

Programmed cell death pathway in epithelial ovarian cancer: Brief review of a novel immunotherapeutic perspective

Tiberiu-Augustin Georgescu^{1,2}, Antonia Carmen Lisievici¹, Lucian Pop^{3,4}, Nicolae Bacalbasa³, Irina Balescu⁵, Corina Grigoriu^{4,6}, Roxana Elena Bohiltea^{4,7}, Claudia Stoica^{8,9}

¹Department of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

²Department of Pathology, "Alessandrescu-Rusescu" National Institute for Mother and Child Health, Bucharest, Romania

³Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

⁴Department of Obstetrics and Gynecology, "Alessandrescu-Rusescu" National Institute for Mother and Child Health, Bucharest, Romania

⁵Department of Visceral Surgery, Ponderas Academic Hospital, Bucharest, Romania

⁶Department of Obstetrics and Gynecology, University Emergency Hospital, Bucharest, Romania

⁷Department of Obstetrics and Gynecology, Filantropia Clinical Hospital, Bucharest, Romania

⁸Department of Anatomy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

⁹Department of Surgery, Ilfov County Emergency Hospital, Bucharest, Romania

ABSTRACT

During the past several decades, ovarian carcinoma has remained the leading cause of death among gynecologic malignancies. The current therapeutic guidelines recommend, in most cases, a combination of surgery and chemotherapy based on platinum agents and taxanes. Despite the increasing usage of newer drug categories, such as bevacizumab and PARP inhibitors, and the expansion of patient groups for these drugs, ovarian cancer is characterized by multiple recurrences, particularly in the form of peritoneal implants. In this article, we perform a short incursion into the programmed cell death pathway in epithelial ovarian cancer and conduct a brief review of literature regarding this novel immunotherapeutic perspective.

Keywords: ovarian cancer, PD-1, immunotherapy, programmed cell death

INTRODUCTION

Ovarian carcinoma affects roughly 1 in 70 women worldwide and is characterized by presentation at late stages of disease, resulting in poor clinical outcome, with only 45% of patients surviving 5 years after diagnosis. Current therapeutic guidelines for ovarian cancer include surgery and cytotoxic chemotherapy. Although approximately 80% of patients may achieve complete or at least partial clinical remission using this regimen, the majority of women will experience multiple recurrences, de-

velop chemotherapy resistance and eventually die due to cancer-associated morbidity. Therefore, although chemotherapy remains the main strategy for ovarian cancer management, innovative treatments are needed to improve the clinical outcome for patients with this disease.

Fortunately, during the past several years there has been increasing evidence indicating that ovarian cancer is a rational target for immunotherapy. Several studies showed that ovarian cancer is capable of inducing spontaneous anti-tumor immune responses, and that cytotoxic T-cell infiltration in

ovarian cancer correlates with significant improvements in overall survival.

In this article, we perform a short incursion into the programmed cell death pathway in epithelial ovarian cancer and conduct a brief review of literature regarding this novel immunotherapeutic perspective.

CONNECTION BETWEEN MICRORNAS, LNCRNAS AND PD-1/PD-L1 IN CANCER

MicroRNAs are small non-coding RNAs that function as oncogenes or tumor suppressors in the cell cycle, through the down regulation of the expression of beta-catenin (1). Studies have shown that its expression is dysregulated in patients with carcinoma through abnormal transcription, amplification and deletion of microRNA genes or even epigenetic defects. In tumors, these alterations can be translated as increased cell death resistance, a sustained proliferative signaling pathway and diverse mechanisms, which can promote tissue invasion and the ability to metastasize (2).

PD-1/PD-L1 expression has been thoroughly researched in the last decade, with most research papers focusing on its expression in lung carcinoma. Recent research papers have demonstrated that in non-small cell lung carcinoma, microRNA-138 can activate the immune response and inhibit tumor growth by down-regulating the tumors capacity to proliferate and by increasing the number of tumor-infiltrating dendritic cells (DC). Furthermore, the expression of PD-1/PD-L1 on dendritic cells and T lymphocytes is down regulated through microRNA-138 (3).

Boldrini et al. have studied the relationship between microRNA-33a and PD-1 expression in lung adenocarcinoma and have observed an inverse correlation between the levels to the two molecules. Patients with high levels of microRNA-33a also had low levels of PD-1, had a better prognosis, and were more commonly encountered in female patients and in patients with low-grade tumors. These results emphasize the potential use of microRNA-33a as a prognostic marker (4).

Similarly, studies have shown that microRNA-4717 levels were significantly decreased in the T lymphocytes of patients with chronic hepatitis and hepatocellular carcinoma that were related to hepatitis B virus infection. This observation has a therapeutic significance, because in patients with chronic HBV infection (rs 10204525, genotype GG), microRNA-4717 was shown not only to increase the IFN- γ and TNF- α levels, but also to decrease PD-1 expression in lymphocytes (5).

Incorvaia et al. have identified a subset of microRNA, called “lymphocyte miRNA signature”,

which was silenced in patients with metastatic clear cell carcinoma of the kidney, but well expressed in patients who had a favorable response to nivolumab therapy. Moreover, nivolumab has been shown to induce a high expression of microRNA-22, a fraction that is usually down regulated in patients with renal clear cell carcinoma. Also, an attenuated tumor immune response was observed in patients with low levels of microRNA-339 and high levels of PD-L1 (6).

Chen et al. have demonstrated on a mouse model, that the cancer progression can be delayed by suppressing the epithelial-mesenchymal transition through microRNA-200, which targets PD-L1 (7). Taken into account this information, the immune response to tumors can be regulated also by targeting the expression of microRNA, which influences the immune checkpoint mRNAs. The microRNA-33a down regulates the expression of PD-1 and PDL-1, by binding to the target mRNA of both genes, at the 3'UTR region (4,8). Thus, in selected cases of non-small cell carcinoma of the lung and in clear cell carcinoma of the kidney, micro-RNA could serve as a predictive tumor biomarker.

Long non-coding RNAs (lncRNA) are defined as an RNA with more than 200 nucleotides, which lacks the ability to translate into proteins. The functions of lncRNA are still incompletely understood, but most of them involve regulation of cell differentiation and development, as well as metabolic balance and modulation of the immune response (9).

In diffuse large B cell lymphoma, the lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) can influence microRNA-195, ending in high levels of PD-L1, which helps elude the host immune system by regulating proliferation and apoptosis of CD8+ lymphocytes. Thus, a therapeutic target in diffuse large B cell lymphoma could be the inhibition of MALAT1 (10). Additionally, Zhao et al. have observed that in diffuse large B cell lymphoma, another lncRNA, called SNHG14 (small nucleolar RNA host gene 14) is up regulated and can promote tumoral progression and immune evasion, through regulation of PD-1/PD-L1 checkpoint. SNHG14 does so by sponging miR-5590-3p to up regulate ZEB1 (Zinc Finger E-Box Binding Homeobox 1), a protein coding gene, which transcriptionally activates SNHG14 and PD-L1, leading to immune evasion of tumoral cells (11).

In esophageal squamous carcinoma, the expression of PD-L1 can also be increased though the presence of small nucleolar RNA host gene 20 (3). The latter can modulate the ATM/JAK-PD-L1 pathway, thus serving as a carcinogen and promoting cellular proliferation and metastatic potential (12). In pancreatic cancer, the lncRNA LINC00473 is highly expressed and also leads to high levels of PD-L1 by

sponging miR-195-5p. Consequently, the inhibition of LINC00473 could augment the CD8⁺ T lymphocytes and subsequently suppress the development of neoplasia (12).

In lung carcinoma two main lncRNA can be co-expressed: NKX2-1-AS1 and NKX2-1. Both can have an influence on the gene CD274, which encodes PD-L1, albeit different. NKX2-1-AS1 can have a favorable effect, by reducing the production of CD274 mRNA, thus down regulating PD-L1, while NKX2-1 can actually promote the transcription of CD274. Consequently, NKX2-1-AS1 could be used as a favorable feature for the inhibition of the immune evasion (13).

PD-1/PD-L1 IN OVARIAN CANCER

Ovarian cancer is the third most frequent type of gynecological cancer after cervical and uterine cancer (14). It also has the worst prognosis and the highest mortality rate among gynecological cancers (15). Although it is less frequent than breast cancer, it is three times more lethal (16). Moreover, global cancer statistics anticipate that the mortality rate of this cancer will increase considerably in the following decades (14). The high mortality rate of patients with ovarian cancer is due to the presence of non-specific symptoms or complete absence of symptoms in the early stages of disease (17). It is also caused by the lack of specific diagnostic markers for ovarian carcinoma (18). It is estimated that approximately 90% of patients diagnosed with stage I and stage II ovarian cancer according to FIGO (International Federation of Gynecologists and Obstetricians) are cured. The 5-year survival rate in this group is as high as 70%. However, it decreases to about 30% in patients with advanced stages of disease (III and IV according to FIGO) (17). Unfortunately, in more than half of the patients, ovarian cancer is diagnosed in FIGO stage III and IV (19). In 80% of patients the disease goes into remission, however within 18 months in more than 60% of patients it relapses. Therefore, it is important to design new methods of treatment of ovarian cancer, including immunotherapies (17).

PD-L1 expression has been demonstrated in several types of cancer, including ovarian malignancy. In these patients, PD-L1 expression was demonstrated within neoplastic cells, as well as on B and T lymphocytes, macrophages and dendritic cells isolated from the primary tumor and loco regional lymph nodes. Hamanishi et al. demonstrated that high PD-L1 expression correlates with shorter survival rate in ovarian cancer patients. Moreover, the authors demonstrated a correlation between high PD-L1 expression and a lower percentage of cytotoxic (CD8⁺) tumor infiltrating lymphocytes (19).

The findings published by Webb et al., who discovered PD-L1 expression mainly in CD68⁺ macrophages, are somewhat different. Their study reveals a positive relationship between PD-L1 expression and the presence of tumor infiltrating regulatory T cells and/or lymphocytes with CD8⁺ CD103⁺ PD-1⁺ phenotype. The level of PD-L1 expression in the different histopathological subtypes of ovarian cancer varied greatly. Webb et al. demonstrated the highest expression of PD-L1 in serous ovarian carcinoma (57.4%), followed by mucinous ovarian carcinoma (26.7%) and endometrioid ovarian carcinoma (24%). The lowest expression of PD-L1 was found in clear cell ovarian carcinoma (16.2%) (20). The authors also demonstrated a positive correlation between PD-L1 expression and the overall survival of patients with high-grade ovarian serous carcinoma (HGSC) (20). Other studies have shown a relationship between high PD-L1 expression in the peritoneal fluid of ovarian cancer patients and the formation of metastases within the peritoneal cavity (21).

PD-1 AND PD-L1 IMMUNOTHERAPY IN OVARIAN CANCER

Understanding the programmed cell death pathway associated with the PD-1 receptor allowed the design of clinical trials for treating ovarian cancer using antibodies against the PD-1 receptor (nivolumab, pembrolizumab) and the PD-L1 ligand (avelumab, BMS - 936559, durvalumab, atezolizumab).

Hamanishi et al. (22) conducted the first study on the use of nivolumab in the treatment of ovarian cancer. Nivolumab is a fully humanized IgG4 monoclonal antibody that binds to the PD-1 receptor. This inhibits its binding to PD-L1 or PD-L2 (22) and may increase the antitumoral activity of T lymphocytes (23). In 2015, the results of the second phase of the study were published, including 20 patients with platinum-resistant advanced ovarian cancer. Nivolumab was administered intravenously every two weeks at doses of 1 or 3 mg/kg in two parallel cohort studies (10 patients each). The best overall response to treatment was 15%, while the disease control rate in all 20 patients was 45%. Two subjects showed a sustained (> 350 days) response to treatment (in the 3 mg/kg cohort). Interestingly, in one case, clear cell ovarian carcinoma had a worse prognosis than the more common serous ovarian carcinoma (24). The dose of 3 mg / kg was found to be more advantageous than the dose of 1 mg / kg because it has a better pharmacological profile, being more effective, without increasing toxicity significantly. In patients enrolled in the clinical trial, the median progression-free survival was 3.5 months and the median overall survival was 20 months. Clinical efficacy of nivolumab has been

demonstrated in patients with platinum-resistant ovarian cancer; however, the authors of the study emphasize the need for larger-scale clinical trials (25,26).

Pembrolizumab is another humanized anti-PD-1 monoclonal antibody which prevents interaction between PD-1 and PD-L1 / L2 ligands. Varga A et al. published interim results from a Phase Ib study including 26 patients with advanced ovarian cancer, fallopian tube cancer and primary peritoneal cancer (27). The prerequisite for eligibility was PD-L1 expression in neoplastic cells ($\geq 1\%$) and failure of prior therapy. Pembrolizumab was administered intravenously at a dose of 10 mg / kg every two weeks for two years, or until the patient had to be excluded from the study because disease progression or unacceptable toxicity was confirmed. The overall response rate was 11.5% and the disease control rate was 34.6%. In the study group, 85% of patients have received therapy against recurrent or disseminated ovarian cancer in the past, 38.5% of whom have undergone 5 or more treatments. Complete response to treatment was obtained in 1 patient; partial response occurred in 2 patients, disease stabilization was confirmed in 6 patients (27,28).

PD-L1 is the second immunotherapy target in ovarian cancer. Avelumab is a fully humanized anti-PD-L1 IgG1 monoclonal antibody, which inhibits

PD-L1 interaction with PD-1 receptor (29). The study conducted with avelumab is the largest study to date involving the programmed death pathway (29). The percentage of objective responses in the group of 124 women with recurrent or refractory ovarian cancer was 9.7%, and the disease control rate was 54%. PD-1 expression was assessed in 74 cases and 57 women (77%) showed increased PD-L1 expression. In this group of patients, the objective response rate was 12.3%, while in the group of women without excessive PD-L1 expression it was 5.9%.

Studies were also carried out with the use of another monoclonal anti-PD-L1 antibody: BMS-936559. This study included 17 patients with ovarian cancer, among which 5.9% responded partially to treatment and 17.6% achieved disease stabilization. All patients received doses of 10 mg/kg. There are also studies on the effectiveness of durvalumab - a monoclonal antibody directed against the PD-L1 protein (30).

CONCLUSION

A role for the immune system in modulating ovarian cancer development and progression has been established by multiple investigators, providing a strong rationale to pursue immune-based therapeutic strategies for women with this disease.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

- Zhu C, Zhao Y, Zhang Z, Ni Y, Li X, Yong H. MicroRNA-33a inhibits lung cancer cell proliferation and invasion by regulating the expression of β -catenin. *Mol Med Rep.* 2015 May;11(5):3647-51.
- Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal Transduct Target Ther.* 2016 Jan 28;1:15004.
- Song N, Li P, Song P, Li Y, Zhou S, Su Q, Li X, et al. MicroRNA-138-5p Suppresses Non-small Cell Lung Cancer Cells by Targeting PD-L1/PD-1 to Regulate Tumor Microenvironment. *Front Cell Dev Biol.* 2020 Jul 10; 8:540.
- Boldrini L, Giordano M, Niccoli C, Melfi F, Lucchi M, Mussi A, Fontanini G. Role of microRNA-33a in regulating the expression of PD-1 in lung adenocarcinoma. *Cancer Cell Int.* 2017 Nov 17;17:105.
- Zhang G, Li N, Li Z, Zhu Q, Li F, Yang C, Han Q, Lv Y, Zhou Z, Liu Z. microRNA-4717 differentially interacts with its polymorphic target in the PD1 3' untranslated region: A mechanism for regulating PD-1 expression and function in HBV-associated liver diseases. *Oncotarget.* 2015 Aug 7;6(22):18933-44.
- Incorvaia L, Fanale D, Badalamenti G, Brando C, Bono M, et al. A "Lymphocyte MicroRNA Signature" as Predictive Biomarker of Immunotherapy Response and Plasma PD-1/PD-L1 Expression Levels in Patients with Metastatic Renal Cell Carcinoma: Pointing towards Epigenetic Reprogramming. *Cancers (Basel).* 2020 Nov 16;12(11):3396.
- Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn YH, Byers LA, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nat Commun.* 2014 Oct 28;5:5241.
- Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005 Jan 14;120(1):15-20.
- Zhou Y, Zhu Y, Xie Y, Ma X. The Role of Long Non-coding RNAs in Immunotherapy Resistance. *Front Oncol.* 2019 Nov 28;9:1292.
- Wang QM, Lian GY, Song Y, Huang YF, Gong Y. LncRNA MALAT1 promotes tumorigenesis and immune escape of diffuse large B cell lymphoma by sponging miR-195. *Life Sci.* 2019 Aug 15;231:116335.
- Zhao L, Liu Y, Zhang J, Liu Y, Qi Q. LncRNA SNHG14/miR-5590-3p/ZEB1 positive feedback loop promoted diffuse large B cell lymphoma progression and immune evasion through regulating PD-1/PD-L1 checkpoint. *Cell Death Dis.* 2019 Sep 30;10(10):731.
- Zhang C, Jiang F, Su C, Xie P, Xu L. Upregulation of long noncoding RNA SNHG20 promotes cell growth and metastasis in esophageal squamous cell carcinoma via modulating ATM-JAK-PD-L1 pathway. *J Cell Biochem.* 2019 Feb 14.
- Kathuria H, Millien G, McNally L, Gower AC, Tagne JB, Cao Y, Ramirez MI. NKX2-1-AS1 negatively regulates CD274/PD-L1, cell-cell interaction genes, and limits human lung carcinoma cell migration. *Sci Rep.* 2018 Sep 26;8(1):14418.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018 Nov;68(6):394-424.
- Coburn SB, Bray F, Sherman ME, Trabert B. International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. *Int J Cancer.* 2017 Jun 1;140(11):2451-2460.
- Yoneda A, Lendorf ME, Couchman JR, Multhaupt HA. Breast and ovarian cancers: a survey and possible roles for the cell surface heparan sulfate proteoglycans. *J Histochem Cytochem.* 2012 Jan;60(1):9-21.

17. Zhu X, Lang J. Programmed death-1 pathway blockade produces a synergistic antitumor effect: combined application in ovarian cancer. *J Gynecol Oncol*. 2017 Sep;28(5):e64.
18. Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, George AJ, Ghaem-Maghami S. Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. *Cancer Immunol Immunother*. 2014 Mar;63(3):215-24.
19. Hamanishi J, Mandai M, Konishi I. Immune checkpoint inhibition in ovarian cancer. *Int Immunol*. 2016 Jul;28(7):339-48.
20. Webb JR, Milne K, Kroeger DR, Nelson BH. PD-L1 expression is associated with tumor-infiltrating T cells and favorable prognosis in high-grade serous ovarian cancer. *Gynecol Oncol*. 2016 May; 141(2):293-302.
21. Abiko K, Mandai M, Hamanishi J, Yoshioka Y, Matsumura N, et al. PD-L1 on tumor cells is induced in ascites and promotes peritoneal dissemination of ovarian cancer through CTL dysfunction. *Clin Cancer Res*. 2013 Mar 15;19(6):1363-74.
22. Hamanishi J, Mandai M, Iwasaki M, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A*. 2007;104(9):3360-3365.
23. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012 Jun 28;366(26):2443-54.
24. Crotzer DR, Sun CC, Coleman RL, Wolf JK, Levenback CF, Gershenson DM. Lack of effective systemic therapy for recurrent clear cell carcinoma of the ovary. *Gynecol Oncol*. 2007 May;105(2):404-8.
25. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *J Clin Oncol*. 2015 Dec 1;33(34):4015-22.
26. Georgescu TA, Cirstoiu M, Costache M, Lazaroiu A, Dumitru A, Sajin M. Histopathological, Immunohistochemical and Therapeutical Assessment of Premalignant Endometrial Lesions in a Hospital Based Series of Cases. *Maedica (Bucur)*. 2016 Jun;11(2):115-121.
27. Varga A, Piha-Paul SA, Ott PA, et al. Antitumor activity and safety of pembrolizumab in patients (pts) with PD-L1 positive advanced ovarian cancer: Interim results from a phase Ib study. *J Clin Oncol*. 2015;33 (Suppl):Abstr 5510.
28. Gadducci A, Guerrieri ME. Immune Checkpoint Inhibitors in Gynecological Cancers: Update of Literature and Perspectives of Clinical Research. *Anticancer Res*. 2017 Nov;37(11):5955-5965.
29. Disis ML, Patel MR, Pant S, et al. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with previously treated, recurrent or refractory ovarian cancer: A phase Ib, open-label expansion trial. *J Clin Oncol*. 2015;33(Suppl):Abstr 5509.
30. Blümcke I, Giencke K, Wardelmann E, Beyenburg S, et al. The CD34 epitope is expressed in neoplastic and malformative lesions associated with chronic, focal epilepsies. *Acta Neuropathol*. 1999 May;97(5):481-90.