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## Clinical Trial

# Autologous dendritic cells pulsed with allogeneic tumour cell lysate induce tumour-reactive T-cell responses in patients with pancreatic cancer: A phase I study



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**KEYWORDS**

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**Abstract Background:** Pancreatic ductal adenocarcinoma (PDAC) is notorious for its poor prognosis even after curative resection. Responses to immunotherapy are rare and related to inadequate T-cell priming. We previously demonstrated the potency of allogeneic lysate-dendritic cell (DC) vaccination in a preclinical model. Here we translate this concept to patients.

**Methods:** In this phase I study, patients with resected PDAC were included when they demonstrated no radiologic signs of recurrence after standard-of-care treatment. Allogeneic tumour lysate-loaded autologous monocyte-derived DCs were injected at weeks 0, 2, 4 and at months 3 and 6. Objectives are feasibility, safety and immunogenicity of allogeneic tumour-DCs. The presence of tumour antigens shared between the vaccine and patient tumours was investigated. Immunological analyses were performed on peripheral blood, skin and tumour.

**Results:** Ten patients were included. DC production and administration were successful. All patients experienced a grade 1 injection-site and infusion-related reaction. Two patients experienced a grade 2 fever and 1 patient experienced a grade 3 dyspnoea. No vaccine-related serious adverse events were observed. Shared tumour antigens were found between the vaccine and patient tumours. All evaluated patients displayed a vaccine-induced response indicated by increased frequencies of Ki67+ and activated PD-1+ circulating T-cells. In addition, treatment-induced T-cell reactivity to autologous tumour of study patients was detected. Seven out of ten patients have not experienced disease recurrence or progression at a median follow-up of 25 months (15–32 months).

**Conclusion:** Allogeneic tumour lysate-DC treatment is feasible, safe and induces immune reactivity to PDAC expressed antigens.

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## 1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related death and its incidence is rising [1–3]. Prognosis is poor and the 5-year survival is less than 10% [1]. The majority of patients with PDAC present with either locally advanced or metastatic disease, with only 10–20% of patients eligible for curative-intent surgery [4,5]. However, even after surgical resection, long-term survival is exceptional in the majority of patients [6–8]. Therefore adjuvant chemotherapy is now considered standard-of-care. However, the median overall survival of patients with resected PDAC after receiving adjuvant gemcitabine treatment is 19.8 months [9]. However, this regimen is nowadays considered out of date as the ESPAC-4 trial demonstrated that adjuvant gemcitabine with capecitabine is superior to gemcitabine monotherapy [10]. In 2018, the PRODIGE-24/CCTG PA.6 trial showed that adjuvant FOLFIRINOX is superior to adjuvant gemcitabine [11]. In the era of these improved multi-agent systemic therapy improvements in survival have been achieved, however, still 70–80% of patients will develop tumour recurrence within 5-years and therefore novel treatment modalities are still urgently needed [12]. Although immunotherapy demonstrated impressive results in various malignancies, immune-checkpoint inhibitors like PD-1 failed to show the improvement of survival [8,13,14] and as such PDAC is considered a non-immunogenic tumour [15–17]. Recent seminal studies implementing rational

immunotherapeutic strategies achieved disease control in PDAC demonstrating the importance of resurrecting immunogenicity [18,19]. In PDAC, T-cell dysfunction and exclusion have been proposed to be paramount [20,21].

Dendritic cells (DCs) are potent activators of the immune system and can successfully be used to induce tumour immunity [22]. DC paucity in PDAC leads to dysfunctional immune surveillance, and it has been shown that restoring DC numbers in early PDAC lesions reinvigorates anti-tumour T-cell immunity [23]. Several DC-vaccination trials have previously demonstrated clinical and immunological responses in PDAC [24–26]. These DC-based vaccines exploit synthetic peptides, purified proteins or DNA/RNA making the detection of immunodominant epitopes imminent. We have demonstrated the rationale, safety and clinical efficacy of an allogeneic-tumour lysate-based DC therapy (MesoPher) in patients with malignant mesothelioma (MM) [22]. An allogeneic tumour lysate has several advantages as this is an off-the-shelf source of various tumour-associated antigens that can be shared across different tumour types, it eliminates the need for obtaining autologous tumour material, a known major logistical hurdle, and it provides treatment standardisation across patients. Also, the use of lysate containing a broad repertoire of tumour-associated antigens including cancer-testis and tumour-differentiation antigens may avoid tumour-immune escape which has been described for single-peptide strategies [27,28]. The

MesoPher platform consists of autologous DCs loaded with an allogenic tumour cell lysate generated from MM cell lines [22]. It has been demonstrated that PDAC and MM share tumour antigens like mesothelin, WT1, Survivin [29–31]. We recently showed that the mesothelioma lysate-loaded DCs induced clinically effective tumour-specific T-cell responses in a murine PDAC model due to shared tumour antigens across MM and PDAC [32]. Therefore, in this study, we investigated this allogeneic lysate-DC strategy for feasibility and immunogenicity in patients with PDAC.

Immunologically, the detrimental survival of PDAC is markedly accounted for the formation of ubiquitous acellular matrix present in the solid tumour. The desmoplastic stroma is able to physically exclude and impair trafficking of T cells, thereby impeding its effector function [33]. We postulate that DC therapy is able to induce adequate anti-tumour immunity against occult metastatic disease before the process of desmoplasia has been initiated. Therefore, in this study, we exclusively focused on patients with surgically resected PDAC who are clinically and radiologically free of local disease recurrence.

Here, we report on feasibility, safety and immune-reactivity of MesoPher treatment in patients with resected PDAC. We determined overlap in tumour antigens between MesoPher and the autologous tumours of patients with resected PDAC. Furthermore, we analysed therapy-induced T-cell activation, and an *in vitro* co-culture assay was performed to assess the induction of autologous tumour-specific T cells.

## 2. Methods

### 2.1. Study design and participants

The REACTiVe (Rotterdam pancrEAtic Cancer Vaccination) Trial is a single-centre, non-randomised, open-label safety phase I study for patients aged 18 years or older with surgically resected and histologically proven PDAC who have completed standard-of-care treatment. Additional eligibility criteria were an Eastern Cooperative Oncology Group performance status score of 0–2, normal organ function and adequate bone marrow reserve (absolute neutrophil count  $> 1.0 \times 10^9/L$ , platelet count  $> 100 \times 10^9/L$ , and Hb  $> 6.0$  mmol/L), and a positive delayed-type hypersensitivity (DTH) skin test (induration  $> 2$  mm after 48 h) against the positive control antigen tetanus toxoid. Patients were excluded if: residual disease was present at the time of inclusion, previously treated with immunomodulatory anticancer drugs, a history of autoimmune disease, organ allograft, malignancy (except adequately treated basal cell or squamous cell skin cancer, superficial or in situ bladder

cancer or other cancer for which the patient has been 5-years disease-free) or used immunosuppressive therapy. A detailed list of inclusion and exclusion criteria can be found in the clinical trial protocol attached in the supplementary material.

The study was approved by the Central Committee on Research involving Human Subjects (NL67169.000.18) 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 Netherlands Trial Register, NL7432. A informed written consent was obtained from each subject.

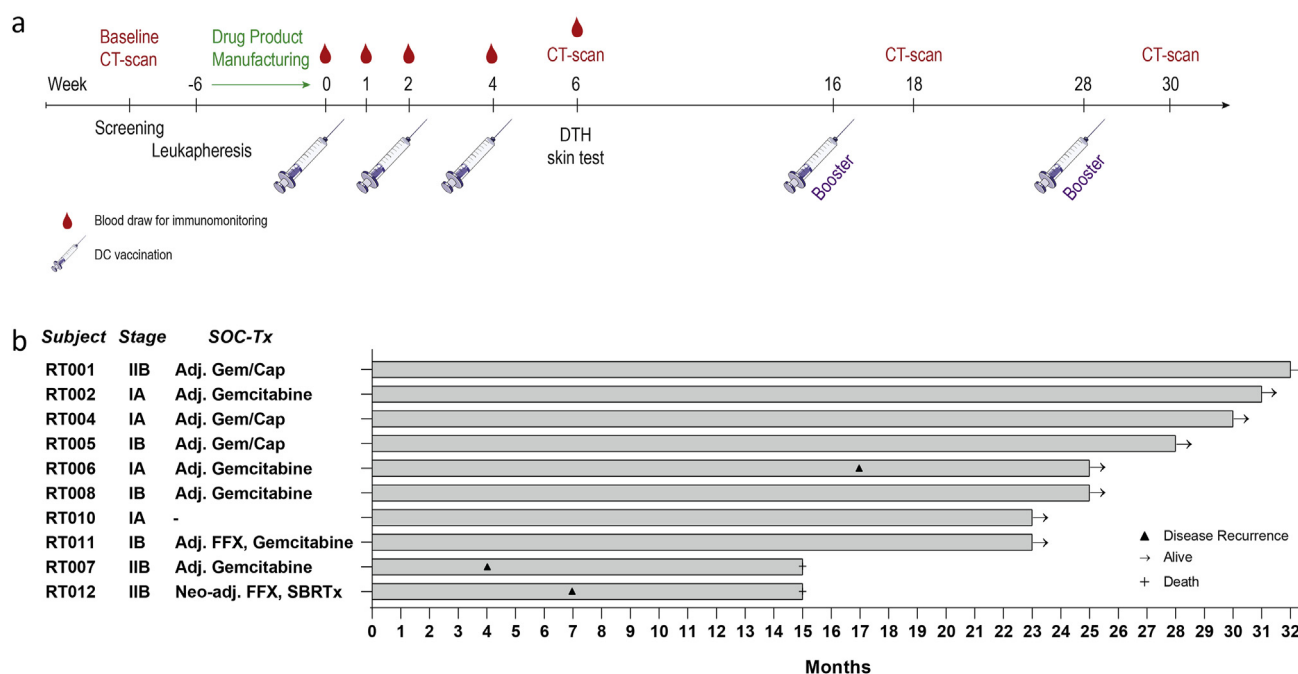
### 2.2. Procedures

Monocytes for DC (mo-DC) production were retrieved via leukapheresis. Every vaccination consists of  $25 \times 10^6$  autologous mo-DCs pulsed with the allogeneic tumour cell line lysate PheraLys, all produced under good manufacturing practice-certified conditions, as described previously [22]. MesoPher is injected 3 times every 2 weeks. After the 3rd injection, a DTH skin test was performed with MesoPher ( $4 \times 10^6$  DCs), and booster vaccinations are given after 3 and 6 months. Therapy is administered two-thirds intravenously and one-third through intradermal injection, as proposed earlier [34]. Blood draws for immunomonitoring done before every main vaccination and one week after the first vaccination and 2 weeks after the third vaccination (Fig. 1a).

Tumour load was radiographically assessed with a CT-thorax/abdomen every three months starting from screening until the end of the study by the radiologist and reported per RECIST v1.1 criteria. Patients underwent follow-up using CT scans examinations every six months or when recurrence was suspected. Safety assessments were done at each study visit including vital signs and laboratory testing. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.03.

### 2.3. Outcomes

The primary objective is feasibility of MesoPher vaccination, as determined by the success of leukapheresis and MesoPher production, and the ability to vaccinate according to the predefined study schedule. Secondary objectives were clinical outcome as determined by overall and progression-free survival, safety according to National Cancer Institute Common Terminology Criteria for Adverse Events, and immunogenicity as detected by DTH skin reactions, peripheral blood T-cell activation and the capacity of T cells to respond to stimulation with MesoPher and/or autologous tumour cell-derived antigens.



**Fig. 1. Treatment schedule REACTiVe Trial and swimmer plot of study patients.** (a) Red droplets indicate blood draws for immunomonitoring. Syringes indicate DC vaccination. Blood draws were taken before immunotherapy. (b) Swimmer plot representing survival of patients since date of inclusion. Tumour stage according to the AJCC Cancer Staging Manual (8th Edition) and standard-of-care treatment is presented per individual patient. Stable disease is depicted as a square, disease recurrence with a triangle, death with a cross and alive with an arrow. Abbreviations: SOC-Tx, standard-of-care treatment; Adj., adjuvant; Neo-adj., neo-adjuvant; Gem/Cap, gemcitabine/capecitabine; FFX, FOLFIRINOX; SBRTx, stereotactic-body radiotherapy. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

## 2.4. Statistical analysis

The primary objective was feasibility. The study was considered positive when eight out of ten patients were able to undergo the whole treatment. Paired Wilcoxon signed-ranks tests were used to test for significance between baseline measurements and other time points. Flow-cytometry data were normalised for baseline. Figures were made using GraphPad Prism software v8.0. Gene-expression data were corrected for multiple testing using Benjamini–Hochberg procedure. Progression-free survival and overall survival were calculated from inclusion to the first documented event. Survival data were plotted as Kaplan–Meier survival curves, and log-rank testing was performed to compare cohorts. In all cases, a  $p$ -value of 0.05 and below was considered significant (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*) as highly significant.

Material and methods concerning the immunological experiments can be found in the supplementary.

## 3. Results

### 3.1. Patient characteristics

Ten patients with surgically resected PDAC who had completed standard-of-care treatment were recruited

between February 2019 and February 2020. Study patients were treated as indicated in Fig. 1a. Patient characteristics are summarised in Table 1. The median age at study entry was 64 years (range 47–81 years). In eight of ten patients the performance status score was 0, and two others had a performance status score of 1 and 2. All patients had a tumour stage of I or II, and all except one patient had a microscopically margin-negative (R0) resection. Eight patients received adjuvant chemotherapy and one neoadjuvant chemoradiation therapy. One patient did not receive (neo) adjuvant treatment as she was deemed unfit for chemotherapy by the treating oncologist. The median time from finalising standard-of-care treatment to inclusion was 3.5 months (range 1–12 months).

### 3.2. Feasibility

Feasibility was assessed for all ten patients. For eight of the ten patients, one leukapheresis was required to produce all five MesoPher vaccinations. In two patients (RT002, RT004), the first leukapheresis had to be interrupted because of venous flow problems. RT002 required a third leukapheresis to produce the 4th and 5th vaccine. All drug products passed quality control and sterility testing (Sup. Table 4). Also, intravenous and intradermal administration of study

drug were performed successfully (Sup. Table 5). Nine of the ten patients received all five treatments. RT012 received four vaccinations due to disease progression.

### 3.3. Safety and toxicity

Safety and toxicity was assessed for all ten patients. No significant clinical changes in vital signs within 2 h after MesoPher administration were observed (Sup. Fig. 1). In some patients, a non-clinically relevant drop in systolic or diastolic blood pressure was observed. This was potentially due to the 2 h of obligatory inactive

observation period. No complaints or clinical signs of distress were reported during vaccination or in the observation period. After vaccination, all patients experienced a grade 1 injection-site reaction (ISR) consisting of erythema (100%), local pruritus (60%), local pain (10%), skin induration (100%), and/or warmth (20%) (Sup. Table 6). All patients also experienced an infusion-related reaction (IRR). Grade 1 IRRs consisted of chills (80%), fatigue (100%), fever (70%), headache (10%), hot flashes (10%), malaise (20%), myalgia (50%), pruritus (10%), vertigo (10%), vomiting (10%). One patient experienced grade 3 dyspnoea following study treatment. Two patients had a grade 2 fever after vaccination. In general, ISR and IRR events lasted for 1–2 days.

No serious adverse events related to MesoPher treatment were reported during the study. One patient experienced a study treatment-unrelated serious adverse events (dyspnoea) requiring hospitalisation. The patient is known with a history of chronic obstructive pulmonary disease and the event (exacerbation of chronic obstructive pulmonary disease) occurred between study treatments. All adverse events during the study are listed in Sup. Table 6.

### 3.4. Clinical outcome

Median overall survival and progression-free survival were not reached at the time of data cut-off (November 2021). No local disease recurrence or any tumour progression was observed in seven of the ten patients with a median follow-up of 25 months (range 15–32 months). Eight patients were disease-free at 12 months (Fig. 1b). Three patients experienced recurrence of disease at the time of data cut-off. RT007 and RT012 died 11 and 8 months after disease progression, respectively. RT006 is 8 months alive after progression without subsequent treatment. During the follow-up of RT002, after completing MesoPher treatment, a solitary pulmonary nodule with a diameter of 7.2 mm was found for which a resection was performed. Retrospectively, this nodule was present at baseline with a diameter of 2.3 mm. Pathological examination revealed that this lesion was a metastatic lesion. Currently, 12 months after video assisted thoracic surgery, RT002 shows no evidence of recurrent disease.

### 3.5. MesoPher and PDAC share known tumour antigens

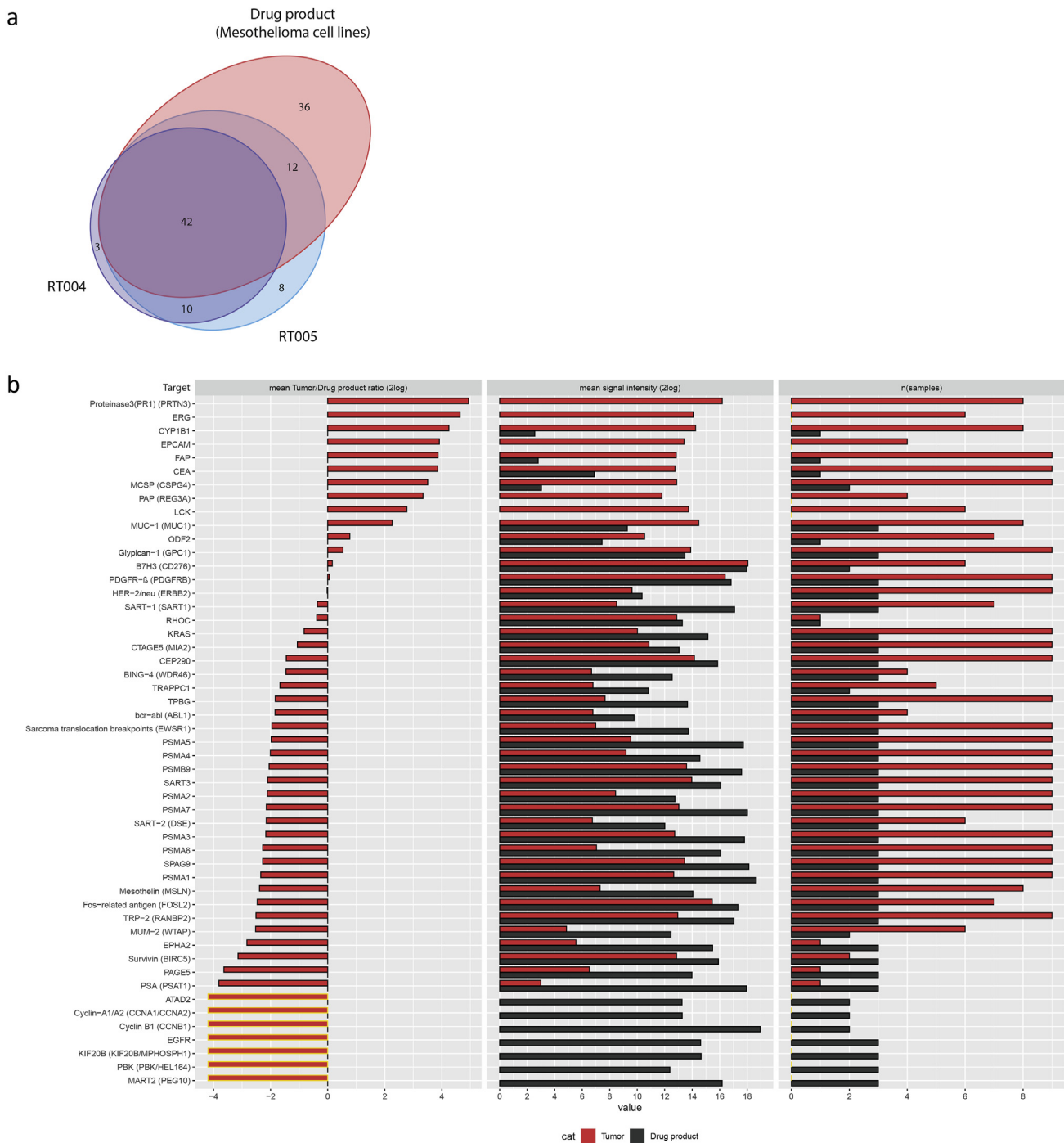
To explore the presence of shared tumour antigens between the drug product and PDAC, we compared the mRNA expression of known tumour antigens (Sup. Table 2) between the five mesothelioma cell lines utilised in MesoPher and autologous tumour cells of study subjects. A total of 111 known tumour antigens were detected (Fig. 2a, Sup. Table 7), 42 of which were shared between the cell lines and patient

Table 1

Demographic and baseline characteristics of patients in the study population.

| Characteristics                       | Patients (N = 10) |
|---------------------------------------|-------------------|
| Age (year)                            |                   |
| Median                                | 64                |
| Range                                 | 47–81             |
| Gender (N)                            |                   |
| Male                                  | 4                 |
| Female                                | 6                 |
| Ethnicity (N)                         |                   |
| Caucasian                             | 9                 |
| Arab-Berbers                          | 1                 |
| ECOG performance status score (N)     |                   |
| 0                                     | 8                 |
| 1                                     | 1                 |
| 2                                     | 1                 |
| Tumour stage (N)                      |                   |
| IA                                    | 4                 |
| IB                                    | 3                 |
| IIA                                   | 0                 |
| IIB                                   | 3                 |
| Pancreatic tumour location (N)        |                   |
| Head                                  | 6                 |
| Body                                  | 1                 |
| Tail                                  | 3                 |
| CA 19-9 at the time of inclusion (N)  |                   |
| <90 U/ml                              | 10                |
| ≥90 U/mL                              | 0                 |
| Surgery (N)                           |                   |
| Pancreaticoduodenectomy               | 7                 |
| Distal pancreatectomy and splenectomy | 3                 |
| Status of surgical margins (N)        |                   |
| R0                                    | 9                 |
| R1                                    | 1                 |
| Additional treatment (N)              |                   |
| Neo-adj. FOLFIRINOX/SBRTx             | 1                 |
| Adj. Gemcitabine                      | 4                 |
| Adj. gemcitabine/capecitabine         | 3                 |
| Adj. gemcitabine/FOLFIRINOX           | 1                 |
| Time since SOC treatment (mos.)       |                   |
| Median                                | 3.5               |
| Range                                 | 1–12              |

Abbreviations: ECOG, Eastern Cooperative Oncology Group; CA19-9, Carbohydrate antigen 19-9; FOLFIRINOX, 5-fluorouracil + leucovorin + irinotecan + oxaliplatin; SBRTx, Stereotactic Body Radiation Therapy; SOC, Standard-of-Care. Tumour stage was assessed according to the AJCC Cancer Staging Manual, 8th Edition.



**Fig. 2. Autologous tumours of study patients and MesoPher demonstrated shared tumour antigens.** (a) Venn diagram of number of identified tumour antigens on transcriptome level between RT004, RT005 and mesothelioma tumour cell lines used in MesoPher. (b) Mass spectrometry analysis on nine tumour samples; Waterfall plot of mean Tumour lysate/Drug product ratio (left), mean measured signal intensity of the tumour antigen (mid), and number of samples in which tumour antigens was identified (right). Yellow marking indicates that no peptide of the tumour antigen was detected above the threshold of  $S/N \geq 10$  for PDAC samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article). PDAC, pancreatic ductal adenocarcinoma.

tumours. Subsequently, the presence of shared tumour antigens was evaluated at protein level analysing MesoPher and PDAC samples of nine patients. The presence of 51 known tumour antigens (Fig. 2b; Sup. Table 8), within 163 identified peptide

sequences (Sup. Fig. 2), was detected. In total, 39 of the 51 proteins were shared between autologous tumours and MesoPher (Fig. 2b right column), confirming the potential of the vaccine to induce PDAC-reactive immune reactivity.

### 3.6. MesoPher vaccination induces T-cell activation

All patients developed a positive delayed-type hypersensitivity skin reaction to MesoPher post-vaccination (Fig. 3).

We first performed broad gene-expression profiling of peripheral blood cells to evaluate the induction of specific immune responses following therapy. This demonstrated the upregulation of various T-cell activation markers (e.g. *CD28*, *ICOS*, *TNFRSF4*) after the first vaccination (Fig. 4a, b). Other genes upregulated two weeks after treatment include *CCR4*, *TCF7*, *USP9Y*, *JAK3*, *CCR7*, *FLT3LG*, and *IL11RA*. To confirm the presence of T-cell activation at protein level, we performed multi-parameter flow cytometry in circulating immune cells at multiple time points (Sup. Fig. 3). A transient increase in absolute numbers of CD3+ and CD4+ T cells was observed after DC vaccination (Fig. 4c). The percentages of CD4+ non-regulatory T cells expressing HLA-DR+, ICOS+, Ki67+ and/or PD-1+Ki67+ frequencies increased early after vaccination (Fig. 4d), while the percentage of CD4+ T cells expressing markers of T-cell inhibition (i.e. TIM-3, CTLA-4, LAG-3) did not (Sup. Fig. 4). No overt changes were found in the proportions of naïve, memory, and effector-cell subpopulations (Fig. 4e). To demonstrate vaccine-specific activation, analysis of T cell receptor (TCR)- $\beta$  repertoires of the T cells isolated from both MesoPher-challenged skin and blood at week 5 of 3 patients was performed. This revealed an increase in the fraction of shared TCRs post vaccination. Although not observed in CD8+ T-cells, an enrichment of shared TCRs was found in the CD4+PD-1+ (activated T cells) non-Treg compartment compared to CD4+PD-1– T-cell fractions (Fig. 4f; Sup. Table 9).

### 3.7. MesoPher stimulated T cells recognise autologous tumour-derived antigens

Six patients had sufficient study material (i.e. mo-DCs, PBMCs, and autologous tumour material) to perform an *in vitro* co-culture assay to assess treatment-directed T-cell responses (Fig. 5a). To investigate the vaccine and tumour reactivity induced by study treatment, peripheral blood lymphocytes isolated before and after treatment were stimulated *in vitro* with MesoPher, autologous tumour lysate-loaded DCs or with non-loaded DCs, and reactivated overnight with DCs. It has been demonstrated that CD137 accurately identifies tumour-specific T-cells [35,36]. In all six tested patients, a MesoPher-specific CD4+ T-cell response, indicated by increased frequencies of CD137+ cells, was detected post-therapy (i.e. week 2, 6) but not before vaccination (i.e. week 0) when peripheral blood lymphocytes were co-cultured with MesoPher and reactivated overnight with MesoPher compared to reactivation with control non-loaded DCs (Fig. 5b). Also, MesoPher-specific

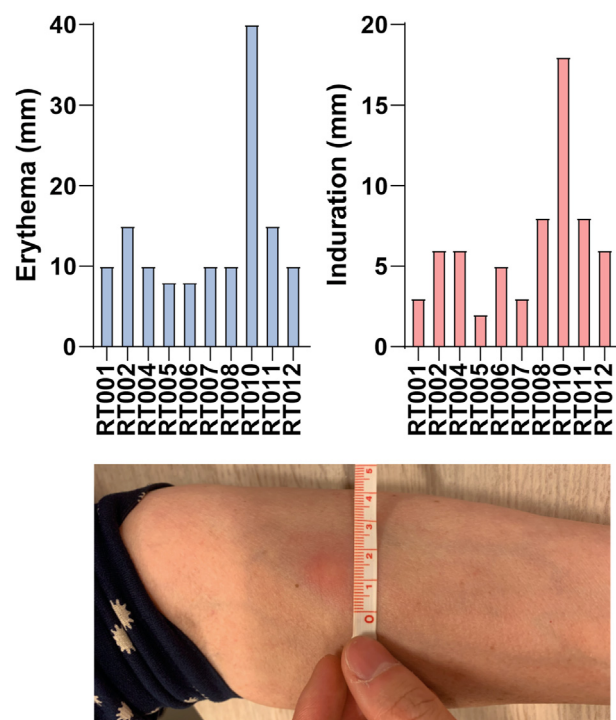
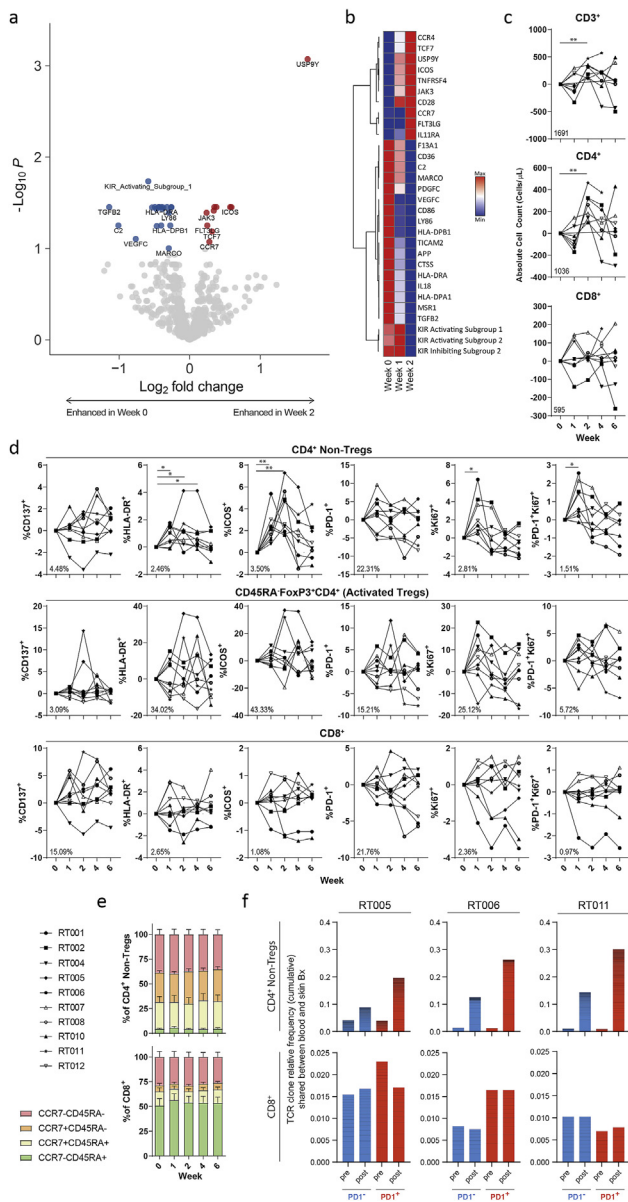


Fig. 3. **Positive delayed-type hypersensitivity skin test following DC vaccination.** A DTH skin test with MesoPher is performed after the third DC vaccination. Bar graphs display erythema and induration following DTH skin test per patient in mm. Photograph illustrates a positive reaction. DC, dendritic cell.

CD8+ T-cell responses were detected in four out of six patients (RT005, RT006, RT008, RT009). When post-treatment PBMCs were co-cultured with MesoPher or autologous tumour-loaded DCs, increased CD137+ frequencies could be observed in three out of five patients (RT006, RT008, RT009) when reactivated with autologous tumour-DCs compared to reactivation with non-loaded DCs. In none of the patients such a response was observed in the pre-treatment samples, or when post-treatment PBMCs were co-cultured with control non-loaded DCs before they were reactivated with autologous tumour-DCs, underlining the presence of a tumour-specific T-cell response.

## 4. Discussion

This is the first-in-human clinical trial, driven by pre-clinical observations, treating patients with PDAC after surgical resection with allogeneic tumour lysate-DC vaccination. MesoPher vaccination therapy was found to be feasible and safe, in line with the previously reported safety data of MesoPher in mesothelioma patients [22]. The primary end-point was reached as all patients were able to receive the three DC vaccinations as planned. This opened the way to an expansion cohort which is currently enrolling to formally assess clinical efficacy [NL67169.000.18].

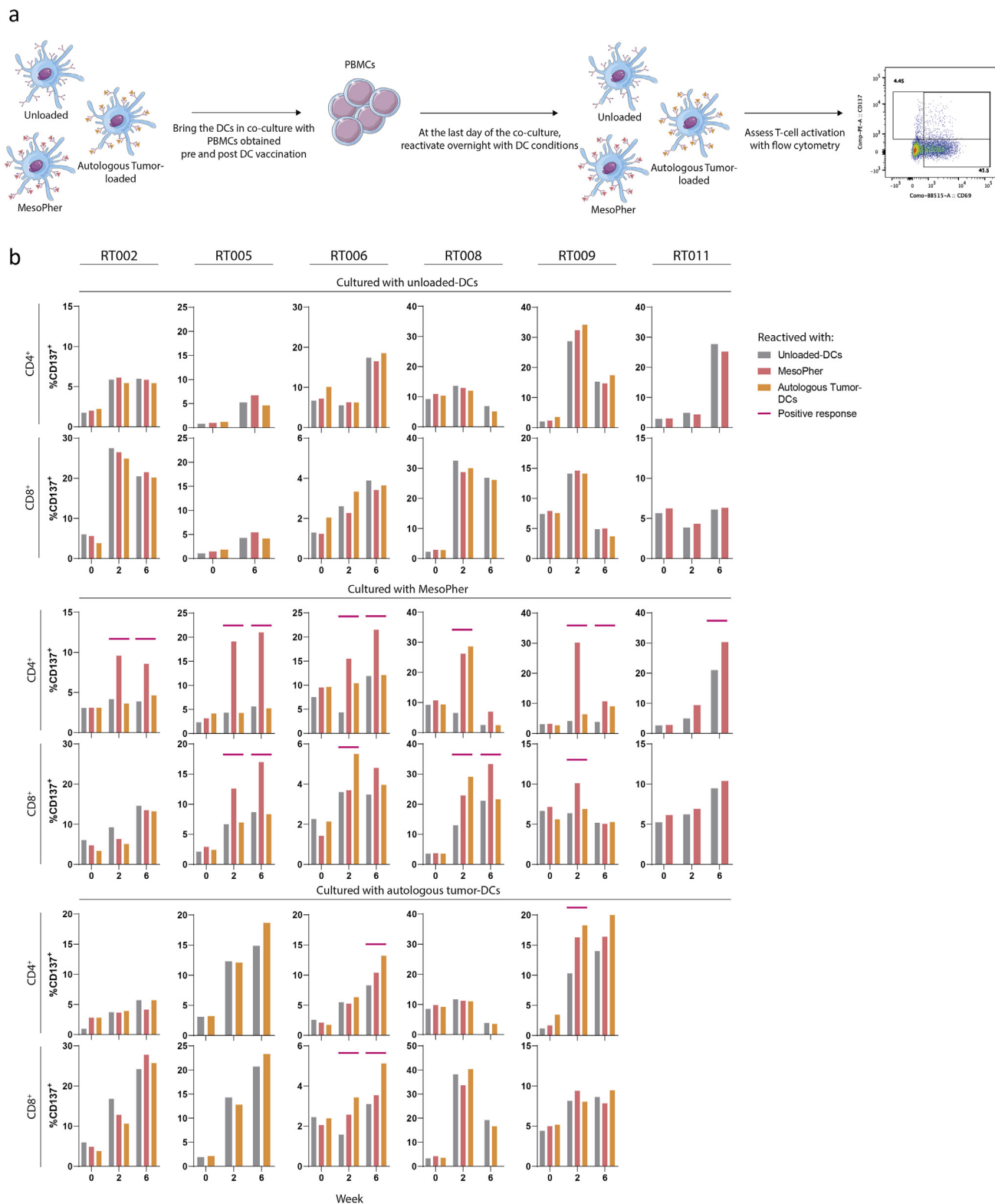


**Fig. 4. MesoPher vaccination induces T-cell activation.** (a) Volcano plot demonstrating genes upregulated at baseline versus 2 weeks after vaccination. Genes with a Benjamini-Hochberg-corrected  $p$ -value  $< 0.1$  were highlighted. (b) Significantly differentially expressed genes (BH-corrected  $p$ -value  $< 0.1$ ) between baseline, week 1 and week 2 are visualised. (c) Number of CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> T cells per uL blood. (d) Percentage of CD137<sup>+</sup>, HLA-DR<sup>+</sup>, ICOS<sup>+</sup>, PD-1<sup>+</sup>, Ki67<sup>+</sup> and PD-1<sup>+</sup>Ki67<sup>+</sup> subsets of CD4<sup>+</sup> Non-Tregs, CD45RA<sup>+</sup>FOXP3<sup>+</sup>CD4<sup>+</sup> Tregs, and CD8<sup>+</sup> cells.  $N = 10$  per group. Data is normalised for baseline (week 0) and paired per patient. Percentage in left corner represent the average frequency at baseline. Significance was determined using the paired Wilcoxon signed-rank test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . (e) Percentage of CCR7<sup>+</sup>CD45RA<sup>+</sup>, CCR7<sup>+</sup>CD45RA<sup>+</sup>, CCR7<sup>+</sup>CD45RA<sup>+</sup> and CCR7<sup>+</sup>CD45RA<sup>+</sup> subsets of CD4<sup>+</sup> Non-Tregs and CD8<sup>+</sup> T cells in peripheral blood. (f) Detection in skin biopsies of TCR $\beta$  clones corresponding to PD-1<sup>+</sup> and PD-1<sup>-</sup> cells in the CD4<sup>+</sup> and CD8<sup>+</sup> T cell compartment shared with blood before (week 0) and after treatment (5 weeks).

Next to feasibility, treatment-induced immunological responses were assessed. MesoPher vaccination was reactogenic as indicated by a positive DTH skin reaction to MesoPher in all patients. Comprehensive multicolour flow cytometry of peripheral blood showed increases in the frequencies of predominantly activated CD4<sup>+</sup> T cells [37]. We also revealed that various memory T-cell subsets displayed a vaccine-induced increase in PD-1<sup>+</sup>Ki67<sup>+</sup> cell frequencies, which may potentially be clinically beneficial as this double-positive population correlates with clinical outcome after immunotherapy [38]. The central memory CD4<sup>+</sup> non-regulatory T-cell compartment showed the strongest response to vaccination. This is favourable in the context of tumour vaccines since central memory T cells can sustain the activation of new effector cells [39,40]. Notably, the upregulation of *TCF7* post-vaccination may indicate the formation of T cells with stem-like properties which are sensitive for immune checkpoint blockade [41]. The combination of *FLT3LG*, *CCR7* and *JAK3* upregulated post-therapy may indicate maturation and migration of DCs [42]. DCs capture, process and (cross-)present tumour antigens and are critical for robust T-cell immunity [43]. Among the different types of DCs that can be distinguished, specifically the rare population of cDC1's seems indispensable for the induction of proper tumour-reactive T-cell responses in the cancer immune cycle during different types of cancer therapies [44]. Although it is possible to successfully use low numbers of cDC1's for vaccine therapy in murine models [44], it yet is still difficult to translate this to the clinic and a reason for us to utilise mo-DCs as antigen-presenting cells. Also, the activation of mo-DC, when correctly triggered, is sufficient to induce effective tumour immunity [44]. This is also stressed by studies showing that disrupting differentiation of mo-DCs leads to diminished effect of chemo and immunotherapy [45].

Shared clones between skin-test infiltrating activated (PD-1<sup>+</sup>) T cells and post-vaccination circulating activated CD4<sup>+</sup>PD-1<sup>+</sup> T cells were also detected, suggesting that the MesoPher-driven changes in circulating activated CD4<sup>+</sup> T-cell frequencies reflect the response of MesoPher-specific T cells. These analyses at transcriptome and protein level back-to-back substantiate the presence of bona fide T-cell responses specifically induced by treatment.

The REACTiVe Trial was initiated on the promises that mesothelioma and PDAC share tumour characteristics and antigens. Indeed, the identification of a selection of known tumour antigens on both transcriptome as protein level on the drug product and autologous tumours derived from study patients showed a large overlap. As only known antigens were analysed by targeted mass spectrometry, a greater repertoire of shared tumour antigens is not unlikely.



**Fig. 5. MesoPher and autologous tumour-directed responses can be measured *in vitro*.** (a) Schematic overview of the *in vitro* co-culture system. PBMCs were co-cultured with various DC conditions at start and reactivated with various DC conditions to assess specific responses. (b) Percentages of CD137+ subsets of CD4+ and CD8+ circulating T cells at baseline (week 0) and post-treatment (week 2, 6) after stimulation with unloaded DCs, MesoPher or autologous tumour-loaded DCs and reactivated overnight with unloaded DCs, MesoPher or autologous tumour-loaded DCs in RT002, RT005, RT006, RT008, RT009 and RT011. Autologous tumour reactivity was not evaluated for RT011 due to lack of material. A positive response to DCs with the antigen indicated is defined as a 50% or higher increase in the percentage of T cells expressing the indicated activation marker when compared to antigen-control cells (unloaded-DC). Positive responses are marked with a red stripe. A vaccine-induced response is defined as a positive response after vaccination which was not present before vaccination and not present when cultured with unloaded-DCs at start. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article). DC, dendritic cell.

Indeed, our *in vitro* co-cultures showed that MesoPher vaccination was able to activate CD4+ and/or CD8+ T cells able to respond to autologous tumour-lysate loaded DCs, which was also found in a preclinical PDAC tumour model [32].

Limitations common to phase I trials should be noted, including small sample size, lack of control group and potential selection bias. Our study population consisted of patients with an ECOG performance status score of 0–2, tumour stage I–II, and free of local disease, representing a population with a potential advantageous clinical outcome compared to resected patients who finished standard-of-care treatment.

With all caveats of the small sample, median progression-free survival and overall survival have not been reached, and seven out of the ten patients have not yet experienced local disease recurrence or new metastatic lesions at a median follow-up of 25 months (range 15–32 months). The study cohort had a favourable survival compared to patients with resected pancreatic cancer who survived for at least 1 year [46]. Furthermore, patients with PDAC with disease recurrence usually results in poor prognosis and rapid death. Interestingly, the study patients with disease recurrence did not demonstrate rapid tumour dissemination or early death. This has also been described for cancer vaccines in other malignancies [47].

In established PDAC disease, immunotherapy may offer new treatment opportunities if one takes into account the hurdles posed by the intricate tumour micro-environment [48] as demonstrated in recent trials with rationally combined treatment strategies [18,19]. We have previously demonstrated that improved systemic T-cell immunity following DC therapy was able to restrain murine PDAC tumour growth when given prophylactically but not therapeutically, unless DC therapy was combined with CD40 agonistic antibody therapy [32].

In conclusion, we demonstrated the feasibility and safety of MesoPher in PDAC patients and showed that the MesoPher vaccine induced a T-cell response. Furthermore, shared tumour antigens between the vaccine and PDAC, allowing MesoPher to induce PDAC-reactive T cells. Future results in larger cohorts must demonstrate whether MesoPher-induced immune responses translate into robust clinical efficacy of DC vaccination in patients with resected pancreatic cancer after standard-of-care systemic treatment.

## Disclaimer

Amphera B.V. is a spin-off company from the Erasmus University Medical Center and had no access to patient-related data. They were not responsible for and did not participate in the study design, data collection, analysis, and interpretation, and writing of the manuscript.

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## Author contributions

S.P.L. designed the study and all laboratory studies, performed experiments, analysed the data, and wrote the manuscript. L.K., M.V., J.D. and L.W. performed laboratory experiments. K.B. oversaw MesoPher production. A.v.K. and R.S. contributed to the bioinformatics analyses concerning the RNA sequencing data. M.D. staged the tumours of study patients. W.d.K. and A.S. provided help in data and statistical analysis. H.V. and D.M. participated in data analysis and interpretation. R.v.d.B., J.B.N. and N.F.C.C.d.M. performed the TCR- $\beta$  repertoire analyses. C.S. and T.L. designed, performed, and analysed the mass spectrometry experiments. S.H.v.d.B., J.G.A., and C.v.E. oversaw design of the study and all laboratory studies, data analysis and interpretation, and wrote the manuscript. All authors contributed to the editing of the final report. All authors agreed to all of the content of the submitted manuscript.

## Data and materials availability

All data relevant to the study are included in the article or uploaded as supplementary information and all data are available upon reasonable request.

## Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J.G.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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at [10.1016/j.ejca.2022.03.015](https://doi.org/10.1016/j.ejca.2022.03.015).

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