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Safety and tumour-specific immunological responses of combined dendritic cell vaccination and anti-CD40 agonistic antibody treatment for patients with metastatic pancreatic cancer: protocol for a phase I, open-label, single-arm, dose-escalation study (REACtiVe-2 trial)

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ABSTRACT

Introduction The prognosis of patients with advanced pancreatic ductal adenocarcinoma (PDAC) is dismal and conventional chemotherapy treatment delivers limited survival improvement. Immunotherapy may complement our current treatment strategies. We previously demonstrated that the combination of an allogeneic tumour-lysate dendritic cell (DC) vaccine with an anti-CD40 agonistic antibody resulted in robust antitumour responses with survival benefit in a murine PDAC model. In the Rotterdam PancrEAtic Cancer Vaccination-2 trial, we aim to translate our findings into patients. This study will determine the safety of DC/anti-CD40 agonistic antibody combination treatment, and treatment-induced tumour-specific immunological responses.

Methods and analysis In this open-label, single-centre (Erasmus Univsersity Medical Center, Rotterdam, Netherlands), single-arm, phase I dose finding study, adult patients with metastatic pancreatic cancer with progressive disease after FOLFIRINOX chemotherapy will receive monocyte-derived DCs loaded with an allogeneic tumour lysate in conjunction with a CD40 agonistic antibody. This combination-immunotherapy regimen will be administered three times every 2 weeks, and booster treatments will be given after 3 and 6 months following the third injection. A minimum of 12 and a maximum of 18 patients will be included. The primary endpoint is safety and tolerability of the combination immunotherapy. To determine the maximum tolerated dose, DCs will be given at a fixed dosage and anti-CD40 agonist in a traditional 3+3 dose-escalation design. Secondary endpoints include radiographic response according to the RECIST (V.1.1) and iRECIST criteria, and the detection of antitumour specific immune responses.

Ethics and dissemination The Central Committee on Research Involving Human Subjects (CCMO; NL76592.000.21) and the Medical Ethics Committee (METC; MEC-2021-0566) of the Erasmus M.C. University Medical Center Rotterdam approved the conduct of the trial. Written informed consent will be required for all participants. The results of the trial will be submitted for publication in a peer-reviewed scientific journal.

Trial registration number NL9723.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The 3+3 design allows us to investigate dose-limiting toxicities of an anti-CD40 agonist (mitazalimab) within the DC/anti-CD40 agonist combination immunotherapy regimen for patients with metastasized pancreatic cancer.
⇒ Longitudinal blood sampling will be performed to investigate the immune responses in the peripheral blood on different time points during treatment, both on RNA and protein level.
⇒ Pretreatment and post-treatment tumour biopsies are being performed to investigate the induced immune responses in the tumour microenvironment.
⇒ The limited sample size and non-randomised nature of the study does not allow us to investigate clinical efficacy.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related deaths and carries a grim prognosis with a 5-year survival rate of less than 5%.[1] The

majority of PDAC patients present with advanced disease not eligible for surgery.[2] The current standard-of-care treatment for locally advanced and metastasised pancreatic cancer is FOLFIRINOX chemotherapy, including fluorouracil, leucovorin, irinotecan and oxaliplatin. However, even with this intensive chemotherapy regimen median overall survival is 24.2 months and 11.1 months for locally advanced and metastatic PDAC, respectively, with no superior alternatives available.[3 4] In addition, more than half of the patients experience FOLFIRINOX-related toxicity which could lead to early termination of treatment.[5] Therefore, we are in need of new treatment modalities to tackle unresectable pancreatic disease.

Immunotherapy, like immune checkpoints inhibitors, delivered impressive results in various malignancies, and changed the treatment strategy for solid tumours like non-small cell lung cancer and melanoma.[6–9] Cellular immunotherapies, including chimeric antigen receptor (CAR) T-cells, for haematological malignancies also demonstrated promising results leading to US Food and Drug Administration approval of multiple CAR T treatments.[10–13] Unfortunately, outcomes with immune checkpoint blockers and CAR-T cells in PDAC have been disappointing.[14–17] PDAC is considered an immunological 'cold' tumour with a highly immunosuppressive microenvironment lacking the presence of effector T-cells.[18] Nonetheless, recent studies showed promising results with rational immune-combination strategies demonstrating that comprehensive understanding of the immune composition and tumour biology of PDAC is imperative for successful treatment.[19 20]

Dendritic cells (DCs) play a fundamental role in the antitumour response. They capture, process and present tumour antigens and can subsequently induce tumour-specific effector T cells. It has been demonstrated that DC paucity in PDAC impairs immune surveillance, and resurrection of DCs in early PDAC lesions reinvigorates antitumour T-cell immunity.[21] We have investigated the use of allogeneic-mesothelioma lysate DC vaccination (MesoPher) for patients with resected pancreatic cancer (Rotterdam PancrEAtic Cancer Vaccination Trial, REACtiVe Trial; NL7432). Ideally, a personalised lysate of the autologous tumour would be able to redirect the lymphocyte response to the specific disease of the patients. However, in most PDAC patients, it is not possible to collect sufficient tumour material for the production of a tumour lysate. Also sampling differences between patients will result in different quality of lysates. As a reliable alternative, the use of an allogeneic tumour lysate avoids the need for autologous tumour material and standardises treatment across patients. MesoPher demonstrated clinical activity in mesothelioma patients and mesothelioma and PDAC share various tumour antigens (eg, mesothelin, WT-1, MUC-1, Survivin).[22] In the REACtiVe trial, we have demonstrated the induction of PDAC-specific T cells following MesoPher treatment (Lau *et al*, 2022, Eur J Cancer, Manuscript accepted). However, the tumour microenvironment of established PDAC encompass dense desmoplastic stroma able to exclude effector T cells.[23] CD40 is a surface molecule on various immune cells, including B cells, monocytes/macrophages and DCs.[24 25] Its ligand, CD154, is expressed primarily on activated T cells.[25] Because of their expression, CD40-CD154 interaction plays an important role in both humoral and cellular immunity. It has been demonstrated that CD40-agonists are able to induce stromalysis in PDAC by matrix metallo-proteases produced by tumour-associated macrophages.[26 27] Tumour regression was found when CD40 agonist combined with the chemotherapeutic gemcitabine was given and the antitumour effect was annihilated when macrophages were depleted.[26] In addition, we have previously demonstrated in a PDAC murine model that although CD40-agonists improved intratumoural T-cell infiltration, T cells displayed hallmarks of exhaustion.[28] The addition of DC vaccination improved T-cell phenotype, and DC/anti-CD40 combination therapy led to survival benefit compared with monotherapy (DC vaccination or anti-CD40) treated animals. Finally, CD40 targeting also licenses endogenous (and administered) DCs to cross-present tumour antigens to T cells, boosting the spontaneously activated tumour immunity.[29 30] By rationally combining DC vaccination and an anti-CD40 agonist antibody, we could convert the classically immunological 'cold' PDAC to a 'hot' and immunotherapy-sensitive tumour. These preclinical results lay the foundation for this clinical trial.

We hypothesise that this bimodal-treatment regimen, using DCs to induce tumour-specific T cells and an anti-CD40 agonist to promote introduction of T cells into the tumour, may lead to effective antitumour responses in PDAC patients. In the REACtiVe-2 trial, we will investigate the maximum tolerable dose of anti-CD40 agonist antibody in combination with allogeneic-tumour lysate-DC vaccination in patients with metastasised pancreatic cancer after failure of first-line FOLFIRINOX treatment.

METHODS AND ANALYSIS

Study design and treatment

The Rotterdam PancrEAtic Cancer Vaccination-2 (REACtiVe-2) trial is an open-label, dose-finding, single-centre (Erasmus University Medical Center, Rotterdam, Netherlands), single-arm, phase I study consisting of three parts; screening, bridging and treatment phase. A traditional 3+3 design is implemented to investigate dose-limiting toxicities (DLTs) of an anti-CD40 agonist (mitazalimab) within the DC/anti-CD40 agonist combination-immunotherapy regimen for patients with pancreatic cancer. A minimum of 12 and a maximum of 18 patients will be included.

The study was approved by the Central Committee on Research involving Human Subjects (NL76592.000.21) 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 is registered with the

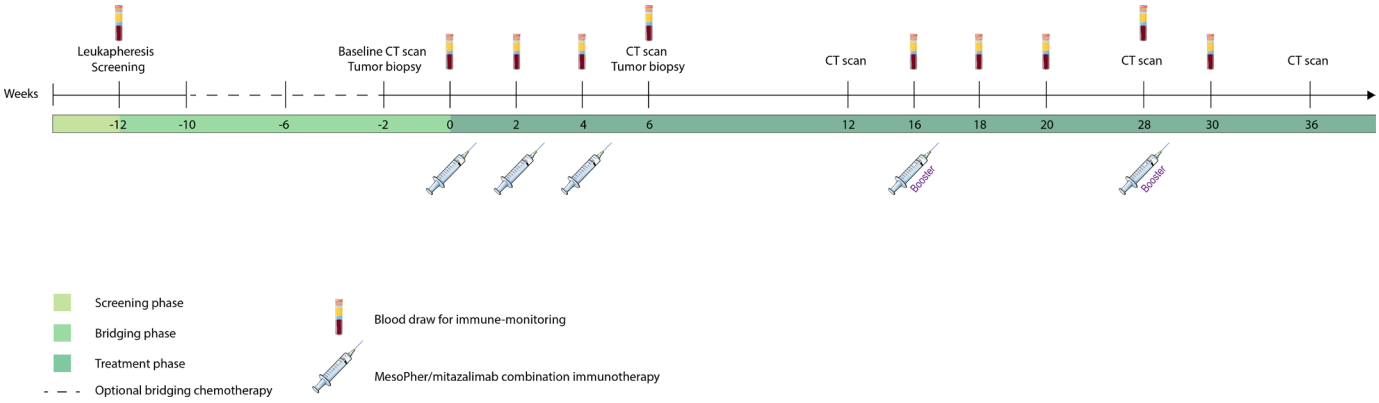

Figure 1 Treatment scheme after screening, a leukapheresis is performed for the production of allogeneic-tumour lysate loaded dendritic cells. The length of the bridging phase can vary between patients, depending on whether patients receive chemotherapy or not. Study patients receive combination immunotherapy on week 0, 2, 4 and booster vaccinations are given at week 16 and 28. A tumour biopsy is taken before and after three administrations of the study treatment. Blood for immune-monitoring is drawn at various time points.

Dutch Trial Register, NL9723. Trial registration details are described in online supplemental table 1.

Screening phase

Patients with metastatic pancreatic cancer with progression on first-line (modified) FOLFIRINOX are screened for eligibility for the study. Screening will start after 2 weeks after the last cycle of chemotherapy (figure 1).

Bridging phase

Included patients will start off with a leukapheresis during the bridging phase. A leukapherisis is performed in order to generate monocyte-derived DCs (mo-DC) for MesoPher production. The production of MesoPher is performed according to DC immunotherapy protocols that are approved by the ethics committee (NL24050.000.08, NL44330.000.14, NL62105.000.17, NL67169.000.18, NL76592.000.21). Every vaccination consists of around $25{\times}10^6$ autologous mo-DCs pulsed with the allogeneic mesothelioma tumour cell line lysate, all produced under Good Manufacturing Practice (GMP)-certified conditions, as described earlier.[22 31] Quality control testing will be performed before MesoPher release. The manufacturing process of MesoPher takes approximately 6 weeks. During this bridging phase, patients who experience symptoms from their disease or those that are considered to be rapidly progressive can receive two bridging chemotherapy cycles with gemcitabine and Nab-Paclitaxel or monotherapy gemcitabine, by decision of the treating oncologist. After the optional and patient-dependent bridging therapy, a baseline CT-scan and a biopsy of an accessible tumour lesion will be performed.

Treatment phase

Within 2 weeks after the bridging-chemotherapy and regardless of response, all fit-for-treatment patients will start with immunotherapy. MesoPher and mitazalimab will be administered consecutively in 1 day, three times, biweekly. After the third treatment, booster vaccines will be given after three and 6 months. MesoPher is administered at a fixed dosage of $25 * 10\wedge 6$ DCs, and two-thirds will be injected intravenously and one-third intradermally. Mitazalimab will be infused at a cohort-dependent dosage. A follow-up CT scan and tumour biopsy will be performed after three study treatments. Subsequent CT scans to monitor clinical activity will be performed every 6–8 weeks. Response will be evaluated according to the Response Evaluation Criteria in Solid Tumours (RECIST) (V.1.1) and iRECIST criteria.[32] Study treatment will be halted prematurely when patients have radiological and clinical progressive disease during treatment or if unacceptable toxicity occurs. Some radiological progression without clinical deterioration can allow for continuation of the study treatment, in the absence of other treatment options. Peripheral blood collection will be done at baseline and several time points following treatment for immunomonitoring.

A traditional 3+3 design will be used to determine the maximum tolerated dose (MTD) of mitazalimab within the MesoPher/mitazalimab combination treatment (figure 2). In short, DLTs will be evaluated in three dose-level cohorts. This rule-based design allows dose escalation if no DLT is found in three patients, or if one DLT is found in six patients. In all other cases, dose escalation is stopped and the MTD is found in the previous cohort. Furthermore, the MTD cohort will include at least six evaluable patients. When two DLTs are found in the first three patients in the starting cohort (Dose level 1), de-escalation is required. The first cohort starts at a dose of 300 µg/kg mitazalimab, and depending of found toxicity dose is halved or doubled (table 1). In this study, a minimum of 12 and a maximum of 18 subjects will be included.

The first cohort will start at dose level 1. When more than one DLT is found at the first level, we will go to level −1. When 0/3 or 1/6 patients experience a DLT, we will proceed to the next level.

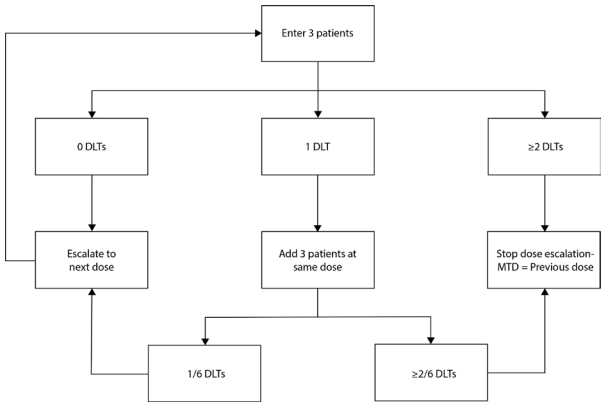

Figure 2 3+3 dose-escalation study design. DLTs, dose-limiting toxicities; MDT, maximum tolerated dose.

Eligibility criteria

Written informed consent according to International Conference on Harmonisation/Good Clinical Practice (ICH-GCP), together with a trained physician, must be given before study treatment is started. The informed consent form, written in Dutch, is provided as online supplemental appendix A. Adult patients with pancreatic cancer with radiologically suspect metastatic lesions and progressive disease on first-line (modified) FOLFIRINOX are eligible for inclusion. Also, an accessible metastatic lesion for histological tissue analysis and immunomonitoring is required and patients must have a WHO performance status of 0–1. Exclusion criteria include abdominal ascites, (previous) use of anti-CD40 agonistic antibodies and/or antitumour vaccinations, use of immunosuppressive drugs, autoimmune disease, organ allograft or active infection. All inclusion and exclusion criteria are listed in online supplemental table 2.

Study end points

The primary objective of this study is determining the toxicity and tolerability of MesoPher/mitazalimab combination immunotherapy for patients with progressive metastatic pancreatic cancer. This will be determined by the frequency of DLTs. Toxicity will be scored according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.[33] Toxicities occurring within 6 weeks after the first vaccination will be considered a DLT (ie, the DLT observation period). All grade 3 or higher adverse events are considered a DLT, except for the toxicities listed in Box 1. Secondary endpoints include radiological responses as defined by RECIST V.1.1 and iRECIST criteria, and the assessment of immune responses. The detection of immune responses will be assessed on multiple levels; vaccine-induced delayed type hypersensitivity (DTH) testing, immune-monitoring of various peripheral immune cell subsets on transcriptomic and protein level, and the detection of antitumour responses.

Box 1 Grade 3 toxicities not considered as dose-limiting toxicity (DLT)

Any grade 3 or higher toxicity will be considered a DLT with the exception of the following toxicities

Haematological toxicity

⇒ Thrombocytopaenia grade 3 lasting less than 7 days days.

⇒ Neutropenia grade 3 lasting less than 7 days days without neutropenic fever.

⇒ Alanine aminotransferase increased grade 3 resolved within 7 days days to grade 1.

⇒ Alkaline phosphatase increased grade 3 resolved within 7 days days to grade 1.

⇒ Aspartate aminotransferase increased grade 3 resolved within 7 days days to grade 1.

⇒ Blood bilirubin increased grade 3 resolved within 7 days days to grade 1.

Non-haematological toxicity

⇒ Grade 3/4 diarrhoea, nausea, vomiting, hypertension if not adequately treated.

Immune-related toxicity

⇒ Cytokine release syndrome (CRS)/infusion-related reactions (IRR) will be scored according to the ASTCT guidelines*. Any grade 3 or higher CRS/IRR will be considered a DLT. Except for grade 3 CRS/IRR if resolved to a lower grade within 24 hours after the onset of symptoms.

⇒ For immune-related toxicities, we will exclude hypo/hyperthyroidism as a DLT.

⇒ Immune-related skin toxicity that is adequately treated with topical therapy will not be considered a DLT.

Laboratory assessments

⇒ Any grade 3 laboratory abnormalities that are asymptomatic and clinically not significant are not considered DLT.

*Grade 1 = Fever, with or without constitutional symptoms. Grade 2 = Hypotension responding to fluids. Hypoxia responding to <40% FiO2. Grade 3 = Hypotension managed with one pressor. Hypoxia requiring ≥40% FiO2. Grade 4 = Life-threatening consequences; urgent intervention needed

Table 1 MesoPher and mitazalimab treatment doses

Dose level	MesoPher (DCs)	Mitazalimab (µg/kg)
–2	25*10^6	75
–1	25*10^6	150
1	25*10^6	300
2	25*10^6	600
3	25*10^6	1200

DC, dendritic cell.

Vaccine-specific response

Keyhole limpet haemocyanin (KLH) is part of the DC vaccine and is known to induce a specific adaptive immune response readily detectable in serum and peripheral blood mononuclear cells (PBMCs) of vaccinated individuals. Humoral responses after vaccination will be detected using a ELISA. Cellular responses to KLH will be measured in vitro. KLH pulsed DCs will be co-cultured with PBMCs taken before-and-after treatment. After a 24 hours coculture, T cells will be stained for activation-,

cytotoxic- and degranulation markers and measured by flow cytometry.

Immune-monitoring of peripheral immune cell subsets

Phenotypical analysis of PBMCs will be conducted with Aurora spectral flow cytometry. Liquid nitrogen-stored PBMCs will be stained with antibodies and measured by flow cytometry. These experiments allow to investigate treatment-induced changes in the frequencies of immune cell subsets that represent distinct lineages and/or express different levels of activation, differentiation and cosignalling markers. In addition, 1 mL of whole blood will be freshly measured by flow cytometry to characterise different immune cell populations before and after treatment.

Modulation of gene expression levels

Gene expression of 770 immune-related genes will be investigated. RNA pellets of PBMCs will be measured by Nanostring Technologies using the PanCancer Immune Profiling Panel to investigate treatment induced changes in the RNA levels.

Antitumour responses

We will perform paired biopsies of all patients at baseline and after three treatments, preferably from the same tumour location, to detect antitumour responses. Two biopsies will be taken at one timepoint. One will be formalin-fixed and paraffin-embedded (FFPE) by our pathology department. The pathologist will determine if there are cancerous cells, and post-treatment signs of treatment effect will be evaluated. FFPE tissues will be used to measure RNA expression levels using Nanostring Technologies (PanCancer Immune Profiling Panel) to investigate treatment related effects at tumour site on RNA level. Also, we will use the Digital Spatial Profiler by Nanostring Technologies to investigate immune-infiltration in the tumour on protein level. Another biopsy will be freshly processed to single cell suspensions and will be freshly measured using flow cytometry. In addition, in patients where we are not able to perform a post-study treatment biopsy, a DTH reaction to MesoPher will be assessed. When this DTH skin test is positive (≥2 mm induration), a skin biopsy will be taken. Biopsies will be used for in situ immunostainings of that is, DCs, myeloid derived suppressor cells and CD8 +T cells.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research

Ethics and dissemination

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (64th version, October 2013) and are consistent with the ICH/GCP guidelines, applicable regulatory requirements. The investigator must also comply with all applicable privacy directives and regulations (eg, EU Data Protection Directive 95/46/EC). Both the Central Committee on Research Involving Human Subjects (CCMO; NL76592.000.21) and the Medical Ethics Committee (METC; MEC-2021-0566) of the Erasmus MC University Medical Center Rotterdam approved the conduct of the trial. Protocol version 3, date 27 May 2021 was approved. Substantial changes in trial conduct will be proposed to the ethical committee with a substantial protocol amendment. The ethical committee needs to approve this amendment before changes in trial conduct will be implemented. The results of this clinical trial will be submitted for publication in a peer-reviewed scientific journal. All data will be collected, captured and analysed according to the rules of the Erasmus MC University Medical Center Rotterdam. A Trial Master File and an Investigator Site File is kept. Data will be captured in the cloud-based clinical data management platform Castor. The database is accessible for the researchers, the trial monitor and data mangers. All serious adverse events will be reported to the Ethical Committee and to Alligator Bioscience, producer of the mitazalimab. Serious adverse events that are considered to be related to MesoPher treatment will be reported to Amphera. The investigators will provide a monthly update to Alligator Bioscience and Amphera about the trial conduct. Written informed consent will be required for all participants.

Trial timeline and status

Dutch law (WMO = Medical Research Involving Human Subjects Act) states that it is mandatory to obtain ethical approval for clinical trials before start of study. Since a special Advanced Therapy Medicinal Product is investigated in the REACtiVe-2 trial, approval first from the central CCMO followed by the local METC is required. Date of approval from the central and local committee is 13 July 2021 and 20 July 2021, respectively. The REACtiVe-2 trial is prospectively registered at the WHO-acknowledged Netherlands Trial Register (NTR). The NTR is currently transitioning to the CCMO register. Our official date of approval/registration as determined by Dutch law is 20 July 2021. We are currently recruiting the first patients. We aim to include all patients by the end of 2022. The first safety data will be available the same year.

DISCUSSION

Although DC-based platforms may introduce tumour-specific T cells able to mount effective immune responses against occult disease lacking desmoplastic stroma, established PDAC requires a rational multimodal treatment regime. The REACtiVe-2 trial was initiated on the promises of preclinical immune and survival results. In this study, we will determine the MTD of mitazalimab in the MesoPher/mitazalimab combination treatment in patients with metastasised pancreatic cancer with progressive disease on first-line (modified) FOLFIRINOX. In addition, clinical responses through radiological assessment and the detection of treatment-induced immune

responses will be evaluated. This is the first clinical trial investigating anti-CD40 agonistic antibodies combined with DC vaccination in PDAC patients. In a previous dose-escalation trial, we have demonstrated that MesoPher should be administered at an amount of 25 ∗ 10∧6 DCs.[22] At this dose, clinical activity was found in mesothelioma patients. This number of DCs has also been implemented in the REACtiVe Trial treating resected PDAC patients. Although it has not been demonstrated that this dosage is optimal for PDAC patients, we do find promising results in the REACtiVe Trial. At this dosage, we found vaccine-induced tumour-specific T-cell response. Moreover, we did not observe any serious toxicity. It is common practice in DC immunotherapy to inject the cells both intravenously and intradermally. In our previous DC vaccination trial, vaccinations were also given both intradermally and intravenously. This strategy induced robust immune responses[22] (Lau *et al*, 2022, Eur J Cancer, manuscript accepted). Two different routes of administration are used in an attempt to maximise the interaction between T cells and DCs in different lymphoid compartments and to maximise the subsequent homing patterns of the activated T-cells to increase the quality and quantity of the antitumour immune response. Therefore, this dosage and route of administration will be adopted in the REACtiVe-2 trial. In the phase 1 dose-escalation study for mitazalimab, intravenous doses up to 1200 µg/kg were considered well tolerated with manageable side effects in patients with advanced solid tumours.[34] Since this trial did not include PDAC patients and prior combination with antitumour vaccinations has not been done, we will titrate mitazalimab in this immunotherapy combination regimen for PDAC patients.

It should be noted that patients with cancer treated with immunotherapy may demonstrate initial transient tumour growth as a result of intratumoural immune cell influx and inflammation.[35] This process called pseudo-progression does not reflect true disease progression and may lead to premature discontinuation of effective treatment. Therefore, we will also incorporate the iRECIST criteria and initial radiographical progression can allow for continuation of study treatment in the absence of clinical deterioration.

A limitation of this study is the relatively small number of patients we will include and the single-armed nature of the trial which complicates analysing clinical efficacy. However, this design and sample size should be sufficient for dose finding. We are aware that finding a MTD for this combination therapy may differ from the minimal effective dose given the pleiotropic nature of CD40 stimulation. When the combination treatment is safe, we will progress to a larger phase II clinical trial to further investigate the immunological responses and clinical efficacy.

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Contributors SPL and FRv'tL drafted this manuscript. SPL, FRv'tL, SHvdB, MYVH, MPL, JGJVA and CvE drafted the original study protocol and revised the manuscript. JGJVA and CvE acquired funding for implementation of the trial protocol. MPL is the principal investigator. All authors contributed to the final manuscript and agreed to all of the content of the submitted manuscript.

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Competing interests MPL: Grants to Institute: JnJ, Astellas, MSD, Sanofi. Consulting or Advisory Role: Roche, Bayer, Amgen, JnJ, Sanofi, Servier, Pfizer, Incyte, Novartis, Pan-Cancer T. J.G.J.V.A: Stock or other Ownership: Amphera. Consulting or Advisory Role: Eli-Lilly, MSD Oncology, Bristol-Myers Squibb, Roche, AstraZeneca. Rest of the authors have no relationship to disclose in relation to the submitted work.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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