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Inflammation and immunity in ovarian cancer

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ABSTRACT

The standard first-line therapy for ovarian cancer is a combination of surgery and carboplatin/paclitaxel-based chemotherapy. Patients with longer survival and improved response to chemotherapy usually present T-cell inflamed tumours. The presence of tumour-infiltrating T cells (TILs) notably varies among the different subtypes of ovarian tumours, being highest in high-grade serous ovarian carcinoma, intermediate in endometrioid tumours, and lowest in low-grade serous, mucinous and clear cell tumours. Interestingly, the presence of TILs is often accompanied by a strong immunosuppressive tumour environment. A better understanding of the immune response against ovarian cancer and the tumour immune evasion mechanisms will enable improved prognostication, response prediction and immunotherapy of this disease. This article provides an overview of some ovarian cancer cell features relevant for antitumour response, such as tumour-associated antigens, including neoantigens, expression of inhibitory molecules, and other mechanisms of immune evasion. Moreover, we describe relevant immune cell types found in epithelial ovarian tumours, including T and B lymphocytes, regulatory T cells, natural killer cells, tumour-associated macrophages, myeloid-derived suppressor cells and neutrophils. We focus on how these components influence the burden of the tumour and the clinical outcome.

1. Introduction

Epithelial ovarian cancer (EOC) represents more than 95% of ovarian neoplasms [1]. Histologically, EOC is classified into 5 main subtypes: high-grade serous (HGSC), low-grade serous (LGSC), endometrioid, mucinous and clear cell ovarian carcinoma. All these subtypes have different presentation patterns and clinical outcomes, with HGSC being the most common subtype (~70%) and representing the majority of deaths [1]. Non-EOCs account for up to 5% of ovarian cancers and mainly include germ cell and sex-cord stromal cancers, as well as rare, small cell carcinoma and ovarian sarcoma [1]. Given the high incidence and mortality of EOC in relation to other ovarian cancer histologies, this review will focus on EOC.

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More than 75% of patients with EOC are diagnosed at late stages owing to the lack of symptoms and/or specific diagnostic biomarkers [2]. EOC primarily disseminates within the peritoneal cavity and is only superficially invasive. After surgical debulking and cytoreduction, 80% of patients experience recurrence with limited treatment options and poor survival.

EOC can be considered an immunogenic tumour because spontaneous antitumour immune response can be detected in around 55% of patients [3]. Interestingly, patients with somatic mutations in breast and ovarian cancer susceptibility protein (*BRCA*)*1/2* (*BRCA*-disrupted tumours) exhibit a higher mutational load and a unique inflammatory signature with increased number of tumour-infiltrating lymphocytes (TILs), as well as elevated expression of programmed cell death (PD-1) or its ligand (PD-L1) compared with homologous recombination (HR)–proficient tumours [4,5]. In addition, patients with T-cell–rich tumours experience longer progression-free and overall survival [6], whereas immune evasion mechanisms are associated with poor survival [7,8]. All these evidences taken together suggest that patients with EOC could potentially benefit from immunotherapy. However, the monotherapy with antibodies inhibiting the cytotoxic T lymphocyte–associated protein 4 (CTLA-4) or PD-1 or PD-L1 axis yielded only modest results in EOC [9,10]. The lack of success of immunotherapy in ovarian cancer can be attributed to dynamic interaction of the transformed cells with the unique tumour microenvironment (TME) that hosts this malignancy. Therefore, understanding the complex immunosuppressive network present in the TME of this neoplasm is crucial in the development of more efficient immunotherapeutic treatments.

2. Tumour antigens and spontaneous antitumour immune response in patients with EOC

The control of tumour progression by the immune system hinges on the ability of T cells to recognize tumour-associated antigens (TAAs) and kill tumour cells. TAAs can be classified into different categories as follows: tissue differentiation antigens (Ag), normal proteins overexpressed by cancer cells, cancer-testis (CT) Ags (also known as tumor germline Ags), viral proteins and tumour-specific mutated Ags. Among the tissue differentiation, Ags expressed in EOC is the epithelial cell adhesion molecule expressed on epithelial cells. Overexpressed Ags are proteins normally produced in very low quantities but whose production is dramatically increased in tumour cells (e.g.: Erb-B2 receptor tyrosine kinase 2, commonly referred to as HER2/neu) (Table 1). Tissue differentiation Ags and overexpressed proteins are poorly antigenic because they are subject to central tolerance mechanisms. Since they are also expressed in normal tissues, therapies targeting these Ags may lead to severe on-target, off-tumour toxicities.

CT Ags are proteins with normal expression restricted to germ cells in testis, female reproductive organs and trophoblast. Because germ cells do not express HLA molecules, they are ignored by the immune system. CT Ags are often expressed aberrantly in many different types of tumours. Some of them skip central tolerance mechanisms becoming highly antigenic. This, together with their tumour specificity, makes them significant targets for cancer immunotherapy. Several CT Ags have been described in EOC (Table 1) and have been proposed as immunotherapy targets, such as New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) and melanoma-associated antigen 3. Other Ags highly specific of ovarian tumours are surface glycoproteins that have an abnormal structure in tumour cells [Mucin (MUC)-1, MUC-2 and MUC-16, also known as ovarian carcinoma antigen CA125].

Altered proteins that result from non-synonymous mutations within the cancerous cells (neoantigens, NeoAgs) are highly antigenic and specific of tumour tissues. Some NeoAgs are the products of driver mutations and are shared by several types of tumours and patients (tumor protein P53, TP53), whereas the majority are private Ags (each patient's particular Ags) that result from passenger somatic mutations.

The likelihood of neoAgs directly correlates with the tumour mutational burden (TMB), being high in tumour with mutational loads higher than 10 somatic mutations/Mb and occasional in tumour with less than 1 somatic mutations/Mb [11]. Comprehensive genomic profiling of EOC revealed low overall TMB among subtypes: HGSC (3.6), LGSC (2.7), endometrioid (2.7), mucinous (2.7) and clear cell (2.7) tumours [12]. Only a small percentage of patients had a significant TMB (20 or more mutations per Mb) [12]. Tumours with deficient HR machinery, owing to mutations in *BRCA1/BRCA2* or other genes involved in HR, have higher predicted neoAg load [4]. Increased TMB has been also observed in tumours with defects in DNA mismatch repair [12]. Loss of expression of mismatch repair genes (mutS homologue 6 [MSH6], MSH2, MLH1, and PMS1 homologue 2, mismatch repair system component [PMS2]) is a characteristic of Lynch syndrome and is found in nearly 8% of Lynch syndrome–associated endometrioid and clear cell ovarian cancers [13]. This subgroup of patients presents significantly higher number of TILs, particularly PD-1 positive TILs, and expressed PD-L1 in tumour cells and/or intraepithelial or peritumoral immune cells.

Around 55% of patients with EOC show spontaneous antitumour immune response [3]. These patients usually present T-cell inflamed tumours and longer survival, as well as improved response to chemotherapy [6,14–18]. This spontaneous immune response can be detected in peripheral blood, tumours and ascites. T cells specific for NY-ESO-1 [19], folate receptor (FR)-α [20,21], Her2 [22,23], insulin-like growth factor binding protein 2 [24], TP53 [25] have been found in patients with EOC. Despite being a tumour with a low average of TMB [26], T cells specific for private neoAgs have been detected in patients with EOC [27–30]. These cells preferentially accumulate in tumours [27]. Interestingly, T cells specific for TP53 driver mutations have also been observed in patients with EOC [29,31]. In addition, antibodies against FR-α, MUC-1, TP53, NY-ESO-1 and mesothelin are frequently found in patients with EOC [32–34]. Multivariate analyses have shown that the presence of anti-TP53 antibodies is an independent predictor of survival [34]. Interestingly, anti-MUC-1 antibodies are also present in healthy women and the levels of anti-MUC-1 antibodies correlate inversely with the risk of EOC [35,36].

Collectively, these data show that immune response is naturally induced against TAA in EOC and impacts the clinical

Table 1 – TAA in Ovarian cancer.

Tissue-specific proteins	Overexpressed proteins	Cancer–testis Ags	Altered glycoprotein	NeoAgs
EPCAM	HER2/neu	NY-ESO-1	MUC-1	TP53
	FR-α	MAGE	MUC-2	'private' NeoAgs
	IGFBP-2	BAGE	MUC-16 (CA125)	
	MSLN	LAGE		
		GAGE		
		SP17		
		SSX		

The table shows example of TAA for each category.
BAGE, B melanoma antigen; LAGE, L antigen family member 3; GAGE: G antigen 1; SP17, sperm protein 17; SSX, synovial sarcoma X; TAAs, tumour-associated antigens; EpCAM, epithelial cell adhesion molecule; MAGE-A3, melanoma-associated antigen 3; FR, folate receptor; IGFBP-2, insulin-like growth factor binding protein 2; MSLN, mesothelin.

outcome. However, as explained in the following paragraph, this spontaneously induced antitumour response is attenuated by a hostile immunosuppressive TME.

3. Prognostic effect of different subsets of TILs in ovarian cancer

TILs are lymphocytes that have left the bloodstream and migrated into the tumour. They can be found in the stroma and within the tumour itself. TILs are a heterogeneous population that includes $CD8^+$ and $CD4^+$ T cells and B cells ($CD20^+$) in variable proportions, T cells being the most abundant cells. The presence of $CD8^+$ TILs is associated with increased survival in most solid tumours [37]. A better comprehension of the TIL subsets will allow a better prognosis, prediction of response and immunotherapy against EOC.

In 2003 Zhang et al. [6] showed that $CD3^+$ T cell into ovarian tumour was associated with improved survival. More recently, Sato et al. [14] have found that patients with high number of intraepithelial $CD8^+$ T cells, as opposed to those with TILs mainly in the stroma, exhibit favourable prognosis, suggesting that CD8 TILs in direct contact with tumour cells are important for mediating the antitumour immune response. In addition, factors associated with cytotoxic T cell response, such as interferon (IFN)-γ, granzyme B, TIA-1 (cytotoxic granule–associated RNA binding protein) and major histocompatibility complex (MHC) class I and II expression by tumour cells, show a positive correlation with survival [15,38].

Recent studies have shown that tumour-specific CD8 TILs in EOC express multiple inhibitory receptors such as PD-1, LAG-3 (lymphocyte-activation gene 3), T-cell immunoglobulin and mucin domain 3 (Tim-3) and CTLA-4 [17,19,39]. These so-called immune checkpoints normally serve as brakes on immune cell overactivity and prevent autoimmune reactivity. The acquisition by tumour cells or immune cells within the TME of the ligands of these immune checkpoints leads to immune evasion and tumour progression.

Intraepithelial CD8 TILs in ovarian tumours express CD103 [16], an integrin protein that binds E-cadherin on epithelial cells. CD103 is also expressed by intraepithelial lymphocytes and tissue-resident memory (TRM) cells, important mediators of adaptive immunity in peripheral tissues [40,41]. CD8 $CD103^+$ TILs in HGSC exhibit an activated phenotype ($HLA\text{-}DR^+$, $Ki\text{-}67^+$, $TIA\text{-}1^+$) and express PD-1. In fact, CD103 and PD-1 are highly coexpressed on intraepithelial CD8 TILs. Interestingly, they are able to elicit a robust IFN-γ and TNF-α response after ex vivo stimulation, indicating that they retain functional competence [17]. Importantly, both $CD103^+$ and $PD\text{-}1^+$ TILs are associated with patient survival in HGSC [42]. Recently, it has been reported that the coexpression of CD103 and CD39 also identify tumour-resident CD8 T cells in EOC [39]. CD39 is an ectonucleotidase that hydrolyses extracellular ATP and ADP into AMP, which is then processed by CD73 into adenosine, a potent inhibitor of T-cell activation [43]. $CD103^+CD39^+$ CD8 TILs display a TRM gene signature and high level of PD-1, CTLA-4 and TIM-3 expression [39]. The TCR repertoire analysis indicates that these cells have experienced clonal expansion. In addition, $CD103^+CD39^+$ CD8 TILs show increased frequencies of cells expressing CD137 (4-1BB) and Ki-67. The activated phenotype of this subset and their skewed TCR repertoire strongly suggest that these cells are recognizing their cognate Ag and proliferating within the tumour. Although there is still no evidence in EOC, the high frequency of $CD103^+CD39^+$ CD8 TILs is associated with a better overall survival in other tumours, such as head and neck squamous cell carcinoma, as well as lung cancer [39].

The prognostic value of $CD8^+$ TILs is enhanced by the presence of $CD4^+$ TILs with $CD25^+FoxP3^-$ phenotype (effector CD4 T cells) [18] but diminished by T regulatory cells (Tregs, $CD4^+CD25^+FoxP3^+$) [7,14]. Effector CD4 TILs in ovarian tumours express high levels of PD-1, TIM-3 and LAG-3 and are functionally exhausted exhibiting deficient cytokine production ex vivo [18].

The prognostic value of $CD8^+$ TILs in EOC is further enhanced by the presence of B cells ($CD20^+$) in the malignant epithelium [44]. These $CD20^+$ TILs are Ag-experienced B cells, as evidenced by immunoglobulin class-switching, somatic hypermutation and oligoclonality, and exhibit an atypical $CD27^-$ memory phenotype [44]. They do not appear to show correlation with serum antibodies to TAA [44]. By contrast, they frequently cluster with $CD8^+$ and $CD4^+$ TILs and express MHC class I and II, CD40, CD80, and CD86, suggesting that they may have some role in Ag presentation [44]. Interestingly, in about 25% of patients with EOC, tertiary lymphoid structures (TLSs) that resemble lymph nodes (LNs) can be observed in

tumour stroma. These structures contain a germinal center, with follicular dendritic cells (DCs) and proliferating B cells, a T cell zone, with mature DCs and T cells, and high endothelial venules [45]. The role of TLS in EOC is not well known but TLS has been associated with beneficial effects in several cancers [45] and might reflect the generation of an adaptive immune response in close proximity to the tumour.

Although belonging to the innate immune system, NK lymphocytes have also been detected in the infiltrate of EOC, but their frequency is much lower than that of T and B cells. The prognostic value of NK cells in EOC is controversial, while higher NK cell activity in the peripheral blood at the time of surgery is predictive of better survival [46], increased number of NK cells in peritoneal and pleural exudates of metastatic EOC has been associated with poorer prognosis [47].

4. Factors associated to TIL density in ovarian cancer

The presence of TIL varies markedly between the different subtypes of EOC. Milne et al. [15] assessed leucocyte infiltration and immune marker expression in a large group of patients with EOC, including 199 HGSC, 132 clear cell, 125 endometrioid and 31 mucinous EOC. They found that HGSC exhibited the highest frequency of $CD45^+$ cells and also more frequently contained other immune cell expressing FoxP3, CD25 and CD20, compared with the other subtypes. Tumours with endometrioid histology had the second highest and clear cell and mucinous subtypes had lower percentages with infiltrates overall. This study underlines the possibility that different EOC subtypes may require different immunotherapeutic approaches.

Among patients with HGSC, it is also common to find differences in TIL density. Consequently, HGSC has been histologically categorized as 'inflamed (hot)', 'immune-excluded' or 'non-inflamed (cold)' tumours [48]. The first one is characterized by a high density of $CD8^+$ T cells in the tumour bed. These tumours present high expression of genes coding MHC class I and II molecules, as well as components of the MHC class I Ag processing machinery [48,48], suggesting that Ag presentation may be an important determinant of T-cell infiltration (Table 2). In accord with this notion, tumours with loss or mutation of the *BRCA1/2* or TP53 genes have an increased density of TILs [5]. This suggests that defective DNA repair and the ensuing genomic instability in tumours may lead to the generation of neoAgs that trigger host T-cell responses. T-cell inflamed tumours are characterized by high expression of gene related to TCR signalling, IFN response, cytotoxicity, chemokines and chemokine receptors, inflammation and CT Ags, as well as by the presence of mechanisms of immune suppression with high expression of PD-L1, PD-1, LAG-3 and other inhibitory receptors [49–54].

'Immune-excluded' tumours are distinguished by the presence of T cells in the normal tissue surrounding the tumour and few intratumoral T cells. 'Immune-excluded' HGSC tumours are characterized by high hypoxia and the presence of myofibroblasts, vascular endothelial cells, pericytes, extracellular matrix and angiogenesis.

Finally, cold tumours are characterized by the absence of T cells in the tumour beds and at tumour edges. The hallmark of 'non–T-cell inflamed' HGSC tumours is WNT/β-catenin signalling that impairs T cell priming. These tumours also express genes involved in proliferation, extracellular matrix, and exhibit low expression of immune cell genes, mucins, kallikreins and reduced membrane localization of E-cadherin [52], which could impair their ability to recruit $CD103^+$ TIL. Tumours with low CD8 T-cell infiltration frequently express CXCR6, a chemokine receptor that has been associated with metastasis in several types of tumours, including epithelial cancers [53].

5. Molecular mechanism of immune evasion and suppression in EOC

Downregulation or loss of expression of genes coding MHC I and II molecules or those involved in Ag processing and presentation is an immune hallmark of cancer that is exploited by many tumours to evade immune recognition [55,56], including EOC [57–59]. These impairments reduce TAA presentation, which leads to the lack of recognition and elimination of the tumour by cytotoxic T lymphocytes. High expression of peptide transporter 1 (TAP1), TAP2, HLA class I heavy chain, beta-2 microglobulin, HLA-DR and HLA-DMB, correlates with improved survival in EOC [57–59].

Table 2 – Factors associated with TIL density in ovarian cancer.

T-cell inflamed	Intermediate T-cell inflamed	Non–T-cell inflamed
• IFN response (IRF-1, CXCL-9, -10, –11) • Chemokine receptors (CXCR3) • Ag processing/presentation (β2 microglobulin, TAP transporters, MHC-I and II, CD74) • TCR signalling (CD3D) • Inflammation (IL-15, IL-32, IL-6R, VAV1, complement) • Cytotoxicity (Granzyme B and TNFSF10) • CT Ags (ZNF165, CEP55, ATAD2, MAGEA3, CTAGE5, TTK, PBK, PRAME, CXorf48) •*BRCA1*-disruption	• High hypoxia • Myofibroblasts • Vascular endothelial cells • Pericytes • Extracellular matrix genes •Angiogenesis genes (VEGF)	• WNT/β-catenin signalling • Cell cycle genes, • Extracellular matrix genes • Low expression of immune cell genes (MHC-I and II), mucins (MUC-1, -16), kallikreins • Low E-cadherin • CXCR6 •Angiogenesis genes (VEGF, ETBR, ET-1)

IRF-1, IFN regulatory factor 1; VEGF, vascular endothelial growth factor; *BRCA*, breast and ovarian cancer susceptibility protein; MAGE-A3, melanoma-associated antigen 3; IFN, interferon; MHC, major histocompatibility complex; ETBR, endothelin-B receptor.

DCs are the most effective Ag-presenting cells (APC) for T-cell activation and among all the different DC subsets, type 1 DC (DC1) is essential for trafficking TAA to LNs and priming tumour-specific CD8 T cells. In several tumours, including EOC, it has been found a strong correlation between activation of the WNT/β-catenin signalling pathway and absence of T cells [52,60–62]. It has been shown that the Wnt/β-catenin inhibits CCL4 expression by tumour cells and limits recruitment of DC1 [63]. Therefore, the increased WNT/β-catenin signalling activity may be a tumour-intrinsic mechanism by which ovarian tumours may impair the induction of a T-cell response.

The expression of inhibitory molecules on cancer cells, including PD-L1 and indoleamine 2,3-dioxygenase (IDO), are important mechanism to evade immune cell attack. Increased tumour infiltration of $CD8^+$ T cells is associated with high PD-L1 expression probably as a result of an adaptive response where $CD8^+$ TIL secretes gamma interferon (IFNγ) that subsequently induces PD-L1 expression in cancer cells. PD-L1 engages PD-1 expressed on activated T cells, which transduces a signal that inhibits T-cell proliferation, cytokine production, and cytolytic function [64]. In the case of EOC, around 10–33% of tumours express PD-L1 [65,66]. High expression of PD-L1 on tumour cells correlates with poorer prognosis in EOC [8], although others have suggested that high PD-1/PD-L1 expression in primary tumours may be associated with a favourable progression-free survival [66]. Similar to many other cancers, EOC tumours express NKG2D ligand, which is an indicator of poor prognosis and could promote T-cell dysfunction in the TME [67].

The enzyme IDO is often overexpressed by cancer cells but also by DCs and macrophages in the TME [68,69]. IDO catabolizes tryptophan, which leads to cell cycle arrest or apoptosis T cells and skewed differentiation of Tregs [70,71]. Positive staining for IDO, observed in 24–57% of patient samples, is associated with poor prognosis of HGSC, decreased $CD8^+$ TILs, as well as resistance to chemotherapy [72].

Different mechanisms regulate the tumour endothelial barrier and prevent T cells from crossing the tumour vasculature. Vascular endothelial growth factor A (VEGF-A) plays an important role in the establishment of this physical barrier. VEGF-A attenuates endothelial T-cell adhesion through deregulation of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells [73]. In addition, the endothelin-B receptor (ETBR) is also positively regulated in ovarian tumours lacking intraepithelial TILs [74] and its overexpression is associated with poor survival. Endothelin 1 (ET1) signals through ETBR and blocks the adhesion of T cells to the endothelium by suppressing the clustering of ICAM-1 on endothelial cell membranes [74]. ET1 is also overexpressed in EOC [74]. Recently it has been shown that angiogenic growth factors, such as VEGF-A, IL-10 and prostaglandin E2 (PGE2), cooperatively induce FasL expression on the tumour endothelium, which acquired the ability to kill effector $CD8^+$ T cells but not Treg cells, revealing a new mechanism of immune evasion [75].

6. Immune suppressor cells in the TME

Several immune cells have been reported to contribute to wane the T-cell response and promote tumour cell growth in EOC.

6.1. *Dendritic cells*

As already mention, ovarian tumours may impair the recruitment of DC1, the most effective APC for T-cell activation. DCs in ovarian tumour frequently present an immature phenotype. Immature DCs mediate tumour tolerance, inducing anergy of effector T cells and/or expansion of Treg cells. VEGF impairs DC differentiation from $CD34^+$ haematopoietic precursors and DC maturation [76]. Moreover, VEGF induces robust expression of PD-L1 on DC [77]. Other tumour-derived mediators, such as transforming growth factor β (TGF-β), interleukin (IL)-10, macrophage colony–stimulating factor (M-CSF), IL-6, hypoxia, lactic acid and PGE2, can also promote a semimature, tolerogenic phenotype of tumour DCs. DCs in ovarian tumour express IDO, as well as arginase, nitric oxide (NO) and reactive oxygen species (ROS), which impair T-cell activation and function [78–80]. It has been also shown that ovarian tumours secrete large amounts of IL-10, which promotes differentiation of DC to $CD14^+$ $CD1a^-$ macrophage-like cells with reduced T-cell activation properties [54]. Although rare in the blood, plasmacytoid DCs and their precursors (preDC2) preferentially accumulate in ovarian tumour [81]. PreDC2 cells express CXCR4 and are attracted into the TME by tumour-derived stroma-derived factor 1, also known as CXCL12. CXCL2 is overexpressed in metastatic sites of papillary ovarian carcinomas [82]. PreDC2 cells recruited into the ovarian TME induce T cells to release large amounts of IL-10, preventing local T-cell activation [81].

6.2. *Regulatory T cells*

$FoxP3^+$ CD4 Tregs negatively regulate antitumour responses through IL-10 and TGF-β production and cell–cell interactions. CD4 Tregs accumulate and are more frequently present in tumours, with a shift in the median ratio of Tregs to TILs from 3 to 8% in healthy tissue to 18–25% in all analysed cancers, including EOC [75]. A seminal study revealed the negative prognostic influence of $CD4^+FoxP3^+CD25^+$ Tregs in EOC [7], a finding that has been confirmed by others [14,18]. Recently, a link between Treg recruitment and hypoxia was discovered in EOC. Hypoxic ovarian cancer cells upregulate CCL28, which recruit Tregs through the cognate receptor CCR10 [83]. $CD8^+$ Tregs have been also described in EOC [84]. They are found in the stroma and intraepithelial areas of EOC tumours. $CD8^+$ Tregs are characterized by the expression of FoxP3, CTLA-4, and CD25 but decreased expression of CD28 [84]. *In vitro*, tumour-derived TGF-β1 induces $CD8^+$ Treg. In addition, seric TGF-β1 levels positively correlate with $CD8^+$ Treg percentages in EOC. $CD8^+$ Tregs were able to convert effector $CD8^+$ T cells into suppressor cells [84]. $CD8^+$ Tregs exert their suppressive function through the secretion of TGF-β1.

6.3. *Myeloid-derived suppressor cells*

Tumour-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) constitute up to 20% of the TME in ovarian tumours and are known to promote an immunosuppressive TME [85]. MDSCs are defined as immature myeloid cells that suppress T cell responses. They include

myeloid progenitors and immature myeloid cells [86]. Because MDSC markers overlap with other cell populations, phenotyping combined with demonstration of T cell suppression is necessary for identification of MDSCs [87]. MDSCs produce arginase-1, NO, ROS, and reactive nitrogen species that dampen T-cell responses [88]. MDSCs also deplete cysteine, induce Tregs, attenuate the cytolytic ability of NK cells, and trigger a M2 macrophage phenotype [85]. The frequencies of $CD11b^{+}$ $CD14^{+}$ CD33+ $CXCR4^{+}$ MDSCs in EOC ascites correlated with CXCL12 and PGE2 levels [89]. MDSCs derived from patients with EOC also increased gene expression of cancer stem cells, sphere formation and metastasis of tumour cells [90]. The density of MDSCs correlated with poor patient prognosis and elevated levels of IL-6 and IL-10 [91]. VEGF expression in ovarian tumours induced MDSCs recruitment [92]. EOC cells also attract myeloid cells by producing adenosine [93].

6.4. *Tumour-associated macrophages*

TAMs are the most abundant immune cells in EOC tissue and ascites [94]. They exhibit an immunosuppressive M2 phenotype characterized by the expression of CD163, CD204, CD206 [94] and their presence correlates with tumour progression [95]. M2 TAMs produce colony-stimulating factor 1 that contributes to tumour growth, invasion, and metastasis [95]. They also secrete the chemokine CCL22 enabling the trafficking of Tregs to the tumours [96]. TAMs as well as tumour cells express B7−H4 molecule [97], a member of the B7 family that exerts a deep inhibitory effect on the proliferation and effector functions of T cells. B7−H4 expression in macrophages correlate with Treg cell numbers in the tumour and with poor patient outcome [97]. Tregs in the TME induce B7−H4+ TAMs to produce IL-10 and IL-6 [97], further supporting an immunosuppressive milieu. In addition, monocyte-derived macrophages in EOC exhibit defective antibody-dependent cell-mediated cytotoxicity and phagocytosis, two important functions for the antitumour response of macrophages [98]. Importantly, two studies evaluating M1 (HLA-DR, iNOS) and M2 (CD163, VEGF) phenotypes have shown that higher M1/M2 TAMs ratio in tumours was associated with a favourable overall survival [99,100], suggesting that immunotherapeutic approaches aiming to switch TAM phenotypes could help the evolution of antitumour responses and improve patient outcome.

6.5. *Neutrophils*

Neutrophils can have considerable heterogeneity and plasticity, with the potential to enhance or suppress antitumour immunity [99,100]. Tumour-associated neutrophils can be broadly divided into N1 (antitumorigenic) or N2 (suppressive and protumorigenic) populations, with distinct transcriptional profiles and functional properties [101]. A study has associated neutrophils with ovarian tumour progression and metastasis [102]. The authors show that patients with advanced EOC and high preoperative neutrophil-to-lymphocyte ratio in peripheral blood had decreased overall survival compared with patients with low NLR. Recently, it has been shown that circulating neutrophils from patients with EOC are not suppressive but acquired suppressor functions (defined as the capacity to reduce T-cell proliferation) after ascites supernatant exposure [103]. The same happens when circulating neutrophils from healthy donors were exposed to ascites supernatants from patients with newly diagnosed advanced EOC. Inhibition of complement C3 activation and neutrophils effector functions, including CR3 signalling, protein synthesis, and vesicular trafficking, abrogated the neutrophils suppressor phenotype [104]. In addition, ovarian tumour−derived inflammatory factors, including mitochondrial DNA and similarly other DAMPs, stimulate neutrophils to mobilize and extrude chromatin webs called neutrophil extracellular traps (NETs) [104,105]. NETs have been detected in the omentum of women with early-stage ovarian cancer [105]. NETs and fibrin filaments trap free-floating tumour cells and enhance seeding to the serosa and local dissemination within the peritoneal cavity [103]. In preclinical mouse models, blockade of NET formation using a PAD4 (enzyme that is essential for NET formation) pharmacologic inhibitor decreased peritoneal cavity colonization [105].

7. Conclusions and future perspectives for therapy in EOC

EOC has traditionally been considered a low immunogenic tumour. However, the fact that tumour-specific T lymphocytes are detected in patients with EOC, and the existence of multiple immune evasion mechanisms in these tumours contradicts this statement. Importantly, *BRCA1/2*-mutated HGSCs exhibit a higher mutational load and a unique genetic signature with increased number of TILs and high expression of PD-1 and PD-L1 ligand in tumour-associated immune cells, compared with HR-proficient tumours. In addition, patients with T-cell−rich tumours experience longer progression-free and overall survival than those with 'immune-excluded' tumours. Although these data suggest that immunotherapy may have a rationale in EOC, single agent immune checkpoint blockades (ICBs) have shown only modest results in this malignancy, even in the best clinical setting. ICBs exert their effect by revitalizing exhausted effector T cells. The lack of efficacy of ICBs in ovarian tumours may be considerably dictated by other immune evasion mechanisms prevailing in ovarian tumours, such as those driving T-cell exclusion, angiogenesis, molecular mechanisms involved in suppression of effector T cells (IDO, arginase, etc) and the presence of highly immunosuppressive cells (such as Tregs, MDSC and TAM) in the TME. Accumulating evidence suggests that the efficacy of chemotherapy and the promising technique of immunotherapy can be improved through the modulation of TME [106]. Other articles in this special issue will address some of these topics, including the exploration of promising immunotherapies for EOC that are currently under investigation.

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Conflict of interest statement

No potential conflicts of interest were disclosed.

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