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Dendritic Cell–Based Immunotherapy in Patients With Resected Pancreatic Cancer

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ABSTRACT

PURPOSE Immunotherapies have shown limited responses in patients with advanced pancreatic cancer. Recently, we reported that dendritic cell (DC)–based immunotherapy induced T-cell responses against pancreatic cancer antigens. The primary objective of this study was to determine the efficacy of DC-based immunotherapy to prevent recurrence of disease.

METHODS This was a single-center, open-label, single-arm, combined phase I/II trial. The primary end point was the 2-year recurrence-free survival (RFS) rate. A 2-year RFS rate of ≥60% was defined as a clinically meaningful improvement. We included patients with pancreatic cancer after resection and completion of standard-of-care (SOC) treatment without recurrent disease on cross-sectional imaging. Patients were treated with autologous DCs pulsed with an allogeneic mesothelioma tumor cell lysate, comprising antigens also expressed in pancreatic ductal adenocarcinoma.

RESULTS Thirty-eight patients were included in the analysis of the primary end point (47% male, 53% female). The median age was 62 years (IQR, 55-68). Twenty-eight patients (74%) received five DC vaccinations and completed the study protocol. Three patients (8%) received four vaccinations, and seven patients (16%) received three vaccinations. After a median follow-up of 25.5 months, 26 patients (68%) had not developed recurrence of disease. The estimated 2-year RFS was 64%. Vaccination led to the enrichment of circulating activated CD4+ T cells and the detection of treatment-induced immune responses in vitro. T-cell receptor-sequencing analyses of a resected solitary lung metastasis showed influx of vaccine-specific T cells.

CONCLUSION This study reached its primary end point of a 2-year RFS rate of ≥60% following pancreatectomy after SOC treatment and adjuvant DC-based immunotherapy in patients with pancreatic cancer. These results warrant a future randomized trial.

INTRODUCTION

After curative intended resection for pancreatic cancer followed by adjuvant treatment with multiagent chemotherapy, recurrence rates are high and long-term survival remains rare.[1,2] Moreover, although neoadjuvant chemoradiotherapy was superior over immediate surgery in the randomized PREOPANC trial, 2-year recurrence-free survival (RFS) rate in this arm was only 40%.[3]

Immunotherapies have shown limited responses in advanced pancreatic cancer.[4-6] In the absence of an immunosuppressive immune microenvironment of established pancreatic ductal adenocarcinoma (PDAC) after resection, immunotherapy might be able to eradicate remnant micrometastatic disease and prevent disease recurrence.

Dendritic cells (DCs) play a pivotal role in the cancer-immunity cycle by priming and activating antigen-specific effector T cells.[7,8] However, DCs are often excluded from PDAC tumors[9]; hence, they may not be able to pick up tumor antigens and trigger tumor-specific immunity. Restoring DC immunity, therefore, could improve outcome.[10] We performed a phase I trial with DC-based immunotherapy in

CONTEXT

Key Objective
What is the value of adjuvant dendritic cell (DC)–based immunotherapy in patients with resected pancreatic cancer after standard-of-care systemic treatment?

Knowledge Generated
DC therapy was able to induce a vaccine-specific immune response in patients with pancreatic cancer after resection and standard-of-care systemic treatment. The cohort demonstrated a promising 2-year recurrence-free survival rate of 64%.

Relevance (E.M. O'Reilly)
This trial adds to the increasing body of evidence that immune-based therapies in resected pancreas cancer, using a variety of different strategies, may not only induce a potent immune response but also can induce an early oncologic signal worthy of further evaluation.*

*Relevance section written by *JCO* Associate Editor Eileen M. O'Reilly, MD.

patients with resected PDAC after standard-of-care (SOC) treatment.[11] We demonstrated safety and vaccine-induced immune responses against the tumor.

The current phase II expansion cohorts' primary objective was to investigate the efficacy of adjuvant DC therapy.

METHODS

Study Design and End Points

This was a single-center, open-label, single-arm, combined phase I/II trial. Safety and feasibility was demonstrated in the phase I trial. After completing the phase I study,[11] an expansion cohort was enrolled to investigate efficacy. At the outset of the study, the phase II expansion cohort was designed. The primary end point was the 2-year RFS rate. Given an expected 2-year RFS rate of 40%, we defined a 2-year RFS rate of ≥60% as a clinically meaningful improvement. Explorative end points were peripheral blood T-cell activation and treatment-induced vaccine-specific immune responses in vitro.

Patients

Key inclusion criteria were (1) histopathologically proven resected pancreatic cancer, (2) completed or offered SOC treatment, (3) no signs of disease recurrence on imaging, (4) normal organ function and adequate bone marrow reserve, (5) an Eastern Cooperative Oncology Group performance status of ≤2,[12] and (6) a positive delayed-type hypersensitivity (DTH) skin test (ie, erythema >2 mm after 48 hours) against tetanus toxoid and no response to infusion medium. Key exclusion criteria were (1) current or previous immunotherapeutic treatment, (2) use of steroids, (3) other malignancies, (4) concomitant disease or active infections, and (5) a shellfish allergy. Inclusion and exclusion criteria are listed in detail in the trial protocol (Data Supplement, online

only). The study and protocol amendments were 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 was registered in the Netherlands Trial Register (NL67169.000.18). Each patient provided written informed consent.

Procedures

Patients underwent a leukapheresis to harvest CD14+ monocytes, which were differentiated into immature DCs, loaded with allogeneic mesothelioma tumor cell lysate, and further matured into monocyte derived DCs in a 9-day culture period. The vaccine is an advanced therapy medicinal product. The production process has been described previously.[13] After 6 weeks, patients started treatment. Patients received three biweekly vaccinations with approximately 25×10^6 DCs per vaccination. Two thirds of the volume was delivered intravenously, and one third was delivered intradermally. Patients received fourth and fifth vaccinations three and 6 months after the third vaccination, respectively, in case of no recurrence. After three vaccinations, a DTH skin test was performed with the DC vaccine, the lysate, and infusion medium (negative control). After a positive reaction after 48 hours to the vaccine or the lysate (induration ≥2 mm) and no reaction to infusion medium, a 3-mm skin biopsy was taken. Figure 1A illustrates the treatment regimen. The experimental methods and definitions are added as appendices (Data Supplement, Appendix S1-S5, Table S1, Fig S1).

Statistical Analysis

For the 2-year RFS benchmark, we selected a subgroup of patients in the PREOPANC trial.[3,14] Patients who received
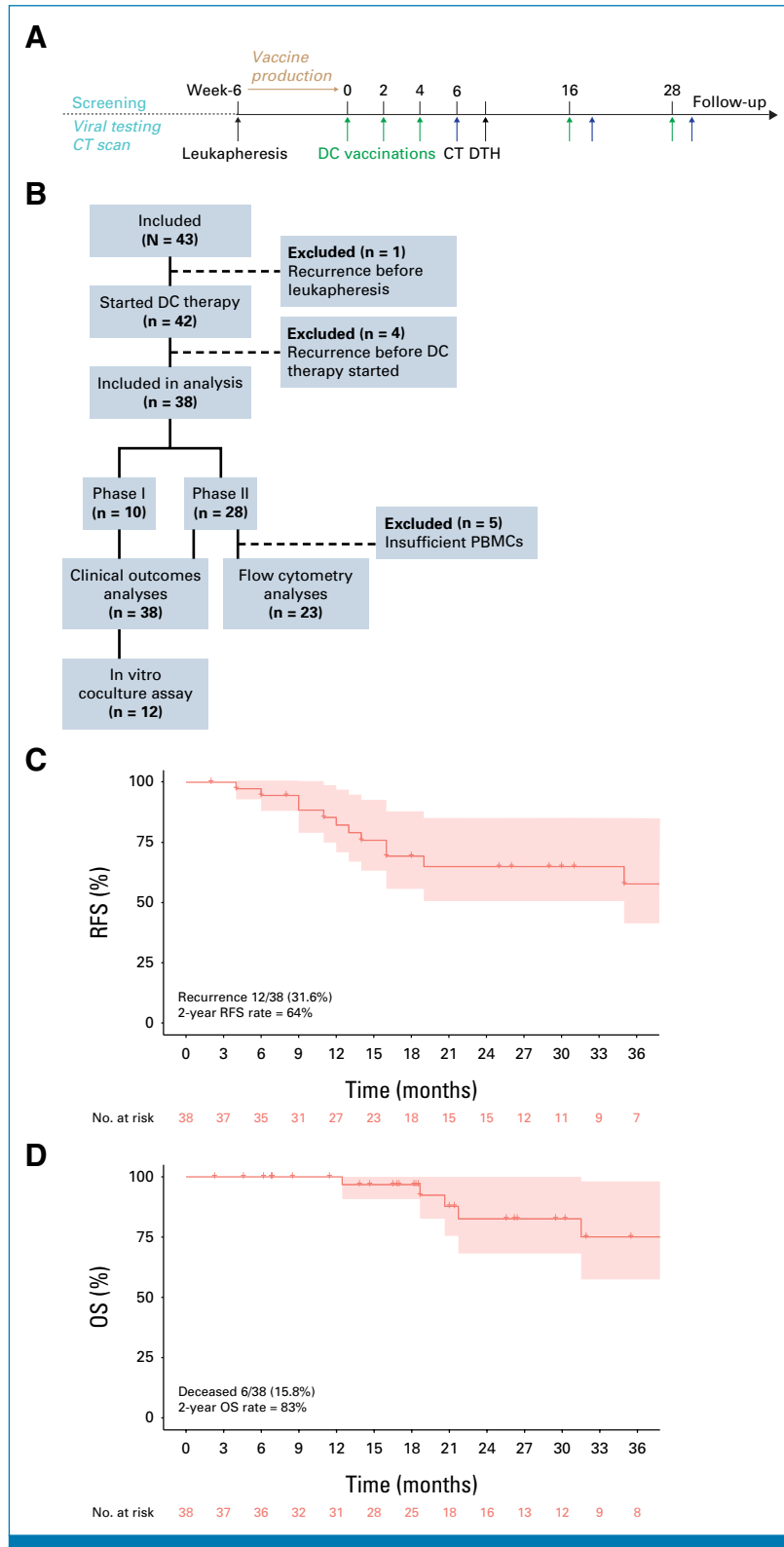
FIG 1. Clinical study summary. (A) Schematic treatment overview. (B) Flowchart of patient inclusion and analyses. (C) RFS from the date of resection, estimated by the Kaplan-Meier method. (D) OS from the date of resection, estimated by the Kaplan-Meier method. CT, computed tomography; DC, dendritic cell; DTH, delayed-type hypersensitivity; OS; overall survival; PBMC, peripheral blood mononuclear cell; RFS, recurrence-free survival.

neoadjuvant chemoradiation, underwent a resection, and started adjuvant chemotherapy (76%) were selected. In that group, the 2-year RFS rate was 40%. We hypothesized that by adding adjuvant DC therapy, this could be improved from 40% to 60%. Using Fleming's procedure,[15] with a significance level of 0.050 and a power of 80%, 38 patients needed to be included. Follow-up from the date of resection was calculated using the reverse Kaplan-Meier method. Repeatedly, data maturity was calculated.[16] If data maturity fulfilled two criteria, the 2-year RFS rate was estimated by the Kaplan-Meier method. The first criterion uses a pre-specified acceptable decrease in the survival curve for an extra event (5%, for example). For the second criterion to hold, this decrease should not be larger than the width of the one-sided 95% CI in case of no censoring. The RFS and overall survival (OS) were calculated from the date of resection until the date of recurrence or date of death. For flow cytometry analyses, a Wilcoxon signed-rank test was used. We did not correct for multiple testing as markers were selected on the basis of previous data.[11] To visualize the immune-monitoring data, we used GraphPad Prism v8.0. Statistical analyses were performed using IBM SPSS Statistics, version 28.0.1.0, and R, version 4.1.0.

RESULTS

Patients

Between February 2019 and November 2022, 43 patients were screened. Of those, one patient (RT035) developed metastases before leukapheresis and four patients were excluded from the analysis because of recurrence before starting treatment (RT003, RT009, RT015, and RT031). In best interest of the patients, they were treated with DC therapy out of the protocol. Thus, 38 patients (47% male, 53% female) were included in the primary end point analysis (Fig 1B). The first 10 were included in the previously published phase I safety trial. An additional 28 patients were included in the phase II expansion cohort. Since most patients had received adjuvant chemotherapy before entering the trial, the median interval between resection and inclusion was 8 months (IQR, 3-13). Table 1 shows detailed patient, disease, and treatment characteristics.

Study Treatment

Twenty-eight patients (74%) received five vaccinations and completed the study protocol, three patients (8%) received four vaccinations, and seven patients (16%) received three vaccinations. Of those who received fewer than five vaccinations, 8/10 (80%) developed disease recurrence, and in 2/10 (20%), not enough DCs were cultured for five vaccinations. In consultation with these patients, no second leukapheresis was done to produce additional vaccinations.

Safety and Tolerability

The DC vaccine was well tolerated. All patients were discharged after every vaccination without symptoms or relevant alterations in vital signs (data not shown). Thirty-seven (97%) patients experienced a grade 1−related adverse event (AE), seven (18%) a grade 2−related AE, and one (3%) a grade 3−related AE (ie, possibly related grade 3 dyspnea). All related and unrelated AEs are reported in the Data Supplement (Table S2).

Clinical Outcomes

After a median follow-up of 25.5 months (95% CI, 15.6 to 35.5), the estimated 2-year RFS and OS rates were 64% and 83%, respectively (Figs 1C and 1D). The median OS and RFS could not be calculated. Of patients who developed recurrence of disease (n = 12), six (50%) had local recurrence, four (33%) had distant recurrence, and two (17%) had both local and distant recurrence. After disease recurrence, 9/12 patients (75%) started palliative chemotherapy. In patients with recurrence, six (50%) had died and the median OS from the date of recurrence was 10.8 months (95% CI, 7.2 to 14.3).

Peripheral Blood T-Cell Activation

In the expansion cohort (n = 28), only the peripheral blood T-cell activation was investigated, since extensive immune monitoring in the phase I study was performed and reported previously.[11] Because of logistics, fresh enumeration of leukocytes was performed in 20/28 patients (71%). After the first and third DC vaccinations, no changes were found in absolute numbers of CD3+, CD4+, and CD8+ cells per μL blood (Data Supplement, Fig S2A). Because of insufficient numbers of peripheral blood mononuclear cells (PBMCs) in five patients, a total of 23/28 patients (Data Supplement, Table S3) were included in the flow cytometric analysis of cryopreserved PBMCs. We found increased frequencies of HLA-DR+ and PD-1+ CD4+ T cells after three vaccinations (Fig 2A). Expression of these markers correlated with each other (Figs 2B), but they were not coexpressed by the same CD4+ T cell (data not shown). Neither regulatory CD4+ T cells (Data Supplement, Fig S2B) nor CD8+ T cells were activated after vaccination (Data Supplement, Fig S2C). Most apparent changes occurred in frequencies of activated CD4+ central memory T (Tcm) cells. Within this population, increased frequencies of HLA-DR+, ICOS+, Ki-67+, PD-1+, Ki-67+PD-1+, and CD39+ cells were observed (Fig 2C). Upregulation occurred 1 week after the first vaccination for all aforementioned markers and remained present after the third vaccination in case of human leukocyte antigen−DR isotype and PD-1. Moreover, we found increased frequencies of HLA-DR+ and CD39+ CD4+ effector memory T cells after three vaccinations (Fig 2D). Finally, we found increased

TABLE 1. Patient, Disease, and Treatment Characteristics

Characteristic	Overall Cohort (N = 38)	Safety Cohort (n = 10)	Expansion Cohort (n = 28)
Patient characteristics			
Age, years, median (IQR)	62 (55-68)	64 (57-74)	60 (55-67)
Male sex, No. (%)	18 (47)	4 (40)	14 (50)
BMI (kg/m^2), median (IQR)	23 (22-26)	24 (20-26)	23 (22-25)
ECOG-PS ≥1, No. (%)	11 (29)	2 (20)	9 (32)
Clinical disease characteristics			
CA 19-9 (U/mL), diagnosis, median (IQR)	89 (25-254)	63 (23-206)	121 (25-317)
Missing, No. (%)	13 (34)	3 (30)	10 (36)
CA 19-9 (U/mL), inclusion, median (IQR)	13 (8-25)	28 (20-36)	11 (7-17)
Tumor size (mm), diagnosis, median (IQR)	25 (20-36)	28 (16-36)	25 (20-38)
Missing, No. (%)	8 (21)	2 (20)	6 (21)
Tumor location, No. (%)			
Head	29 (76.3)	7 (70)	22 (78.6)
Body/tail	9 (24)	3 (30)	6 (21)
Histopathologic disease characteristics			
Tumor size, mm, median (IQR)	23 (17-31)	22 (18-25)	24 (14-35)
pT stage, No. (%)			
pT0-1	15 (40)	5 (50)	10 (36)
pT2-4	23 (60)	5 (50)	18 (64)
pN stage, No. (%)			
pN0	19 (50)	6 (60)	13 (46)
pN1-2	19 (50)	4 (40)	15 (54)
Disease stage, No. (%)			
Stage I to II	30 (79)	10 (100)	20 (71)
Stage III	8 (21)	0 (0)	8 (29)
Margin status, No. (%)			
R0	18 (47)	5 (50)	13 (47)
R1	20 (53)	5 (50)	15 (53)
Perineural invasion, No. (%)	26 (68)	7 (70)	19 (68)
Lymphangioinvasion, No. (%)	22 (58)	5 (50)	17 (61)
Treatment characteristics			
Resection, No. (%)			
Pancreatoduodenectomy	28 (74)	7 (70)	21 (75)
Distal pancreatosplenectomy	10 (26)	3 (30)	7 (25)
Chemotherapy setting, No. (%)			
Neoadjuvant only	10 (26)	1 (10)	9 (32)
Adjuvant only	17 (45)	8 (80)	9 (32)
Neoadjuvant and adjuvant	6 (16)	0 (0)	6 (22)
None	5 (13)	1 (10)	4 (14)
Type of neoadjuvant chemotherapy, No. (%)			
Gemcitabine-based chemoradiotherapy	3 (8)	0 (0)	3 (11)
(m)FOLFIRINOX	13 (34)	1 (10)	12 (43)
None	22 (58)	9 (90)	13 (46)
Duration, months, median (IQR)	3 (2-5)	3	3 (2-5)
Type of adjuvant chemotherapy, No. (%)			
Gemcitabine monotherapy	5 (13)	3 (30)	2 (8)
(m)FOLFIRINOX	14 (37)	1 (10)	13 (46)
Gemcitabine + capecitabine	4 (11)	4 (40)	0 (0)
None	15 (39)	2 (20)	13 (46)
Duration, months, median (IQR)	5 (3-6)	6 (5-6)	4 (1-5)

TABLE 1. Patient, Disease, and Treatment Characteristics (continued)

Characteristic	Overall Cohort (N = 38)	Safety Cohort (n = 10)	Expansion Cohort (n = 28)
Radiotherapy, No. (%)			
Chemoradiotherapy	3 (8)	0 (0)	3 (11)
Stereotactic radiotherapy	3 (8)	1 (10)	2 (7)
None	32 (84)	9 (90)	23 (82)
Interval resection and inclusion in study, months, median (IQR)	8 (3-13)	11 (7-14)	7 (3-10)

NOTE. TNM staging according to the AJCC staging manual, 8th edition; margin status was defined as R0 in case of >1 mm tumor clearance from any, except the anterior margin and R1 in case of ≤1 mm tumor clearance from any, except the anterior margin according to the AJCC guidelines.
Abbreviations: AJCC, American Joint Committee on Cancer; CA, carbohydrate antigen; ECOG-PS, Eastern Cooperative Oncology Group Performance Score.

frequencies of CD28+, CD39+, and LAG-3+ Temra T cells after vaccination (Fig 2E). In conclusion, we found treatment-induced activation of peripheral blood CD4+ T cells (primarily CD4+ Tcm cells), without concurrent activation of regulatory CD4+ T cells.

Vaccine-Specific Immune Responses In Vivo and In Vitro

Thirty-eight patients (100%) developed a positive DTH skin test against the DC vaccine, indicating that all patients were able to respond to the vaccine. Because of sample availability, samples from 12/38 patients (Data Supplement, Table S4) were included in the in vitro coculture assay to establish vaccine-specific immunity. Coculturing PBMCs in the presence of the vaccine led to increased activation of CD4+ and CD8+ T cells, mostly in PBMCs isolated after three vaccinations (Figs 3A and 3B). Concomitantly, vaccination led to increased expression of CD107a by both T-cell subsets and IFN-γ by CD8+ T cells (Fig 3C). Finally, increases in CD8+ T cells coexpressing CD107a and IFN-γ were detected after vaccination (Fig 3C). These data indicate that DC treatment resulted in activation of vaccine-specific cytokine-producing CD4+ and CD8+ T cells.

Lobectomy of a PDAC Lung Metastasis After DC Therapy

After DC therapy, patient RT002 underwent a lobectomy for a slowly growing solitary lung nodule. Retrospectively, this lesion developed almost 2 years before the lobectomy. The lobectomy was performed 34 months after pancreatectomy and 11 months after the last DC vaccination, without development of other metastatic sites. Molecular analyses showed identical *KRAS* (exon 2: c.35G>A; p.G12D), *TP53* (exon 7: c.722C>G; p.S241C), and *SF3B1* (exon 15: c.2098A>G; p.K700E) mutations in the pancreas and the lung tumor, supporting the lung lesion was a metastasis. However, single-nucleotide polymorphism (SNP) analyses showed similar and different variants between the primary pancreas tumor and the tumor in the lung. There were no signs of recurrence 57 months after the pancreatectomy and 22 months after the lobectomy (Figs 4A and 4B). To study the T-cell receptor (TCR) repertoire and reactivity of the T cells, TCR sequencing was performed. The

primary tumor before DC therapy, the DTH skin test against the vaccine after three vaccinations, and the lung metastasis after five DC vaccinations were used (Fig 4C). We found 86 TCR clonotypes unique to the primary tumor, 202 TCR clonotypes unique to the lung metastasis, and four overlapping between the primary tumor and the lung metastasis. Of the latter, two were also shared with the skin biopsy. This not only suggests that these T cells are responding to vaccine antigens but also that they were able to infiltrate PDAC metastases. Thirteen TCR clonotypes overlapped between the skin biopsy and lung metastasis, which were absent in the primary tumor. One of these TCR clonotypes was the third most abundant one in the lung metastasis (TCR sequencing reads are added in the Data Supplement). Immunohistochemical staining of the lung metastasis revealed infiltration of CD4+ and CD8+ T cells without upregulation of PD-L1 on tumor cells (Fig 4D). Cells expressing CD8 were mainly found at the periphery of the tumor, showing a moderately dense infiltration of cytotoxic T cells.

DISCUSSION

This single-arm, single-center, open-label phase I/II trial sought to determine efficacy of DC therapy in preventing disease recurrence after resection and SOC treatment in patients with PDAC. Thirty-eight patients were included. Treatment demonstrated to be safe. We found an estimated 2-year RFS rate of 64%, meaning that the primary end point was met. Vaccination led to increased percentages of activated peripheral blood CD4+ T cells and detection of treatment-induced vaccine-specific immune responses in vitro. In one patient with remarkably prolonged disease-free survival after resection of a lung metastasis, influx of vaccine-specific T cells was detected.

Despite PDAC being a tumor notoriously resistant to immune therapies, recently, several studies demonstrated the potential of adjuvant vaccination strategies. A phase I study found immune responses against a personalized neoantigen vaccine in half of the patients when used in combination with immune checkpoint–blocking antibodies and chemotherapy.[17] Immune responses correlated with clinical outcomes. Another recent phase I study investigated lymph node–targeted vaccination
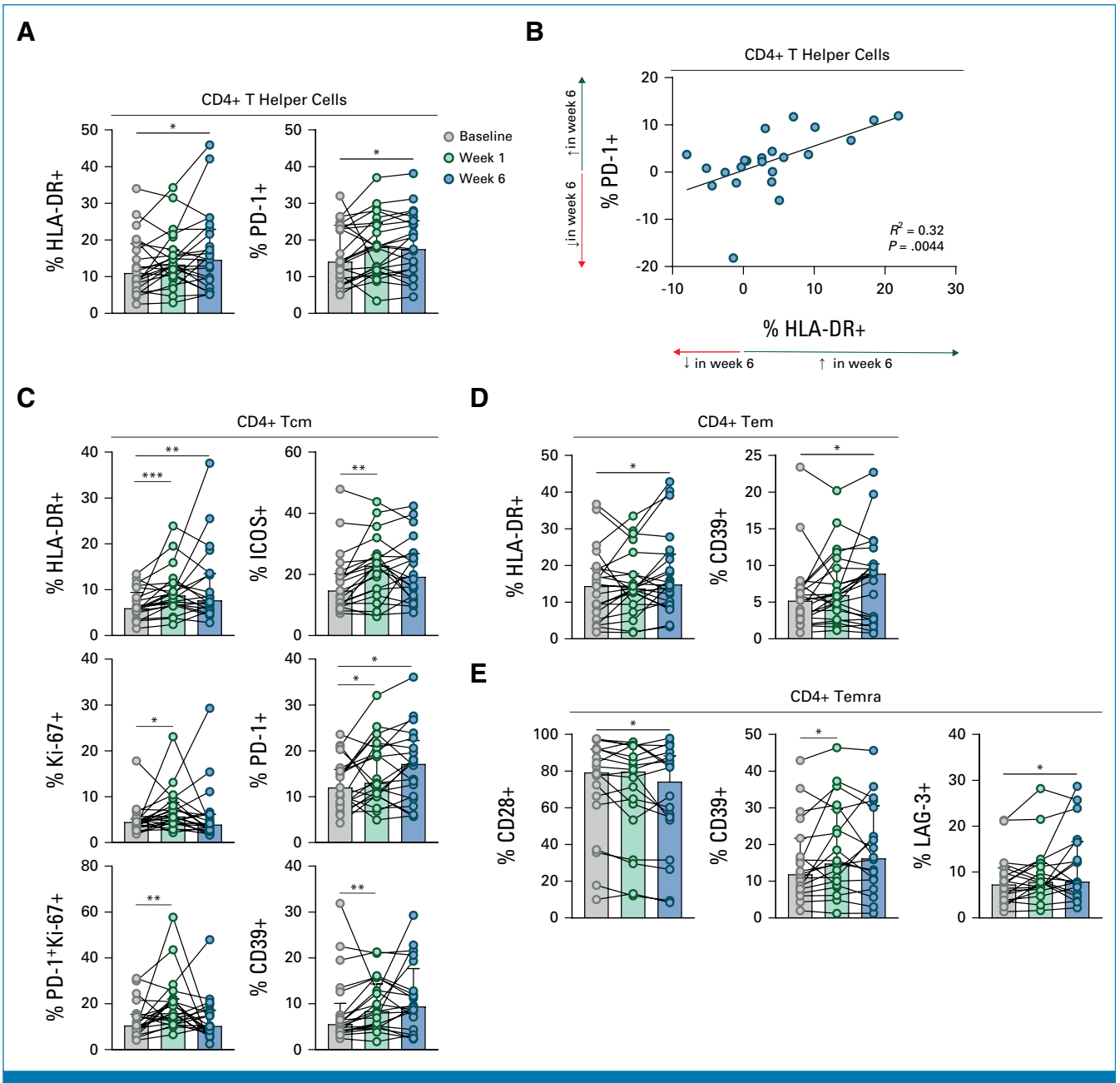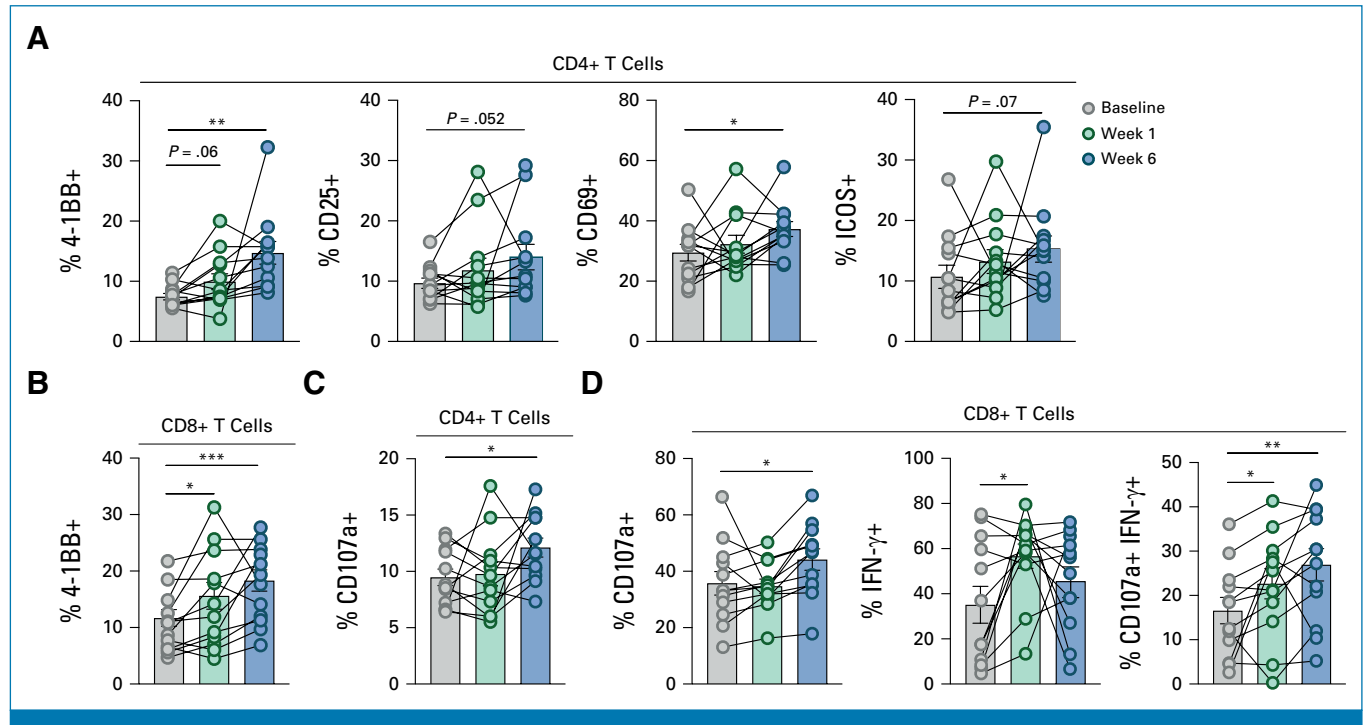
FIG 2. Flow cytometry analysis revealed peripheral blood T-cell activation after DC therapy. (A) Increased frequencies of HLA-DR− and PD-1−positive CD4+ T helper cells after DC therapy (n = 23). (B) Frequencies of HLA-DR− and PD-1−positive CD4+ T helper cells correlated (R^2 = 0.32; P = .0044) in week 6 (n = 23). (C) Increased frequencies of HLA-DR−, ICOS-, Ki-67−, PD-1−, PD-1− and Ki-67−, and CD39-positive CD4+ central memory T cells after DC therapy (n = 21). (D) Increased frequencies of HLA-DR− and CD39-positive CD4+ effector memory T cells after DC therapy (n = 23). (E) Increased frequencies of CD28-, CD39-, and LAG-3−positive CD4+ Temra T cells after DC therapy (n = 19). *P ≤ .05; **P ≤ .01; ***P ≤ .001. DC, dendritic cell; HLA-DR, human leukocyte antigen-DR isotype; ICOS, inducible T-cell costimulator.

containing G12D and G12R *mKRAS* long peptides after resection of PDAC or colorectal cancer.[18] Most patients developed a *mKRAS*-specific T-cell response.

Advantages of DC vaccines over other recently used mRNA and long peptide vaccine approaches are multifactorial. DCs are the most professional antigen-presenting cells to stimulate both CD4 and CD8 tumor-reactive T cells. Other vaccines require target antigens to be expressed (DNA, RNA),

ingested for processing (DNA, RNA, long peptides), and presented on MHC class I (for which long peptide vaccine are less suited) and MHC class II by DCs. This important and in vivo sometimes indistinct process is overcome when generating DC-based vaccines. Furthermore, the injected DCs may serve as donor cells that transfer antigens to endogenous cross-presenting DCs, which are key to inducing tumor-reactive CD8 T-cell reactivity.[19] In contrast to a personalized neoantigen vaccine, usage of an allogeneic

FIG 3. Flow cytometry analysis revealed peripheral blood T-cell activation and cytokine production upon coculture with the DC vaccine. (A) Increased frequencies of 4-1BB−, CD25-, CD69-, and ICOS-positive CD4+ T cells after a coculture with the DC vaccine (n = 12). (B) Increased frequencies of 4-1BB−positive CD8+ T cells after a coculture with the DC vaccine (n = 12). (C) Increased frequencies of CD107a-positive CD4+ T cells after a coculture with the DC vaccine (n = 12). (D) Increased frequencies of CD107a-positive, IFN-γ−positive, and co-expressing CD8+ T cells after a coculture with the DC vaccine (n = 12). *$P \leq .05$; **$P \leq .01$; ***$P \leq .001$. DC, dendritic cell; ICOS, inducible T-cell costimulator.

tumor cell lysate implies that vaccine development can start before surgery and be administered shortly after resection, which might be the most optimal scenario. Another advantage of DC therapy over other cellular therapies is the safety profile without the need of lymphodepleting chemotherapy and risk of cytokine release syndrome.

In this study, after a median follow-up of 25.5 months (95% CI, 15.6 to 35.5) following pancreatectomy, the estimated 2-year RFS rate was 64%. In other cohorts, this percentage is around 20%-25%.[20,21] A study found that the expected 2-year OS rate in patients who had survived for 1 year after the resection was 55%.[22] In this study, the estimated 2-year OS rate was 83%.

In line with our previous data, treatment was associated with minimal toxicity.[13] This is critical, considering the morbidity associated with pancreatic resection[23,24] and multiagent chemotherapeutic treatment.[1,25] Most patients developed fever (n = 28, 68%) and injection site reactions (n = 37, 97%) after the second, third, fourth, or fifth vaccination, reflecting activation of the immune system by the vaccine. This was corroborated by the fact that all patients developed a positive DTH skin test.

Similar to the previously reported safety cohort,[11] we found activation of peripheral blood CD4+ T helper cells after DC

therapy in the expansion cohort. Importantly, CD4+ regulatory T cells were not activated. In line with previous data in mice and humans, we did not observe activation in circulating CD8+ T cells.[11,26] The most apparent activation occurred in CD4+ Tcm cells. In addition, in vitro, we found a strong vaccine-specific CD4+ and CD8+ T-cell response. Cytokine production occurred after a limited time of exposure to the vaccine, indicating the presence of vaccine-specific effector and/or memory T cells.

We showcased a woman who displayed a remarkably slow-growing solitary PDAC lung metastasis and a long disease-free survival. TCR sequencing revealed only four overlapping TCR clonotypes between the primary tumor and the lung metastasis, whereas 86 distinct TCR clonotypes were present in the primary tumor and 202 in the lung metastasis. This variation in the TCR repertoire between the primary tumor and the metastatic site was also previously found in patients with metastatic gastroesophageal adenocarcinoma.[27] It is known that tissue-resident lymphocytes differ between organs,[28,29] possibly resulting in the influx of different lymphocyte populations. We acknowledge the fact that patients with lung-only recurrence might resemble a subgroup of patients with PDAC with distinct cancer biology and relatively good prognosis.[30,31] Nevertheless, 13 TCR clonotypes were overlapping between the DTH skin test and the lung metastasis after DC therapy. The absence of these TCR
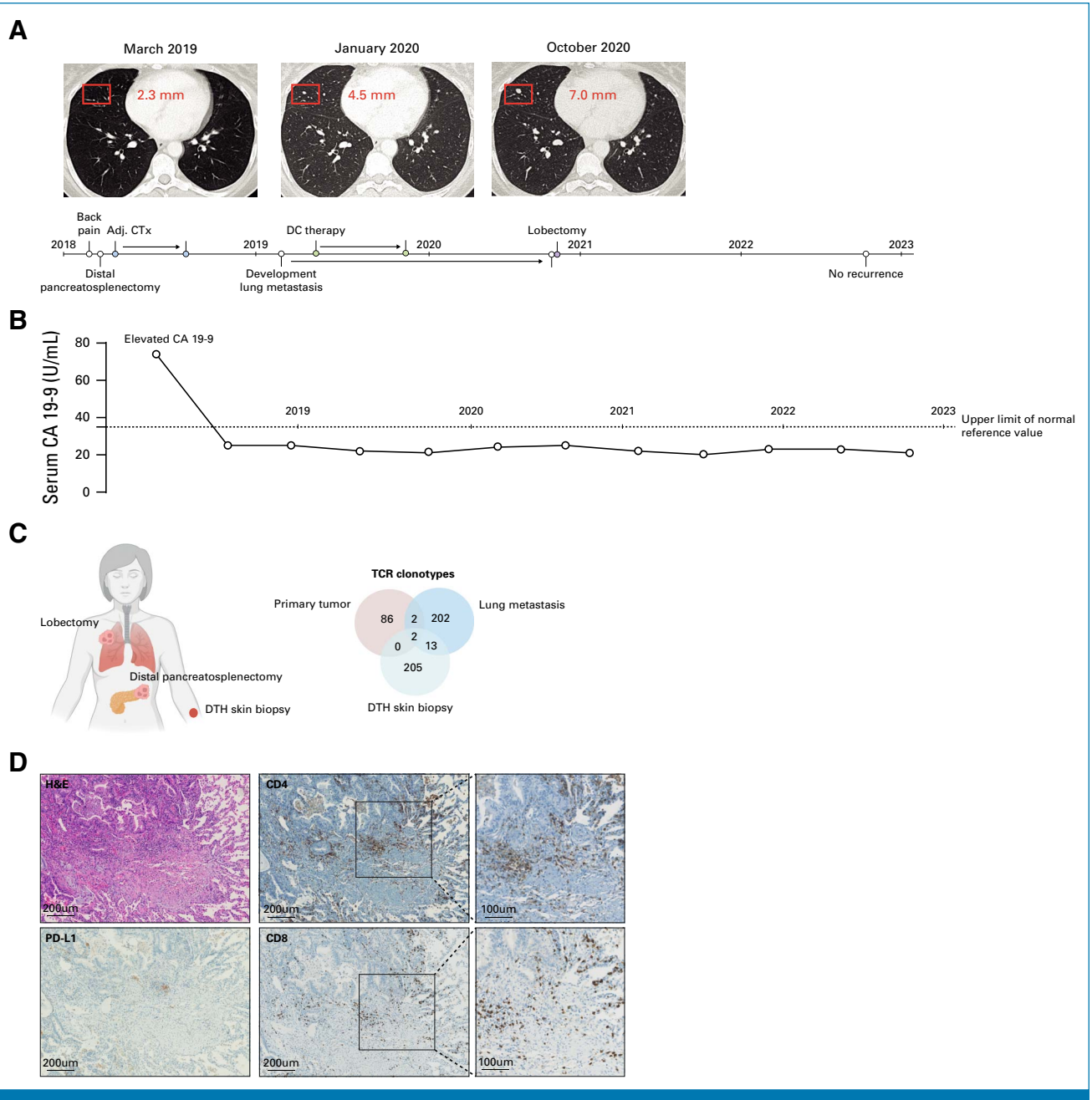
FIG 4. Case report of RT002. (A, B) Clinical course of patient RT002. Patient underwent a distal pancreatosplenectomy, adjuvant chemotherapy, and DC therapy. Later, the patient underwent a lobectomy for a solitary lung metastasis. At data cutoff, she is alive without recurrence 57 and 22 months after pancreatectomy and lobectomy, respectively. (C) T-cell receptor sequencing of the primary tumor, the metastasis, and a DTH skin test and biopsy against the vaccine revealed 13 overlapping TCR clonotypes between the skin biopsy and the lung metastasis after DC therapy. (D) Immunohistochemistry staining revealed high infiltration of CD4+ T cells and, to a lesser extent, infiltration in CD8+ cells. DC, dendritic cell; DTH, delayed-type hypersensitivity; TCR, T-cell receptor.

clonotypes in the primary tumor before treatment suggests a treatment-induced influx of vaccine-activated T cells. Notably, one of the DTH skin test TCR clonotypes was the third most abundant clonotype in the lung metastasis, suggesting a clinically relevant response. Despite infiltration of vaccine-specific T cells, the lung metastasis did grow slowly over time. We have several hypotheses for this observation. First,

the CD8+ cells appear to be stuck at the border of the tumor. Second, the phenotype of tumor-infiltrating CD4+ T cells may be (partly) immune-suppressive regulatory T cells. Third, other immunosuppressive cells may be present. Finally, although the lung tumor was a metastasis from the primary pancreatic cancer, SNP analyses revealed similar but also different variants between both tumors.

As single-arm studies with time-to-event outcomes are challenging to interpret because of patient selection bias and variability in the natural course of the disease, this study has several limitations. First, a selected cohort (ie, after resection) of patients was included, resembling a minority of patients with PDAC. However, although all patients had undergone resection, the cohort was heterogeneous regarding stage of disease, pretreatment, and comorbidities, which could affect outcomes. Second, the cohort was subject to biases. The noted survival and lead-time bias in calculating RFS from the time of pancreatectomy rather than study enrollment is duly acknowledged. Furthermore, patients were enrolled after a median of 8 months (IQR, 3-13) following pancreatectomy, effectively screening out the high-risk population for early recurrence, which introduces immortal time bias.[32] Third, a historical subgroup for defining a 2-year RFS benchmark is challenging since this study was conducted in a selected cohort of patient who have not recurred or died for some nontrivial time after resection. Fourth, in the analyses of vaccine-induced immunity, it is unknown against which antigens or which epitope of antigens the immune response was directed. Therefore, it is unknown which antigens were immunogenic. Also, immune responses against keyhole limpet hemocyanin, which is used as a carrier protein in the vaccine, could be present. Fifth, because of the single-armed design and treatment of patients without active disease, discriminating between treatment effects and tumor biology remains challenging. Further improvements could be made using DC therapy as an adjuvant. First, earlier postoperative treatment might be more efficient, and vaccines could already be made preoperatively. Second, although we hypothesize that antigen-specific T cells remain in the body and a rapid response can be initiated upon re-exposure to the vaccine, it is likely that systemic immune activation wears off over time. In our future trial, we will investigate the role of the booster vaccinations in the immune response. Moreover, we aim to continue with booster vaccinations until disease recurrence.

In conclusion, in patients with pancreatic cancer, we observed a 2-year RFS rate of 64% after pancreatectomy, SOC treatment, and adjuvant DC-based immunotherapy. Translational research supported the induction of immune activation by the vaccine. Despite the limitations of the trial, we argue that these results warrant a future randomized trial investigating the efficacy of adjuvant DC therapy in patients with resected pancreatic cancer. A follow-up phase II/III trial with adjuvant DC therapy is currently being developed within the Dutch Pancreatic Cancer Group, which will start after all patients have been included in the PREOPANC-3 study (ClinicalTrials.gov identifier: NCT04927780).

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DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI https://doi.org/10.1200/JCO.23.02585. The data that support the findings of this study are available from the corresponding author, C.v.E., upon reasonable request.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Dendritic Cell–Based Immunotherapy in Patients With Resected Pancreatic Cancer

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