hematoma, which was surgically evacuated. Exploratory laparotomy was performed in another patient, resulting in the diagnosis of jejunitis without additional resections. A detailed description of organ resections during cytoreduction and postoperative complications is provided in online supplemental table 1.

### Feasibility

Feasibility was determined based on the proportion of patients who were able to undergo leukapheresis with successful production of MesoPher and who received the first three adjuvant vaccinations. Four out of 16 patients underwent leukapheresis after CRS-HIPEC. For three patients this was because of inclusion in the trial after CRS-HIPEC was performed in another hospital. For one patient the leukapheresis was postponed due to pancytopenia after induction chemotherapy prior to CRS-HIPEC. The median time from leukapheresis to CRS-HIPEC was 4 weeks (2–5) for patients undergoing leukapheresis before CRS-HIPEC (table 2). In none of the patients SAEs or delay to CRS-HIPEC due to leukapheresis was reported. One patient underwent a second leukapheresis procedure after the administration of the first three vaccinations, as the yield of monocytes from the first procedure was not sufficient to produce all five DC vaccinations. All patients were sufficiently recovered to undergo DC therapy within 10 weeks after surgery and were able to undergo the first three DC treatments according to protocol. Five patients (31.3%) showed progressive disease at first response evaluation and did therefore not receive all five DC vaccinations.

### Safety of DC treatment

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

### Clinical outcomes

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

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

Immune cell profiling was performed for 14 out of the 16 patients who were treated with DCBI. The FlowSOM

**Table 1** Baseline and perioperative characteristics

| | N=18 |
|---|---|
| Age at inclusion in trial (range) | 59 (30–75) |
| Gender | |
| Male | 14 (77.8) |
| Female | 4 (22.2) |
| History of asbestos exposure | |
| Yes | 2 (11.1) |
| No | 11 (61.1) |
| Unknown* | 5 (27.8) |
| Epithelioid morphology | 18 (100) |
| Ki67 index (range)† | 8 (1–70) |
| Germline BAP1 mutation‡ | 2 (11.1) |
| Prior therapy | |
| Systemic chemotherapy§ | 5 (27.8) |
| Systemic immunotherapy | 1 (5.6) |
| PIPAC | 1 (5.6) |
| Prior surgery¶ | 3 (16.7) |
| CRS-HIPEC | 16 (89) |
| PCI (range)** | 39 (19–39) |
| Chemotherapy regimen** | |
| Cisplatin/doxorubicin | 14 (87.5) |
| Mitomycin-C | 2 (12.5) |
| CRS-HIPEC duration (minutes, range)†† | 494 (194–679) |
| Blood loss (liters, range)‡‡ | 1.5 (0.2–5.4) |
| Perioperative blood transfusion**§§ | 2 (12.5) |
| Organ resections (range)** | 4 (0–9) |
| Completeness of cytoreduction** | |
| CC-0 | 7 (43.8) |
| CC-1 | 3 (18.8) |
| CC-2 | 0 (0) |
| CC-3 | 6 (37.5) |
| In-hospital length of stay** | 16 (10–16) |
| Any postoperative complication** | 13 (81.3) |
| Severe postoperative complications** | 3 (18.8) |
| Ileus¶¶*** | 1 (6.3) |
| Intra-abdominal hematoma¶¶*** | 1 (6.3) |
| Other infection ¶¶ | 1 (6.3) |
| Malposition JJ-stent ††† | 1 (6.3) |
| Reoperation**‡‡‡ | 2 (12.5) |

Continuous variables are shown as median (IQR) unless otherwise specified. Frequencies are shown as N (%).
*Reported as unknown by the patient.
†Available for 17 out of 18 patients, for patients who received induction chemotherapy (n=3) Ki67 before chemotherapy was available for 2 patients.
‡Germline mutational analysis performed in 3 out of 8 patients with a BAP1 deficiency.
§All patients received a combination of pemetrexed and a platinum-based chemotherapeutic agent.
¶Surgery with resections for peritoneal disease.
**Out of 16 patients who underwent CRS-HIPEC.
††Data available for 12 patients.
‡‡Data available for 13 patients.
§§Both patients underwent leukapheresis before CRS-HIPEC.
¶¶Clavien Dindo grade 3b.
***These complications were present in the same patient.
†††Clavien Dindo grade 3a.
‡‡‡Evacuation of an intra-abdominal hematoma (n=1) and exploratory laparotomy resulting in the diagnosis jejunitis (n=1).
CC, completeness of cytoreduction score; CD, Clavien-Dindo; CRS, cytoreductive surgery; HIPEC, hyperthermic intraperitoneal chemotherapy; PCI, Peritoneal Cancer Index; PIPAC, pressurized intraperitoneal aerosolized chemotherapy.

Table 2 Leukapheresis and treatment with DC vaccination

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

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

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

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

In addition to lymphocyte proliferation, the expression of a variety of co-stimulatory and co-inhibitory receptors on peripheral T cells was assessed (figure 4A). Co-stimulatory molecules, including ICOS, HLA-DR and CD28 were significantly upregulated on Th cells, specifically on memory or proliferating cells. This upregulation was most dominant after one vaccination in the total Th population but remained present two weeks after the third vaccination on the memory and proliferating cells. In addition, the expression of co-inhibitory molecules such as CD39 and LAG-3 changed with similar dynamics on Th cells.

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

## DISCUSSION

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

For patients with pleural mesothelioma and pancreatic adenocarcinoma, DCBI treatment has been proven feasible and safe.[10 14] The current trial shows that DCBI is also feasible after major surgery for MPM. The cut-off value for feasibility (ie, 75% of patients treated with DCBI after CRS-HIPEC) was based on the rate of patients with colorectal carcinoma who are not able to undergo adjuvant systemic therapy due to severe complications after CRS-HIPEC (20–30%).[15 16] The sample size was calculated at 20 patients, but inclusion was stopped when 16 patients were successfully treated with adjuvant DCBI thereby meeting the feasibility endpoint. Severe complications after surgery were reported in 19% of patients. Despite these complications, all patients received their first three vaccinations within the protocol time frame (for patients treated according to protocol). For none of the patients leukapheresis resulted in delay of surgery. The two

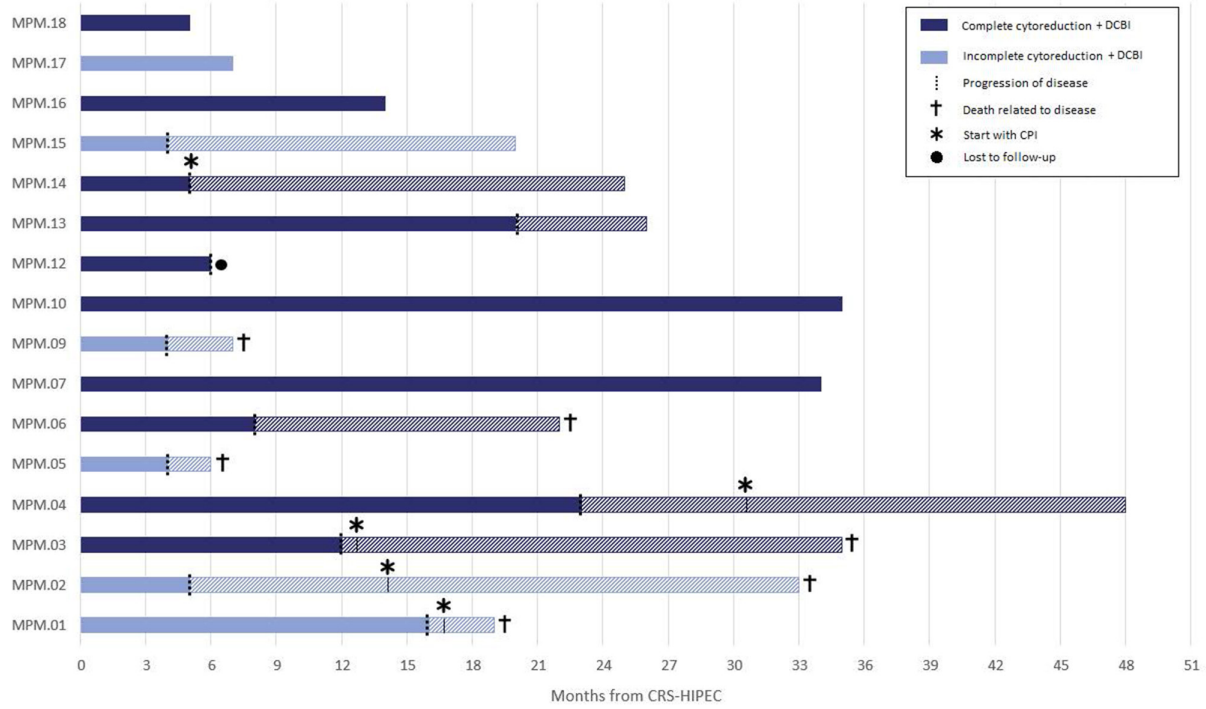


**Figure 3** Progression-free survival (PFS) and overall survival (OS) for patients with malignant peritoneal mesothelioma (MPM) treated with adjuvant dendritic cell-based immunotherapy (DCBI) after cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). The filled bars represent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

patients who dropped out of the trial before CRS-HIPEC was performed were not included in the feasibility determination, as the reason for exclusion was unrelated to DCBI treatment, but due to ineligibility for CRS-HIPEC.

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

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

Unlike the effect of DCBI treatment on Th-cell proliferation, the effect on CTLs was less profound. A slight upregulation of co-stimulatory molecules on CTLs after DCBI treatment was seen for all patients. For patients who had a complete cytoreduction (feasible in 63%) median PFS was 20 months, compared with 4 months for patients with an incomplete cytoreduction. CTL activation and proliferation seemed to be more pronounced in those patients who had a long PFS. This enhanced CTL response might be affected by the tumor load, which was lower in patients with a complete cytoreduction and is in line with earlier studies in mice.[8] The effect of the tumor load on the CTL response might be explained by the tumor microenvironment (TME).[20] Several studies have explored the TME of mesothelioma and reported that these tumors show variable degrees of T-cell infiltration.[24–26] The TME also consists of regulatory and inhibitory cells, among which

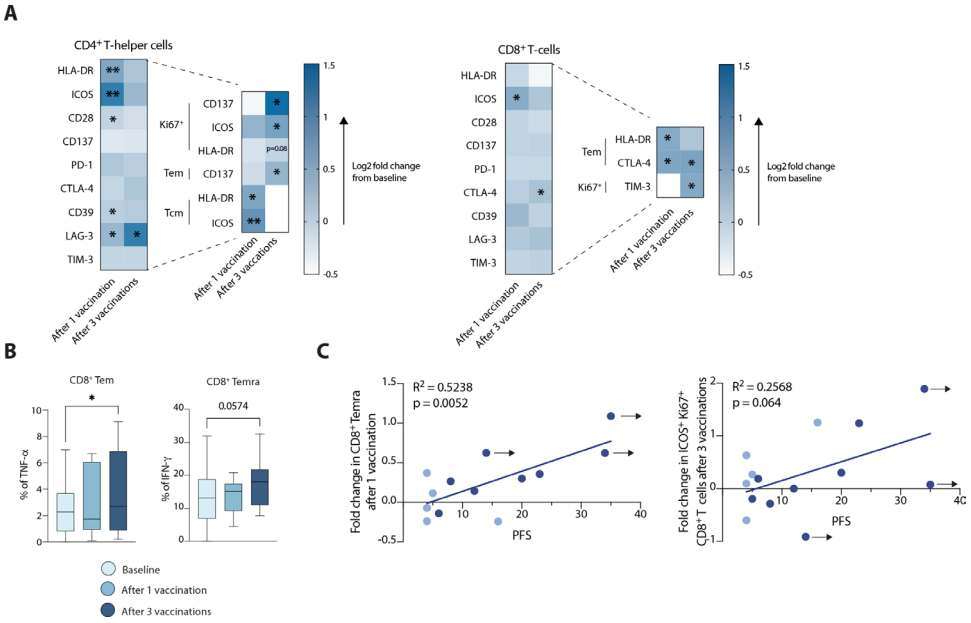


**Figure 4** DC vaccination alters the phenotype of circulating CD4+ and CD8+ T cells. (A) Log2 fold change from baseline in stimulatory (HLA-DR, ICOS, CD28, CD137, PD-1) and inhibitory (PD-1, CTLA-4, CD39, LAG-3, TIM-3) by CD4+ Th-cells and CD4+ Th-cell subsets (left) and by CD8+ T cells and CD8+ T-cell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

regulatory T cells, M2-like macrophages and myeloid-derived suppressor cells, that can hamper an effective antitumor response.[24 27–31] This supports the rationale for the combination of DCBI treatment with cytoreduction.

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

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

This trial has some limitations, including small sample size and limited follow-up time. Therefore, robust statements about the effect of adjuvant DCBI after CRS-HIPEC on survival cannot be made. Another limitation were the protocol violations regarding the time of inclusion for some patients. Inclusion of three patients was performed after CRS-HIPEC, as it was hypothesized that these patients might still benefit from adjuvant DCBI.

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

## CONCLUSIONS

The current trial shows that treatment with adjuvant DCBI after CRS-HIPEC in patients with MPM is feasible and safe, and showed promising survival outcomes. DCBI

has an immune modulatory effect on lymphoid cells, mainly Th-cells, and induces memory T-cell activation. Complete cytoreduction and an increase in CD8+ Temra cells seemed to lead to better patient outcomes. Future research should be done to investigate the effect of DCBI on survival outcomes and identify possible combination treatment strategies to optimize the effect of DCBI.

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