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Exploring the feasibility of anti-PD-1/PD-L1 immunotherapy in endometriosis-associated ovarian cancer

Epithelial ovarian carcinoma (EOC) is the most common form of ovarian cancer and the deadliest gynecologic cancer in women (1), with a particularly poor prognosis because most cases are diagnosed as late-stage invasive disease (2). Despite advances in surgery and treatment, the 5-year survival rate is <50% for all EOC types (3). Epithelial ovarian carcinoma is classified into five major histologic subtypes, each of which has different cellular origins and molecular profiles. High-grade serous ovarian carcinoma is the most common and aggressive form of EOC (2), followed by endometrioid ovarian carcinomas (EnOC) and ovarian clear cell carcinomas (OCCC), which are both grouped under the term "endometriosis-associated ovarian carcinoma" (EAOC). Clinicopathologic and epidemiologic findings suggest that endometriosis, a common gynecologic disease affecting 10%–15% of reproductive-age women (4), is the precursor of both EnOC and OCCC. Among the histologic types of ovarian endometriosis, atypical endometriosis (AE) has been postulated as the transitioning entity from benign lesions to malignant variants in 60%–80% of all EAOC (5).

The gold standard for EOC treatment consists of cytoreductive surgery followed by taxane/platinum-based chemotherapy, although in many cases, patients relapse or develop resistance to the treatment. Should refractory or resistance to platinum-based therapy appear, the life expectancy reduces to <1 year, calling for the development of new treatment options to significantly increase their response rate and survival (3).

Successful development of new therapies based on immune checkpoint blockade has been raised as a promising new option for cancer treatment. Immune checkpoints are immunologic synapsis composed of molecules acting as receptors or ligands that are present on the surface of distinct immune cells and mediate the transduction of either inhibitory or stimulatory signals on target recognition. Under normal physiologic conditions, immune checkpoints are essential to maintain self-tolerance and to protect tissues after a response to pathogens. However, in the tumor microenvironment, the immune response could be suppressed drastically in intensity, quality, and duration, because tumors express suppressor molecules as a mechanism of immune resistance, particularly to evade the action of tumor-specific T cells (3). In this context, the pharmacologic interruption of this process by immune checkpoint inhibitors has improved the prognosis of affected patients with multiple types of cancer (e.g., non–small cell lung carcinoma and melanoma) (2).

For instance, the PD-1 receptor, expressed on the surface of T cells, natural killer cells, B cells, macrophages, and several subsets of dendritic cells, is inactivated after binding of its ligand, PD-L1. For this reason, the up-regulation of PD-L1 observed in tumor cells serves as a self-protection mechanism against immune attack (1). Additionally, tumors mimic the action of antigen-presenting cells by expressing B7 on their cell surface, which is capable of inhibiting T lymphocytes by appropriate binding to CTLA-4 receptors. In this context, the blockade of the PD-L1/PD-1 and/or B7/CTLA4 interaction by specific antibodies can restore T cell function, leading to enhanced antitumor immune responses. Accordingly, several antibodies targeting CTLA-4 (e.g., ipilimumab), PD-1 (e.g., pembrolizumab), and PD-L1 (e.g., atezolizumab) have been approved for the treatment of several malignancies.

The activity of anti-CTLA-4 was demonstrated in patients with melanoma, but the results showed higher toxicity and lower response rates than treatment with anti-PD-1/PD-L1 monoclonal antibodies. Therefore, the latter are now approved as monotherapy in various cancer types, including second-line treatment in advanced melanoma patients who have progression after ipilimumab and BRAF inhibitors, and in small cell lung cancer patients with progression after at least one platinum-based chemotherapy regimen (3).

Although the expression and functions of tumor-infiltrating lymphocytes (TILs) and the PD-1/PD-L1 pathway have been studied in many cancers, including high-grade serous ovarian cancer, few studies have focused on EAOC. With that aim, in the current issue of *Fertility and Sterility*, Nero et al. (5) performed a study including patients with a histological diagnosis of early EAOC (FIGO stage IA–IIB; n = 55) and patients with different types of benign endometriosis: ovarian endometriosis (OE; n = 55), AE (n = 12), and deep endometriosis (n = 44). From formalin-fixed, paraffin-embedded ovary samples, lymphocyte subpopulations ($CD3^+$, $CD4^+$, and $CD8^+$) and PD-1 and PD-L1 expression were analyzed by immunostaining. PD-L1 was assessed with the use of two different scores: combined positive score and tumor proportion score, which express the percentage of PD-L1 staining cells in the total number of viable tumor cells, and the percentage of viable tumor cells with a partial or complete membrane staining, respectively. Interestingly, these scores had been used previously for PD-L1 assessment in cancer, but not in endometriosis, in which müllerian type epithelium and endometrial type stroma were considered as PDL-1 staining tumor cells for calculating these two scores.

The study by Nero et al. (5) is of great interest for several reasons. It showed that stromal TIL markers ($CD3^+$, $CD4^+$, and $CD8^+$) and PD-1/PD-L1 molecules are expressed differently within EAOC, OE, AE, and deep endometriosis cases. Regarding TIL count, differences were especially significant between EOAC and OE cases, noting a lower presence in EAOC of TILs in general and, specifically, of $CD4^+$ T

Supported by the ISC III and "Fondo de Desarrollo Regional: una manera de hacer Europa" (PI17/01945), by the Department of Innovation, Universities, Science and Digital Society of the Valencian Community (GV/2020/200, APOSTD/2019/087 and ACIF/2020/216) and by the General Hospital Research Foundation University of Valencia (FIHGUV 2019 Award).

lymphocytes. Moreover, they observed a higher PD-1/PD-L1 combined positive score in the EAOC group than all other groups.

Interestingly, it was also observed that one-third of patients with OE showed a PD-1/PD-L1 expression profile similar to EAOC cases. This finding is of great interest because it suggests that patients with a benign condition might suffer expression changes characteristic of a malignant condition (EAOC), to which they are likely to progress to. Accordingly, an accurate follow-up of these patients would be deserved to determine whether they finally develop EAOC. This discovery, transferred to the clinic, would allow earlier diagnosis of EAOC in patients with OE who present with a PD-1/PD-L1 expression profile characteristic of EAOC by establishing specific detection protocols in this high-risk population.

Additionally, the investigators observed differential expression of PD-1 and PD-L1 between EAOC and their proposed precursor lesion, AE. However, when they focused the study on a cohort of EAOC patients with AE lesions in the contralateral ovary, the results showed no differences between EAOC and AE samples from the same patients, reinforcing the hypothesis that AE is a precursor of EAOC. Considering that an important limitation of the study is the small number of subjects included with AE (n = 12) and EAOC patients with AE lesions (n = 8), these promising observations would need to be tested in a large number of patients to draw evidence-based conclusions.

To date, clinical studies of immunotherapies with antibodies against PD-1 and PD-L1 have presented modest responses in patients with EOC, partly because of a low mutation burden of the tumor and also because of the redundancy of checkpoints by which tumor cells overcome the blockade. In this scenario, the study by Nero et al. (5) lays the foundation for a putative biomarker to implement the use of immunotherapies in EAOC. In this sense, promising (albeit limited) data have been observed in several clinical trials. On one hand, the UMIN000005714 study, a phase II trial with nivolumab (anti-PD-1) as monotherapy, showed an overall response rate of 15% in patients with EOC. Interestingly, 2 of a total of 20 patients had a complete response, one of them having OCCC. On the other hand, in the phase II KEYNOTE-100 study (NCT02674061) using pembrolizumab (anti-PD-1) in monotherapy, the overall response rate was 8% in patients with EOC, but 16% in patients with OCCC (3). These results abrogate for a better response to immunotherapy in OCCC among other EOC.

Currently, several clinical trials using monoclonal antibodies targeting PD-1 or PD-L1 in patients with EAOC are in progress. Specifically, two trials (HEARTBEAT-OV [NCT04510584] and Re-VOLVE [NCT05065021]) have been approved for EnOC patients and are currently in the patient recruitment phase. The HEARTBEAT-OV study is intended to evaluate a combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) as maintenance treatment in patients with a *TP53* mutation. The latter (Re-VOLVE) is addressed to patients with progression after treatment with PARP inhibitors, testing the use of the anti-PD-1 dostarlimab in different combinations with niraparib (PARP inhibitor) and bevacizumab to optimize the combination treatment algorithm for these patients. Regarding patients with OCCC, a clinical trial (NCT03355976 or BrUOG) using nivolumab (anti-PD-1) in monotherapy or in combination with ipilimumab is now in the patient recruitment phase.

In conclusion, the observations made by Nero et al. (5) and the emerging data from clinical trials postulate a putative role for the use of immunotherapy in EAOC, with a possible benefit compared with EOC in general. Preliminary data indicate that EAOC subtypes might show an enhanced response to immune checkpoint inhibitors addressed to PD-1/PD-L1, reinforcing the need for studying the immune mechanism of PD-1/PD-L1 blockade in EAOC and the combination of this blockade with other immunotherapies, which may increase the effect and improve treatment responses.

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https://doi.org/10.1016/j.fertnstert.2021.11.007

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