Feasibility, safety, and efficacy of stereotactic body radiotherapy combined with intradermal heat-killed mycobacterium obuense (IMM-101) vaccination for non-progressive locally advanced pancreatic cancer, after induction chemotherapy with (modified)FOLFIRINOX: the LAPC-2 trial

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Original Article

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ABSTRACT

Background and purpose: In this phase I/II trial, non-progressive locally advanced pancreatic cancer (LAPC) patients after (modified)FOLFIRINOX therapy were treated with stereotactic body radiotherapy (SBRT) combined with heat-killed mycobacterium (IMM-101) vaccinations. We aimed to assess safety, feasibility, and efficacy of this treatment approach.

Materials and methods: On five consecutive days, patients received a total of 40 Gray (Gy) of SBRT with a dose of 8 Gy per fraction. Starting two weeks prior to SBRT, they in addition received six bi-weekly intradermal vaccinations with one milligram of IMM-101. The primary outcomes were the number of grade 4 or higher adverse events and the one-year progression free-survival (PFS) rate.

Results: Thirty-eight patients were included and started study treatment. Median follow-up was 28.4 months (95 %CI 24.3 – 32.6). We observed one grade 5, no grade 4 and thirteen grade 3 adverse events, none related to IMM-101. The one-year PFS rate was 47 %, the median PFS was 11.7 months (95 %CI 11.0 – 12.5) and the median overall survival was 19.0 months (95 %CI 16.2 – 21.9). Eight (21 %) tumors were resected, of which 6 (75 %) were R0 resections. Outcomes were comparable with the outcomes of the patients from the previous LAPC-1 trial, in which LAPC patients were treated with SBRT, without IMM-101.

Conclusion: Combination treatment with IMM-101 and SBRT was safe and feasible for non-progressive locally advanced pancreatic cancer patients after (modified)FOLFIRINOX. No improvement in the progression-free survival could be demonstrated by adding IMM-101 to SBRT.

Pancreatic ductal adenocarcinoma (PDAC) is a notorious disease because of its poor prognosis. The 5-year survival rate for all stages of disease is less than 5 % and has only increased marginally over the last decades.[1] At diagnosis, around 35 % of patients have locally advanced pancreatic cancer (LAPC) (Stage III), determined by the anatomical staging of the disease on radiological imaging.[2] Local blood vessel involvement of the tumor prevents upfront oncological resection in these patients, thus chemotherapy is the first treatment option.[2] Systemic chemotherapy with FOLFIRINOX is the preferred treatment for patients with LAPC who have a sufficient clinical performance status.[3–7] Treatment with induction chemotherapy can provide systemic control of the disease and provides the opportunity to select patients with favourable tumor biology for subsequent locoregional treatment.[8] Since long-term survival is only probable after resection of the tumor,[9] an exploration and possible resection is recommended in carefully selected LAPC patients. A systematic review found that approximately 28 % of LAPC tumors could be resected after induc-

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tion chemotherapy with FOLFIRINOX.[5] However, even after radical resection of the tumor, the cancer often recurs in the majority of patients.[10] Mostly the disease recurs at distant sites,[10] indicating that even in supposedly localized disease, systemic micrometastatic spread has already occurred. The latter highlights the importance of systemic control of the disease.

For those patients who do not show progressive disease during chemotherapy, stereotactic body radiotherapy (SBRT) can be added to the regimen in an attempt to further downstage the tumor and increase the R0 resection rate.[11] Furthermore, in multiple small studies, SBRT alone or chemotherapy followed by SBRT have shown promising locoregional control of the disease.[12–17] Besides the direct anti-cancer effect, radiation therapy can act as an *in-situ vaccine* upon tumor cell destruction and consequent antigen shedding. Radiation therapy has demonstrated to be able to increase the expression of cell surface receptors, to increase tumor antigen presentation, and possibly to induce anti-tumor cytotoxic T cell responses.[18–21] In this study we added the immunological adjuvant IMM-101 to SBRT treatment. This vaccine containing a heat-killed mycobacterium-obuense has demonstrated to induce activation and maturation of dendritic cells.[22] In patients with metastasized PDAC, Gemcitabine and IMM-101 combination therapy already suggested a beneficial effect on survival.[23] Therefore, we expected that the combination of IMM-101 with SBRT could induce an innate and adaptive immune response against pancreatic cancer, and thereby could improve systemic control of the disease.

In this phase I/II, single centre, non-randomized trial we investigated safety, feasibility, and efficacy of adding IMM-101 to SBRT in LAPC patients who already have been treated with (modified) FOLFIRINOX. By combining SBRT and IMM-101 we aimed to provide not only local disease control, but also induce a systemic immune response to inhibit distant disease progression. Safety and efficacy were compared with those of a previous clinical trial (LAPC-1)[24] in which LAPC patients received SBRT without IMM-101 vaccination after systemic treatment with (modified) FOLFIRINOX.

Materials and methods

Patients

In this open-label, non-randomized, single-arm, single-centre, phase I/II clinical trial we included biopsy-proven LAPC patients who did not show signs of progressive disease after having received at least 4 cycles of (modified)FOLFIRINOX. Resectability was determined at time of diagnosis according to the Dutch Pancreatic Cancer Group guidelines for resectability, and tumors were classified as LAPC in case of > 90° arterial contact and/or > 270° venous contact and/or venous occlusion.[25] At our institution (Erasmus MC Cancer Institute), we performed a diagnostic laparoscopy (DLS) at time of diagnosis to rule out occult metastatic disease. During the same procedure, we placed a port-a-cath for the systemic treatment. This was not standard of care treatment in referral centers, and therefore a DLS at time of diagnosis was not mandatory. Inclusion criteria were an age above 18 and below 75 years, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and an American Society of Anesthesiologists (ASA) classification of I or II.[26,27] Another criterion was a maximum tumor diameter of seven centimetre in sagittal, transverse and coronal plane and no direct contact of the tumor with the stomach, colon or small bowel. In addition, patients needed to have an adequate renal function, normal liver tests and normal bone marrow function. Exclusion criteria were prior treatment with radiotherapy or chemotherapy other than FOLFIRINOX, as well as previous pancreatic resection and current or previous treatment with immunotherapeutic drugs. All in- and exclusion criteria are listed in detail in **Supplementary** Table 1. The study was approved by the Central Committee on Research involving Human Subjects (NL68762.078.19) as defined by the Medical Research Involving Human Subjects Act. Procedures followed were in accordance with the ethical standards of these committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The trial was registered in the Netherlands Trial Registry (NL7578). Written informed consent was obtained from each subject.

Sample size calculation

The study consisted of two consecutive phases. The primary objective of the first phase was to determine the safety of adding IMM-101 to SBRT. The previously observed grade 4 toxicity rate related to SBRT in the setting of LAPC was 10 %.[24] Using the binomial exact method, we calculated that a sample size of 20 patients would enable to estimate a toxicity rate of 10 % within a 95 % confidence interval of 1.2 % and 31.7 %. In effect, this implied that we would accept at most 6/20 patients with grade 4 adverse events related to the intervention, before moving on to the second phase. During the second phase we aimed to investigate the efficacy. In the previous LAPC-1 trial, the one-year progression-free survival (PFS) rate of the patients who received SBRT was 45 %. We hypothesized that by adding IMM-101 to SBRT, the one-year PFS rate could be improved to 65 %. According to Fleming's procedure, with significance level of 0.05 and a power of 80 %, we needed a total of 38 patients to test this hypothesis.[28].

SBRT and IMM-101 vaccination

Prior to the radiation, the gastro-enterologist placed three radio-opaque markers (fiducials) in or near the tumor (<3 cm distance from the tumor) with endoscopic ultrasound guidance.[29] Patients received a total of 40 Gy of SBRT over five consecutive days with 8 Gy per fraction. The tumors were irradiated with the CyberKnife (Accuray, Sunnyvale, USA). For the radiation, patients were placed in supine position. Patients were prepared for radiotherapy with a dedicated CT scanner in treatment position, with an immobilisation device. To verify the motion of the bowel and stomach during each fraction, an expiration CT scan was acquired just before every treatment fraction in treatment position. The clinical target volume (CTV) included the gross target volume (GTV), plus possible tumor extension of 5 mm. The planning target volume (PTV) included the CTV, plus 2 mm margin. Dose constrains for organs at risk were a maximum of 50 Gy in equivalent dose in 2 Gy per fraction (EQD2) ($\alpha/\beta = 3$) to the spinal cord, and 35 Gy in 5 fractions to stomach and bowel ($\alpha/\beta = 3$). The mean kidney dose was not allowed to exceed 18 Gy in EQD2 ($\alpha/\beta = 2.5$), and 700 cc of the liver was not allowed to receive more than 20 Gy (absolute dose). Radiation started at week 2.

In addition, intradermal vaccinations containing one milligram IMM-101 were given. Pre-labeled vials of IMM-101 were shipped by Immodulon Therapeutics ltd. (Uxbridge, UK). The vaccine was injected intradermally over the deltoid muscle by the standard Mantoux intradermal injection technique. A 27 gauge needle was used. Adequate injection technique resulted in immediate formation of a raised papule. IMM-101 was administered at week 0, week 2, week 4, week 8, week 10 and week 12.

Response evaluation and follow-up

Three months after SBRT, we performed the first response evaluation (i.e., clinically, radiographically and biochemically). This interval between radiotherapy and response evaluation was used,

since the radiological response after SBRT can occur after several months. In a multidisciplinary tumor board, we decided which patients would be candidates for an explorative laparotomy and a possible resection. The decision to perform an explorative laparotomy, was made based on the patients' condition (ECOG 0–1), tumor biology (CA 19–9 levels and evolution), and surgical technical considerations (e.g., extent of vessel involvement, the change of successful divestment of the artery, the need for an arterial resection with or without reconstruction, and the probability of a radical resection). Patients with stable disease who did not qualify for an exploration, were offered to receive maintenance vaccinations with IMM-101 (i.e., a monthly vaccination with 0.5 mg IMM-101) for 12 months or until disease progression. After completion of the study treatment, patients went into routine follow-up for at least five years after SBRT. Regular follow-up CT scans were made. After disease progression, patients were referred to the medical oncologist for the decision to restart systemic treatment with chemotherapy. Fig. 1 illustrates the treatment schedule.

Objectives

The primary objective of the phase I study was to investigate safety and feasibility of adding IMM-101 to SBRT. Feasibility was defined as the number of patients receiving SBRT and the IMM-101 vaccinations at the designated time points. Safety was defined as grade 4 and 5 adverse events which were considered to be possibly related to therapy.[30] The primary objective of the phase II study was to investigate efficacy of the treatment, assessed by the one-year PFS rate.

Statistical analysis

Data analyses were performed using IBM SPSS Statistics 25 and R version 4.1.2. and R-studio. Patient characteristics are summarized using the median and interquartile range for continuous variables and using counts and percentages for categorical variables. A Mann-Whitney U test was performed to compare medians. Categorical variables were compared using a Pearson Chi-Squared test. Follow-up time was calculated using the reverse Kaplan-Meier method. Survival analyses were performed using the Kaplan-Meier method. Follow up time, OS and PFS were calculated from the start of FOLFIRINOX until an event (i.e., date of death or date of progression of disease respectively). Patients with no event were censored at the last follow-up date. In all analyses, a two-sided p-value < 0.050 was considered statistically significant.

Results

Between November 2019 and January 2021, a total of 38 patients were included and started the study treatment. In most cases (n = 27; 71 %) the tumor was located in the head of the pancreas. Eighteen (47 %) patients needed biliary drainage at diagnosis. Moreover, a diagnostic laparoscopy at time of diagnosis was performed in 13 (34 %) patients. The median number of cycles of FOLFIRINOX was 8 (IQR 8–8). Twenty-seven (71 %) patients had radiographically stable disease after induction chemotherapy with (modified)FOLFIRINOX according to RECIST 1.1.[31] Ten (26 %) patients had a partial response and one patient showed a complete radiological response. The median time elapsed between the last cycle of (modified)FOLFIRINOX and inclusion in the study, and starting study treatment was 4 (IQR 2–4), and 6 (IQR 5 – 7) weeks, respectively. Table 1 shows detailed patient characteristics and the comparison of important variables with those of the previous LAPC-1 trial.

All patients received the scheduled 40 Gy of SBRT. Thirty-five (92 %) patients who started with the study treatment received all scheduled vaccinations; three received fewer vaccinations due to disease progression. The administration of IMM-101 was not associated with significant changes in vital signs (**Supplementary** Fig. 1). None of the patients reported discomfort or symptoms after vaccination. Twenty-six (68 %) patients developed grade 1 injection site reactions upon vaccination (**Supplementary** Fig. 2). During the study period we observed one grade 5, no grade 4 and thirteen grade 3 adverse events in 9 (24 %) patients. Seven adverse

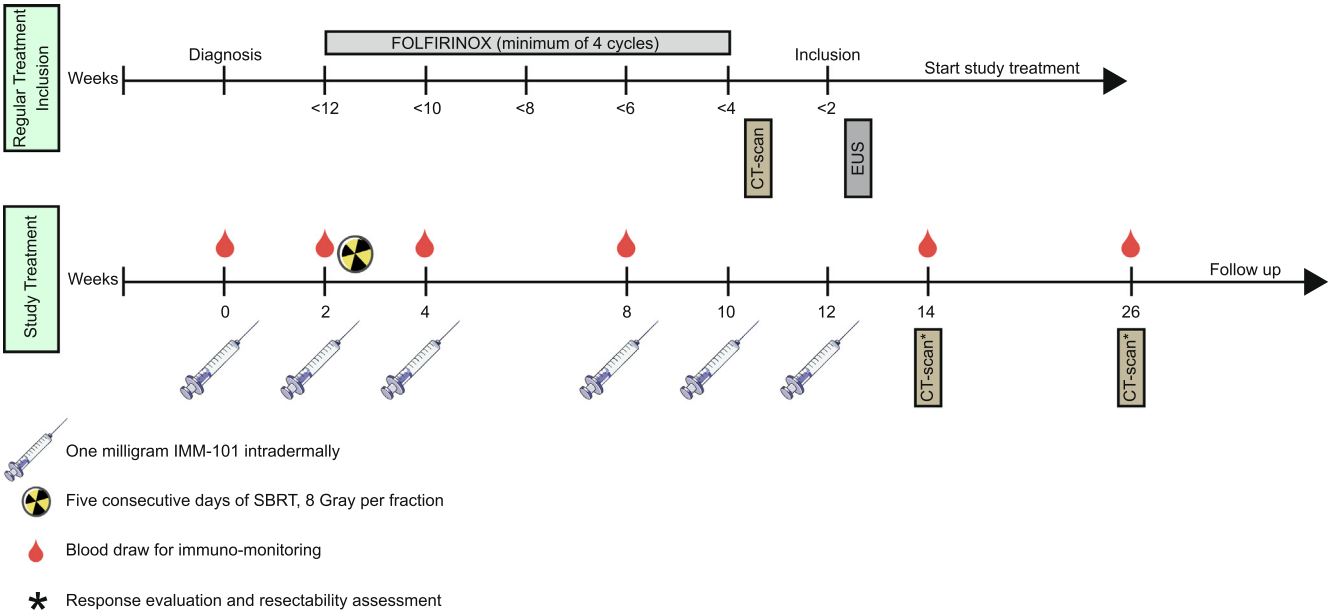

Fig. 1. Schematic treatment schedule. Before inclusion in the trial, patients had received at least four cycles of FOLFIRINOX. After inclusion, an endoscopic ultrasound (EUS) is performed to place the radio-opaque fiducials in or near the tumor. Patients received three bi-weekly intradermal vaccinations of IMM-101. At week two, patients started with five consecutive days of treatment with stereotactic body radiotherapy. Every fraction was 8 Gray (Gy). From week four to eight, patients received no treatment. From week eight to twelve, patients received the second course of three bi-weekly vaccinations. At week fourteen, the first response assessment was done. Patients with progressive disease were referred to the oncologist. Some patients were offered an explorative laparotomy to pursue a potential curative resection.

Table 1
Patient, disease, and treatment characteristics.

Patient characteristics	LAPC-2 n = 38	LAPC-1 n = 39	p value
Age (years), *median (IQR)*	63 (59–71)	60 (52 – 64)	0.059[a]
Male sex, *n (%)*	16 (42)	19 (49)	0.560[b]
BMI (kg/m^2), *median (IQR)*	24 (21 – 27)	24 (22 – 28)	0.697[a]
ECOG performance status, *n (%)*			
0	12 (32)	-	-
1	26 (68)	-	-
Disease characteristics			
CA 19–9 (U/ml; diagnosis), *median (IQR)*	508 (126–1331)	200 (64 – 923)	0.092[a]
CA 19–9 (U/ml; inclusion), *median (IQR)*	113 (34–206)	-	-
CEA (μg/L; diagnosis), *median (IQR)*	5.37 (3.53–9.80)	4.2 (3.00 – 18.00)	0.862[a]
CEA (μg/L; inclusion), *median (IQR)*	4.4 (3.4–6.4)	-	-
Tumor location, *n (%)*			0.231[b]
Pancreatic head	27 (71)	22 (58)	
Pancreatic body/tail	11 (29)	16 (42)	
Tumor size (mm; diagnosis), *median (IQR)*	37 (30 – 46)	39 (32 – 45)	0.480[a]
Tumor size (mm; inclusion), *median (IQR)*	31 (25 – 40)	-	-
Vessel involvement (inclusion), *n (%)*			
Arterial contact			-
≤ 90 degrees	5 (13)	-	
> 90 degrees	33 (87)	-	
Venous contact			-
≤ 270 degrees	24 (63)	-	
> 270 degrees and/or occlusion	14 (37)	-	
Treatment characteristics			
Diagnostic laparoscopy (diagnosis), *n (%)*	13 (34)	39 (100)	**< .001[b]**
Biliary drainage (diagnosis), *n (%)*	18 (47)	-	-
FOLFIRINOX chemotherapy, *n (%)*	38 (100)	39 (100)	-
Number of cycles, *median (IQR)**	8 (8 – 8)	8 (6 – 8)	**< .001[a]**
Radiological response after FOLFIRINOX, *n (%)***			-
Stable disease	27 (71)	-	
Partial response	10 (26)	-	
Complete response	1 (3)	-	

Abbreviations: IQR, interquartile range; BMI, body mass index, ECOG, Eastern Cooperative Oncology Group; CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen. Statistics: a, Mann-Whitney U test; b, Pearson Chi-Squared test. Arced p-value represent a significant p-value (i.e., <0.050). Definitions: Arterial contact represents abutment of the tumor with the superior mesenteric artery, the celiac trunk and / or the hepatic artery; venous contact represents abutment of the tumor with the portal vein or mesenteric veins; *Range: 8 – 13 cycles for LAPC-2 and 2 – 8 for LAPC1. **According to RECIST criteria version 1.1.

events were biliary events (i.e., cholestasis or cholangitis) related to the underlying cancer or biliary drainage. We observed five gastro-intestinal (GI) bleedings during the study period, in four different patients. Of those, four were classified as grade 3 bleedings, and one as a grade 5 bleeding. As GI-bleedings can be a complication of radiotherapy, they are discussed in more detail below. The first grade 3 GI-bleeding occurred in patient IMM003. This bleeding occurred four months after the SBRT, and could be managed conservatively with three packed cells. Dmax to stomach, duodenum, and bowel were 35.8 Gy, 7 Gy, and 27 Gy respectively. Endoscopy showed tumor ingrowth into the D2 of the duodenum, and ulceration distally from the tumor. There was no active source of bleeding. Second grade 3 GI-bleeding occurred in patient IMM006. It occurred two months after the SBRT. It was managed successfully conservatively, with two packed cells. Dmax to stomach, duodenum, and bowel were 35.7 Gy, 35.3 Gy, and 32.9 Gy respectively. During endoscopic evaluation, the mucosa of the stomach and duodenum (up to pars descendens) showed no abnormalities. In patient IMM007, the third and fourth grade 3 GI-bleeding occurred, one and three weeks after the SBRT. Dmax to stomach, duodenum, and bowel were 15.0 Gy, 35.6 Gy, and 15.5 Gy, respectively. The patient had a metal choledochobulbostomy, which occluded six days after SBRT. The occluded choledochobulbostomy was removed and two double pigtails stents were placed. Two days later, the pigtails migrated and caused the first bleeding. Two covered self-expandable metal stents (SEMS) were placed. Two weeks later, a re-bleed occurred. Endoscopic evaluation revealed oozing blood loss, located at the SEMS. Lastly, the fifth GI-bleeding classified as grade 5. Patient IMM026 was admitted to the hospital 6 weeks after SBRT treatment. Dmax to stomach, duodenum, bowel, and gallbladder were 29.6 Gy, 38.8 Gy, 37.4 Gy, and 22.7 Gy, respectively. She presented with a perforated cholecystitis, which was managed conservatively with drainage and antibiotics. CT scan revealed diffuse liver metastases. Two days later, another CT scan revealed perforation of a liver abscess into the abdominal cavity. Same day the patient developed a fatal massive bleeding. No endoscopic evaluation was performed because of an infaust prognosis. Table 2 describes all grade 3 or higher adverse events, and the presumed relation of the adverse event to the study treatment.

At a median follow-up of 28.4 (95 %CI 14.3 – 32.6) months, 34/38 (90 %) patients had progression of disease (i.e., local, distant, or both), and 29/38 (76 %) patients had died. The one-year PFS rate was 47 %. The one-year OS rate was 82 %. The median PFS was 11.7 (95 %CI 11.0 – 12.5) months. The median OS was 19.0 (95 %CI 16.2 – 21.9) months. The median time to locoregional progression was 15.1 (95 %CI 12.7 – 17.5) months and median time to distant progression (i.e., lung, liver, peritoneal, or omental metastasis) was 12.2 (95 %CI 10.8 – 13.6) months. Three months after SBRT, 15/38 (39 %) patients showed progressive disease, 20/38 (53 %) had stable disease, and 3/38 (8 %) showed a partial response. Of the 15 patients who had progressive disease three months after SBRT, 6 (40 %) had distant progression, 6 (40 %) had local progression, and 3 (20 %) had both distant and local progression. After disease progression, 22/34 (64 %) patients started with palliative chemotherapy. Of those, 2/22 (9 %) received gemcitabine, 4/22 (18 %) received FOLFIRINOX, 14/22 (64 %) received gemcitabine with nab-paclitaxel, and in 2/22 (9 %) the type of chemotherapy

Table 2
All grade 3 or higher adverse events and the relation to the study treatment.

Adverse event term	SBRT			IMM-101			Unrelated		
	3	4	5	3	4	5	3	4	5
Cholestasis	-	-	-	-	-	-	3	-	-
Cholangitis	-	-	-	-	-	-	4	-	-
GI-Bleeding	1	-	-	-	-	-	3	-	1
Duodenal obstruction	-	-	-	-	-	-	1	-	-
Vomiting	-	-	-	-	-	-	1	-	-

Abbreviations: GI, gastro-intestinal; SBRT, stereotactic body radiotherapy.
The most probable cause of the adverse events, according to the investigators, were reported (SBRT, IMM-101, unrelated to the treatment). Grading of the adverse events was done according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.

was unknown. Reasons for not starting palliative chemotherapy were watchful waiting (n = 1), patient preference (n = 3), and poor performance status (n = 8).

Ten (26 %) out of all 38 patients underwent an explorative laparotomy, in 8/38 (21 %) patients a resection was performed. After resection, patients did not receive adjuvant treatment. Seven (88 %) patients underwent a (extended)pancreatoduodenectomy and one (12 %) patient underwent a total pancreatectomy. In 5 (63 %) patients a venous resection and in one (12 %) patient an arterial resection and reconstruction was performed. Major morbidity (i.e., Clavien Dindo grade 3A or higher) occurred in three patients and two patients eventually died because of complications from the operation. One patient developed a pancreatic fistula which was treated successfully with drainage and antibiotics. IMM002 developed a shock liver caused by a portal vein thrombosis, after an extended pancreatoduodenectomy with portal vein wedge resection and segmental resection with primary reconstruction of the superior mesenteric vein. The patient died from the complications. IMM021 suffered from a blow-out of the arterial anastomosis on postoperative day 13, after a total pancreatectomy with a venous resection and reconstruction, and resection of a branch of the superior mesenteric artery with primary reconstruction to the common hepatic artery. Initially, the bleeding could be managed with a bypass (donor graft) between the superior mesenteric artery and the common hepatic artery. Unfortunately, a re-bleed occurred, and in the absence of surgical options, the artery was coiled with risk of liver ischaemia. The patient died the same day due to a shock liver. Fig. 2 shows a swimmers plot of the resected patients. The resection rate of 21 % (n = 8) was comparable with the resection rate of 18 % (n = 7) from the previous LAPC-1 trial. Moreover, histopathological variables of the resection specimens from the LAPC-2 and LAPC-1 trial, were equally distributed. Detailed pathology reports are shown in Table 3.

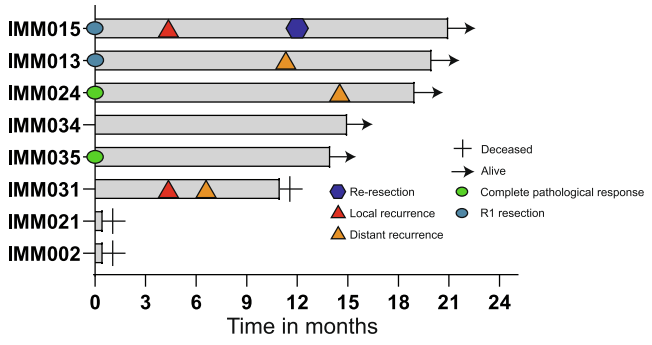

Fig. 2. Swimmers plot after resection. At t = 0 the resection was performed. Each bar represents one subject in the study. Two subjects, 001IMM002 and 001IMM021 died from complications of the operation. One subject, 001IMM015, developed local recurrence and responded to systemic chemotherapy. In absence of disease progression, a re-resection was performed, and 9 months later there were no signs of disease recurrence.

Discussion

In this phase I/II trial non-progressive LAPC patients after (modified)FOLFIRINOX were treated with SBRT, combined with intradermal IMM-101 vaccinations. All included patients finished the scheduled SBRT and 35/38 (92 %) patients received all scheduled vaccinations. Most of the adverse events, such as biliary adverse events, were considered to be related to the underlying condition. During the study period, five GI-bleedings (four grade 3, one grade 5) occurred in four patients. GI-bleedings after (stereotactic)radiotherapy to the pancreas have been described previously.[24,32] All patients who suffered from a GI-bleeding had received acceptable doses to the organs at risk. The bleeding from IMM006 was considered to be likely caused by the SBRT, since endoscopic evaluation revealed no other sufficient explanation. Other bleedings were likely caused by disease progression (IMM003, IMM026) and migrated bile duct stents (IMM007). After treatment with IMM-101, no systemic toxicity was observed. Only grade 1 injection site reactions, were observed. We may conclude that SBRT + IMM-101 in this study population was a safe and feasible treatment approach. After SBRT/IMM-101 combination treatment, the one-year PFS rate was 47 %. In the previous LAPC-1 trial, in which patients were treated with SBRT without IMM-101, this was 45 %. Therefore, the primary objective of the study, and improvement in the one-year PFS rate from 45 % to 65 %, was not achieved.

The resection rate was comparable between both cohorts (LAPC-2, 21 % vs LAPC-1, 18 %). This resection rate is in line with the resection rate of LAPC reported in literature after induction chemotherapy.[5] Histopathological examination of the resection specimens after treatment in the LAPC-2 or LAPC-1 trial, revealed no significant differences. For another study (*analysis in progress, data not shown*) we performed RNA-sequencing analyses, comparing tumors of patients treated with FOLFIRINOX + SBRT (n = 12) with tumors of patients treated in the LAPC-2 trial with FOLFIRINOX + SBRT + IMM-101 (n = 8). Differentially expressed genes (DEGs) analyses revealed limited DEGs between both treatment groups. This implied limited effect of IMM-101 in the tumor after intradermal vaccinations. Moreover, we recently reported data on the immuno-modulating effects of SBRT + IMM-101 in the peripheral blood of the first 20 patients included in the current trial.[33] This study found transient lymphodepletion and immune activation. The changes in the immune system, combined with the timing of the vaccinations and SBRT, indicated that the observed effects were likely caused by the radiotherapy. In the absence of a control group, an effect of IMM-101 could not be ruled out completely.

Besides minor differences, the cohorts of the LAPC-1 and LAPC-2 trial were reasonably comparable. Patients in the LAPC-2 trial

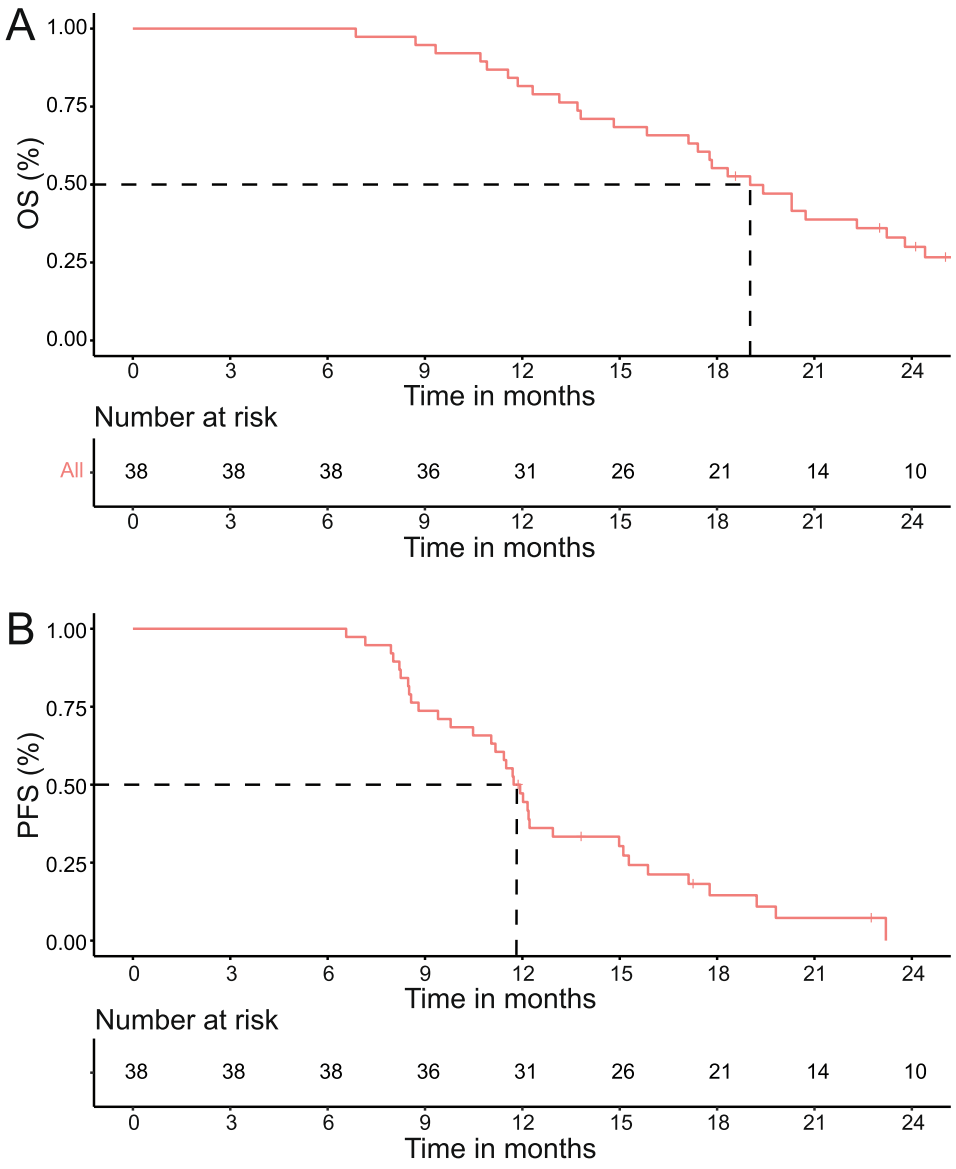

Fig. 3. The Kaplan-Meier curves showing OS (Fig. 3a) and PFS (Fig. 3b) probability. The median OS was 19.0 (95%CI 16.2 – 21.9) months. The median PFS was 11.7 (95%CI 11.0 – 12.5) months.

received significantly more chemotherapy, which could have improved their outcomes. On the other hand, only 34 % of the patients in the LAPC-2 trial underwent a staging laparoscopy compared to all patients in the LAPC-1 trial. Previously we have reported a 19 % rate of occult metastatic disease in the context of LAPC.[34] The latter finding suggests that patients in the LAPC-2 trial were probably under staged compared to patients in the LAPC-1 trial, which could have influenced the outcomes of the current trial. Although there is no statistically significant difference, under staging of patients in the LAPC-2 trial could also be reflected by the tumor marker CA 19–9 at diagnosis.

In a previously conducted randomized clinical trial, patients with advanced pancreatic cancer were randomized to receive gemcitabine monotherapy or gemcitabine with IMM-101.[23] In a predefined subgroup of metastatic PDAC patients, adding IMM-101 to gemcitabine improved overall survival with 4.4 to 7.0 months (p = 0.01).[23] This demonstrated the potential for IMM-101 in combination with chemotherapy to provide improved systemic control of the disease. In this study we could not demonstrate that adding intradermal IMM-101 vaccinations to SBRT improved the systemic control of the disease compared to patients treated with SBRT alone; the median time to distant progression was comparable between both cohort (LAPC-2: 12.2 (95 %CI 10.8 – 13.6) months, LAPC-1: 11 (95 %CI 9 – 13) months).

Interestingly, 6/8 (75 %) and 6/7 (86 %) resections in the LAPC-2 and LAPC-1 trial respectively, were R0. This high rate of radical resections is important because the resection margin status is a relevant prognostic factor for disease recurrence.[35,36] The high rate of radical resections after treatment with SBRT, which has also been reported in literature,[11] demonstrates the possible benefit of SBRT, or radiotherapy in general, in the treatment of localized pancreatic cancer. In previous studies with stage I/II pancreatic cancer, preoperative radiotherapy was associated with a high margin negative resection rate, possibly minimizing the risk for locoregional recurrence.[37,38] Another possible explanation for this observation is that by treating patients with SBRT after FOLFIRINOX, the pre-operative treatment time is prolonged. Response evaluation was done three months after SBRT. This test of time could theoretically improve the selection of favourable tumor biology, before the eventual resection.

Table 3
Pathological outcomes of resected tumors in LAPC-2, and the previous LAPC-1 trial.

	LAPC-2 n = 8	LAPC-1 n = 7	p-value
Tumor size (mm), *median IQR*	10 (2 – 22)	1 (0 – 15)	0.409[a]
Margin status, *n (%)*			0.605[b]
R0	6 (75)	6 (86)	
R1	2 (25)	1 (14)	
pT stage, *n (%)*			0.605[b]
pT0-1	6 (75)	6 (86)	
pT2-3	2 (25)	1 (14)	
N stage, *n (%)*			0.133[b]
pN0	5 (62)	6 (86)	
pN1-2	3 (38)	0 (0)	
Missing	0 (0)	1 (14)	
Perineural invasion, *n (%)*			0.460[b]
Present	1 (12)	0 (0)	
Absent	7 (88)	4 (57)	
Missing	0 (0)	3 (43)	
Lymphangioinvasion, *n (%)*			0.460[b]
Present	1 (12)	0 (0)	
Absent	7 (88)	4 (57)	
Missing	0	3 (43)	
HTRG, *n (%)*			0.057[b]
HTRG 0–1	4 (50)	5 (71)	
HTRG 2	4 (50)	0 (0)	
Missing	0 (0)	2 (29)	

a, Mann-Whitney U Test; b, Pearson Chi-Squared Test; R0 $\geq$ 1 mm, R1 < 1 mm, according to the College of American Pathologist guidelines, 2017; HTRG, Histological Tumour Regression Grade; HTRG 0, no viable tumor cells; HTRG 1, < 5 % viable tumor cells; HTRG 2, $\geq$ 5 % viable tumor cells, according to the College of American Pathologist guidelines, 2017; TNM staging according to American Joint Committee on Cancer, Cancer staging Manual, 8th edition.

A strength of the study, is the comparability with the previous LAPC-1 study. This resulted in a relative robust understanding of the effect of adding IMM-101 to SBRT treatment, without the need to conduct a large randomized trial. A randomized trial would strengthen our conclusions, however based on the results of this trial we would not suggest a randomized clinical trial with SBRT in combination with IMM-101 intradermally alone. A limitation of the study, is the lack of immunological data from the previous LAPC-1 trial. However, the data from the immuno-monitoring of the phase I LAPC-2 trial,[33] is highly suggestive for an effect caused by SBRT, rather than IMM-101. Therefore we anticipate that a randomized trial will not deliver new insights. In the phase I LAPC-2 study,[33] we found upregulation of the immune checkpoint CTLA-4, on the circulating T cells. This endorses the combination of the current strategy with checkpoint blocking antibodies in future trials. This approach was also suggested previously by others.[39] Furthermore, conceptually administering IMM-101 into the tumor prior to SBRT, with the accompanying inflammatory response and influx of immune cells might be a better option and should be further explored.

Conclusions

Intradermal Heat-killed mycobacterium obuense vaccinations (IMM-101) in combination with stereotactic body radiotherapy is a safe and feasibly treatment option for locally advanced pancreatic cancer patients after treatment with (modified)FOLFIRINOX. An improvement in PFS by adding IMM-101 to SBRT, could not be demonstrated. Combining the current treatment strategy with immune checkpoint blocking antibodies, or intra-tumoral administration of IMM-101, should be further explored in future trials.

Author contributions

FRvtL and DL drafted this manuscript. CvE, JN, MYVH and DL, among others, wrote the study protocol. CvE is the principal investigator. FRvtL, DL and CvE included patients in the trial and provided patient care. MM was the research nurse, administered most vaccinations, planned most appointments and was the main contact person for the patients. KB, BAB, JSDM, EvdH, PPLOC, JHW and GPvdS referred patients for inclusion to our institution. All authors contributed to the final manuscript and agreed with all of the content of the submitted manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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