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Review

Immune Checkpoint Inhibitors in Gynecological Cancers: Update of Literature and Perspectives of Clinical Research

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Abstract. *The presence of tumor infiltrating lymphocytes (TILs) influences the clinical outcome of cancer patients and immune checkpoint inhibitors (ICPI) have been approved for treating different types of malignancies. In this review, we assess the scanty data from literature and the perspectives of clinical research about the use of ICPI in gynecological cancers. These agents have obtained objective response rates ranging from 5.9% to 15% in early phase Ib-II trials, including patients with platinum-resistant ovarian cancer, whereas only anecdotal data are available for patients with recurrent, heavily pretreated endometrial cancer. Several ongoing trials are investigating ICPI alone or in combination with chemotherapy or with other biological agents in untreated and recurrent ovarian cancer, advanced and recurrent endometrial cancer, as well as advanced and recurrent cervical cancer. Breast cancer (BRCA)-mutated high-grade serous ovarian cancers, clear cell ovarian cancers with microsatellite instability (MSI), POLE ultramutated and MSI hypermutated endometrial cancers are likely to be sensitive to programmed cell death (PD-1)/PD-ligand 1 (PD-L1) pathway blockade, since these tumors show increased neoantigen load, increased CD8$^+$ TIL number and PD-1 and PD-L1 overexpression. ICPI could have a role as maintenance treatment in patients with persistent, recurrent or metastatic cervical cancer in response after chemotherapy.*

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There is a large body of evidence supporting the crucial role of tumor immune microenvironment in carcinogenesis (1). The presence of tumor infiltrating lymphocytes (TILs) correlates with the clinical outcome of patients with different malignancies, such as melanoma, breast cancer, prostate cancer, renal cell cancer, esophageal cancer and colorectal cancer (2-8). The pooled analysis of 33 studies including several types of cancer revealed that high CD3$^+$ TIL number, high CD8$^+$ TIL number and high CD8$^+$TIL//CD4$^+$CD25$^+$FoxP3$^+$ regulatory TIL (T-reg) ratio had a positive impact on overall survival (OS) with a hazard ratio (HR) of 0.58 (95% confidence interval (CI)=0.43-0.78), 0.71 (95%CI=0.62-0.82) and 0.48 (95%CI= 0.34-0.68), respectively (9). T-reg cells are a type of CD4$^+$ T cells that inhibit immune responses characterized by lack of expression of effector cytokines, such as interferon (IFN)-γ and the production of inhibitory cytokines, such as transforming growth factor (TGF)-β, interleukin (IL)-10 and IL-35 (10). Beside T-reg cells and type-2 tumor-associated macrophages (TAM), the blockade of the T-cell response against tumor cells may be caused by immune checkpoint receptors expressed on the T-cell surface, such as cytotoxic T lymphocyte-antigen 4 (CTLA-4) and programmed cell death (PD-1) receptor (10-14). CTLA4 counteracts the activity of the T cell co-stimulatory receptor CD28 for binding to the ligands B7.1 and B7.2 expressed on antigen-presenting cells and, therefore, CTL4 functions as a negative immune regulator. Similarly, the linkage between PD-1 on T cells and the programmed-death ligand (PD-L1) on tumor cell surface inhibits T-cell proliferation and increases T-reg cell number, thus resulting in Tcell function inhibition (11-14). Whereas CTLA-4 predominately regulates T cell activation within secondary lymphoid organs, PD-1 regulates T cell function within peripheral tissues and tumor microenvironment (10, 12, 15).

Monoclonal antibodies (mAbs) targetingPD-1 (*i.e.* pembro-lizumab and nivolumab) and PD-L1 (*i.e.* avelumab, atezolizumab and durvalumab), as well as mAbs targeting CTLA-4 (ipilimumab and tremelimumab), have been

approved by Food and Drug Administration (FDA) for several malignancies, such as melanoma, non-small cell lung cancer, renal cell cancer, urothelial cancer and lymphoma (16-20). Anti-CTLA mAbs and anti-PD-1/PD-L1 mAbs cause immune-related adverse events (AEs) in 60% and 40% of cases, respectively, but severe AEs are more frequent with anti-CTLA therapy (21, 22). The management of these AEs depends on their severity and ranges from immune checkpoint inhibitor therapy suspensionto the administration of corticosteroids and, also, to the use of anti-tumor necrosis factor (TNF)-α mAbs, mycophenylate mofetil, tacrolimus and cyclosporine (23).

Four distinct patterns of response to immune checkpoint inhibition have been observed: (i) regression of baseline lesions; (ii) stable disease followed by a slow decline in tumor burden; (iii) delayed response after an initial increase in tumor burden; and (iv) response after the appearance of new lesions (24). The three latter patterns of response havenot been reportedwith chemotherapeutic agents (25). Novel immune-related response criteria, different from the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and modified World Health Organization (WHO) criteria (24), have been suggested for immune checkpoint inhibitors (25, 26).

Tumors can be classified into four groups based on PD-L1 expression and T cell infiltration (27). Type I tumors (PD-L1-positive, TILs+) exhibit an adaptive immune resistance and are likely to respond to immune checkpoint inhibitors, whereas type II tumors (PD-L1-negative, $TILs^-$) display no detectable immune reaction and are likely to be unresponsive to single- agent checkpoint blockade. Type III tumors (PD-L1-positive, $TILs^-$) exhibit intrinsic expression of PD-L1 with no immune reactivity, which suggests that PD-L itself is not apredictive biomarker of response to anti-PD-1/PD-L1 mAbs. Type IV tumors (PD-L1-negative, TILs+) may be amenable to targeting of other non-PD-1/PD-L1 checkpoint receptors.

The present manuscript reviews the few data availablein literature on the use of immune checkpoint inhibitors in the management of gynecological cancers.

Epithelial Ovarian Cancer

The number and type of TILs influence the clinical outcome of patients with epithelial ovarian cancer (EOC) (28-33). For instance, in the study of Zhang *et al*. (28), the presence of TILs was detected in 54.8% of 186 frozen specimens from women with this malignancy and found to be correlated with increased expression of IFN-γ, IL-2 and lymphocyte-attracting chemokines within the tumor. Median progression-free survival (PFS) and median OS were 22.4 months and 50.3 months, respectively, for patients whose tumors contained TILs *versus* 5.8 months and 18.0 months for those whose tumors did not contain TILs ($p<0.001$ for both). The meta-analysis of 10 studies, including 1,815 patients with EOC, confirmed that lack of intraepithelial TILs correlated with worse OS (pooled HR=2.24, 95% CI=1.71-2.91) (29).

High $CD8^+$ T cell infiltration was an independent favorable prognostic factor in a series of 203 patients with EOC: the mortality risk decreased by 18% with each doubling of $CD8^+$ T cell density (HR=0.82, 95%CI=0.73-0.92) (33). The immunohistochemical analysis of paraffin-embedded specimens from 117 women with EOC showed that patients with higher frequencies of intraepithelial $CD8^+$ T cells had a better clinical outcome compared with those with lower frequencies (median OS=55 *versus* 26 months, HR=0.33, 95%CI=0.18-0.60) (31). It is noteworthy that median OS was 57.6 months for patients with high $CD8^+$ T cell/T-reg cell ratios *versus* 22.6 months for those with low ratios (HR=0.31, 95%CI=0.17-0.58).

Hamanishi *et al*. (34) found strong immunostaining for PD-L1 in 68.5% of tissue samples from 70 patients with EOC. There was a significant inverse correlation between intraepithelial $CD8^+$ T cell count and PD-L1 expression, thus suggesting that PD-L1 on tumor cells could block the intraepithelial invasion of tumor-specific $CD8^+$ T cells.

Conflicting data emerge from the literature about the prognostic relevance of PD-1 and PD-L1 expression in EOC and these discordant results may reflect different techniques for PD-1 and PD-L1 assessment, different cut-off values for tumor cell staining, as well aspatients' heterogeneity (34-38).

PD-1/PD-L1 blockade caused tumor regression in a syngeneic EOC mouse model, thus supporting the relevance of this regulatory pathway in ovarian carcinogenesis (38).

Some phase Ib-II trials have investigated the activity and safety of anti-PD1 or anti-PD-L1 mAbs in platinum-resistant EOC, with objective response (OR) (complete response (CR)+ partial response (PR)) rates ranging from 5.9% to 15% (22, 39-41) and with grade (G)≥3 AE rates ranging from 3.8% to 40% (Table I). Nivolumab obtained an OR and a disease control (CR+PR+stable disease (SD)) in 15% and 45% of 20 patients who had received at least two prior chemotherapy lines (39) (Table I). Immunohistochemical expression of PD-L1 was high in 16 patients (80%) and low in 4 (20%); however, this variable did not correlate with OR rate.

Varga *et al*. (40) reported an OR rate of 11.5% and a disease control rate of 34.6% in 26 women with PD-L1-positive advanced EOC treated with pembrolizumab (Table I). Eighty-five per cent of the patients had received prior therapies for recurrent/metastatic disease, whereas 38.5% had received five or more therapies.

Avelumab achieved an OR in 9.7% and a disease control in 54.0% of 124 women with recurrent or refractory EOC (41) (Table I). PD-L1 expression was evaluable in 74 cases. Using a ≥1% cut-off for tumor cell staining, 57 women (77.0%) were PD-L1+, with OR rate being 12.3% in PD-L1-positive *versus* 5.9% in PD-L1-negative patients.

Table I. *Phase I-II studies of anti-programmed cell death (PD)1 or anti-programmed cell death ligand (PD-L)1 antibodies in platinum-resistant epithelial ovarian cancer.*

Reference	Agent	Patients	CR	PR	OR	SD	AEs	
							Any G	G≥3
39	Nivolumab 1 or 3 mg/kg q14	20	2	1	3 (15%)	6 (30%)	19^ (95%) (40%)	8
40	Pembrolizumab 10 mg/kg q14	26	1	2	3 (11.5%)	6 (23.1%)	18^^ (69.2%)	1 (3.8%)
41	Avelumab 10 mg/kg q14	124	0	12	12 (9.7%)	55 (44.3%)	82+ (66.1%)	8 (6.5%)
22	BMS-936559 0.3-10mg/kg q14	17	0	1	1 (5.9%)	3 (17.6%)	81++/207° (39.1%)	10/207° (4.8%)

^Most common: increased serum transaminases, hypothyroidism, lymphocytopenia, decreased serum albumin, fever, rash, arthralgia, arrhythmia, fatigue, anemia; ^^Most common: fatigue,anemia, decreased appetite; +Most common: fatigue, infusion reactions, diarrhea; ++Most common: fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, headache; °toxicity was evaluated on the total of the 207 patients included in the study (75 with non-small-cell lung cancer, 55 with melanoma, 18 with colorectal cancer, 17 with renal-cell cancer, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer and 4 with breast cancer. CR, Complete response; PR, partial response; OR, objective response; SD, stable disease; AEs, adverse events; G, grade.

In a phase I trial, Brahmer *et al*. (22) administered the anti-PD-L1 mAbBMS-936559 to 207 patients with selected advanced cancers (Table I). Among the 17 women with EOC, 1 (5.9%) had a PR and 4 (23.5%) had a disease control, all at the 10-mg dose.

Combining the nivolumab and avelumab studies, it has been observed that 4 of the 5 patients who experienced durable responses had clear cell EOC (CCOC), which is a chemoresistant histologic type. Alterations of the phosphatidylinositol 3-kinase (PIK3CA)/AKT/mammalian target of rapamycin (mTOR) pathway have been detected in 15%-75% of CCOCs (42-49) and preclinical data from non-small cell lung cancer have suggested that activation of this pathway correlates with an increased expression of PD-L1 in tumor cells (50).

The administration of ipilimumab after vaccination with irradiated, autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor showed antitumor effects also in patients with stage IV EOC (51). In a phase II study, 40 patients with recurrent platinum-sensitive EOC were scheduled to receive ipilimumab (10 mg/kg) every 3 weeks for 4 cycles (induction phase) followed by ipilimumab (10 mg/kg) every 12 weeks until progression or unacceptable toxicity (52). Thirty-eight patients (95%) did not complete the induction phase, mainly because of progression or toxicity. The OR rate was 10.3% and G≥3 AEs occurred in 50% of the patients.

Table II shows the ongoing trials of checkpoint inhibitors alone or in combination with chemotherapy in EOC.

Immune checkpoint inhibitors have demonstrated remarkable activity against hypermutated cancers, such as melanoma and lung cancer, and against tumors with mismatch repair (*MRR*) gene alterations, such as colon cancer, which harbor more tumor-specific neoantigens (53-56). This higher antigen load enhances the recruitment of an increased number of TILs, which is counterbalanced by PD-1 or PD-L1 overexpression. Strickland *et al*. (57) subdivided 245 tumor samples from patients with HGSOC in three groups: *BRCA1-2* mutated (n=54), wild-type (wt)-*BRCA1-2* with homologous recombination (*HR*) deficiency (HRD) (n=69) and wt-BRCA1-2 without *HRD* (HR-proficient tumors).Wt-*BRCA1-2* with *HRD* tumors harbored mutations in several HR genes, such as Fanconi anemia, *RAD50, RAD51, RAD54L, ATM* and *ATR*, tensin homolog deleted on chromosome (*PTEN*) deletion, EMSY amplification or mutation and promoter hypermethylation of *BRCA1* or *RAD51C*. Neoantigen load was higher in the *BRCA1-2* mutated subset compared with HR-proficient subset (p=0.008), as well as in wt-BRCA1-2 with HRD cohort compared with HR-proficient cohort (p=0.003). Moreover, *BRCA1-2* mutated tumors showed a significantly increased $CD3^+$ and $CD8^+$ TIL number anda significantly higher expression of PD-1 and PD-L1 compared with HR-proficient tumors. Therefore, BRCA1-2-mutated HGSOCs could represent a subset of tumors fit for treatment with immune checkpoint inhibitors alone or in combination with poly(adenosine diphosphate (ATP-ribose) polymerases (PARP) inhibitors (57-59). Moreover, Strickland *et al*. (60) found that 3 out of 30 (10%) CCOCs exhibited microsatellite instability (MSI) and that CCOCs with MSI had a higher number of $CD3^+$ TILs and higher number of PD1-positive TILs compared with both microsatellite stable (MSS) CCOCs

Table II. *Ongoing trials of checkpoint inhibitors alone or in combination with chemotherapy in epithelial ovarian cancer.*

Study number	Study phase	Study design
NCT02498600	2	Nivolumab with or without ipilimumab in treating patients with persistent or recurrent EOC, PPC or FTC
NCT02718417	3	Avelumab in previously untreated patients with EOC.
JAVELIN OVARIAN 100		This is an open-label, efficacy and safety study of avelumab in combination with and/or following platinum-based chemotherapy.
NCT02580058	3	A study of avelumab alone or in combination with PLD *versus*
JAVELIN OVARIAN 200		PLD alone in patients with platinum resistant/refractory EOC

EOC, Epithelial ovarian cancer; PPC, primary peritoneal cancer; FTC, fallopian tube cancer; PLD, pegylated liposomal doxorubicin.

and HGSOCs. Therefore, CCOCs with MSI may represent an immunogenic subset of tumors likely to respond to anti-PD-1/PD-L1 mAbs.

Several phase I-II studies are currently testing immune checkpoint inhibitors in combination with other biological agents, such as PARP inhibitors or anti-angiogenic agents, in patients with recurrent EOC.

The NCT02571725 trial will assess the side-effects and best dose of tremelimumab when given together with the PARP inhibitor olaparibin patients with recurrent platinum-sensitive or platinum-resistant EOC, fallopian tube cancer (FTC) or primary peritoneal cancer (PPC) and germline *BRCA1* or *BRCA2* mutation.

The NCT02734004 trial will evaluate the safety, tolerability, pharmacokinetics and antitumor activity of durvalumab in combination with olaparib in patients with different advanced solid tumors, including those with recurrent platinum-sensitive EOC and germline *BRCA* mutation.

The NCT02953457 trial will study the side-effects and best dose of olaparib when given together with durvalumab and tremelimumab, as well as the activity of this combination in treating patients with recurrent platinum-sensitive or -resistant or -refractory EOC, FTC or PPC and germline or somatic *BRCA1* or *BRCA2* mutation.

The NCT02484404 study will test the safety and activity of durvalumab in combination with olaparib and/or the vascular endothelial growth factor (VEGF) receptor inhibitor cediranib in patients with different advanced solid tumors, including those with recurrent platinum-sensitive, -resistant or -refractory EOC, FTC or PPC and germline *BRCA1* or *BRCA2* mutation.

NCT02657889 will assess the safety and efficacy of combination treatment with the PARP inhibitor niraparib and pembrolizumab in patients with triple-negative breast cancer who have received ≤3 lines of prior chemotherapy in the metastatic setting or with recurrent, platinum-resistant EOC who have received ≤4 lines of prior chemotherapy for advanced disease.

Endometrial Cancer

The dualistic model for endometrial cancer (EC) subdivides this malignancy in two main categories: type-I estrogen-dependent endometrioid carcinomas and type-II estrogen-independent non-endometrioid carcinomas (61-63). Major molecular alterations of type-I carcinomas include *PTEN* silencing, *PIK3CA* mutations, *MMR* defects, *MSI* and *K-RAS* or β-catenin (*CTNNB*) mutations, whereas type II-carcinomas often show *p53* mutations, *p16* inactivation, low E-cadherin expression, Her-2/neu overexpression, *STK15* amplification and loss of heterozygosity (LOH) on several chromosomes (62-67).

The loss of PTEN function with consequent activation of PIK3CA/AKT/mTOR pathway is an early and common event in the pathogenesis of endometrioid carcinomas, occurring approximately up to 80% of these malignancies and 55% of endometrial hyperplasias (68, 69). Beta-catenin mutations with nuclear protein accumulation, *MSI*, and *K-RAS* mutations have been detected in 25% to 38%, 25% to 35% and 10% to 30% of endometrioid carcinomas, respectively (62, 63, 68-70). Whereas *MSI, PTEN* alterations or *K-RAS* mutations may coexist in many cases, these molecular abnormalities are not usually associated with β-catenin mutations. However, this dualistic model does not take into account the molecular, biological and pathological heterogeneity among each category and recently a novel classification system has been proposed by The Cancer Genome Atlas Research Network (TCGA) (71, 72). The integrated genomic, transcriptomic and proteomic characterization of 373 ECs using array- and sequencing-based technologies has suggested to classify these malignancies into four categories: polymerase ε (*POLE*) ultramutated, *MSI* hypermutated, copy number low (endometrioid) and copy number high (serous-like).

POLE is a catalytic subunit of DNA polymerase involved in DNA replication, DNA repair and cell cycle control (73). *POLE* ultramutated tumors display very high mutation rates

(232×10^{-6} mutations/Mb) and are characterized by hotspot mutations in the exonuclease domain of *POLE*, high frequency of base substitutions and large excess of cytosine → adenine transversions, elevated incidence of *PTEN, PIK3CA, FBXW7* and *K-RAS* mutations, strong association with endometrioid histology and MSS (71, 72, 74). Although *p53* mutations can be detected in approximately one third of the cases, it is important not to misclassify these tumors as serous carcinomas (75). Tumor grade ranges from 1 to 3 but more frequently is 3, with prognosis beingexcellent also in cases with high histological grade (75-79). *POLE* mutations were detected in 6.1% of 788 ECs enrolled in PORTEC-1 and-2 randomized trials. Women with *POLE*-mutant ECs had fewer relapses (6.2% *versus* 14.1%) and tumor-related deaths (2.3% *versus* 9.7%) (76). It is noteworthy that among the 109 grade 3 tumors, disease relapsed in none of 15 POLE-mutant carcinomas *versus* 30.9% of 94 *POLE* wild-type carcinomas. Meng *et al*. (77) identified *POLE* mutations in 15% of 53 grade 3 endometrioid carcinomas compared with 0% of 25 serous carcinomas, 0% of 16 clear cell carcinomas and 0% of 5 de-differentiated carcinomas. None of the patients with *POLE* mutated tumors in this study (77), as well as in the study of Hussein *et al*. (75), developed recurrent disease. McCornery *et al*. (79) detected *POLE* mutations in 9.6% of 406 ECs. *POLE*-mutated women were younger and frequently had stage I disease (92%), tumor grade 3 (62%), endometrioid histology (82%) and lymphovascular space involvement (LVSI) (49%). *POLE* mutation was an independent favorable prognostic variable for PFS (HR=0.34, 95%CI=0.15-0.73) and disease-specific survival (DSS) (HR=0.35, 95%CI=0.13-0.92).

MSI hypermutated ECs show high mutation rates (18×10^{-6} mutations/Mb), recurrent *RPL22* frameshift deletions, frequent *PTEN* and *K-RAS* mutations, as well asendometriod histology (71, 72). Tumor grade ranges from 1 to 3, with prognosis beingusually good but less favorable than that of *POLE* ultramutated EC (intermediate prognosis). Alterations of *MMR* genes are involved in the pathogenesis of the hypermutated tumors.

Copy number low ECs include MSS grade 1-2 endometrioid tumors with low mutation rates (2.9×10^{-6} mutations/Mb), frequent *PTEN, PI3KCA* and β-catenin (*CTNNB*) mutations and intermediate prognosis, whereas copy number high (serous-like) ECs show extensive copy number aberrations, low mutation rates (2.3×10^{-6} mutations/Mb), very frequent *p53* mutations and MSS (71, 72) . Histological type can be serous, grade 3 endometrioid, as well asmixed, serous and endometrioid, with poor prognosis.

The immune system plays an important role both in the physiological changes of endometrium and inendometrial carcinogenesis (80, 81). de Jong *et al*. (82) assessed $CD8^+$ TILs and T-reg cells on paraffin-embedded tissue specimens from 368 patients with Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage I-IV EC. A high number of $CD8^+$ T cells and/or a high $CD8^+$ T cell /T-reg cell ratio correlated with early stage, low histologic grade, superficial myometrial invasion, lack of LVSI, negative lymph nodes and absence of distant metastases at the time of diagnosis. A high number of $CD8^+$ T cells was an independent favorable prognostic variable for OS in the entire cohort (HR=0.48, 95%CI.=0.26-0.89) and in type II ECs (HR=0.17, 95% CI=0.08-0.36), whereas a high $CD8^+$ T cell/T-reg cell ratio was an independent good prognostic factor for OS in type I ECs (HR=0.44, 95%CI=0.23-0.84). The analysis of paraffin-embedded specimens from 53 women with EC revealed thatT-reg cell count and T-reg cell/$CD8^+$ T cell ratios were significantly higher in patients with advanced, poorly differentiated carcinomas and with LVSI than in those with early, well-differentiated carcinomas and without LVSI (83). Disease-free survival (DFS) of patients with high T-reg cell count and high T-reg cell/$CD8^+$ T cell ratio was significantly worse than that of patients with low T-reg cell count and low T-reg cell/$CD8^+$ T cell ratio.

PD-1 and PD-L1 have been detected in EC tissues (84, 85). The immunohistochemical analysis of tumor samples from 301 ECs showed positive immunostaining for PD-1 and PD-L1 in 77.9% and 39.7%, respectively, of endometrioid carcinomas, 68.2% and 10.2% of serous carcinomas, 69.2% and 23.1% of clear cell carcinomas and 80.0% and 22.2% of carcinosarcomas (84).

Howitt *et al*. (86), who assessed tumor specimens from 63 patients with EC, found that median neoantigen load per sample was 8,342 (range=628-20,440) in *POLE* ultramutated tumors, 541 (range=146-8,063, $p<0.001$) in *MSI* hypermutated tumors and 70.5 (range=7-1,877, $p<0.001$) in MSS tumors. Similarly, *POLE* ultramutated and *MSI* hypermutated ECs had significantly higher numbers of $CD3^+$ TILs and $CD8^+$ TILs compared with MSS ECs; this high immunogenicity could be an explanation of their favorable prognosis. Moreover, PD-1 was overexpressed in TILs and peritumoral lymphocytes of *POLE* and *MSI* tumors, whereas PD-L1 expression was infrequent in tumor cells but common in intraepithelial immune cells, especially in *POLE* and *MSI* tumors. Therefore, *POLE* ultramutated and *MSI* hypermutated ECs show high neoantigen load and high number of TILs, counterbalanced by PD-1 and PD-L1 overexpression. Such features could represent a strong rationale for testing immune checkpoint inhibitors in these cancer subgroups (86-88).

Santin *et al*. (89) reported a remarkable clinical response to nivolumab intwo patients with recurrent, heavily pretreated ultramutated or hypermutated endometrial cancer.

The ongoing trials of checkpoint inhibitors in EC are shown in the Table III. In the NCT02630823 trial, the mechanism of action of pembrolizumab on tumor environment will be investigated by examining both pre-treatment endometrial biopsy and the definite surgical specimen after two cycles of pembrolizumab.

Table III. *Ongoing trials of checkpoint inhibitors in endometrial cancer and cervical cancer.*

Study number	Study phase	Study design
NCT02549209	2	Pembro/carbo/taxol in endometrial cancer (EC) This is a single-arm, open-label study for women with measurable advanced/recurrent EC using pembrolizumab in combination with CBDCA+TAX chemotherapy.
NCT02630823	1	MK-3475 (pembrolizumab) immunotherapy in EC Patients with grade3 endometrioid, serous, clear cell or mixed high-grade EC will undergo endometrial biopsy followed by 2 cycles of pembrolizumab (3 weeks apart). Then the standard of care surgical resection will take place followed by standard of care adjuvant therapy. The standard chemotherapy will consist of CBDCA+TAX q21 for 6 cycles. The decision to administer radiotherapy will be per the treating physician. For patients with high-risk features and advanced stage, pembrolizumab will be given q21 after completion of adjuvant therapy for a maximum of 4 doses post-surgery.
NCT02257528	2	A phase II evaluation of nivolumab in the treatment of persistent or recurrent CC
NCT01693783	2	Ipilimumab in treating patients with metastatic or recurrent HPV-related CC of either squamous cell or adenocarcinoma histologies
NCT01711515	1	Chemoradiation therapy and ipilimumab in treating patients with stages IB2-IIB or IIIB-IVA CC
NCT02488759	1-2	Non-comparative, open-label, multiple cohort, phase 1-2 study of nivolumab monotherapy and nivolumab combination therapy in patients with virus-positive and virus-negative solid tumors

EC, Endometrial cancer; CBDCA, carboplatin; TAX, paclitaxel; CC, cervical cancer; HPV, human papilloma virus.

Cervical Cancer

The goal of treatment of women with recurrent, persistent or metastatic cervical cancer (CC) not amenable to surgery or radiotherapy is palliation of symptoms and prolongation of survival and the combination of paclitaxel (TAX) + cisplatin (CDDP) has been long considered as the standard of care (90). The Gynecologic Oncology Group (GOG) 240 phase III trials randomized 452 patients with stage IVB, recurrent or persistent CC to receive combination chemotherapy with TAX + CDDP (every 3 weeks) or topotecan + TAX (every 3) weeks with or without bevacizumab (BEV) (15 mg/kg every 3 weeks) (91). Topotecan + TAX showed a significantly higher risk of progression and a trend to a higher risk of death when compared with CDDP + TAX either with or without BEV. With the data for the two regimens combined together, the addition of BEV significantly improved median PFS (8.2 *versus* 5.9 months; HR=0.67, 95% CI=0.54-0.82) and median OS (17.0 *versus* 13.3 months; HR=0.71, 95% CI=0.54-0.95) compared with chemotherapy alone.

The treatment after platinum failure is a big challenge and the currently available single-agents, such as topotecan (92), vinorelbine (93), gemcitabine (94), pemetrexed (95), capecitabine (96), docetaxel (97) and nab-paclitaxel (98), have shown unsatisfactory activity. Therefore, the assessment of novel drugs, such as immunecheckpointinhibitors, is strongly warranted in this clinical setting (99-102).

Both PD-1 and PD-L1 have been detected in CC tissues (101, 103-106). Heeren *et al*. (106) assessed PD-L1 expression in tumor specimens from two cohorts of CC patients: primary tumor samples from cohort I (squamous cell carcinoma (SCC), n=156 and adenocarcinoma (AD), n=49) and primary and paired metastatic tumor samples from cohort II (SCC, n=96 and AD, n=31). PD-L1 positivity was observed in >5% of the tumor cells in 54% of SCCs and in 14% of ADs ($p<0.001$), PD-L1-positive TAMs were present in 53% of the former and 12% of the latter ($p<0.001$) displaying an M2-like phenotype characterized by pro-tumorigenic properties (107). DFS and DSS were significantly poorer in SCC patients with diffuse PD-L1 expression than in those with marginal PD-L1 expression in primary tumors ($p=0.022$ and $p=0.046$, respectively). Moreover, DSS was worse in AD patients with PD-L1-positive TAMs than in those without PD-L1-positive TAMs ($p=0.014$).

More dense cordons of PD-L1-positive immune cells were found surrounding the metastases compared with the paired primary tumors in both SCC and AD. These findings appear to provide a rationale for therapeutic targeting of the PD-1/PD-L1 pathway in CC.

Several phase I-II trials are currently testing immune checkpoint inhibitors in CC but no results have been releasedyet (Table III).

In the NCT02257528 trial,immune infiltration-related biomarkers (*i.e.*, CD4+T, $CD8^+$ T, T-reg) in tumor specimens and PD-1 and PD-L1 expression in TILs and CC cells will be related to OR , PFS and OS of patients with persistent, recurrent or metastatic CC treated with nivolumab.

NCT02488759 is a phase 1-2 study investigating the safety and efficacy of nivolumab alone andin combination with ipilimumab or BMS-986016 anti- lymphocyte activation gene-3 (LAG-3) mAb or daratumumab (anti-CD38 mAb) in patients with different tumors, including squamous cell carcinoma of the cervix, vagina, or vulva.

Longoria and Tewari (100) have proposed a clinical trial of combined anti-angiogenic therapy and immunotherapy in metastatic, recurrent or persistent CC. Patients with evidence of response and no unacceptable toxicity after combination chemotherapy (CDDP+TAX or carboplatin+TAX or topotecan+ TAX) plus BEV (15 mg/kg every 3 weeks) for 7 cycles will be randomly allocated to receive either chemotherapy +BEV + nivolumab (3 mg/kg) every 3 weeks or chemotherapy + BEV + placebo every 3 weeks until progression or unacceptable toxicity.

Conclusion

Very limited data are currently available about the activity of immune checkpoint inhibitors in gynecological cancers; however, immune checkpoint inhibitors, and especially anti-PD-1 and anti-PD-L1 mAbs, could represent a novel pharmacological tool for the management of gynecological malignancies, although is yet to be defined which patients could draw a greater clinical benefit from these agents and when immunecheckpointinhibitors should be incorporated into the therapeutic strategy (37, 108, 109). Moreover, reliable biomarkers predictive of response have not been identified yet.

BRCA1-2-mutated HGSOC and CCOC with MSI could have an elevated probability of response to these agents because these tumors show increased neoantigen load, increased $CD3^+$ and $CD8^+$ TIL number, as well as PD-1 and PD-L1 overexpression. Immune checkpoint inhibitors in combination with PARP inhibitors are currently investigated in patients with recurrent EOC and mutated *BRCA1-2*.

As far as EC is concerned, *POLE* ultramutated tumors have a median number of neo-antigensalmost 15-fold higher than *MSI* hypermutated tumors,with the latter having a median number of neo-antigens 7-fold higher than MSS tumors. *POLE* ultramutated and *MSI* hypermutated ECs exhibit a high number of TILs counter balanced by PD-1 and PD-L1 overexpression and, therefore, they are likely to be sensitive to PD-1/PD-L1 pathway blockade. Immune check-point inhibitors could have a role as maintenance treatment in patients with persistent, recurrent or metastatic CC in response after chemotherapy.

References

1 Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser R, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM and Housseau F: The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov *5*: 43-51, 2009.

2 Vesalainen S, Lipponen P, Talja M and Syrjanen K: Histological grade, perineural infiltration, tumour-infiltrating lymphocytes and apoptosis as determinants of long-term prognosis in prostatic adenocarcinoma. Eur J Cancer *30*: 1797-1803,1994.

3 Marrogi AJ, Munshi A, Merogi AJ, Ohadike Y, El-Habashi A, Marrogi OL and Freeman SM: Study of tumor infiltrating lymphocytes and transforming growth factor-beta as prognostic factors in breast carcinoma. Int J Cancer *74*: 492- 501,1997.

4 Halpern AC and Schuchter LM: Prognostic models in melanoma. Semin Oncol *24(Suppl 4)*: S2-S7,1997.

5 Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H and Ohtani H: CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res *58*: 3491-3494,1998.

6 Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, Suzuki Y, Shintaku I, Nagura H and Ohtani H: Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. Cancer Res *61*: 5132-5136, 2001.

7 Schumacher K, Haensch W, Roefzaad C and Schlag PM: Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. Cancer Res *61*: 3932-3936, 2001.

8 Mittal D, Gubin MM, Schreiber RD and Smyth MJ: New insights into cancer immunoediting and its three component phases – elimination, equilibrium and escape. Curr Opin Immunol *27*: 16-25, 2014.

9 Gooden MJ, de Bock GH, Leffers N, Daemen T and Nijman HW: The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer *105*: 93-103, 2011.

10 Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer *12*: 252-64, 2012.

11 Chen DS and Mellman I: Oncology meets immunology: the cancer-immunity cycle. Immunity *39*: 1-10, 2013.

12 Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V and Bowman MR: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J. Exp. Med *192*: 1027-1034, 2000.

13 Keir ME, Francisco LM and Sharpe AH: PD-1 and its ligands in T-cell immunity. Curr Opin Immunol *19*: 309-314, 2007.

14 Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK and Sharpe AH: PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J. Exp. Med *206*: 3015-3029, 2009.

15 Fife BT and Bluestone JA: Control of peripheral T-cell tolerance and autoimmunity *via* the CTLA-4 and PD-1 pathways. Immunol Rev *224*: 166-182, 2008.

16 Huang J, Zhang Y, Sheng J, Zhang H, Fang W, Zhan J, Zhou T, Chen Y, Liu L and Zhang L: The efficacy and safety of nivolumab in previously treated advanced non-small-cell lung cancer: a meta-analysis of prospective clinical trials. Onco Targets Ther *9*: 5867-5874, 2016.

17 Comin-Anduix B, Escuin-Ordinas H and Ibarrondo FJ: Tremelimumab: research and clinical development. Onco Targets Ther *9*: 1767-76, 2016.

18 Wang J, Chmielowski B, Pellissier J, Xu R, Stevinson K and Liu FX: Cost-Effectiveness of pembrolizumab *versus* ipilimumab in ipilimumab-naïve patients with advanced melanoma in the United States. J Manag Care Spec Pharm *23*: 184-194, 2017.

19 Martin-Liberal J, Ochoa de Olza M, Hierro C, Gros A, Rodon J and Tabernero J: The expanding role of immunotherapy. Cancer Treat Rev *54*: 74-86, 2017.

20 Liu KG, Gupta S and Goel S: Immunotherapy: incorporation in the evolving paradigm of renal cancer management and future prospects. Oncotarget *10*: 17313-17327, 2017.

21 Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A and Urba WJ: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med *363*: 711-723, 2010.

22 Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A and Wigginton JM: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med *366*: 2455-2465, 2012.

23 Spain L, Diem S and Larkin J: Management of toxicities of immune checkpoint inhibitors. Cancer Treat Rev *44*: 51-60, 2016.

24 Wolchok JD, Hodi FS, Weber JS, Allison JP, Urba WJ, Robert C, O'Day SJ, Hoos A, Humphrey R, Berman DM, Lonberg N and Korman AJ: Development of ipilimumab: a novel immunotherapeutic approach for the treatment of advanced melanoma. Ann NY Acad Sci *1291*: 1-13, 2013.

25 Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, Maio M, Binder M, Bohnsack O, Nichol G, Humphrey R and Hodi FS: Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res *15*: 7412-7420, 2009.

26 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*: 228-247, 2009.

27 Teng MW, Ngiow SF, Ribas A and Smyth MJ: Classifying Cancers Based on T-cell Infiltration and PD-L1. Cancer Res *75*: 2139-2145, 2015.

28 Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC and Coukos G: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med *348*: 203-213, 2003.

29 Hwang WT, Adams SF, Tahirovic E, Hagemann IS and Coukos G: Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol *124*: 192-198, 2012.

30 Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L and Zou W: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med *10*: 942-949, 2004.

31 Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ and Odunsi K: Intraepithelial CD8$^+$ tumor-infiltrating lymphocytes and a high CD8$^+$/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci USA *102*: 18538-18543, 2005.

32 Vermeij R, de Bock GH, Leffers N, Ten Hoor KA, Schulze U, Hollema H, van der Burg SH, van der Zee AG, Daemen T and Nijman HW: Tumor-infiltrating cytotoxic T lymphocytes as independent prognostic factor in epithelial ovarian cancer with wilms tumor protein 1 overexpression. J Immunother *34*: 516-523, 2011.

33 Bachmayr-Heyda A , Aust S, Heinze G, Polterauer S, Grimm C, Braicu EI, Sehouli J, Lambrechts S, Vergote I, Mahner S, Pils D, Schuster E, Thalhammer T, Horvat R, Denkert C, Zeillinger R and Castillo-Tong DC: Prognostic impact of tumor infiltrating CD8$^+$ T cells in association with cell proliferation in ovarian cancer patients – a study of the OVCAD consortium. BMC Cancer *13*: 422, 2013.

34 Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, Honjo T and Fujii S: Programmed cell death 1 ligand 1 and tumor-infiltrating CD8$^+$ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA *104*: 3360-3365, 2007.

35 Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, George AJ and Ghaem-Maghami S:Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. Cancer Immunol Immunother *63*: 215-224, 2014.

36 Darb-Esfahani S, Kunze CA, Kulbe H, Sehouli J, Wienert S, Lindner J, Budczies J, Bockmayr M, Dietel M, Denkert C, Braicu I and Jöhrens K: Prognostic impact of programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor-infiltrating lymphocytes in ovarian high grade serous carcinoma. Oncotarget *7*: 1486-1499, 2016.

37 Hamanishi J, Mandai M and Konishi I: Immune checkpoint inhibition in ovarian cancer. Int Immunol *28*: 339-348, 2016.

38 Webb JR, Milne K, Kroeger DR and Nelson BH: PD-L1 expression is associated with tumor-infiltrating T cells and favorable prognosis in high-grade serous ovarian cancer. Gynecol Oncol *141*: 293-302, 2016.

39 Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, Kanai M, Mori Y, Matsumoto S, Chikuma S, Matsumura N, Abiko K, Baba T, Yamaguchi K, Ueda A, Hosoe Y, Morita S, Yokode M, Shimizu A, Honjo T and Konishi I: Safety and antitumor activity of anti-PD- 1 antibody, nivolumab, in patients with platinum- resistant ovarian cancer. J Clin Oncol *33*: 4015-4022, 2015.

40 Varga A, Piha-Paul SA, Ott PA, Mehnert JM, Berton-Rigaud D, Johnson EA, Cheng JD, Yuan S, Rubin EH and Matei DE: 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 *33*: abstr 5510, 2015.

41 Disis ML, Patel MR, Pant S, Hamilton EP, Lockhart AC, Kelly K, Beck JT, Gordon MS, Weiss GJ, Taylor MH, Chaves J, Mita AC, Kevin M, Chin KM, von Heydebreck A, Cuillerot JM and Gulley JL: Avelumab (MSB0010718C; anti-PD- L1) in patients with recurrent/refractory ovarian cancer from the JAVELIN Solid Tumor phase Ib trial: Safety and clinical activity. J Clin Oncol *34*: abstr 5533, 2016.

42 Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, Senz J, McConechy MK, Anglesio MS, Kalloger SE, Yang W, Heravi-Moussavi A, Giuliany R, Chow C, Fee J, Zayed A, Prentice L, Melnyk N, Turashvili G, Delaney AD, Madore J, Yip S, McPherson AW, Ha G, Bell L, Fereday S, Tam A, Galletta L, Tonin PN, Provencher D, Miller D, Jones SJ, Moore RA, Morin GB, Oloumi A, Boyd N, Aparicio SA, Shih IeM, Mes-Masson AM, Bowtell DD, Hirst M, Gilks B, Marra MA and Huntsman DG: ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med *363*: 1532-1543, 2010.

43 Ayhan A, Mao TL, Seckin T, Wu CH, Guan B, Ogawa H, Futagami M, Mizukami H, Yokoyama Y, Kurman RJ and ShihIe M: Loss of ARID1A expression is an early molecular event in tumor progression from ovarian endometriotic cyst to clear cell and endometrioid carcinoma. Int J Gynecol Cancer *22*: 1310-1315, 2012.

44 Jones S, Wang TL, Shih IeM, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr, Vogelstein B, Kinzler KW, Velculescu VE and Papadopoulos N: Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science *330*: 228-231, 2010.

45 Yamamoto S, Tsuda H, Takano M, Tamai S and Matsubara O: Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations. Mod Pathol *25*: 615-624, 2012.

46 Xiao W, Awadallah A and Xin W: Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. Int J Clin Exp Pathol *5*: 642-650, 2012.

47 Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Nakayama N, Ishikawa M, Ishibashi T, Iida K, Kobayashi H, Otsuki Y, Nakayama S and Miyazaki K: Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. Mod Pathol *25*: 282-288, 2012.

48 Gadducci A , Lanfredini N and Tana R: Novel insights on the malignant transformation of endometriosis into ovarian carcinoma. Gynecol Endocrinol *30*: 612-617, 2014.

49 Friedlander ML, Russell K, Millis S, Gatalica Z, Bender R and Voss A: Molecular profiling of clear cell ovarian cancers: Identifying potential treatment targets for clinical trials. Int J Gynecol Cancer *26*: 648-654, 2016.

50 Lastwika KJ, Wilson W 3rd, Li QK, Norris J, Xu H, Ghazarian SR, Kitagawa H, Kawabata S, Taube JM, Yao S, Liu LN, Gills JJ and Dennis PA: Control of PD-L1 expression by oncogenic activation of the AKT–mTOR pathway in non-small cell lung cancer. Cancer Res *76*: 227-238, 2016.

51 Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, Kruse A, Macrae S, Nelson M, Canning C, Lowy I, Korman A, Lautz D, Russell S, Jaklitsch MT, Ramaiya N, Chen TC, Neuberg D, Allison JP, Mihm MC and Dranoff G: Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci USA *105*: 3005-3010, 2008.

52 NCT01611558: Phase II Study of Ipilimumab Monotherapy in Recurrent Platinum- sensitive Ovarian Cancer – Study Results. https://clinicaltrials.gov/ct2/show/results/NCT01611558. Accessed 24 May 2016.

53 Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM and Sznol M: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med *366*: 2443-2454, 2012.

54 Xiao Y and Freeman GJ: The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. Cancer Discov *5*: 16-18, 2015.

55 Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B and Diaz LA Jr.: PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med *372*: 2509-2520, 2015.

56 Hussein YR, Weigelt B, Levine DA, Schoolmeester JK, Dao LN, Balzer BL, Liles G, Karlan B, Kobel M, Lee CH and Soslow RA: Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. Mod Pathol *28*: 505-514, 2015.

57 Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, Garber JE, Chowdhury D, Wu CJ, D'Andrea AD, Matulonis UA and Konstantinopoulos PA: Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. Oncotarget *7*: 13587-13598, 2016.

58 McAlpine JN, Porter H, Köbel M, Nelson BH, Prentice LM, Kalloger SE, Senz J, Milne K, Ding J, Shah SP, Huntsman DG and Gilks CB: BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. Mod Pathol *25*: 740-750, 2012.

59 Mittica G, Genta S, Aglietta M and Valabrega G: Immune checkpoint inhibitors: a new opportunity in the treatment of ovarian cancer? Int J Mol Sci *17*: 1169, 2016.

60 Strickland K, Howitt BE, Rodig SJ, Matulonis UA and Konstantinopoulos P: Immunogenicity of clear cell ovarian cancer: Association with ARID1A loss, microsatellite instability and endometriosis. J Clin Oncol *34*: abstr 5514, 2016.

61 Bokhman JV: Two pathogenetic types of endometrial carcinoma. Gynecol Oncol *15*: 10-17, 1983.

62 Matias-Guiu X, Catasus L, Bussaglia E, Lagarda H, Garcia A, Pons C, Muñoz J, Argüelles R, Machin P and Prat J: Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol *32*: 569-577, 2001.

63 Prat J, Gallardo A, Cuatrecasas M and Catasús L: Endometrial carcinoma: pathology and genetics. Pathology *39*: 72-87, 2007.

64 Moreno-Bueno G, Sánchez-Estévez C, Cassia R, Rodríguez-Perales S, Díaz-Uriarte R, Domínguez O, Hardisson D, Andujar M, Prat J, Matias-Guiu X, Cigudosa JC and Palacios J: Differential gene expression profile in endometrioid and nonendometrioid endometrial carcinoma: STK15 is frequently overexpressed and amplified in nonendometrioid carcinomas. Cancer Res *63*: 5697-702, 2003.

65 Santin AD, Bellone S, Van Stedum S, Bushen W, Palmieri M, Siegel ER, De Las Casas LE, Roman JJ, Burnett A and Pecorelli S: Amplification of c-erbB2 oncogene: a major prognostic indicator in uterine serous papillary carcinoma. Cancer *104*: 1391-1397, 2005.

66 Catasus L, Gallardo A, Cuatrecasas M and Prat J: PIK3CA mutations in the kinase domain (exon 20) of uterine endometrial adenocarcinomas are associated with adverse prognostic parameters. Mod Pathol *21*: 131-139, 2008.

67 Yalta T, Atay L, Atalay F, Caydere M, Gonultas M and Ustun H: E-cadherin expression in endometrial malignancies: comparison between endometrioid and non-endometrioid carcinomas. J Int Med Res *37*: 163-168, 2009.

68 Hayes MP, Wang H, Espinal-Witter R, Douglas W, Solomon GJ, Baker SJ and Ellenson LH: PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. Clin Cancer Res *12*: 5932-5935, 2006.

69 Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, Weng LP and Eng C: Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst *92*: 924-930, 2000.

70 Lax SF, Kendall B, Tashiro H, Slebos RJ and Hedrick L:The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer *88*: 814-824, 2000.

71 Murali R, Soslow RA and Weigelt B: Classification of endometrial carcinoma: more than two types Lancet Oncol *15*: e268-278, 2014.

72 Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding L, Zhang W, Mills GB, Kucherlapati R, Mardis ER and Levine DA: Integrated genomic characterization of endometrial carcinoma. Nature *497*: 67-73, 2013.

73 Pursell ZF and Kunkel TA: DNA polymeras epsilon:polymerase of unusual size (and complexity). Prog Nucleic Acid Res Mol Biol *82*: 101-145, 2008.

74 Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, Gorman M, Martin L, Howarth KM, Hodgson SV, NSECG Collaborators, Kaur K, Taylor J and Tomlinson IP: DNA polymerase ε and δ exonuclease domain mutations in endometrial cancer. Hum Mol Genet *22*: 2820-2828, 2013.

75 Hussein YR, Weigelt B, Levine DA, Schoolmeester JK, Dao LN, Balzer BL, Liles G, Karlan B, Köbel M, Lee CH and Soslow RA: Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. Mod Pathol *28*: 505-514, 2015.

76 Church DN, Stelloo E, Nout RA , Valtcheva N, Depreeuw J, ter Haar N, Noske A, Amant F, Tomlinson IP, Wild PJ, Lambrechts D, Jürgenliemk-Schulz IM, Jobsen JJ, Smit VT, Creutzberg CLand Bosse T:Prognostic significance of POLE proofreading mutations in endometrial cancer. J Natl Cancer Inst *107*: 402, 2014.

77 Meng B, Hoang LN, McIntyre JB, Duggan MA, Nelson GS, Lee CH and Köbel M: POLE exonuclease domain mutation predicts long progression free survival in grade 3 endometrioid carcinoma of the endometrium. Gynecol Oncol *134*: 15-19, 2014.

78 Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, Leary A, Edmondson RJ, Powell ME, Crosbie EJ, Kitchener HC, Mileshkin L, Pollock PM, Smit VT and Creutzberg CL: Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. Mod Pathol *28*: 836-844, 2015.

79 McConechy MK, Talhouk A, Leung S, Chiu D, Yang W, Senz J, Reha-Krantz LJ, Lee CH, Huntsman DG, Gilks CB and McAlpine JN: Endometrial Carcinomas with POLE Exonuclease Domain Mutations Have a Favorable Prognosis.Clin Cancer Res *22*: 2865-2867, 2016.

80 Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK and Ochiel DO: Sex hormone regulation of innate immunity in the female reproductive tract: the role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. Am J Reprod Immunol *63*: 544-565, 2010.

81 Vanderstraeten A, Tuyaerts S and Amant F: The immune system in the normal endometrium and implications for endometrial cancer development. J Reprod Immunol *109*: 7-16, 2015.

82 de Jong RA, Leffers N, Boezen HM, ten Hoor KA, van der Zee AG, Hollema H and Nijman HW: Presence of tumor-infiltrating lymphocytes is an independent prognostic factor in type I and II endometrial cancer. Gynecol Oncol *114*: 105-110, 2009.

83 Yamagami W, Susumu N, Tanaka H, Hirasawa A, Banno K, Suzuki N, Tsuda H, Tsukazaki K and Aoki D: Immunofluorescence-detected infiltration of $CD4^{+}FOXP3^{+}$ regulatory T cells is relevant to the prognosis of patients with endometrial cancer. Int J Gynecol Cancer *21*: 1628-1634, 2011.

84 Herzog T, Arguello D, Reddy S and Gatalica Z: PD-1 and PD-L1 expression in 1599 gynecological malignancies - implications for immunotherapy. Gynecol Oncol *137*: 204-205, 2015.

85 Vanderstraeten A, Luyten C, Verbist G, Tuyaerts S and Amant F: Mapping the immunosuppressive environment in uterine tumors: implications for immunotherapy. Cancer Immunol Immunother *63*: 545-557, 2014.

86 Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, Stover E, Strickland KC, D'Andrea AD, Wu CJ, Matulonis UA and Konstantinopoulos PA: Association of Polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. JAMA Oncol *1*: 1319-1323, 2015.

87 Longoria TC and Eskander RN: Immunotherapy in endometrial cancer - an evolving therapeutic paradigm. Gynecol Oncol Res Pract *2*: 11, 2015.

88 Gargiulo P, Della Pepa C, Berardi S, Califano D, Scala S, Buonaguro L, Ciliberto G, Brauchli P and Pignata S:Tumor genotype and immune microenvironment in POLE-ultramutated and MSI-hypermutated Endometrial Cancers: New candidates for checkpoint blockade immunotherapy? Cancer Treat Rev *48*: 61-68, 2016.

89 Santin AD, Bellone S, Buza N, Choi J, Schwartz PE, Schlessinger J and Lifton RP: Regression of chemotherapy-resistant polymerase ε (POLE) ultra-mutated and MSH6 hypermutated endometrial tumors with nivolumab. Clin Cancer Res *22*: 5682-5687, 2016.

90 Monk BJ , Sill MW, McMeekin DS, Cohn DE, Ramondetta LM, Boardman CH, Benda J and Cella D: Phase III trial of four cisplatin-containing doublet combinations in stage IVB, recurrent, or persistent cervical carcinoma: a Gynecologic Oncology Group study. J Clin Oncol *27*: 4649-4655, 2009.

91 Tewari KS, Sill MW, Long HJ 3rd, Penson RT, Huang H, Ramondetta LM, Landrum LM, Oaknin A, Reid TJ, Leitao MM, Michael HE and Monk BJ: Improvedsurvival with bevacizumab in advancedcervical cancer. N Engl J Med *370*: 734-743, 2014.

92 Abu-Rustum NR, Lee S and Massad LS: Topotecan for recurrent cervical cancer after platinum-based therapy. Int J Gynecol Cancer *10*: 285-288, 2000.

93 Muggia FM, Blessing JA, Method M, Miller DS, Johnson GA, Lee RB and Menzin A: Evaluation of vinorelbine in persistent or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol *92*: 639-643, 2004.

94 Schilder RJ, Blessing J and Cohn DE: Evaluation of gemcitabine in previously treated patients with non-squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol *96*: 103-107, 2005.

95 Miller DS, Blessing JA, Bodurka DC, Bonebrake AJ and Schorge JO: Evaluation of pemetrexed (Alimta, LY231514) as second line chemotherapy in persistent or recurrent carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol *110*: 65-70, 2008.

96 Garcia AA, Blessing JA, Darcy KM, Lenz HJ, Zhang W, Hannigan E and Moore DH: Phase II clinical trial of capecitabine in the treatment of advanced, persistent or recurrent squamous cell carcinoma of the cervix with translational research: a gynecologic oncology group study. Am J Clin Oncol *30*: 428-431, 2007.

97 Garcia AA, Blessing JA, Vaccarello L and Roman LD: Phase II clinical trial of docetaxel in refractory squamous cell carcinoma of the cervix: a Gynecologic Oncology Group Study. Am J Clin Oncol *30*: 428-431, 2007.

98 Minion LE, Chase DM, Farley JH, Willmott LJ and Monk BJ: Safety and efficacy of salvage nano-particle albumin bound paclitaxel in recurrent cervical cancer: a feasibility study. Gynecol Oncol Res Pract *3*: 4, 2016.

99 Tewari KS and Monk BJ: New strategies in cervical cancer: from angiogenesis blockade to immunotherapy Clin Cancer Res *20*: 5349-5358, 2014.

100 Longoria TC and Tewari KS: Pharmacologic management of advancedcervical cancer: antiangiogenesis therapy and immunotherapeutic considerations. Drugs *75*: 1853-1865, 2015.

101 Boussios S, Seraj E, Zarkavelis G, Petrakis D, Kollas A, Kafantari A, Assi A, Tatsi K, Pavlidis N and Pentheroudakis G: Management of patients with recurrent/advanced cervical cancer beyond first line platinum regimens: Where do we stand? A literature review. Crit Rev Oncol Hematol *108*: 164-174, 2016.

102 Lee SJ, Yang A, Wu TC and Hung CF: Immunotherapy for human papillomavirus-associated disease and cervical cancer: review of clinical and translational research J Gynecol Oncol *27*: e51, 2016.

103 Karim R, Jordanova ES, Piersma SJ, Kenter GG, Chen L, Boer JM, Melief CJ and van der Burg SH: Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res *15*: 6341-6347, 2009.

104 Yang W, Song Y, Lu YL, Sun JZ and Wang HW: Increased expression of programmed death (PD)-1 and its ligand PD-L1 correlates with impaired cell-mediated immunity in high-risk human papillomavirus-related cervical intraepithelial neoplasia. Immunology *139*: 513–522, 2013.

105 Mezache L, Paniccia B, Nyinawabera A and Nuovo GJ: Enhanced expression of PD L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol *28*: 1594-1602, 2015.

106 Heeren AM, Punt S, Bleeker MC, Gaarenstroom KN, van der Velden J, Kenter GG, de Gruijl TD and Jordanova ES: Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix. Mod Pathol *29*: 753-763, 2016.

107 Chanmee T, Ontong P, Konno K and Itano N: Tumor-associated macrophages as major players in the tumor microenvironment. Cancers (Basel) *6*: 1670-1690, 2014.

108 Ring KL, Pakish J and Jazaeri AA: Immune Checkpoint Inhibitors in the Treatment of Gynecologic Malignancies. Cancer J *22*: 101-107, 2016.

109 Emens LA, Kok M and Ojalvo LS: Targeting the programmed cell death-1 pathway in breast and ovarian cancer. Curr Opin Obstet Gynecol *28*: 142-147, 2016.

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