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

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ABSTRACT

Background. The ImmunoPeCa trial investigated the use of intraperitoneal MOC31PE immunotoxin as a novel therapeutic principle for the treatment of peritoneal metastasis from colorectal cancer (PM-CRC). We here report long-term outcome from the trial.

Methods. This was a dose-finding trial aiming to evaluate safety and toxicity (primary endpoint) upon a single dose of intraperitoneal MOC31PE in patients with PM-CRC undergoing CRS-HIPEC with mitomycin C. Overall survival (OS) and disease-free survival (DFS) were secondary endpoints. Twenty-one patients received the study drug at four dose levels on the first postoperative day, including six patients constituting an expansion cohort.

Results. With a 34-month follow-up, the median OS was not reached and the estimated 3-year OS was 78%. Median DFS for all patients was 21 months and the 3-year DFS was 33%, with a median follow-up of 31 months. When excluding patients with potential favorable characteristics from the analysis (n=4), the median DFS was 13 months and the 3-year OS 72%.

Conclusions. The promising long-term outcome combined with low systemic absorbance, high drug concentration and cytotoxic activity in peritoneal fluid support further investigations of clinical efficacy.

KEYWORDS

Peritoneal metastasis; colorectal cancer; immunotoxin; MOC31PE; phase I/II trial, CRS-HIPEC

1. INTRODUCTION

Peritoneal metastasis from colorectal cancer (PM-CRC) is a condition with poor prognosis where palliative therapy most often is the only treatment option [1-3]. Selected patients with resectable disease may however be eligible for cytoreductive surgery (CRS), the removal of all visible intraperitoneal tumor deposits, followed by hyperthermic intraperitoneal chemotherapy (HIPEC) performed at surgery. Patients undergoing this specialized treatment can achieve long-term survival and sometimes even cure [4-9]. However, most patients will experience disease recurrence following CRS-HIPEC, and there is need for better treatment options. The ImmunoPeCa trial was initiated with this in mind, hypothesizing that intraperitoneal immunotoxin therapy would benefit PM-CRC patients. The PRODIGE 7 randomized controlled trial (NCT00769405) has recently challenged the addition of HIPEC to CRS, reporting that oxaliplatin-based HIPEC did not improve survival beyond that of CRS [10], and the timing is ideal for exploring new treatment alternatives.

Immunotoxins are a group of targeted anti-cancer drugs, composed of an antibody linked to a toxin. MOC31PE immunotoxin is a result of researcher-driven drug development at the Norwegian Radium Hospital. The drug is composed of the MOC31 monoclonal antibody conjugated to the potent *Pseudomonas* exotoxin A (PE) [11]. MOC31 binds to the tumor-associated antigen epithelial cell adhesion molecule (EpCAM), which is highly expressed in CRC [12]. Once internalized into the cancer cell, the toxin will cause irreversible inhibition of protein synthesis and cell death [11, 13], and because MOC31PE inhibits protein synthesis, it will kill both dividing and non-dividing cells. When given intravenously, MOC31PE was well tolerated, with transient liver enzyme elevation as dose limiting toxicity (DLT) [14]. In the ImmunoPeCa trial MOC31PE was administered intraperitoneally for the first time, on the first postoperative day following CRS-HIPEC with mitomycin C (MMC).

We have previously shown that the drug was safe and well tolerated, that peritoneal fluid samples had high drug concentrations, that MOC31PE was still cytotoxic after peritoneal exposure, and that systemic drug uptake was low, in the first 15 patients of the ImmunoPeCa trial [15]. We established a dose level for further investigation in an expansion cohort of 6 patients and concluded that MOC31PE seemed ideally suited for local treatment in the peritoneal cavity. We here present for the first time long-term outcome for the ImmunoPeCa trial, as well as data regarding safety and tolerability for the expansion cohort.

2. PATIENTS AND METHODS

2.1 ImmunoPeCa trial

This was an open, dose-finding trial (NCT00769405) primarily investigating safety and toxicity upon intraperitoneal MOC31PE administration the day after CRS-HIPEC for PM-CRC [15]. Pharmacokinetic profile, neutralizing anti-immunotoxin antibody response, overall survival (OS), and disease-free survival (DFS) were secondary endpoints. Safety, toxicity, pharmacokinetics and neutralizing antibody response for the first 15 patients were presented before [15]. The study was carried out at The Norwegian Radium Hospital, Oslo University Hospital (Comprehensive Cancer Center), between September 2014 and September 2016. Adult patients with radiologically suspected PM from histologically verified EpCAM positive CRC could be included if they met the inclusion criteria (Supplementary table 1). MOC31PE was instilled intraperitoneally through two abdominal drainage catheters on the morning after CRS-HIPEC, as previously described [15]. The catheters were clamped for 6h following drug instillation, and then reopened to remove excess intraabdominal fluid and to collect peritoneal fluid samples for analyses. Patients were treated at four dose levels (2.5, 5.0, 7.5 and 10.0 µg/kg), and a 3+3 dose escalation design was applied.

Safety and toxicity were evaluated using the common terminology criteria for adverse events (CTCAE) version 4.0, as previously described [15]. Postoperative complications (30-day morbidity and 100-day mortality) were registered using the Accordion severity grading of postoperative complications [16]. Toxicity grading was performed on days 3, 4, 5 and 9 after CRS-HIPEC, and at follow-up out-patient visits at 4 and 8 weeks, 3, 6, 9 and 12 months, and then biannually for a total of 5 years or until disease progression. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, and was approved by the Norwegian Medicines Agency, the Norwegian Ethics Committee and local health authorities. All patients gave written informed consent before study entry.

Peritoneal fluid samples were collected before drug instillation and at 6h, 24h and 48h. Concentrations of MOC31PE in peritoneal fluid were analyzed using a quantitative sandwich ELISA with a lower detection limit of 10 ng/ml, as previously described [14]. The 800 ng/ml cut-off limit for comparison of high versus low levels of MOC31PE in peritoneal fluid was set after exploring different cut-off values.

2.2 Immunohistochemistry

Specimens were immunohistochemically analyzed for EpCAM protein expression using the Dako FLEX+ System (Agilent/Dako, Glostrup, Denmark). Following deparaffinization, sections were incubated with a 0.3% hydrogen peroxide (H₂O₂) solution for 5 minutes to block endogenous tissue peroxidase activity. Sections were then incubated with a monoclonal anti-EpCAM antibody (clone AUA1; Abcam, Cambridge UK), ready-to-use, antigen retrieval in citrate buffer (pH 6). Sections were thereafter treated with EnVision™ Flex+ mouse linker (15 min) and EnVision™ Flex/HRP enzyme (30 min), stained for 10 min with 3,3

diaminobenzidine tetrahydrochloride (DAB), counterstained with hematoxylin, dehydrated and mounted in Richard-Allan Scientific Cyto seal XYL (Thermo Fisher Scientific). Positive control consisted of breast carcinoma. Membrane staining was scored as positive.

2.3 Statistical analysis

Descriptive statistics was applied; categorical variables were described using frequencies/percentages and continuous variables were described with median/minimum-maximum values. The Kaplan-Meier method was used to estimate OS and DFS, with survival time from CRS-HIPEC to death or censoring date July 16, 2018 (OS) and from CRS-HIPEC to the time of recurrence (intraperitoneal relapse and/or distant metastasis), new primary cancer, death or last follow-up (DFS). The reverse Kaplan-Meier method was used to estimate follow-up time and differences in survival were tested with the log-rank method. Statistical analysis was performed using SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.)

3. RESULTS

3.1 Baseline characteristics

Of the 21 patients treated in the ImmunoPeCa trial, 13 were female (62%) and median age was 58 years (29-73 years). Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 at inclusion, except for 2 patients who had ECOG 1. The primary tumors were located in the appendix (n=3), colon (n=15) and the rectum (n=3), and all were scored as strongly and diffusely (>80% of tumor cells) positive for EpCAM expression. Ten patients had synchronous PM, defined as detection of PM within 6 months of primary surgery, and 9 patients had metachronous PM. Two patients with appendiceal cancer were treated with CRS-HIPEC due to primary tumor perforation. In these patients, no PM was

detected at surgery. Further, no malignancy was detected in the surgical specimen of a third patient with a clinical PCI of 6. The remaining patients had histologically verified PM at the time of CRS-HIPEC (except for one patient with PCI 0 who underwent surgery for bilateral ovarian metastasis (histologically verified), the largest 6 cm in diameter, 3 weeks prior to CRS-HIPEC). Three patients were treated at each of the 3 first dose levels, while 12 patients were treated at dose level 4 (Table 1). In three patients CRS-HIPEC and primary tumor removal were performed in the same session. Median peritoneal cancer index (PCI) was 7 (0-20). Of the 21 patients, five (24%) who had synchronous PM-CRC received adjuvant postoperative systemic chemotherapy following CRS-HIPEC. The concentration of MOC31PE in peritoneal fluid at 6h following study treatment showed large inter-individual differences, with a clear trend to increase with increasing dose level [15] (Supplementary table 1). Thirteen patients had high levels (>800 ng/ml) of MOC31PE, while 8 patients had low (< 800 ng/ml) levels.

3.2 Safety and toxicity for the expansion cohort

There was no grade ≥ 3 laboratory defined toxicities (CTCAE) in the expansion cohort of 6 patients, and no patients experienced grade ≥ 3 postoperative complications according to the Accordion severity grading for postoperative complications. Four patients experienced in total 8 grade ≤ 2 complications. There were no $\geq$ grade 4 complications at any dose level, including no 100-day mortality. Postoperative complications for dose level 4 (n=12) are reported in table 2. Two patients experienced a skin reaction near the abdominal catheter used for study drug instillation. This was diagnosed as erysipelas in one patient and treated with intravenous antibiotics, while in the other patient a topical steroid cream was prescribed, and it was deemed likely non-infectious.

3.3 Long-term outcome

At the time of censoring, 13 of the 21 patients included in the ImmunoPeCa trial had experienced recurrence, and four were dead from recurrent disease (Table 1). The median OS was not yet reached and the estimated 3-year OS was 78% (Figure 1). Median follow-up was 34 months (95% CI 28-40 months). Estimated median DFS was 21 months (95% CI 8-33 months) and the 3-year DFS was 33%, with a median follow-up time of 31 months (95% CI 30-31 months). In univariable analysis, MOC31PE concentrations in peritoneal fluid samples collected 6h after drug instillation was not associated with outcome ($p=0.3$), nor was the use of adjuvant postoperative systemic chemotherapy ($p=0.2$).

4. DISCUSSION

Even though safety was the primary endpoint for the ImmunoPeCa trial, the main ambition for this novel treatment approach is to improve outcome for patients with PM-CRC. With 34 months follow-up, the median OS was not reached and the estimated 3-year OS was 78%, which is considerably longer than what is reported in most studies of patients undergoing CRS-HIPEC for PM-CRC. A recent meta-analysis including 76 papers on PM-CRC reported a 39% 3-year OS for MMC-based HIPEC regimens [17]. Estimated median DFS in the ImmunoPeCa trial was 21 months and the 3-year DFS 33%.

The ImmunoPeCa trial included four patients that theoretically could be expected to have a more favorable prognosis: three patients with appendiceal cancer (signet-ring cell carcinoma ($n=1$), mucinous differentiation ($n=1$), and highly differentiated adenocarcinoma ($n=1$)), which as a group hold a better prognosis than CRC. Additionally, one patient was included where PM was never biopsy verified. When excluding these four patients from survival analysis, the 3-year OS was still very high (72%). The median DFS was 13 months, which is

comparable to the HIPEC-arm of the PRODIGE 7 trial [10], but 5 months longer than we previously reported in a cohort of PM-CRC patients treated in our institution [9]. Although the estimated OS in the ImmunoPeCa study is encouraging, more patients should ideally have been included to improve the power of the study for long-term outcome estimation.

Intraperitoneal administration of MOC31PE was performed without severe toxicity likely to be caused by the drug, also when including analysis of the expansion cohort of 6 patients. Two patients experienced skin reactions in relation to the abdominal catheters used for drug instillation, and there is a possibility that the skin reaction was associated with administration of the study drug. Other immunotoxin studies have reported skin reactions that were easily managed by topical steroids [18]. A challenge in the ImmunoPeCa trial was the complex setting for investigating tolerability. All patients had undergone major surgery and HIPEC, which are both associated with toxicity, and MOC31PE toxicity could potentially have been camouflaged by side effects associated with CRS-HIPEC. All adverse events (AEs) were nonetheless reported, and the results from the expansion cohort support our previous conclusion that intraperitoneal administration of MOC31PE is safe [15].

To date, most of the research in the field of PM-CRC and CRS-HIPEC has focused on describing and investigating the effect of CRS and HIPEC combined, and in the literature these two entities are therefore almost inseparable. The main results from the French prospective randomized phase III multicenter trial PRODIGE 7 (NCT00769405) were presented at the 2018 ASCO annual meeting [10]. Patients were randomized to oxaliplatin-based HIPEC for 30 min or to no HIPEC in the operating room following CRS. With a 64-month follow-up, no difference in median OS was seen between patients undergoing CRS-HIPEC compared to CRS only (41.7 vs 41.2 months, respectively), while patients treated with

CRS-HIPEC were more prone to postoperative complications than those undergoing CRS only. Several questions remain unanswered following PRODIGE 7, among others whether the choice of drug for HIPEC is important, and in particular whether addition of MMC-based HIPEC would influence outcome differently. With these questions unanswered, continued treatment of patients should ideally be conducted in trials designed to answer this and other research questions. Since the benefit of HIPEC has been questioned, it is highly relevant to explore treatment alternatives, and the timing is therefore excellent for investigation of novel therapeutic options, such as MOC31PE.

To conclude, the favorable toxicity profile combined with the promising long-term outcome observed for patients in the ImmunoPeCa trial, are encouraging. Patients show a remarkable 78% 3-year OS following CRS-HIPEC and study treatment. Although this small-scale study is vulnerable to patient selection and results should be interpreted with caution, larger studies to further evaluate the clinical efficacy of MOC31PE are justified.

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CONFLICTS OF INTEREST

Four of the authors (YA, ØF, SD, and KF) are registered as inventors in a patent application owned by Oslo University Hospital (WO 2019/020648) claiming various uses of the product described in this paper for cancer treatment.

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FIGURE LEGENDS

FIG. 1 Overall survival (OS) and disease-free survival (DFS) in the ImmunoPeCa trial

TABLES

TABLE 1 Dose level, dose, PCI, vital status and site of first recurrence for the ImmunoPeCa trial

ID	Dose level (µg/kg)	Dose (µg)	PCI**	Vital status	Site of first recurrence	
					PM	Distant metastasis
2	1 (2,5)	235		Alive	Yes	No
3		165		Alive	Yes	No
4		163		Alive	No	No
6	2 (5,0)	380		Dead	No	Yes
7		453		Alive	No	No
8		470		Alive	Yes	No
9	3 (7,5)	533		Alive	No	No
11		600		Alive	No	Yes
12		668		Alive	Yes	Yes
13	4 (10,0)	730		Alive	No	No
14		720		Alive	No	No
15		775		Dead	Yes	Yes
16		513		Alive	Yes	Yes
18		840		Dead	Yes	No
20		820		Alive	Yes	No
22*		720	7	Alive	No	Yes
23*		760	6	Alive	No	No
24*		810	5	Alive	No	No
25*		610	8	Dead	Yes	Yes
26*		560	12	Alive	No	No
28*		890	2	Alive	Yes	No

*Expansion cohort

** PCI for the first 15 patients published in [15]

PCI peritoneal cancer index, *PM* peritoneal metastasis

TABLE 2 Postoperative complications within 30 days for patients treated at dose level 4 (n=12), according to the Accordion Severity Grading for Postoperative Complications.

Grade	Frequency	Complication (treatment)
No complication	4 (33%)	
Grade 1	9 (75%)	1 Flushing (antihistamines and systemic steroids) 1 Localized skin rash (topical steroids) 2 Pleural effusion (diuretics) 3 Urinary retention (urinary catheter) 1 Wound infection treated at bedside 1 Subileus (NG-tube)
Grade 2	5 (42%)	1 Nutritional problem (tube feeding) 1 Erysipelas (iv antibiotics) 1 E.coli sepsis (iv antibiotics) 2 Pneumonia (iv antibiotics) 1 Urinary tract infection (iv antibiotics)
Grade 3	1 (8%)	1 Pleural effusion (limited therapeutic thoracentesis)

NG nasogastric, *iv* intravenous

There were no $\geq$ grade 4 complications, including no 100-day mortality