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Review

Dendritic Cell Immunotherapy for Ovarian Cancer: An Overview of Our Achievements

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Simple Summary: Epithelial ovarian carcinoma (EOC) remains the fifth leading cause of cancer-related death in women. Its poor prognosis is mainly due to the advanced stage of the disease at which a diagnosis is made. EOC has poor response to immunotherapy by means of immune checkpoint inhibitors, and only a small number of patients with a homologous recombination deficiency benefit from PARP inhibitors in terms of their overall survival. We systematically studied the immune status of patients with EOC at various stages of the disease. We developed and tested an autologous dendritic cell (DC)-based vaccine (DCVAC), which has been shown to be safe and to significantly improve progression-free survival and overall survival in randomized phase II clinical trials enrolling patients at different stages of EOC.

Abstract: Epithelial ovarian carcinoma (EOC) is the fifth leading cause of cancer-related death in women, largely reflecting the early dissemination of this malignant disease to the peritoneum. Due to its immunological features, EOC has poor response to immune checkpoint inhibitors (ICIs), including a limited tumor mutational burden (TMB), poor infiltration by immune cells, and active immunosuppression. Thus, novel strategies are needed to overcome the frequent lack of pre-existing immunity in patients with EOC. We developed and tested an autologous dendritic cell (DC)-based vaccine (DCVAC), which has recently been shown to be safe and to significantly improve progression-free survival (PFS) in two independent randomized phase II clinical trials enrolling patients with EOC (SOV01, NCT02107937; SOV02, NCT02107950). In addition, our exploratory data analyses suggest that the clinical benefits of the DCVAC were more pronounced in patients with EOC with lower-than-median TMBs and reduced $CD8^+$ T cell infiltration. Thus, the DC-based vaccine stands out as a promising clinical tool to jumpstart anticancer immunity in patients with immunologically "cold" EOC. Our findings underscore the need for personalized immunotherapy and the clinical relevance of potential tumor-related biomarkers within the immunotherapy field. Additional clinical trials are needed to address these strategies as well as the potential value of the TMB and immune infiltration at baseline as biomarkers for guiding the clinical management of EOC.

Keywords: ovarian cancer; immunotherapy; dendritic cells; clinical trials

Citation: Bartůňková, J. Dendritic Cell Immunotherapy for Ovarian Cancer: An Overview of Our Achievements. *Onco* **2024**, *4*, 46–55. https://doi.org/10.3390/onco4010004

Academic Editors: Constantin N. Baxevanis, Sotirios P. Fortis and Maria Goulielmaki

Received: 14 February 2024
Revised: 4 March 2024
Accepted: 11 March 2024
Published: 21 March 2024

1. Introduction

Ovarian cancer is a significant health concern, ranking as the eighth most common cancer in women. Ovarian cancer, specifically epithelial ovarian carcinoma (EOC), accounts for approximately 2% of all malignancies affecting women and thus might seem to be a very rare disease; nevertheless, its prognosis remains poor. This translates to the fact that EOC is the fifth leading cause of cancer-related deaths among women. Ovarian cancer is a silent cancer, for which a good prognosis and survival mainly rely on early-stage detection. However, the majority of cases are diagnosed as late-stage disease (III–IV stages, according to the current classification), mainly due to the lack of clinical symptoms and the lack of a specific early diagnostic laboratory marker. From a histological point of view, EOC is classified into five histological subtypes; the most frequent is high-grade serous

ovarian carcinoma (HGSOC). Other subtypes include low-grade serous ovarian carcinoma, endometrioid ovarian carcinoma, mucinous ovarian carcinoma, and finally, ovarian clear cell carcinoma. Each of these subtypes not only differ from the point of view of their cellular origin, but they also have different molecular profiles. Despite advances in the complex management of patients suffering from cancer generally, the prognosis remains poor. For the advanced disease, platinum-based chemotherapy is still the first-choice treatment. The administration of chemotherapy leads to remission in the majority of patients, but recurrence rates are high. The poor outcomes for HGSOC are mainly due to the early dissemination to the peritoneal cavity. Micro- and macro-metastases gaining omentum result in the formation of malignant ascites [1], and at this stage, the disease resists all available approved therapies.

Immunotherapy has revolutionized the treatment of many solid tumors, yet its efficacy in ovarian cancer has been limited [2–4]. Antiangiogenic therapies, such as bevacizumab, have shown restricted effectiveness [5]. Similarly, immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4, which have been approved for various cancers, have not demonstrated significant survival improvement in ovarian cancer [2]. A comprehensive review of 20 clinical trials, including phase I, II, and III studies, found no reported improvement in survival with ICIs, and some trials were terminated early due to toxicity or lack of response. Combining ICIs with chemotherapy, anti-vascular–endothelial growth factor (VEGF) therapy, or poly-ADP ribose polymerase (PARP) inhibitors did modestly improve the response rates and survival, albeit with a worse safety profile [6]. The identification of predictive biomarkers of ICI efficacy and the genomic and immune profiling of ovarian cancer are crucial for developing better treatment options and designing tailored trials [3].

In this review, we describe the contribution of our scientific and clinical team to the field of ovarian cancer. Over the past 20 years, we have tried to understand the immune contexture of ovarian cancer, and we have focused on developing an immunotherapy based on an autologous dendritic cell vaccine. A DC-based vaccine, called DCVAC/OvCa, was then tested in several clinical trials. Our partial achievements, both in basic research and in clinical trials, were published and discussed in depth in the corresponding papers. Here, we provide a comprehensive review of the twenty years of scientific development, from bench to bedside, referring to individual published articles.

2. Dendritic Cell Vaccines in Ovarian Cancer

Dendritic cell (DC)-based immunotherapy has been studied as an approach for treating ovarian cancer for a long time [7]. DC vaccines are generated using autologous DCs derived from peripheral blood monocytes, which are yielded from the patients by means of leukapheresis. Immature DCs (iDCs) are exposed to tumor-associated antigens from different sources (autologous tumor cell lysates, allogenic cells derived from tumor cell lines that have been killed using various methods, tumor-derived mRNA, tumor-derived or synthetic peptides, etc.). The exposition of iDCs to tumor antigens is called pulsation. During this process, several immunostimulatory molecules should be added to induce the maturation of the pulsed immature DCs. The final product, containing mDCs that are pulsed with tumor antigens, are reinfused into patients, most often via subcutaneous administration [8–11]. DC-based vaccines can induce tumor-specific $CD8^+/CD4^+$ T cell responses in vivo [12]. Unfortunately, the immune responses to tumor antigens do not usually reflect their clinical efficacy, meaning that they are often suboptimal as a monotherapy. Nevertheless, the immunogenicity and efficacy of DC vaccines can be augmented by a combinatorial chemo-immunotherapy regimen [13–15]. Further combinations were tested in several clinical trials, which included concomitant therapies consisting of DCs with ICIs, radiation, hormonal therapy, kinase inhibitors, antiangiogenic therapies, and others [16,17].

We conducted a feasibility study on the ex vivo generation of DCs in 2006, using autologous tumor cells for pulsation. The study demonstrated the technical feasibility of preparing individual DC-based vaccines. In vitro-generated DCs were able to induce T lymphocyte responses. From the methodological point of view, we generated monocyte-

derived DCs that were cultivated with a granulocyte–monocyte colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4) and pulsed with autologous tumor-derived apoptotic bodies [18]. Tumor cells were acquired from the ascites of patients with ovarian carcinoma during surgery. The tumor cells were killed by means of UV irradiation. The immature DCs were matured by the addition of poly-inosinic:polycytidylic acid (poly-I:C) and finally cocultured with autologous lymphocytes to test the ability of T cells to proliferate and produce interferon gamma (IFN-γ), detected by means of ELISPOT. Our results showed that the maturation of DCs and induction of a T cell response were achieved in 75% of the tested patients. Thus, we proved a limited feasibility of this approach [18–20].

Obtaining autologous cells from a patient's tumor during surgery can be logistically challenging. To address this issue, we explored the use of allogenic tumor cell lines for DC pulsation. An analysis of patient samples was performed to compare the expression of tumor antigens with available cell lines. In order to select a suitable combination of cancer cell lines as an appropriate source of antigens for dendritic-cell-based immunotherapy of ovarian cancer, we analyzed the expression levels of 21 tumor-associated antigens (BIRC5, CA125, CEA, DDX43, EPCAM, FOLR1, Her-2/neu, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, MUC-1, NY-ESO-1, PRAME, p53, TPBG, TRT, and WT1) in four established ovarian cancer cell lines and in primary tumor cells that were isolated from high-grade serous epithelial ovarian cancer tissue. More than 90% of the tumor samples expressed very high levels of CA125, FOLR1, EPCAM, and MUC-1 and elevated levels of Her-2/neu, which was similar to the OVCAR-3 cell line. The combination of the OV-90, SK-OV3, and OVCAR-3 cell lines showed the highest overlap with the patient samples in terms of their tumor-associated antigen (TAA) expression profile. Finally, we selected the OV-90 and SK-OV cell lines as the most suitable for pulsation of DCs, not only due to their high overlap with the patient samples in their tumor-associated antigen expression profile, but also due to the technical feasibility of obtaining a license for their use [21].

We introduced a new physical modality, high hydrostatic pressure (HHP), as a method for inducing immunogenic cell death (ICD) in tumor cell lines [14,20]. HHP induced a rapid expression of immunogenic markers such as HSP70, HSP90, and calreticulin on the cell surface, as well as the release of "danger signaling" molecules such as high-mobility group B1 (HMGB1) and adenosine triphosphate (ATP). The interaction of DCs with HHP-treated tumor cells led to enhanced DC phagocytosis, the upregulation of maturation and activation of the surface markers CD83, CD86, and HLA-DR, and the release of the proinflammatory cytokines IL-6, IL-12p70, and tumor necrosis factor alfa (TNF-α). DCs that were pulsed with HHP-treated tumor cells induced high numbers of tumor-specific $CD8^+$ and $CD4^+$ T cells, but on the other hand, they produced the lowest number of regulatory T cells (FoxP3+), establishing HHP as a reliable and potent inducer of immunogenic cell death in human tumor cells [14,22].

Additionally, we developed a fast DC protocol by comparing standard DCs (Day 5 DCs) and fast DCs (Day 3 DCs), generated in CellGro media and subsequently activated by means of Poly (I:C) or LPS. We found that Day 3 DCs that were activated using Poly (I:C) were about as potent in most functional aspects as DCs that were produced using the standard 5-day protocol. This fact provides a rationale for faster protocols for DC generation in clinical trials [19].

In order to improve the performance of DC-based vaccines, we are currently working on further modifications of the manufacturing process. These modifications aim to skew the differentiation of DC progenitors and achieve the maturation of DCs into a phenotype that could effectively induce a Th1 response and that could effectively translate into much stronger proliferation of tumor-reactive lymphocytes, namely, the $CD8^+$ T cells. One of these modifications is the implementation of LL-37 into the manufacturing process. LL-37 is an active form of antimicrobial peptide cathelicidin that can either suppress or stimulate immune responses based on the actual conditions. Due to this immunomodulatory duality, we have tested multiple algorithms of its implementation into different phases of the

production of monocyte-derived DCs. We found that the differentiation of monocyte-derived DCs in the presence of LL-37 minimally improved the ability of DCs to induce the proliferation of $CD8^+$ T cells. However, also implementing LL-37 during the phases of the DC antigen pulsation (loading) and maturation markedly enhanced the produced DCs' ability to expand $CD8^+$ T cells, downregulate their expression of PD-1, and significantly enhance the frequency of tumor-cell-reactive $CD8^+$ T cells. These attributes also translated into a superior in vitro cytotoxicity of the expanded cells against tumor cells. These data surprisingly demonstrated that whereas a partial implementation of LL-37 into the cell production process does not have the desired impact on the antitumor performance of the produced DCs, LL-37 implementation into the entire process of their ex vivo production elicits the desired impact, leading to a significantly enhanced antitumor performance of the produced DCs [23]. However, introducing such a change into the GMP manufacturing process requires months of administrative work, and a "new" DC product should be tested from scratch in new clinical trials. Compliance with all legislative requirements has a significant financial impact and substantially slows down the clinical use of the product.

3. Tumor Microenvironment in Ovarian Cancer and Its Impact on Disease Outcome

In parallel with the preparation of manufacturing processes for the generation of a dendritic cell vaccine, we studied immunological markers in the blood and within primary tumors, as well as within metastases, with the aim to better understand the immunological contexture of tumors and its impact on the disease outcome [24–26]. The immunological configuration of ovarian carcinoma was analyzed, highlighting the poor infiltration by immune cells and active immunosuppression within the tumor microenvironment [27]. A comparative analysis of the humoral and cellular features of primary and metastatic epithelial ovarian carcinoma was performed, proposing measures to alter them to increase treatment sensitivity and patient survival [26].

We also described the dynamics of T cell infiltration during the course of ovarian cancer. We studied immune cells that infiltrated the tumor tissues and circulated in the peripheral blood of ovarian cancer patients at different stages of the disease. Patients in the early stages of development of ovarian cancer (stages I–II) were characterized by a strong Th17 immune response. In stage II patients, we observed the recruitment of high numbers of Th1 cells. In disseminated tumors (stages III–IV), we found a dominant population of activated regulatory T cells (Tregs) expressing the molecule Helios, as well as high numbers of myeloid dendritic cells (mDCs) and monocytes/macrophages. The tumor-infiltrating Tregs had a markedly lower expression of the chemokine receptor CCR4 than that of circulating Tregs. The number of tumor-infiltrating Tregs significantly correlated with the amount of chemokine CCL22 in ovarian tumor cell culture supernatants, suggesting their recruitment via a CCR4/CCL22 interaction. We demonstrated that the chemokine CCL22 was mainly produced by tumor cells, monocytes/macrophages, and mDCs in the primary ovarian tumors, and its expression markedly increased in response to IFNγ. On the basis of these experiments, we hypothesize that the recruitment of Tregs was triggered by inflammatory stimuli in the advanced stages of the disease, which finally led to significant immune suppression in the late stages of ovarian cancer. The gradual shift from a Th17/Th1 effector cell response to a predominant infiltration by regulatory T cells in the advanced stages reflects the decline in the effective antitumor immune response to a significant immune suppression, which enables the loss of the immune surveillance against tumors and, consequently, disease progression [28].

Furthermore, we investigated the expression of classic (programmed death receptor/ligand—PD-1/PD-L1) and more recently described coinhibitory checkpoint molecules (TIM-3) in relation to the functional orientation of the immune infiltrate in ovarian cancer [29]. High levels of PD-L1 and high densities of PD-1+ cells in the microenvironment of high-grade serous ovarian cancer were associated with an immune contexture that was characterized by robust Th1 polarization and a cytotoxic orientation, which enabled superior clinical benefits. However, PD-1+TIM-3+$CD8^+$ T cells presented features of func-

tional exhaustion and correlated with a poor disease outcome. The amount of TIM-3+ cells contributed to the patient stratification based on the intratumoral abundance of CD8+ T cells [29].

The potential impact of mature dendritic cells (DCs) in shaping the immune contexture of high-grade serous ovarian carcinoma, their role in the establishment of T cell-dependent antitumor immunity, and their potential prognostic value for HGSC patients were also investigated. A high density of tumor-infiltrating DCs expressing the molecule "dendritic cell lysosomal-associated membrane glycoprotein" (DC-LAMP) was clearly associated with an immune contexture that was characterized by Th1 polarization and cytotoxic activity. Both mature DCs and CD20+ B cells played a critical role in generating a clinically favorable cytotoxic immune response in the HGSC microenvironment. Robust tumor infiltration by both DC-LAMP+ DCs and CD20+ B cells was associated with the most favorable overall survival in two independent cohorts of chemotherapy-naïve HGSC patients [30].

4. Clinical Trials with the DC-Based Vaccine in Patients with Ovarian Cancer

After fulfilling all legislative requirements, including good manufacturing process (GMP) premises and regulatory approval, we conducted the first-in-human investigator-initiated clinical trial phase I of DCVAC/OvCa (EudraCT: 2010–021462-30) in patients with ovarian cancer. This small study included 10 patients with recurrent platinum-sensitive ovarian cancer in stages III-IV. The primary end points were safety and immune response. The study showed that the DC vaccination was safe. The administration of the DCVAC/OvCa led to increased numbers of NY-ESO-1-, MAGE-A1-, and MAGE-A3-specific T cells in the peripheral blood.

In 2010, the biotech company Sotio was founded and took over the further development of this dendritic cell immunotherapy. Sotio sponsored two phase II randomized clinical trials in patients with ovarian cancer. The first study was an open-label, parallel-group, phase 2 trial (NCR02107950) study, which included patients with platinum-sensitive ovarian cancer that relapsed after first-line chemotherapy. The DCVAC/OvCa was administered every 3–6 weeks up to 10 doses to patients who were randomized into arm A, who received the DCVAC/OvCa and chemotherapy. Patients in arm B were treated with chemotherapy alone. The endpoints of this clinical trial included safety, progression-free survival (PFS), and overall survival (OS) (PFS being the primary efficacy endpoint and OS the secondary one).

A total of 71 patients were randomized to chemotherapy in combination with the DCVAC/OvCa or to chemotherapy alone. Adverse events were mainly related to the chemotherapy. Progression-free survival was not improved significantly (hazard ratio 0.73, $p = 0.274$), while the 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 tendency to enhanced antigen-specific T cell activity was seen in patients who were assigned to the chemotherapy+ DCVAC/OvCa treatment [31].

In parallel, another phase II study (NCT02107937) was conducted to assess the safety and efficacy of dendritic-cell-based immunotherapy in patients with recently diagnosed ovarian cancer. The DCVAC/OvCa was added to first-line chemotherapy (carboplatin plus paclitaxel) after debulking surgery. Ninety-nine patients with stage III EOC (serous, endometrioid, or mucinous) who underwent cytoreductive surgery up to three weeks prior to randomization and were scheduled for first-line platinum-based chemotherapy were eligible. The patients were stratified by tumor residuum (0 or <1 cm) and were randomized (1:1:1) to DCVAC/OvCa along with chemotherapy (Group A), DCVAC/OvCa sequentially to chemotherapy (Group B), or chemotherapy alone (Group C). The primary endpoints were safety and progression-free survival (PFS), and the secondary endpoint was overall survival (OS). The modified intent-to-treat population included 31, 29, and 30 patients in Groups A, B, and C, respectively. There were no differences in the baseline characteristics between the three treatment arms. The median PFS was 20.3, not reached, and 21.4 months in Groups A, B, and C, respectively. The hazard ratio for Group A versus Group C was 0.98 (0.48 to 2.00; $p = 0.9483$), and the hazard

ratio for Group B versus Group C was 0.39 (0.16 to 0.96; $p = 0.0336$). The median OS was not reached in any group after a median follow-up of 66 months (34% of events), but a non-significant trend of improved OS in Groups A and B was noted. DCVAC/OvCa application and the process of leukapheresis itself were not associated with significant safety concerns. Overall, DCVAC/OvCa administration showed a good safety profile. Thus, this study found that DCVAC/OvCa administration sequentially to platinum-based first-line chemotherapy led to a statistically significant improvement in the progression-free survival in patients with epithelial ovarian cancer [32].

A deeper analysis of the data from this trial revealed that patients with so-called cold tumors, which typically have a poor prognosis, benefited the most from the DC immunotherapy. We analyzed pretreatment tumor samples taken from primary surgery and pretreatment and post-treatment peripheral blood samples from 82 patients who were enrolled in this trial. The aim was to identify biomarkers that would predict the clinical outcome of patients with epithelial ovarian cancer who were treated with the DCVAC/OvCa. The samples were analyzed using several methods, including immunohistochemistry, flow cytometry, sequencing, and multispectral immunofluorescence microscopy. We found that patients with a low mutational burden and cold tumors (low numbers of $CD8^+$ T cells infiltrating the tumor) benefited from DCVAC/OvCa administration, both in terms of their overall survival, as well as in the induction of antitumor immunity. Patients with hot tumors (characterized by the high numbers of $CD8^+$ T cells that are infiltrating the tumor) had quite a good prognosis with the application of chemotherapy only. Adding immunotherapy by means of the DCVAC/OvCa did not further improve the outcome of the disease in this subgroup of patients. DCVAC/OvCa administration to patients with cold tumors improved their initially poor prognosis to a better prognosis than that of patients with pretreatment hot tumors. Thus, a DC-based vaccination seems to initiate clinically relevant anticancer immune responses in patients with cold tumors. Based on these data, the numbers of $CD8^+$ T cells infiltrating a tumor, combined with the level of the mutation burden, might serve as a good biomarker for the selection of patients who will benefit from immunotherapy by means of DC-based vaccines [33].

The administration of DC vaccines developed by our team also showed efficacy in patients with non-small cell lung cancer in phase II clinical trials [34]. In addition, a subgroup of patients with metastatic castrate-resistant prostate cancer in a phase III clinical trial benefited from this treatment [35]. A list of selected clinical trials with the DCVAC is presented in Table 1.

Table 1. Selected clinical trials with DCVAC.

Clinical Trials with DCVAC/PCa—Prostate Cancer			
Phase III—VIABLE SP005 *Started in May 2014* NCT02111577	Randomized, double-blind, multi-center, parallel-group study of the DCVAC/PCa drug added to the standard of care, in comparison with placebo	Men with metastatic CRPC who were eligible for first-line chemotherapy	[35]
Clinical Trials with DCVAC/OvCa—Ovarian Cancer			
Phase II—SOV01 *Started in November 2013* NCT02107937	Randomized, open-label, three-arm, multi-center phase II clinical trial evaluating the effect of adding DCVAC/OvCa to standard chemotherapy (carboplatin and paclitaxel)	Women with newly diagnosed epithelial ovarian cancer, right after radical debulking surgery	[32]
Phase II—SOV02 *Started in November 2013* NCT02107950	Randomized, open-label, parallel-group, multi-center phase II clinical trial evaluating the effect of adding DCVAC/OvCa to standard chemotherapy (carboplatin and gemcitabine)	Women with 1st relapse of platinum-sensitive epithelial ovarian cancer	[31]
Clinical Trials of DCVAC/LuCa—Lung Cancer			
Phase I/II—SLU01 *Started in December 2014* NCT02470468	Randomized, open-label, three-arm, parallel-group, multi-center phase I/II clinical trial evaluating the safety and efficacy of DCVAC/LuCa added to standard first-line chemotherapy with carboplatin and paclitaxel ± immune enhancers	Patients with stage IV non-small cell lung carcinoma (NSCLC)	[34]

5. Discussion and Conclusions

Despite significant progress in the complex management of oncological diseases, including immunotherapy of different tumors, relatively little progress has been achieved in the management of and prognosis for ovarian cancer. The majority of women with EOC reach complete remission after primary or interval cytoreductive surgery combined with chemotherapy based on a platinum–taxane doublet, but almost all experience a relapse of the disease [36]. Understanding the genetic and molecular background of the disease led only to a partial improvement in the prognosis of patients. This is the case with homologous recombination (HR) defects that are imposed by germline or somatic BRCA1 DNA-repair-associated (*BRCA1*) or *BRCA2* mutations, which are key determinants of platinum sensitivity in EOC patients and provide a strong rationale for maintenance therapy based on PARP inhibitors, which is generally associated with improved progression-free survival (PFS). The hope that an improvement in PFS with PARP inhibitors will translate into an overall survival benefit only concerns the population of HR-deficient patients, who represent a minority of ovarian cancer patients. Even HR-deficient patients do ultimately develop recurrence of the disease and a resistance to PARP inhibitors. The development of novel therapies for patients with ovarian cancer is thus very urgent.

The approval of immune checkpoint inhibitors (ICIs) for the treatment of various tumor types such as melanoma, non-small-cell lung cancer, or renal cancer has also created hope for patients with EOC. Unfortunately, the efficiency expectations have not been met in the case of ovarian cancer. EOC is not very sensitive to ICIs, both when administered as a monotherapy or in combination with chemotherapy or PARP inhibitors [37–39]. The reason for this ineffectiveness of ICIs in EOC is related to an absent or low anticancer immunity in the majority of patients and the highly immunosuppressive tumor microenvironment at baseline. Based on our data and other published scientific articles, multiple mechanisms are involved in the formation of an immunosuppressive environment in EOC. We and others described increased levels of proinflammatory or immunosuppressive cytokines, including interleukins IL-6 and IL-10, macrophage migration inhibitory factor (MIF), and vascular endothelial growth factor A, as well as an increased level of immunosuppressive metabolites such as indoleamine 2,3-dioxygenase 1 (IDO1), lactate, and arginase 1. With the progression of the disease, these factors contribute to the accumulation of immunosuppressive cells, including regulatory T cells, tolerogenic dendritic cells, and various types of myeloid suppressor cells (MDSCs) and tumor-associated macrophages. Targeting these factors and cells by means of various pharmacologic and therapeutic approaches represents attempts to break the immunosuppression in EOC in order to improve the prognosis of patients suffering from this disease [13,40]. The possible approaches in the current treatment of ovarian cancer are shown in Figure 1. Here, the scheme does not include surgery as the primary treatment modality, standard chemotherapy, or local administration of chemotherapeutics such as HIPEC (hyperthermic intraperitoneal chemotherapy).

The figure illustrates the various immunotherapeutic approaches that are employed in the treatment of ovarian cancer, which are as follows: (1) monoclonal antibodies (mAbs) directed against tumor-associated antigens (TAAs), including anti-VEGF and anti-EGFR mAbs, or mAbs that are conjugated with cytotoxic agents (referred to as ADC, antibody–drug conjugate), including ADC-targeting antifolate receptor alpha and ADC-targeting Napi2b; (2) checkpoint inhibitors, including mAbs targeting PD-1, PD-L1, CTLA-4, and TIM-3; (3) bispecific antibodies, such as antibodies that are directed against the epithelial cell adhesion molecule (EpCAM) and the T cell antigen CD3; (4) immunomodulatory radiotherapy; (5) adoptive T cell therapy and therapy with CAR-T cells, including anti-ALPP CAR-T cells and anti-MESO CAR-T cells; (6) cytokines, such as interleukin-2/15; (7) antitumor vaccines, such as peptide vaccines, DC-based vaccines, and recombinant viral vaccines; (8) oncolytic viruses, such as GL-ONC1; (9) agents that affect metabolic processes, such as indoleamine 2,3-dioxygenase (IDO) or small molecules such as PARP or kinase inhibitors. Abbreviations: ADC—antibody–drug conjugate; ALPP—Alkaline Phosphatase Placental; CAR-T—Chimeric Antigen Receptor T cell; CD3—Cluster of Differentiation 3; CTLA-

4—Cytotoxic T Lymphocyte Antigen 4—DC: dendritic cell; EGFR—Epidermal Growth Factor Receptor; EpCAM—epithelial cell adhesion molecule; GL-ONC1—oncolytic virus; IDO—Indoleamine 2,3-Dioxygenase; IL-2—interleukin-2; mAbs—monoclonal antibodies; MESO—Mesothelin; PD-1—Programmed Cell Death Protein 1; PD-L1—Programmed Death Ligand 1; TAAs—tumor-associated antigens; VEGF—vascular endothelial growth factor.

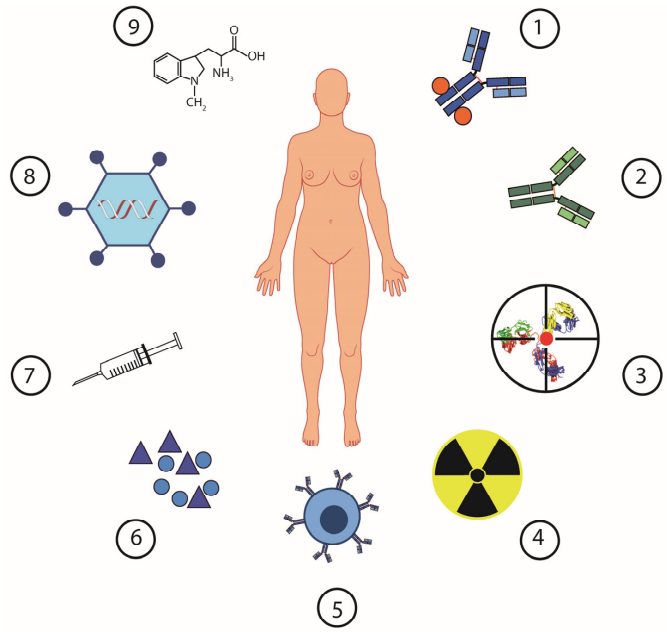

Figure 1. Immunotherapeutic strategies in the treatment of ovarian cancer.

Our research contributed to a better understanding of the roles of different cells of the immune system in the tumor microenvironment. We found that pre-existing immunity in the ovarian TME has a major impact on the sensitivity of EOC to immunotherapy using a dendritic cell vaccine. The identification of other immune biomarkers which might be integrated into common diagnostic assessments will guide appropriate treatment selection in the future.

We developed and tested a DC-based immunotherapy in patients with EOC. Our data showed that the administration of the therapy is safe and translates into clinical benefits both in patients with recurrent ovarian cancer in combination with second-line chemotherapy, as well as in patients with early-stage disease after cytoreductive surgery in combination with the first-line platinum-based chemotherapy. Patients with cold tumors that are characterized by a low mutation burden and low T cell infiltration experienced the most significant clinical benefit from the therapy. Based on these observations and other scientific data, a combination of treatment modalities that respects the individual baseline immune characteristics of the tumor microenvironment seems to be the best therapeutic strategy to reverse the natural course of the disease.

Based on the promising data from phase II clinical studies, we planned to initiate a phase III study in patients with recurrent platinum-sensitive ovarian cancer. However, the registration study for the DC-based vaccine did not commence. While it was initially delayed by the COVID-19 pandemic, subsequent changes in the ownership structure of Sotio led to the decision not to continue the vaccine's development due to the lengthy and risky nature of a phase III trial. The current regulatory environment suggests that a phase III trial would take approximately eight years to yield statistically relevant results for product registration. Consequently, there is no business case for pharmaceutical companies to co-develop such a product, and the investment would be too high and risky for a single sponsor. In light of the recent failure of PARP inhibitors in HR-proficient ovarian cancer patients [41] and the inefficacy of the approved checkpoint inhibitors for this disease, there

is currently no immunotherapy that will be available in the near future for ovarian cancer patients. It is hoped that the right combination, timing, and/or sequence of the current therapies, along with a personalized approach based on relevant biomarkers, will improve the prognosis for patients with this deadly disease.

Funding: This research was partially funded by Ministry of Health of the Czech Republic, grant number NU22-03-00300. All clinical studies were conducted in compliance with GCP.

Conflicts of Interest: The author is a minority shareholder of the biotech company Sotio Biotech, a.s.

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