Immunotherapies in ovarian cancer

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ABSTRACT

Ovarian cancer is the leading cause of death for gynaecological cancer, and new therapies are urgently awaited. Although the presence of tumour-infiltrating lymphocytes has been confirmed to be associated to a better prognosis, immunotherapy is not yet incorporated to the armamentarium in ovarian cancer. This review briefly summarises the strategies that have been tested or are under study for the three different groups of tumours: immune desert, inflamed and immune-excluded ovarian tumours. Finally, a better knowledge of the biology and immune microenvironment is needed for successfully developing new immunotherapy strategies.

1. Introduction

Immunotherapy in ovarian cancer is not so successful as in other cancers so far. The knowledge of ovarian cancer biology is key to develop knew immunotherapy strategies. Furthermore, the interaction between the immune system and tumour cells should be reviewed to understand the mechanisms of novel and old immune targets. Tumours have escape strategies to avoid immune attacks. Cancer cells are able to deregulate antigen presentation to make the tumour invisible to the immune system. In addition, tumours secrete immunosuppression factors that inhibit immune cells, and they can attract immunosuppressive cells [1–3]. Immune microenvironment composition is relevant to get growth tumour control. So, if the ovarian microenvironment is rich in cluster of differentiation 4 T helper 2 (CD4 Th2) lymphocytes, myeloid-derived suppressor cells, T-regulatory (Treg) lymphocytes and M2 tumour-associated macrophages

(TAMs), all these immune cells are not going to control tumour growth properly. Nevertheless, when immune cells as CD8 lymphocytes, CD4 T-cell helper 1 (Th1) lymphocytes, natural killer (NK) cells, dendritic cells (DCs) and M1 tumour-associated macrophages are most cells in the microenvironment, tumours are very well controlled (Fig. 1).

Epithelial ovarian cancer is recognised as a heterogeneous disease in which different subtypes can be distinguished: high-grade serous, low-grade serous, clear cell, endometrioid and mucinous ovarian cancer [4]. In each of these subtypes, several pathway alterations have been described. What is more The Cancer Genome Atlas Research Network (TCGA) in ovarian cancer describes the percentage of different mutations in high-grade serous ovarian cancer (HGSOC) [5]. In addition to this knowledge, it is really key to know how the microenvironment is composed in all these ovarian cancer subtypes. Different studies have demonstrated the prognosis value of tumour-infiltrating lymphocytes (TILs) in ovarian cancer [6–8]. Li et al. [9] have recently published a meta-

analysis confirming that intraepithelial TILs (ieTILs) are predictive biomarkers for the prognosis of patients with ovarian cancer. Interestingly, CD8 TILs and the immunoreactive high-grade serous subtype are associated with the breast cancer 1 gene (BRCA1) mutation and not with BRCA2 mutation [10].

Currently there is a large number of clinical trials trying to settle the right place for the diverse immunotherapy approaches. Although the relevance of the microenvironment in cancer is known, there is not a guideline to solve what is the best immunotherapy strategy to be used according to the different tumour microenvironments.

In 2017, Chen and Mellman [11] described three main cancer immune phenotypes:: the immune-inflamed, the immune-excluded and the immune-desert phenotypes.

The immune-inflamed phenotype is characterised by the presence in the tumour parenchyma of both CD4- and CD8-expressing T cells. This phenotype frequently exhibits staining for programmed death-ligand 1 (PD-L1) on infiltrating immune cells and occasionally tumour cells. Moreover, proinflammatory and effector cytokines can be detected in the samples of this subtype. Thus, immune-inflamed tumours would be more likely to respond to immunotherapy.

The immune-excluded phenotype is characterised by the presence of abundant immune cells. However, these immune cells do not penetrate the parenchyma of these tumours being retained in the stroma surrounding tumour cell nests. Immunotherapy is often ineffective as immune cells are excluded from the tumour.

The third profile, the immune-desert phenotype, is characterised by a paucity of T cells in either the parenchyma or the stroma of the tumour. The most frequent feature of this phenotype is the presence of non-inflamed tumour microenvironment. As expected, these tumours rarely respond to anti−PD-L1 or anti−PD-1 therapy.

The immune-excluded and the immune-desert subtypes are also known as non-inflamed tumours.

Although these phenotypes are not implemented in the clinic, this review will use these phenotypes as structure to better understand the different immunotherapy strategies in ovarian cancer (Table 1).

2. Immunotherapies in immune-desert ovarian tumours

Immune-desert ovarian tumours are non-inflamed tumours that will poorly respond to anti−PD-1/anti−PD-L1 [11]. The objective in this profile should be to convert a non-inflamed tumour into an inflamed tumour [12]. The main strategies to consider in this context are chemotherapy, vaccines, adoptive T-cell transfer, toll-like receptors (TLRs) and Poly ADP (Adenosine Diphosphate)-Ribose Polymerase (PARP) inhibitors (PARPis).

2.1. Chemotherapy

Chemotherapy can induce immunomodulatory effects. Traditionally, it has been considered that apoptosis is non-immunogenic and does not induce an inflammatory response. However, recent studies suggest that certain chemotherapeutic agents may induce immunogenic cell death, a type of apoptosis that stimulates immune response.
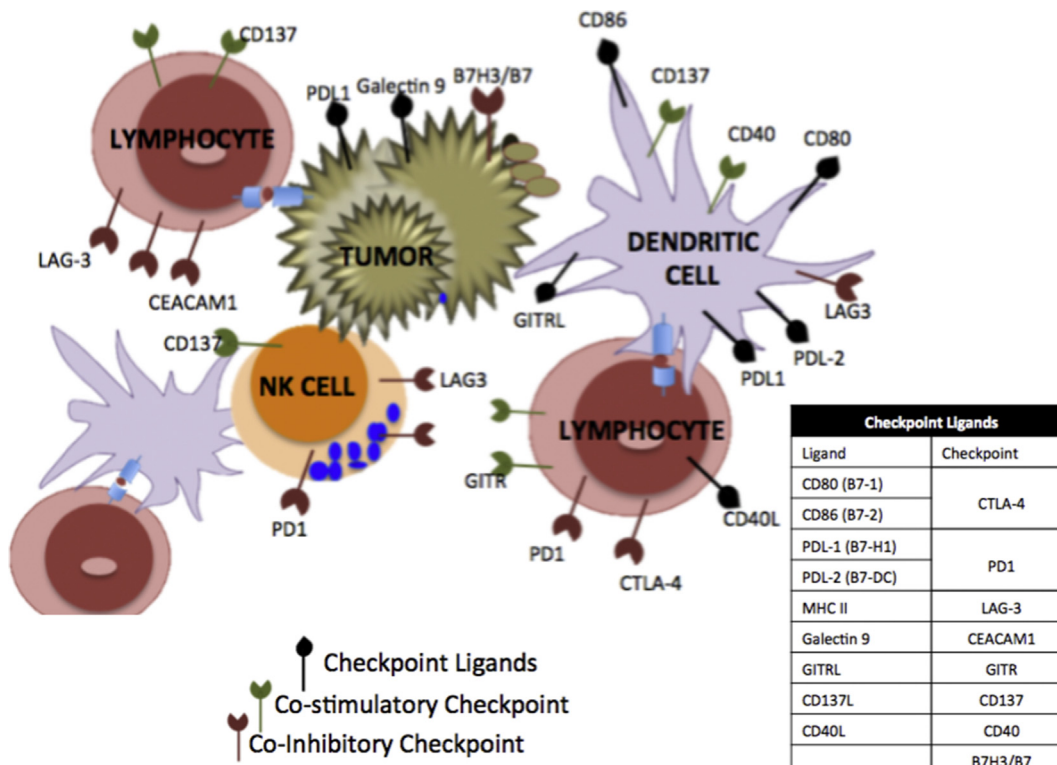

Fig. 1 − Inhibitors and costimulatory checkpoints in ovarian cancer.

This process is characterised by upregulation or secretion of a series of signalling molecules such as calreticulin, adenosine triphosphate and high mobility group box 1 [13].

Recent evidence shows the immunomodulatory effect of chemotherapy in ovarian cancer. Mesnage et al. [14] analysed the stromal TILs (sTILs) and PD-L1 expression before and after neoadjuvant chemotherapy (NACT). An overall increase in median sTILs from 20 to 30% was seen after NACT (p = 0.0005). Moreover, post-NACT sTILs were predictive of platinum-free interval. PD-L1 expression was also increased by the effect of NACT. The proportion of tumours with PD-L1 expression was 30% before NACT and 50% after NACT (p = 0.026). Among those with paired biopsies, 63% with negative PD-L1 expression became positive after NACT. On the multivariate analysis, high sTILs both before and after NACT were independent prognostic factors for progression-free survival (PFS) (hazard ratio [HR] = 0.49, p = 0.02 and HR = 0.60, p = 0.05, respectively). Nevertheless, no prognostic impact of ieTILs or PD-L1 expression was detected.

A more recent study [15] also analysed sTILs and PD-L1 expression and its prognostic impact after NACT in patients with HGSOC. From 113 patients, only 12 (10.6%) had high PD-L1 expression. Again, these high levels were not associated with PFS (p = 0.34) or overall survival (OS) (p = 0.7). Similarly, high stromal TILs after NACT did not show any impact on PFS or OS (p = 0.25 and p = 0.8, respectively). However, post-NACT evaluation of immune biomarkers could be a valuable strategy for defining post-operative therapy in these patients.

Some specific agents have shown different immune changes. For instance, platinum chemotherapy induces DC activity and decreases immunosuppressive tumour's capacity by the signal transducer and activator of transcription 6 (STAT6)-pathway [16]. Trabectedin produces monocytes and TAM cytotoxicity and inhibits chemokine (C–C motif) ligand 2, interleukin-6 (IL-6), vascular endothelial growth factor (VEGF) and tumour necrosis factor [17].

Nonetheless, clinical prospective trials including chemotherapy and immune checkpoint inhibitors have been somehow discouraging. JAVELIN 200 was a randomised clinical trial [18] that compared avelumab versus pegylated liposomal doxorubicin (PLD) versus avelumab + PLD in a population of platinum-resistant or platinum-refractory patients with up to 3 prior platinum lines. A total of 566 patients were included, but unfortunately there were no differences in the primary end-point (PFS). An exploratory analysis according to the expression of PD-L1 showed a benefit in PFS for the combination arm (HR = 0.65; 95% confidence interval = 0.457–0.919) and a trend to a benefit in OS.

Table 1 – Potential immune strategies in ovarian cancer according to immunogenic profile.

Ovarian cancer subtypes		Ongoing studies
Immune desert	Chemotherapy	Paclitaxel, platinum, trabectedin immune effects
	Vaccines	Dendritic cell vaccines
		Whole tumour cell vaccines
		Peptide/protein vaccines
	Adoptive T-cell transfer	RT (P. II): allogenic natural killer cells (NCT00652899, NCT01105650)
		RT (P. I): intraperitoneal natural killer cells (NCT02118285, NCT03213964)
		RT (P. I): CD8 T-cell infusion, aldesleukin and utomilimab (NCT03318900)
	Toll-like receptors	RT: motolimod and PLD (NCT01666444 (P. II), NCT01294293 (P. I))
Inflamed	Monoclonal antibodies	Pertuzumab, farletuzumab, abagovomab, oregovomab, catumaxomab
	Cytokines	RT(P. I-II): tocilizumab (mAb IL-6R) and platinum-based CT (NCT01637532)
		FT(P. II): IL-12 sc weekly, maintenance setting (NCT00016289)
	Checkpoint inhibitor antibodies	Refer Table 3
	Checkpoint immunostimulatory antibodies	RT(P. II): OX-40 agonist and durvalumab, OX-40 agonist and tremelimumab (NCT03267589)
		RT (P. II): CD73 agonist and durvalumab (NCT03267589)
	TGFβ inhibitors	FT - RT (P. I): galunisertib and paclitaxel-carboplatin in carcinosarcoma (NCT03206177)
		RT (P. II): vigil and atezolizumab (NCT03073525)
	IDO Inhibitor	FT (P. II): INCB024360 vs tamoxifen, maintenance setting (NCT01685255)
		FT: (P. I-II): NCB024360 and DEC-205/NY-ESO-1 fusion protein CDX-1401 (NCT02166905)
		RT(P. I): NCB024360 and intraperitoneal natural killer cells (NCT02118285)
		RT(P. I-II): CRS-207 with epacadostat (NCT02575807)
		FT(P. II): epacadostat before surgery (NCT02042430)
Immune excluded	Antiangiogenic therapy	Refer Table 4

RT: relapsed treatment; FT: front-line treatment; P.: phase; PLD: pegylated liposomal doxorubicin; CT: chemotherapy; TGFβ: transforming growth factor beta; IDO: indoleamine 2,3-dioxygenase.

2.2. Vaccines

The vaccines are strategies that attempt to stimulate immunogenic response by increasing antigen exposure. There are different vaccine types such as DC vaccines [19–21], whole-tumour-cell vaccines [22], genetic vaccines [23,24] and peptide/protein vaccines (Table 2).

To date, this strategy is not considered as an alternative in ovarian cancer. However, different strategies to improve vaccine efficacy could convert this in a future approach in ovarian cancer. Such strategies are based on an appropriate selection of the antigens, better patient selection or the combination with appropriate immunomodulatory agents [25].

In this context, in 2018, a Swiss group [26] showed that personalised vaccines, generated by autologous DC pulsed with oxidised autologous whole-tumour cell lysate induced T-cell responses in 25 patients with ovarian cancer.

2.3. Toll-like receptors

TLRs are expressed on the membranes of leukocytes, interestingly in myeloid cells including DCs, macrophages and monocytes and also in NK cells and lymphocytes. These receptors belong to the pattern recognition receptor's family and constitute the first line-line defence. Recent reports describe that targeting TLR signalling generates cancer stem cells inhibition, which is very favourable in cancer treatment due to the ability of these cells to promote tumour progression and metastasis[27]. Activation of TLR stimulates the release of inflammatory mediators including Th1-polarising cytokines [28,29].

Several clinical trials are evaluating the role of TLR in cancer treatment [30]. A randomised phase II study evaluated the efficacy of motolimod (TLR8) in addition to PLD in recurrent ovarian cancer. Unfortunately, the addition of motolimod to PLD failed to improve OS [31]. TLR signalling modulation is a complex issue as this strategy may increase immunosuppression [32].

2.4. PARPis

In the last year, several studies have demonstrated how PARPis may have a dual role inducing immune activation through the stimulator of interferon genes (STING) pathway or increasing the neoantigen rate [33,34] and inducing immune suppression by ATM (ataxia telangiectasia mutated)-ATR (ataxia telangiectasia and Rad3 related)-CHK1 (checkpoint kinase 1) activation that finally induce PD-L1 upregulation [35].

2.5. Immunotherapies in inflamed ovarian tumours

Immune-inflamed tumours have a tumour parenchyma rich in T cells and other immune population, as well as diverse cytokines [11].

These tumours can benefit from multiple therapies based on immune strategies: monoclonal antibodies targeting tumour-associated antigens, cytokines, checkpoint inhibitor antibodies, agonistic monoclonal antibodies of costimulatory receptors, transforming growth factor beta inhibitors, indoleamine 2,3-dioxygenase antagonists and others.

2.6. Monoclonal antibodies targeting tumour-associated antigens

Immuno-inflamed tumours usually present exhausted T cells. Treatment with monoclonal antibodies against tumour-

Antigen	IHC expression	Vaccine	Antibodies
Germline/cancer testes antigens			
NY-ESO-1	19%	NY-ESO-1 NY-ESO-1 OLP NY-ESO-1 protein	
MAGE-A4	57%		
Overexpressed/differentiation antigens			
Mesothelin	71%		Amatuximab
Her2	29–52%	Vaccine HER-2/neu-ICD	Pertuzumab Trastuzumab Seribantumab
Folate receptor alpha	72%–82%		Farletuzumab
MUC-1	90%		Y-muHMFG1 PankoMab-GEX
EpCAM	High		Catumaxumab
Oncofetal antigens			
CA 125	80%		Abagovomab [46] Oregovomab
Mutated antigens			
P53	45–50%	P53 [47] P53-SLP [48,49]	
Other			
		PPV [50] Flt3-L [51]	

Table 2 – Vaccines and different antigens.

NY-ESO-1, New York oesophageal squamous cell carcinoma-1; IHC, Immunohistochemistry.

associated antigens may restimulate immune response in this immunogenic profile.

Pertuzumab, farletuzumab, abagovomab and oregovomab are some of the monoclonal antibodies targeting some of the tumour-associated antigens in ovarian cancer (Table 2). Nevertheless, none of these antibodies have demonstrated better responses than those of standard treatment.

Pertuzumab in combination with chemotherapy, in platinum-resistant patients with low tumour human epidermal growth factor receptor 3, did not show a significant increase in PFS [36]. Nevertheless, the authors recommend further pertuzumab investigation in ovarian cancer.

Abagovomab is a mouse anti-idiotype monoclonal antibody whose epitope mirrors the CA125 tumour antigen. In the MIMOSA trial, abagovomab vs placebo, as maintained treatment after first-line therapy, showed no improvement, neither in recurrence free nor in OS [37].

Catumaxomab is a bispecific (anti-EpCAM and antiCD3) trifunctional (FCγ receptors) antibody that binds epithelial cell CD3 T cells to other cells such as macrophages, DCs and NK cells. This binding produces EpCAM+ cells destruction. Catumaxomab has been approved for the intraperitoneal treatment of malignant ascites in EpCAM+ carcinomas [38]. In addition, in a phase II trial with intraperitoneal catumaxomab as consolidation therapy for patients with relapsed epithelial ovarian cancer in second or third complete remission, catumaxomab showed a PFS survival of 16.7 months. Despite toxicity, 82% of patients completed the planned doses [39].

2.7. Cytokines

Cytokines are a large family of small proteins with a key role in cell signalling. Although many clinical trials are testing their function in cancer treatment, alone or in combination in several cancer types [40], only two interleukins are being checked in ovarian cancer.

Solid preclinical data show IL-6 as a mediator of platinum resistance in ovarian cancer. Furthermore, the inhibition of IL-6/STAT3 axis has been proposed as a strategy to overcome taxol resistance in ovarian cancer [41–43].

IL-12–expressing oncolytic herpes simplex virus promotes anti-tumour activity and immunologic control of metastatic ovarian cancer in mice [44].

2.8. Checkpoint antibodies

Chen and Mellman [45] described in the cancer-immunity cycle how the immune system kills cancer cells. Several cell-surface cosignalling receptors are key to allow the interaction between tumour and immune cells and to regulate activated immune cells. These receptors may produce costimulatory and coinhibitory signals and are known as immune checkpoints [46].

Targeting all of these immune checkpoints is probably the most innovative advance in the immunotherapy field [47,48].

2.9. Checkpoint inhibitor antibodies

Few data are available about efficacy of PD-1 or PD-L1 antagonists in ovarian cancer. PD-1 inhibitors have been tested in very heavily treated patients that received more than 4 lines of chemotherapy. Although complete responses were described in 2% of patients, the overall response rate was 15% [49,50]. Similar data with anti–PD-L1 have been communicated in another study, in which 75 patients with resistant ovarian cancer relapse and more than 3 lines of prior chemotherapies received avelumab regardless of PD-L1 expression, reporting a response rate of 10.7% [51]. Probably, better patient selection (taking into account their immune tumour microenvironment) and drug combination strategy (to avoid primary or adaptive resistance to checkpoint inhibitors) would be the future approach to improve these outcomes. According to the results of the KEYNOTE-100 clinical trial [52], PD-L1 expression could be a potential immune biomarker to this end. In this phase II study, higher PD-L1 expression was associated with higher responses to pembrolizumab in patients with relapsed epithelial ovarian cancer and platinum-free interval of 3–12 months. Investigators reported 4.1% objective response rate (ORR) in patients with PD-L1 combined positive score (CPS) <1, ORR 5.7% CPS ≥1 and ORR 10% for CPS ≥10.

As mentioned previously, PARPis have shown to induce an immune-suppressive effect via ATM (ataxia telangiectasia mutated)-ATR (ataxia telangiectasia and Rad3 related) that leads to an upregulation of PD-L1 [35]. In this context, combining an anti–PD-1/anti–PD-L1 agent would help to overcome these mechanisms of immune evasion in patients treated with PARPis (Table 3).

In fact, several trials have explored this strategy in the clinics. The TOPACIO/KEYNOTE-162 (NCT02657889) phase I/II clinical trial evaluated the niraparib-pembrolizumab combination [53]. Patients with ovarian cancer who had platinum responses to first-line treatment longer than 6 months and resistant relapses with no more of five-line treatment were included. In American Society of Clinical Oncology (ASCO) 2018, Konstantinopoulos et al. [53] communicated a 25% ORR with a 68% clinical benefit rate (CBR). Patients with BRCA mutations responded better to the combination (45% ORR, 73% CBR). In the basket phase II clinical trial (MEDIOLA), 32 patients with BRCA-mutated ovarian cancer were included [54]. Patients received olaparib treatment during 4 weeks followed by the olaparib-durvalumab combination. Drew et al. [54] communicated very good data: disease control rate at 12 weeks was 81%, with 68% overall response rate and 19% complete responses.

The cytotoxic T-lymphocyte–associated protein 4 (CTLA-4 or CD152) plays an essential role in regulating T-cell CD4+ and CD8+ activation. CTLA-4 binds with CD28's ligands B7-1 and B7-2 [55] Although a large amount of data demonstrate the efficacy of anti-CTL4 in patients with melanoma, we are waiting for data in ovarian cancer coming from two phase II clinical studies, not only testing the usefulness in monotherapy but also in combination with PARP inhibition in BRCA-deficient ovarian cancer.

A recent analysis of the NRG Oncology trial (NRG-GY003) [56] suggested that adding the anti-CTLA4 ipilimumab to the immune checkpoint inhibitor nivolumab improved response and PFS in 100 women with recurrent ovarian cancer. Within 6 months of randomisation, 12.2% vs 31.4% responses were seen (odds ratio = 3.28). These results suggest that combining

Table 3 – Randomised phase III trials with immune checkpoint inhibitors in combination with PARP inhibitors.

Trial	Arms	PD-L1 stratification	Status
Front line			
ENGOT Ov43*	BRCAwt • PC-placebo-placebo • PC-pembro-placebo • PC-pembro-olaparib	Yes	Ongoing
ENGOT Ov44 (FIRST)*	BRCAmut • PC-placebo-niraparib • PC-TSR042-niraparib	No	Ongoing
	BRACwt • PC-placebo-placebo • PC-placebo-niraparib • PC-TSR042-niraparib	No	Ongoing
ENGOT Ov45 (ATHENA)	Maintenance after PC • Rucaparib-nivolumab • Rucaparib-placebo • Nivolumab-placebo • Placebo-placebo	No	Ongoing
ENGOT Ov 46 (DUO-O)	BRCAmut • PC-Bev*-durvalumab-olaparib BRCAwt • PC-Bev-placebo-placebo • PC-Bev-durvalumab-placebo • PC-Bev-durvalumab-olaparib		
Recurrent disease			
ENGOT ov41 (ANITA)	Carbo-based + placebo → niraparib + placebo (if no PD) Carbo-based + atezolizumab → niraparib + atezolizumab (if no PD)	Yes	Ongoing

PC: paclitaxel-carboplatin; *Bevacizumab optional; Bev: bevacizumab.

anti-CTL4 and anti−PD-L1 therapies could be a valuable strategy in ovarian cancer.

Further promising checkpoint inhibitor antibodies are being studies in phase I clinical trials including ovarian cancer. B7−H3 (CD276) is an immune checkpoint with a coinhibitory role on T cells, belonging to the B7 family. Besides its relation with other checkpoint markers, non-immunological function in cancer progression has been described. B7−H3's receptor is unknown so far [57]. Zang et al. [58] have demonstrated that ovarian tumours aberrantly express B7−H3 and B7x and that B7−H3-positive tumour vasculature is associated with the high-grade serous histological subtype, increased recurrence and reduced survival.

2.9.1.   Targeted agents modulating lymphocyte activation gene-3 and carcinoembryonic antigen-related cell adhesion molecule 1
Lymphocyte activation gene-3 (LAG-3) is a single transmembrane protein expressed on T cells, Treg lymphocytes, NK cells, DCs and B cells. Huang et al. [59] demonstrated that the compensatory upregulation of PD-1, LAG-3 and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. Interestingly, tumour-infiltrating New York oesophageal squamous cell carcinoma-1 −specific CD8+ T cells were negatively regulated by LAG-3 [60].

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed on T cells, NK cells and tumour cells. Isoform CEACAM1-L is the dominant one and has coinhibitory function [61].

Both LAG-3 and CEACAM1 are promising targets for immune modulation in ovarian cancer.

3.   Immunotherapies in immune-excluded ovarian tumours

The immune-excluded phenotype is characterised by the presence of abundant immune cells, but they are retained in the stroma that surrounds, which limits most of the immunotherapy responses [11]. As defined by Chen and Mellman, [11] in the immune-excluded phenotype, vascular factors play a key role for excluding immune cells from the tumour parenchyma. In this context, the treatment with antiangiogenic agents could be more suitable to increase immune responses.

The relation between angiogenesis and the immune microenvironment has been very recently described. The VEGF induces immunosuppression by decreasing Treg lymphocytes, DC maturation and increasing M2 macrophages. Moreover, the VEGF produces an imbalance in adhesion molecule and several chemokines of the endothelium producing an altered trafficking of immune cells through the aberrant vascularity of tumours.

Few data are available related to antiangiogenic therapy in combination with checkpoint inhibitors so far. Liu et al. [62] published efficacy data of the nivolumab plus bevacizumab combination. A 40% ORR with a 75% CBR in patients with platinum-sensitive relapsed ovarian cancer and 16.7% ORR with 33% CBR in platinum-resistant relapsed ovarian cancer was reported. The PFS was 8.1 months. The durvalumab and

Table 4 – Phase III randomised clinical trials of checkpoint inhibitors in combination with antiangiogenics.

Trial	Arms	PD-L1 stratification	Status
Front line			
GOG 3015/ENGOT Ov39	PC + Bev + placebo	Yes	Closed
	PC + Bev + atezolizumab		
Recurrent disease			
ENGOT ov29 (ATALANTE)	Carbo-based + Bev + placebo	Yes	Ongoing
	Carbo-based + Bev + atezolizumab		
ENGOT ov34 (AGO Ovar 2.29)	PLD or paclitaxel + Bev + placebo	Yes	Ongoing
	PLD or paclitaxel + Bev + atezolizumab		

PC: paclitaxel-carboplatin; Bev: bevacizumab; PLD: pegylated liposomal doxorubicin.

cediranib combination was also effective but very poorly tolerated [63]. Data from several clinical trials are still to be published (Table 4).

4.   Conclusions

Immunotherapy is a promising strategy in ovarian cancer, but the negative clinical results of JAVELIN trials suggest that the understanding of immune processes must be improved. In this context, it is crucial to assess biological samples for biomarker research and to perform quality translational studies to prospectively identify patients more likely to benefit from treatment. The identification of an appropriate predictive biomarker is an important need and is probably the key for the future development of this strategy. Consequently, some ongoing clinical trials such as ENGOT-OV41/GEICO 69-O/ANITA have implemented amendments to introduce mandatory biopsies with the aim of assessing PD-L1 and other potential predictive factors before enrolment. The pending results of the translational study of this and other ongoing trials will be extremely important to confirm the future therapeutic value of immunotherapy in ovarian cancer.

The combination of immunotherapy with other agents, such as PARPis or antiangiogenic agents, can be another potential alternative. Monotherapy is probably an insufficient strategy in non-inflamed ovarian tumours. The exploration of different combos of immunotherapy to overcome the primary resistance of these less immunogenic subtypes is necessary. Nevertheless, biomarker identification remains a crucial issue even with combinations.

Finally, the selection of patients according to microenvironment, such as the immune profile, could be challenging. The heterogeneity of ovarian tumours, both intra- and inter-tumour, is a complex issue that undoubtedly may affect the microenvironment. This is perhaps one of the most relevant challenges in the identification of new biomarkers to drive patient selection.

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