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Dendritic cell-based immunotherapy (DCVAC/OvCa) combined with second-line chemotherapy in platinum-sensitive ovarian cancer (SOV02): A randomized, open-label, phase 2 trial

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HIGHLIGHTS

- Randomized trial of DCVAC/OvCa, dendritic cell-based immunotherapy in platinum-sensitive ovarian cancer.
- The addition of DCVAC/OvCa to second-line chemotherapy had a favorable safety profile.
- DCVAC/OvCa did not improve progression-free survival, but did prolong overall survival by 13.4 months.
- DVCAC/OvCa plus chemotherapy enhanced surrogate T-cell activity.

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ABSTRACT

Objective. DCVAC/OvCa is an active cellular immunotherapy designed to stimulate an immune response against ovarian cancer. We explored the safety and efficacy of DCVAC/OvCa plus carboplatin and gemcitabine in platinum-sensitive ovarian cancer.

Methods. In this open-label, parallel-group, phase 2 trial (ClinicalTrials.gov number NCT02107950), patients with platinum-sensitive ovarian cancer relapsing after first-line chemotherapy were randomized to DCVAC/OvCa and chemotherapy or chemotherapy alone. DCVAC/OvCa was administered every 3–6 weeks (10 doses). Endpoints included safety, progression-free survival (PFS; primary efficacy endpoint) and overall survival (OS; secondary efficacy endpoint).

Results. Between November 2013 and May 2015, 71 patients were randomized to chemotherapy in combination with DCVAC/OvCa or to chemotherapy alone. Treatment-emergent adverse events related to DCVAC/OvCa, leukapheresis and chemotherapy occurred in six (16.2%), two (5.4%), and 35 (94.6%) patients in the DCVAC/OvCa group. Chemotherapy-related events occurred in all patients in the chemotherapy group. Seven patients in the DCVAC/OvCa group were excluded from primary efficacy analyses due to failure to receive ≥1 dose of DCVAC/OvCa. PFS was not improved (hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.42–1.28, $P = 0.274$, data maturity 78.1%). Median OS was significantly prolonged (by 13.4 months) in the DCVAC/OvCa group (HR 0.38, 95% CI 0.20–0.74, $P = 0.003$; data maturity 56.3%). A signal for enhanced surrogate antigen-specific T-cell activity was seen with DCVAC/OvCa.

Conclusions. DCVAC/OvCa combined with chemotherapy had a favorable safety profile in patients with platinum-sensitive ovarian cancer. DCVAC/OvCa did not improve PFS, but the exploratory analyses revealed OS prolongation and enhanced surrogate antigen-specific T-cell activity.

1. Introduction

Cytoreductive surgery followed by platinum and taxane combination is the standard first-line treatment for advanced ovarian cancer [1]. However, most patients diagnosed with advanced ovarian cancer suffer from recurrences [2]. In these patients, survival correlates with the duration of remission after the last dose of platinum-based chemotherapy and platinum-based chemotherapy doublet remains the treatment of choice in patients recurring >6 months after first-line treatment [2]. Although immune checkpoint inhibitors (ICIs) have emerged as another pillar in the systemic therapy of cancer [3], ICIs alone induce clinical responses in <10% of patients with ovarian cancer [4].

Alternative immunotherapeutic approaches include dendritic cell (DC)-based immunotherapies, such as autologous DCs expanded, activated, and loaded with a source of tumor-associated antigens (TAAs) *ex vivo* [5]. DCVAC/OvCa was developed as an active, DC-based immunotherapy for ovarian cancer, harnessing allogeneic tumor cells killed by high hydrostatic pressure as a source of multiple TAAs [6,7]. The rationale for loading DCs with a broad range of TAAs, as opposed to a single antigen, is based on the increased likelihood of generating a polyclonal T-cell response against malignant cells, maximizing the potency of immunization and minimizing the probability of immune evasion *via* antigen loss [8].

We hypothesized that the administration of DCVAC/OvCa would elicit an antitumor immune response capable of delaying disease progression and improving survival. Herewith, we report the results of SOV02, a randomized, phase 2 clinical trial to investigate the safety and preliminary signs of efficacy of second-line DCVAC/OvCa combined with chemotherapy (*vs* chemotherapy alone) in patients with recurrent platinum-sensitive ovarian cancer.

2. Methods

2.1. Ethics and trial oversight

The trial protocol and all its amendments, as well as the information provided to the patients, were reviewed and approved by the appropriate ethics committees. The trial was conducted in accordance with the provisions of the Declaration of Helsinki and International Council for Harmonisation guidelines on Good Clinical Practice. Written informed consent was obtained from each patient. The trial was registered on ClinicalTrials.gov (NCT02107950). An independent data monitoring committee reviewed key safety data and provided recommendations for continuation or termination of the trial.

2.2. Patients and trial design

SOV02 was an international, multicenter, open-label, parallel-group, randomized phase 2 trial that was conducted in the Czech Republic, Poland and Germany.

Eligible patients were aged ≥18 years with histologically confirmed ovarian, fallopian tube, or primary peritoneal carcinoma (serous, endometrioid, or mucinous) classified as stage III or IV according to the criteria of the International Federation of Gynecology and Obstetrics [9]. Patients were required to have experienced a documented complete remission for ≥6 months following first-line platinum-based chemotherapy and radiologically confirmed relapse ≤4 weeks before enrolment.

Key exclusion criteria included: clear cell, non-epithelial, and borderline ovarian carcinoma; prior or concurrent systemic therapy for ovarian cancer except first-line platinum-based chemotherapy with or without bevacizumab; previous or concurrent radiotherapy to the abdomen and pelvis; malignancy other than epithelial ovarian cancer unless in complete remission for ≥3 years (with the exception of curatively treated carcinoma *in situ* of the cervix or non-melanoma skin cancers); evidence of active microbial infection requiring systemic treatment; and clinically significant cardiovascular disease. A complete list of inclusion and exclusion criteria is given in the Supplementary Materials.

2.3. Randomization

Investigators used an automated interactive web-response system to randomize (1:1 ratio in the primary analysis set) eligible patients to receive DCVAC/OvCa plus carboplatin and gemcitabine (DCVAC/OvCa group) or carboplatin and gemcitabine alone (control group). Patients were stratified according to prior bevacizumab use as part of first-line treatment (yes or no) and remission duration (6–12 or > 12 months). Early in the trial, the number of patients who dropped out before starting DCVAC/OvCa was greater than anticipated. In order to ensure an approximately equal number of patients in both groups in the

primary analysis set, the trial protocol was amended to allow the replacement of patients who were randomized to the DCVAC/OvCa group but failed to receive at least one dose of DCVAC/OvCa and the randomization ratio was changed from 1:1 to 2.5:1.

2.4. Procedures

After randomization, all patients in the DCVAC/OvCa group underwent a single leukapheresis session. Leukapheresis and DCVAC/OvCa manufacturing procedures are described in the Supplementary Materials.

Chemotherapy started ≤7 days after leukapheresis (DCVAC/OvCa group) or ≤ 2 weeks after randomization (control group). All patients received carboplatin and gemcitabine in 21-day cycles. In each cycle, carboplatin (AUC 4–5) was intravenously administered on day 1 and gemcitabine (1000 mg/m^2) on days 1 and 8. Patients were scheduled to receive 6, 8, or 10 cycles of chemotherapy according to the investigator's decision.

Patients in the DCVAC/OvCa group received up to 10 doses of DCVAC/OvCa, starting after the second cycle of chemotherapy. On the day of administration, one 1 mL aliquot of DCVAC/OvCa was thawed and diluted with 0.9% saline to a total volume of 5 mL. Two 2.5 mL injections were administered subcutaneously into the axillary and contralateral inguinal lymph node areas. These regions were chosen based on prior phase 1/2 trials in which administration of DCVAC in close proximity to the axillary and contralateral inguinal lymph nodes resulted in efficient migration of the DCVAC-related DCs to lymph nodes with strong potency to prime the anti-tumor T cell response [10,11].

The first five doses of DCVAC/OvCa were administered at 3-week intervals (±3 days) and the other doses at 6-week intervals (±3 days). DCVAC/OvCa was to be continued until all doses had been administered, regardless of tumor radiological progression, or until refusal, intolerance, a protocol violation, death or a decision of the investigator. Patients randomized to the DCVAC/OvCa group who failed to receive ≥1 dose were withdrawn from the trial; these patients could be replaced. Patients randomized to the control group received the same carboplatin and gemcitabine regimen without placebo, hence excluding the need to undergo leukapheresis and avoid unnecessary exposure to an invasive medical procedure.

2.5. Outcomes

Safety was evaluated in terms of adverse events (AEs) graded according to the Common Terminology Criteria for Adverse Events version 4.03 [12], hematology, biochemistry, urinalysis and vital signs. Treatment-emergent AEs (TEAEs) were defined as AEs that started or worsened during trial treatment or ≤ 30 days after the last dose of DCVAC/OvCa (DCVAC/OvCa group) or chemotherapy (control group). Routine safety laboratory assessments were performed before each chemotherapy cycle and DCVAC/OvCa administration, and AE causality was assessed by the investigator.

The primary efficacy endpoint was PFS, defined as the time from randomization to tumor progression or death due to any cause. Secondary efficacy endpoints were OS (defined as the time from randomization until death due to any cause), biological progression-free interval (PFI_{BIO}) based on serum cancer antigen (CA) 125 levels, objective response rate (ORR), and immune responses.

Computed tomography/magnetic resonance imaging scans were done at screening and then every 8 weeks for up to 72 weeks after the start of chemotherapy. The date of progression was determined locally according to modified RECIST 1.1 criteria [13], and classified as slow or non-slow disease progression (Supplementary Methods [Definition of slow/non-slow progression] and Table S1). CA 125 levels were measured every 6–8 weeks.

Blood samples for immune assessments were collected at screening and at weeks 24, 48, and 72, and were analyzed as described in the Supplementary Methods (Lymphocyte subsets and antigen-specific T-cell responses). We chose NY-ESO-1 and MAGE-A1 for this analysis because of their high immunogenicity [14–16] and considering the results of prior studies showing an OS advantage of NY-ESO-1, in particular, in patients with ovarian cancer [14,17].

2.6. Statistical analysis

The main part of the trial was terminated in accordance with the trial protocol at 72 weeks after the last patient initiated second-line chemotherapy on November 21, 2016. All data available at this date were used for primary efficacy analyses. The trial continued to collect OS data through to May 17, 2018 (trial completion) in accordance with the trial protocol.

Safety endpoints were evaluated in all patients who received at least one dose of chemotherapy or DCVAC/OvCa. Efficacy analyses were done using all randomized patients, excluding those who failed to receive at least one dose of DCVAC/OvCa. Because this was an exploratory trial, these patients were excluded from efficacy analyses in order to dissect the potential benefit of DCVAC/OvCa itself on survival outcomes in a relatively small group of patients. In addition, we performed sensitivity analyses of OS for all randomized patients, after retrieving information on survival (through to May 17, 2018) for patients who were withdrawn from the trial due to failure to receive ≥1 dose of DCVAC/OvCa; similar sensitivity analyses of PFS or PFI_{BIO} were not possible because these could not be retrieved.

An unstratified log-rank test was used to compare PFS, PFI_{BIO} and OS between the trial groups. Hazard ratios (HR) with 95% confidence intervals (CI) were determined using Cox proportional hazard regression. For immune parameters, the fold changes (FCs) from the value at screening were determined. Values below the detection limit were set to 0.01. SOV02 was not powered to detect statistically significant differences between treatment groups. Analyses were done using Statistical Analysis Systems (SAS) software version 9.4.

3. Results

3.1. Patients

Between November 2013 and May 2015, a total of 71 patients were randomized, 39 to the DCVAC/OvCa group and 32 to the control group. Of the 39 patients in the DCVAC/OvCa group, two patients did not undergo leukapheresis (one had active hepatitis B and the other experienced an AE); both were excluded from the safety and efficacy analyses. Five patients underwent leukapheresis but did not receive DCVAC/OvCa (three due to manufacturing failure and two died before receiving the first dose); these patients were excluded from efficacy analyses. Of the 32 patients in the control group, one patient did not receive chemotherapy; this patient was included in efficacy but not in safety analyses. Reasons for premature withdrawal from the trial are summarized in Table S2.

Twenty patients in the DCVAC/OvCa group (51.3%) and 11 patients in the control group (34.4%) remained on the trial until the primary analyses on November 21, 2016. Collection of survival data continued until May 17, 2018, when 24 patients in the DCVAC/OvCa group (61.5%) and 25 patients in the control group (78.1%) were known to have died (Fig. 1).

Both groups were balanced regarding demographics and medical history (Table 1). The predominant histologic subtype was serous and all patients had FIGO stage III at diagnosis. Bevacizumab was administered in the first-line setting to 21.9% of patients in the DCVAC/OvCa group and to 18.8% of patients in the control group. Over half of the patients in both groups relapsed >12 months after the last cycle of platinum-based chemotherapy.

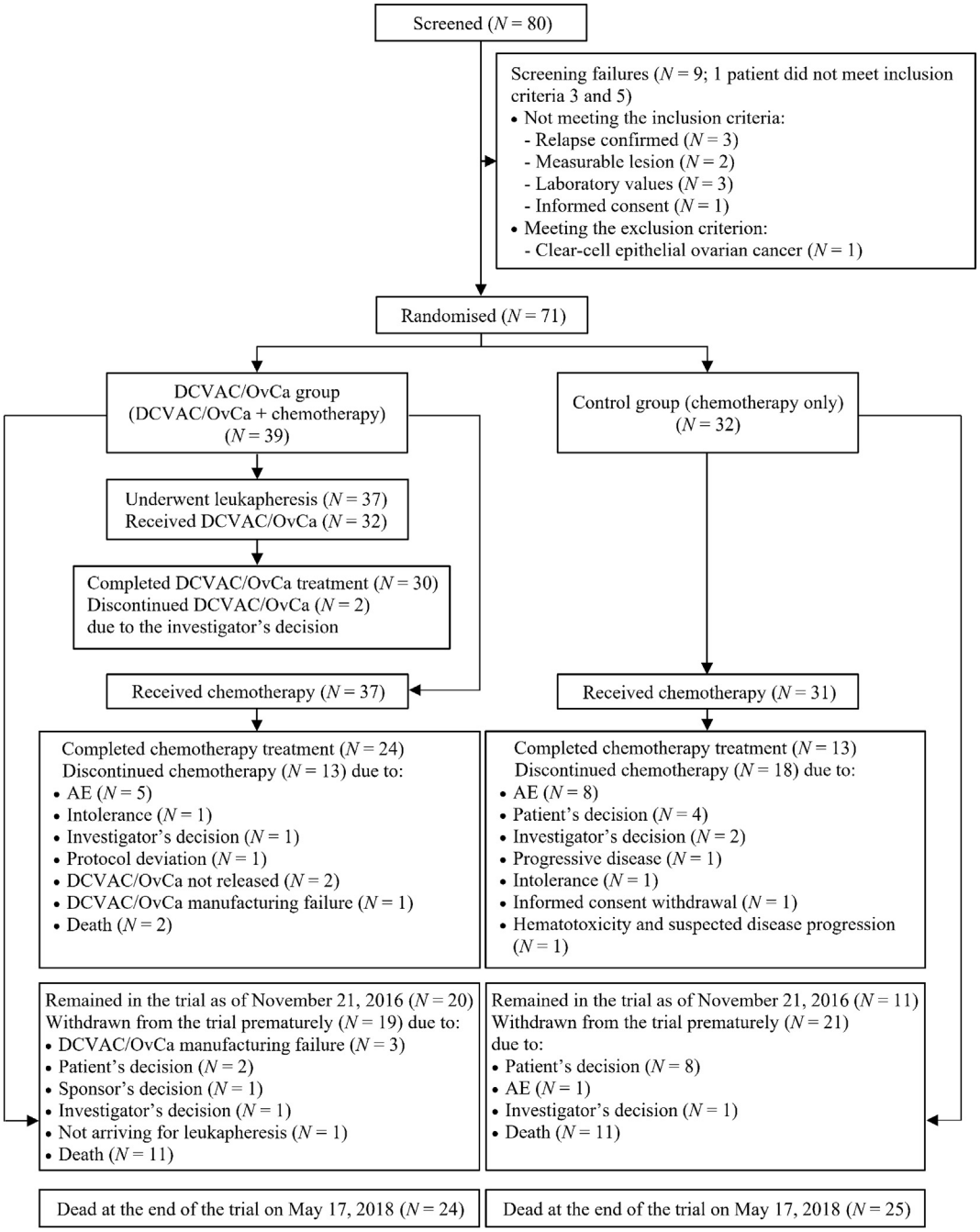

Fig. 1. Patient disposition (randomization and treatment).

3.2. Treatments

The mean number of DCVAC/OvCa doses received was 9.8. Twenty-eight of 37 patients in the DCVAC/OvCa group (75.7%) received all 10 scheduled doses of DCVAC/OvCa; five patients did not receive any doses of DCVAC/OvCa. The median duration of exposure to DCVAC/OvCa was 295.0 days (Table S3). Exposure to chemotherapy was comparable in both groups.

3.3. Safety

Within the DCVAC/OvCa ($n = 37$) and control ($n = 31$) groups, similar proportions experienced TEAEs (97.3% *vs* 100.0%), chemotherapy-related TEAEs (94.6% *vs* 100.0%), and grade ≥ 3 TEAEs (78.4% *vs* 83.9%) (Table 2). Fewer patients experienced serious TEAEs or TEAEs leading to discontinuation of chemotherapy in the DCVAC/OvCa group. TEAEs related to DCVAC/OvCa were reported in six patients (16.2%), of which three were only related to DCVAC/OvCa (abdominal rigidity, injection site erythema, and lymphadenopathy). No TEAE led to discontinuation of DCVAC/OvCa.

The overall incidence of TEAEs appears balanced between the two groups, considering the difference in the duration of the reporting period, which was twice as long in the DCVAC/OvCa group compared with the control group, with median durations of 360 and 150 days, respectively. The most common TEAEs (DCVAC/OvCa *vs* control) were thrombocytopenia, anemia, neutropenia, and leukopenia, all of which

Table 1
Characteristics of the patients at baseline, primary analysis set.

	DCVAC/OvCa group $N = 32$	Control group $N = 32$
Age, years: *n*; median (range)	32; 58.5 (42–80)	32; 60.5 (43–74)
Race: *n* (%)		
White	32 (100.0%)	32 (100.0%)
Weight, kg: *n*; median (range)	32; 77.5 (53–118)	31; 70.0 (48–108)
Height, cm: *n*; median (range)	32; 164.5 (151–178)	31; 164.0 (145–172)
BMI, kg/m^2: *n*; median (range)	32; 29.4 (20–48)	31; 26.6 (18–39)
BSA, m^2: *n*; median (range)	32; 1.81 (1.6–2.3)	31; 1.79 (1.4–2.2)
Type of epithelial cells		
Endometrioid, *n* (%)	2 (6.3%)	2 (6.3%)
Mucinous, *n* (%)	–	1 (3.1%)
Serous, *n* (%)	30 (93.8%)	29 (90.6%)
FIGO stage		
Stage III, *n* (%)	32 (100.0%)	32 (100.0%)
Time from initial diagnosis to randomization, days		
Patients	23	23
Mean	835.5	814.6
Standard deviation	372.70	378.74
Median	771.0	672.0
Q1–Q3	629.0–978.0	538.0–974.0
Range	352–1907	383–1692
Bevacizumab use as first-line treatment		
No	25 (78.1%)	26 (81.3%)
Yes	7 (21.9%)	6 (18.8%)
Duration of remission		
6–12 months	12 (37.5%)	14 (43.8%)
>12 months	20 (62.5%)	18 (56.3%)

BMI, body mass index; BSA, body surface area; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique (International Federation of Gynecology and Obstetrics).

Table 2
Adverse events, safety set.

	DCVAC/OvCa group $N = 37$	Control group $N = 31$
Any TEAE	36 (97.3%)	31 (100.0%)
DCVAC/OvCa-related TEAEs	6 (16.2%)	–
Leukapheresis-related AEs	2 (5.4%)	–
Chemotherapy-related TEAEs	35 (94.6%)	31 (100.0%)
Serious TEAEs	20 (54.1%)	20 (64.5%)
TEAEs leading to death	2 (5.4%)	–
Grade ≥ 3 TEAEs	29 (78.4%)	26 (83.9%)
TEAEs leading to discontinuation of DCVAC/OvCa	–	–
TEAEs leading to discontinuation of chemotherapy	5 (13.5%)	8 (25.8%)
TEAEs leading to trial withdrawal	–	1 (3.2%)
TEAEs reported to be related to DCVAC/OvCa only		
Abdominal rigidity	1 (2.7%)	–
Injection site erythema	1 (2.7%)	–
Lymphadenopathy	1 (2.7%)	–
TEAEs reported in >10% of patients in either treatment group		
Thrombocytopenia	24 (64.9%)	25 (80.6%)
Anemia	24 (64.9%)	21 (67.7%)
Neutropenia	22 (59.5%)	21 (67.7%)
Leukopenia	17 (45.9%)	10 (32.3%)
Fatigue	10 (27.0%)	4 (12.9%)
Nausea	9 (24.3%)	7 (22.6%)
Drug hypersensitivity	9 (24.3%)	5 (16.1%)
Vomiting	7 (18.9%)	5 (16.1%)
Diarrhea	5 (13.5%)	5 (16.1%)
Dyspnea	4 (10.8%)	2 (6.5%)
Arthralgia	4 (10.8%)	1 (3.2%)
Asthenia	4 (10.8%)	1 (3.2%)
Pain in extremity	4 (10.8%)	1 (3.2%)
Upper respiratory tract infection	4 (10.8%)	1 (3.2%)
Hypokalemia	3 (8.1%)	5 (16.1%)
Constipation	2 (5.4%)	5 (16.1%)

Values are *n* (%) of patients.
AE, adverse event; TEAE, treatment-emergent adverse event.

occurred in ≥10 patients in each group. Most of these events were related to chemotherapy. Serious TEAEs and fatal TEAEs are summarized in Tables S4 and S5.

3.4. Efficacy

Thirty-two patients were included in each group for the efficacy analyses, which were performed 72 weeks after the initiation of second-line chemotherapy in the last patient (cut-off date: November 21, 2016). Kaplan–Meier plots of survival outcomes (PFS, PFI_{BIO} and OS) are shown in Fig. 2a–c. Median PFS was 11.3 *vs* 9.5 months in the DCVAC/OvCa *vs* control groups and was not significantly different between the two groups (HR 0.73, 95% CI 0.42–1.28, $P = 0.274$, data maturity 78.1%; Fig. 2a, Table 3). Furthermore, the median PFI_{BIO} was not greater in the DCVAC/OvCa group (10.3 *vs* 10.1 months; HR 0.82, 95% CI 0.47–1.43, $P = 0.478$; Fig. 2b, Table 3).

At the time of the analysis (November 21, 2016) the OS was not significantly different between the two groups, with a median OS of 23.8 months in the DCVAC/OvCa group *vs* 21.5 months in the control group (HR 0.63, 95% CI: 0.26–1.54, $P = 0.303$; Fig. 2c, Table 3). However, the OS data were immature at this point of time. In accordance with the trial protocol, survival data were collected through to May 17, 2018 and exploratory analyses showed a statistically significant increase in the median OS (Fig. 2d), by 13.4 months, in the DCVAC/OvCa group (35.5 *vs* 22.1 months) with an HR of 0.38 (95% CI 0.20–0.74, $P = 0.003$; Table S6). Overall, 36 deaths had been recorded for 64 patients, resulting in 56.3% data maturity. As a sensitivity analysis, we also analyzed OS for all randomized patients (*i.e.*, 39 patients in the DCVAC/OvCa group and 32 patients in the control group; Fig. 2e). In the all-randomized analysis set, in which we were able to collect data on the survival status of 14 of 20 patients who had been withdrawn from the trial, the median OS was 7.3 months longer in the DCVAC/OvCa group (29.5 *vs* 22.2 months; Table S6; data maturity 69.0%).

The ORR was greater in the DCVAC/OvCa group (87.5%, 95% CI 71.0–96.5) compared with the control group (62.5%, 95% CI 43.7–78.9) (Table S7).

To explore the effects of DCVAC/OvCa on immune cells, we isolated PBMCs and analyzed the frequencies of $CD45^+$ cells; $CD3^+$, $CD4^+$, and $CD8^+$ T cells; $CD16^+$ $CD56^+$ NK cells; and $CD19^+$ B cells at weeks 24 and 72. We observed no significant changes in the absolute numbers of these immune cell subsets during treatment in the DCVAC/OvCa group (Fig. S1). DCVAC/OvCa tended to increase the frequencies of $CD4^+$ T cells specific for MAGE-A1 (median FC 1.26 *vs* 0.84) and NY-ESO-1 (median FC 1.11 *vs* 0.8) *vs* the control group (Fig. 3). Conversely, the percentages of $CD8^+$ T cells recognizing MAGE-A1- and NY-ESO-1-derived peptides, as well as the relative proportions of circulating $CD4^+$ and $CD8^+$ T lymphocytes expressing granzyme B in the absence of peptide stimulation did not differ between the DCVAC/OvCa and control groups (Fig. S2).

The Kaplan–Meier curves revealed a possible signal suggesting improved OS in patients with MAGE-A1- and NY-ESO-1-specific $CD4^+$ and $CD8^+$ T cells frequencies above the median, although the number of events was low (Fig. S3). Similarly, the univariate Cox analyses indicated a possible signal suggesting longer OS in patients with enhanced MAGE-A1- and NY-ESO-1-specific $CD4^+$ T-cell activities (Table S8).

4. Discussion

In this randomized, open-label, phase 2 trial, we investigated the safety and efficacy of DCVAC/OvCa in combination with carboplatin

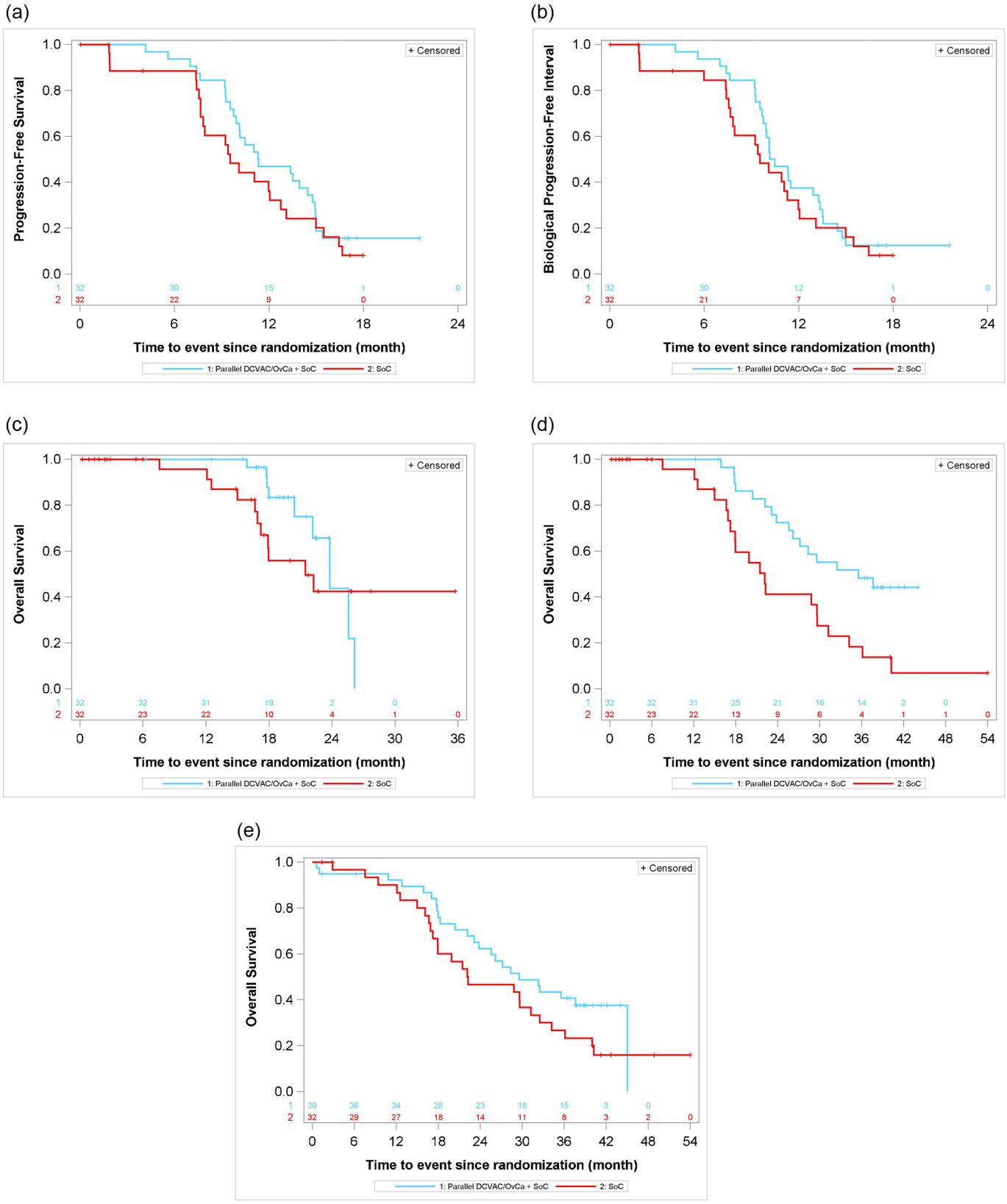

Fig. 2. Kaplan–Meier plots. (a–c) Progression-free survival (PFS; a), biological progression-free interval (PFI_{BIO}; b) and overall survival (OS; c) in the primary analysis set through to November 21, 2016. (d) OS through to May 17, 2018, in the primary analysis set. (e) OS through to May 17, 2018, for all randomized patients. SoC, standard of care chemotherapy.

Table 3
PFS, OS, and PFI_{BIO} (DCVAC/OvCa group over control group), primary analysis set.

	P-value (log-rank test)	HR (95% CI)	Benefit (median, in months)	Data maturity
PFS	0.274	0.73 (0.42–1.28)	11.3 *vs* 9.5	78.1%
PFI_{BIO}	0.478	0.82 (0.47–1.43)	10.3 *vs* 10.1	78.1%
OS	0.303	0.63 (0.26–1.54)	23.8 *vs* 21.5	31.3%

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFI_{BIO}, biological progression-free interval; PFS, progression-free survival.

plus gemcitabine, followed by maintenance DCVAC/OvCa, *vs* carboplatin plus gemcitabine chemotherapy alone in patients with recurrent platinum-sensitive ovarian cancer.

Our principal goal was to collect evidence on the safety of DCVAC/OvCa administered in combination with chemotherapy. Notably, DCVAC/OvCa did not increase the frequency of TEAEs compared with chemotherapy alone. Indeed, the vast majority of TEAEs were considered related to chemotherapy, with only three patients experiencing TEAEs specifically related to DCVAC/OvCa as per investigator's assessment.

From an efficacy perspective, the data revealed a trend towards an improvement in OS in the DCVAC/OvCa group, albeit without prolonging the PFS or PFI_{BIO}. At the final OS analysis, a significant difference in OS was detected, in favor of the DCVAC/OvCa group, with a risk reduction of 62% for death *vs* chemotherapy alone. This difference was observed even though the trial was not specifically powered to compare OS and therefore one should consider these results to be exploratory in nature and interpret them with appropriate caution.

The reason why DCVAC/OvCa prolonged OS, but not PFS, is not fully clear. We hypothesize that DCVAC/OvCa induces a delayed, yet durable anticancer immune response that leads to long-term disease stabilization and slower progression, resulting in prolonged survival, as has been reported in other studies with active cellular immunotherapy [18,19]. The presence of tumor-infiltrating lymphocytes has been demonstrated as an independent prognostic factor in patients with ovarian cancer [20]. The role of $CD4^+$ T cells as key regulators of the cytolytic activity of $CD8^+$ T cells (which is paramount for tumor eradication) has been established in various neoplasms [21]. Moreover, $CD4^+$ T cells influence the differentiation and proliferation of tumor-specific $CD8^+$ T cells, and are critical for generation and maintenance of long-term memory $CD8^+$ T-cell responses. Consistent with this notion, DCVAC/OvCa tended to increase surrogate antigen-specific T-cell activity at week 72, which was accompanied by a trend towards better prognosis. Importantly, this was not observed in unstimulated controls. Similar results were obtained in the first-in-human investigator-initiated clinical trial of DCVAC/OvCa (EudraCT: 2010–021462-30). In this context, significant increases in the numbers of NY-ESO-1-, MAGE-A1-, and MAGE-A3-specific T cells were detected after DCVAC/OvCa administration [22]. These findings are consistent with those of other studies, suggesting a positive impact of TAA-specific T cells on prognosis [10,18,23].

Thus, DC-based immunotherapy as a monotherapy generally fails to sufficiently reverse tumor progression, leading to the development of combination regimens. In this context, some chemotherapeutic agents appear to be an optimal partner by not only decreasing the tumor burden, but also exert immunostimulatory effects. Carboplatin and gemcitabine were reported to improve the function of immune effector

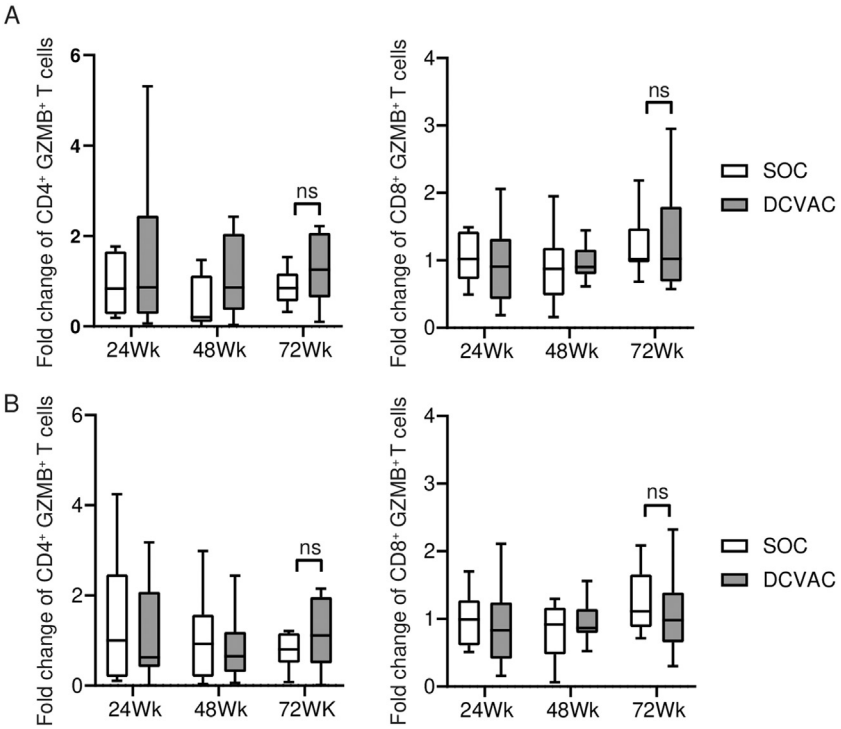

Fig. 3. TAA-specific immune responses, primary analysis set.
Fold-changes (relative to the screening values) for $CD4^+$ $GZMB^+$ and $CD8^+$ $GZMB^+$ T cells isolated from peripheral blood samples from patients in the DCVAC/OvCa (DCVAC) and control (SOC) groups at weeks 24, 48, and 72 upon exposure of the corresponding PBMCs to the peptide mixture spanning MAGE-A1 (A) and NY-ESO-1 (B) as determined by flow cytometry. Box plots: lower quartile, median, upper quartile; whiskers, minimum and maximum values. *P*-values were determined using the Wilcoxon test. GZMB, granzyme B; MAGE-A1, MAGE family member A1; NS, not significant; NY-ESO-1, cancer/testis antigen 1B (CTAG1B); Wk, week.

cells, including DCs and $CD8^+$ T cells, and induce so-called "immunogenic cell death", which is associated with the delivery of multiple adjuvant-like signals for DCs [24–26]. Altogether, these preclinical and clinical findings indicate that carboplatin and gemcitabine may support the functions of DCVAC/OvCa, ultimately favoring an adaptive immune response with therapeutic potential.

The principal limitation of this trial is that it was not sufficiently powered to detect significant differences in efficacy outcomes. Nevertheless, an OS benefit was apparent in the long-term follow-up. The efficacy analyses excluded patients who failed to receive at least one dose of DCVAC/OvCa (essentially a modified intention to treat analysis set) in order to exclude patients for whom DCVAC/OvCa could not be manufactured and avoid any potential bias associated with non-treatment in a small sample size. A review of the baseline characteristics of those patients did not reveal any differences compared with the other enrolled patients that might suggest potentially worse prognosis. By nature, we cannot fully exclude the possibility that the exclusion of these patients could introduce selection bias towards better-responding patients and improved survival, but the OS sensitivity analyses of all randomized patients confirmed a positive trend towards improved OS; therefore, the selection bias, if any, did not affect our conclusions.

In summary, we found that DCVAC/OvCa combined with gemcitabine plus carboplatin as second-line treatment and continued as maintenance therapy had a favorable safety profile in patients with platinum-sensitive ovarian cancer. DCVAC/OvCa did not improve PFS, but exploratory analyses showed it may have prolonged OS and enhanced surrogate antigen-specific T-cell activity. These findings warrant confirmation in appropriately powered randomized studies.

Data availability

The datasets generated during and/or analyzed during the current trial are not publicly available due to commercial requirements, but are available from the corresponding author on reasonable request.

Prior publication

Results of this study were presented at the 50th Annual Meeting of the Society of Gynecologic Oncology March 16–19, 2019, Honolulu, HI, USA (abstract 046).

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Role of the funding source

SOTIO a.s. was involved in trial design and conduct, data analyses, production of DCVAC/OvCa, and drafting of the manuscript. All authors had full and unrestricted access to all of the data, results of the analyses, and took the final responsibility for the decision to submit the article for publication.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2021.07.003.

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