Targetable Immune Regulatory Molecule Expression in High-Grade Serous Ovarian Carcinomas in African-American Women: A Study of PD-L1 and IDO in 112 Cases from the African American Cancer Epidemiology Study (AACES)

Anne M. Mills[1], **Lauren C. Peres**[2], **Alice Meiss**[1], **Kari L. Ring**[3], **Susan C. Modesitt**[3], **Sarah E. Abbott**[2], **Anthony J. Alberg**[4], **Elisa V. Bandera**[5], **Jill Barnholtz-Sloan**[6], **Melissa L. Bondy**[7], **Michele L. Cote**[8], **Ellen Funkhouser**[9], **Patricia G. Moorman**[10], **Edward S. Peters**[11], **Ann G. Schwartz**[8], **Paul D. Terry**[12], **Kristin Wallace**[13], and **Joellen M. Schildkraut**[2]

[1]Department of Pathology, University of Virginia, Charlottesville, VA

[2]Department of Public Health Sciences, University of Virginia, Charlottesville, VA

[3]Department of Obstetrics and Gynecology, University of Virginia, Charlottesville, VA

[4]Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC

[5]Department of Population Science, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

[6]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH

[7]Department of Medicine, Section of Epidemiology and Population Sciences, Baylor College of Medicine, Houston, TX

[8]Department of Oncology and the Karmanos Cancer Institute Population Studies and Disparities Research Program, Wayne State University, Detroit, MI

[9]Division of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL

[10]Department of Community and Family Medicine, Duke University Medical Center, Durham, NC

[11]Epidemiology Program, Louisiana State University Health Sciences Center School of Public Health, New Orleans, LA

[12]Department of Medicine, University of Tennessee Medical Center-Knoxville, Knoxville, TN

[13]Department of Public Health Sciences, Medical University of South Carolina, Charleston, SC

Abstract

African-American (AA) women with high-grade serous ovarian carcinoma (HGSOC) have worse outcomes compared to women of European descent. Although the discrepancy is partially

attributed to differences in access to care, the tumor immune microenvironment may also contribute. Expression of targetable immune regulatory molecules such as PD-L1 and IDO is of particular interest as it may help guide therapy in this population. Using cases from the largest study of AA women with ovarian cancer, the African American Cancer Epidemiology Study, we characterized PD-L1 and IDO expression in 112 HGSOC. Immunohistochemistry for PD-L1, IDO, CD8, FOX3p, and CD68 was performed. PD-L1 and IDO were scored as the percentage of positive tumor cells and tumor-associated immune cells. CD8 and FOX3p counts were averaged across 10 high-power fields. Cox proportional hazards regression was used to evaluate the association between PD-L1 and IDO expression and survival. Tumor cells were positive for PD-L1 and IDO in 29% and 58% of cases, respectively. The majority showed <10% staining, and no cases exceeded 25% positivity. The majority of PD-L1-positive cases co-expressed IDO. PD-L1 and IDO expression was associated with higher CD8 and FOX3p counts (p<0.05). No association was observed between PD-L1 and IDO and survival. In summary, expression of PD-L1 and IDO is seen in a subset of HGSOC from AA women and is correlated with elevated lymphocyte infiltration. While PD-L1 and IDO co-expression suggests a role for dual immunotherapy, diffuse expression of PD-L1 and IDO is rare, invoking caution regarding the potential for immunotherapeutic response.

BACKGROUND

Ovarian cancer is the 5th deadliest malignancy among women, with an estimated 14,080 deaths in 2017.[1,2] The vast majority of deaths are due to high-grade serous ovarian carcinoma (HGSOC). Survival has increased only modestly despite advances in treatment, with a relative 45% 5-year survival among women of European descent diagnosed in 2005–2011 compared to 36% in those diagnosed in 1975–1977.[2] Survival rates remain even lower among African-American (AA) women, with 38% survival for those diagnosed in 2005–2011.[2,3] This disparity can be partially attributed to differences in treatment access and quality of care, but these variables do not fully account for the outcome discrepancy.[4–6]

Improving the prognosis for AA women with ovarian cancer requires a multifocal effort, including not only careful epidemiologic characterization, but also directed study of tumor biology. Immune context is increasingly understood to contribute to tumor behavior.[7,8,9] It may be that racial survival discrepancies in HGSOC could be partially attributable to differences in the immune milieu. AAs have been shown to have elevated inflammatory biomarkers relative to individuals of European descent,[10,11] and differences in inflammatory markers have been linked to altered cancer outcomes.[12,13] Genetic variability in inflammatory genes has also been shown to impact ovarian cancer risk.[14–16]

Understanding the immune context of tumors is also important given the recent rise of immunotherapy.[17–21] Immune checkpoint blockades have proven effective, particularly in the context of an elevated inflammatory milieu.[17,20,22] Targets include programmed cell death-1 (PD-1) and its partner, programmed cell death ligand-1 (PD-L1/CD274), and evidence suggests that inhibiting this axis could be useful in ovarian cancer treatment.[23–27] Another mechanism of immunotherapy is through enzymatic interference. Indoleamine 2,3 dioxygenase (IDO) is an immune modulatory enzyme of interest for ovarian cancer therapy

because it is expressed in over half of ovarian carcinomas, has been correlated with adverse outcomes, and has clinically available antagonists.[28–30]

Immune regulatory molecule expression has not been well investigated in ovarian carcinomas from AA women due to the paucity of studies containing a sufficient proportion of these patients. The African American Cancer Epidemiology Study (AACES) is a multi-center population-based case-control study of ovarian cancer in AA women and represents the largest available cohort of this patient population.[31] This study population therefore represents a unique opportunity to evaluate clinically actionable components of the immune microenvironment of women with HGSOC who are underrepresented in existing literature. We herein complete a directed assessment of PD-L1 and IDO expression alone and in the context of CD8+ cytotoxic T cell and FOX3p+ regulatory T cell infiltrates in HGSOCs from AA women enrolled in the AACES study in order to 1) understand how tumoral immune evasion might contribute to poor prognosis in AA women with this cancer and 2) address potential immunotherapeutic vulnerability in this population.

METHODS

Study Population

Cases were selected from AACES, the largest population based case-control study of AA women with epithelial ovarian cancer. Study enrollment procedures and methods have been discussed elsewhere.[31] Briefly, newly diagnosed cases of invasive epithelial ovarian cancer were identified between December 1, 2010 and December 31, 2015 using a rapid case ascertainment approach at cancer registries and gynecologic oncology departments and hospitals in 11 geographic locations (Alabama, Georgia, Illinois, Louisiana, Michigan, North Carolina, New Jersey, Ohio, South Carolina, Tennessee, and Texas). Patients were eligible for the study if they were 20–79 years of age, self-reported AA race, resided in one of the 11 geographic locations, and were able to complete a baseline telephone interview in English. A total of 601 cases were enrolled in AACES.

We requested formalin-fixed paraffin-embedded (FFPE) tumor blocks for the primary ovarian carcinoma from the diagnosing facility, and for each tissue block received, twenty-five sections were cut, with one section hematoxylin and eosin (H&E) stained. All H&E slides and original pathology reports were centrally reviewed by expert pathologists at Duke University (R.B.) and the University of Virginia (UVA) (A.M.) to confirm diagnosis and stage. Although still ongoing, as of August 2017, the pathology review had been completed for 445 cases and of those, 294 are HGSOCs. For the purposes of this analysis, we selected 124 cases with sufficient tissue for immunohistochemical (IHC) staining; cases were selected chiefly based on the availability of an adequate number of unstained sections for complete analysis.

Vital status was ascertained through an annual systematic collection of follow-up data from cancer registries as well as annual contact with AACES participants. Follow-up time was calculated as the number of days from the date of diagnosis to the date of death or the date last known to be alive.

Immunohistochemistry

All IHC staining was performed at the UVA Biorepository and Tissue Research Facility. Whole section slides of FFPE tumor were stained on a Dako instrument with PD-L1 (Spring Biosciences, SP142, 1:200 dilution) and IDO (Sigma Prestige, HPA 023072, 1:2,000 dilution) antibodies. This PD-L1 clone has been previously validated in this laboratory on a large array of non-small cell lung cancer against results obtained on the Dako 22C3 antibody using the Dako platform with satisfactory results (e.g. no discrepant cases at clinically relevant thresholds). Although clinical standards for IDO expression do not exist, this IDO clone has performed reliably in our research setting in a variety of tissue types. Placental tissue was used as a positive control, with circumferential villous staining without significant background stromal staining required for validity for the PD-L1 immunostain and villous vascular staining required for the IDO immunostain.

PD-L1 was considered positive when circumferential membranous staining was identified. IDO was considered positive when staining was identified in the cytoplasmic compartment. PD-L1 and IDO were scored separately in tumor cells and in tumor-associated immune cells (chiefly lymphocytes and macrophages) and were given a score from 0–5 for each compartment. The 5 scoring categories were generated to include all clinically meaningful cut-offs for all immunotherapy drugs (0: 0%, 1: 1–5%, 2: 6–10%, 3: 11–25%, 4: 26–50%, 5: >50%). These stains were interpreted in concert with a separate slide stained for CD68 (KP1, company: Novus Biologicals, 1:200 dilution) to ensure that tumor vs. macrophage staining was interpreted appropriately.

Dual staining was also performed for the lymphocyte markers CD8 (Dako, C8/144B, 1:100 dilution) and FOX3p (Abcam, clone 236A/E7, 1:50 dilution) to characterize the intratumoral cytotoxic and regulatory T cell populations, respectively. Intratumoral lymphocytes were defined as immunopositive cells touching tumor cells; therefore, peritumoral immune cells located in the stroma but not directly in contact with the tumor were not enumerated. This method was chosen to control for the fact that not all tumor sections contained stroma; some consisted exclusively of tumor cells. CD8 and FOX3p counts were performed manually and averaged across 10 high power fields measuring 0.55mm in diameter ($10\times$ 22mm ocular, $40\times$ objective). The initial field selected for each case was chosen based on the area of highest lymphocyte density, after which fields were selected randomly.

Statistics

The dataset was restricted to include only those cases with data for the immune modulatory markers (PD-L1 and IDO) as well as the lymphocyte makers (CD8 and Fox3p)., N=112. CD8 and Fox3p counts were evaluated categorically using the median count as the cut-point (median value for CD8: 12.95 and Fox3p: 3.20). Due to the small sample size, the scoring categories of PD-L1 and IDO expression were collapsed into 2 levels: positive vs. no staining. We used Chi square tests to evaluate whether PD-L1 and IDO expression differed by counts of CD8 and Fox3p and whether PD-L1 and IDO positivity differed according to demographic and clinical characteristics of AACES patients.

Cox-proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the relationship between PD-L1 and IDO expression and overall survival. The following *a priori* confounders were adjusted for in all models: age at diagnosis (continuous, years), stage (stage I-II and III-IV), region of residence (Northeast: Michigan, Illinois, New Jersey and Ohio, Southeast: Georgia, Tennessee, North Carolina and South Carolina, South: Alabama, Texas and Louisiana), and residual disease (optimal debulking: <1 cm residual tumor diameter after cytoreductive surgery or CA-125 < 35 after adjuvant chemotherapy, suboptimal debulking: 1 cm residual tumor diameter after cytoreductive surgery or CA-125   35 after adjuvant chemotherapy). We also repeated these analyses stratified by high and low CD8 and Fox3p counts to evaluate whether the effects of PD-L1 and IDO on outcome are dependent on the presence of tumor infiltrating lymphocytes. SAS Version 9.4 (Cary, North Carolina) was used to complete all analyses.

RESULTS

A total of 112 women with high-grade serous ovarian carcinomas who were enrolled in AACES were included (Table 1). The mean age at diagnosis was 59.7 ± 10.0 years, and the majority were residents of the Southeast region of the U.S. at the time of diagnosis (69% in Georgia, Tennessee, North Carolina or South Carolina) and were diagnosed with late stage disease (83% Stage III-IV). Treatment data were available for 104 cases; the majority of women underwent an optimal debulking surgery followed by adjuvant chemotherapy. Approximately 42% of the women were deceased and the mean survival duration was 2.9 ± 1.5 years.

PD-L1 Expression

PD-L1 expression was uncommon in tumor cells. The majority of cases (71%) were entirely negative for PD-L1 within the tumor cells, while 29% of cases showed patchy positivity involving 1–10% of cells (Figure 1, Table 2). No cases exceeded 10% staining in tumor cells. Immunopositivity was often concentrated at the infiltrating edge of the tumor and/or in most robust in areas of high lymphocyte infiltration, while other cases showed only rare scattered positive cells (Figure 2–3). Tumor-associated immune staining for PD-L1 was more common, with 73% of cases showing some positivity in tumor-associated lymphocytes and/or macrophages, and 24% displaying >10% positivity. (Figure 4, Table 2) Immune cell staining was most commonly seen in macrophages that were either infiltrating or immediately juxtaposed to the tumor, including but not limited to congregations of macrophages in necrotic areas. (Figure 2–3)

IDO Expression

Tumor cell staining for IDO was more common, although diffuse expression remained rare: tumor cell staining for IDO was identified in 58% of cases, with most showing <10% staining. (Figure 1, Table 2) A small subset (6%) demonstrated positivity in 11–25% of tumor cells, and no cases exceeded the 25% threshold. IDO positivity was enhanced at the infiltrating edge of tumors and in regions of increased lymphocyte density. (Figure 2–3) Tumor-associated immune staining for IDO was identified in 54% of cases. (Figure 4) Most IDO positives had only focal patchy staining involving   10% of lymphocytes and

macrophages, while 2 cases had staining in 11–50% of the immune compartment. (Table 2, Figure 2–4)

PD-L1 and IDO Co-Expression

PD-L1 and IDO were often expressed in concert. Approximately 78% of cases with positive PD-L1 staining in tumor cells also expressed IDO tumor staining and 38% of IDO-positive cases also expressed PD-L1. In the immune compartment, 66% of PD-L1-positive cases also expressed IDO and 89% of IDO-positive cases expressed PD-L1 (data not shown).

CD8+/FOX3p+ Tumor-Associated Lymphocytes

CD8-positive cytotoxic T cells and FOX3p-positive regulatory T cells showed a range of distributions. (Figure 5) The median count of CD8-positive cytotoxic T cells per HPF was 13.0, with a range from 0–203.9. (Table 2) Fewer regulatory FOX3p-positive cells were present (median 3.2/HPF, range 0–39). High CD8 counts were observed more frequently for tumors with positive PD-L1 and IDO staining for both the tumor and immune cell compartments (p< 0.05) (Table 3). However, these differences were more striking among the immune compartment: among women with positive PD-L1 immune staining, 60% had high CD8 counts versus 23% for women with negative PD-L1 immune staining (p=0.0006). Similarly, for IDO, 66% of women with positive immune staining had high CD8 counts compared to 31% with negative staining (p=0.0003). Similar trends were observed for Fox3p staining where tumors with positive staining for PD-L1 and IDO had higher Fox3p counts. However, these differences were only significant for women with PD-L1 expression in the immune compartment (p=0.01).

PD-L1/IDO Expression and Survival

Supplementary Table 1 provides the distribution of participant characteristics by PD-L1 and IDO staining. There were no statistically significant differences in the positivity of these markers by most demographic and clinical characteristics, p>0.05. However, we observed notable differences in outcome, where women with positive PD-L1 and IDO tumor staining were significantly more likely to be alive than women with no PD-L1 and IDO tumor staining (75% vs. 51% and 67% vs. 45%, respectively). This trend was also observed for staining in the immune cell compartment, but the differences were not statistically significant. Similarly, a slightly better mean survival time was observed for women with positive staining, yet not statistically significant.

In multivariable models, adjusting for age at diagnosis, region, stage, residual disease, we restricted the sample to only those women with data on these covariates (N=93). PD-L1 and IDO staining in the tumor and immune compartment were not associated with overall survival (Table 4). However, PD-L1 and IDO tumor staining was suggestive of improved patient outcome, although not statistically significant (HR=0.61, 95% CI=0.26, 1.41 and HR=0.79, 95% CI=0.41–1.51, respectively). Among women with high CD8 counts, a significantly lower risk of mortality was observed for women with positive PD-L1 tumor staining (HR=0.19, 95% CI=0.04–0.94) but not among women with low CD8 counts (HR=0.81, 95% CI=0.26–2.51). No other statistically significant differences in the

relationship between PD-L1 and IDO and overall survival were observed by CD8 and Fox3p counts. These results are based on small sample sizes and should be interpreted with caution.

DISCUSSION

Cancer is increasingly understood to be a disease both of neoplastic cells and of the tumor environment. Existing evidence suggests that there may be significant racial differences in both systemic and intratumoral inflammation, however data are limited given the poor representation of minority populations in the medical literature.[10–16] Data on racial differences in the inflammatory context of ovarian carcinomas are particularly sparse, yet are of considerable interest given the persistent survival differences between ovarian cancer patients of African versus European descent. Potential differences in the immune microenvironment are of particular clinical interest given the emergence of therapeutic targets for a variety of immune modulatory molecules including PD-L1 and IDO. We herein characterize PD-L1 and IDO expression in HGSOCs in AA women from the AACES study to assess potential immunotherapeutic vulnerability in this population.[3–6]

Prior work has suggested a role for PD-1/PD-L1 inhibition in ovarian cancer treatment. PD-L1 expression by monocytes in blood and ascites fluid has been correlated with worsened outcomes in ovarian carcinoma patients[23,24] and can also be identified in peritumoral lymphocytes and macrophages as well as directly on tumor cells.[25–27] Tumoral and peri-tumoral immune PD-L1 positivity is also correlated with increased tumor infiltrating lymphocytes and BRCA1/2 mutations in ovarian cancer,[25,26] suggesting that more immunogenic tumors may have increased vulnerability to PD-1/PD-L1 inhibition. As has been documented in other studies of HGSOC,[25–27] tumor cell staining for PD-L1 was seen in a minority of cases in this series of AA women. When present, tumoral expression of PD-L1 was typically concentrated at the infiltrating edge in areas of greatest lymphocyte infiltration, indicative of an adaptive immune response. It is notable that, at 29% positive, the proportion of cases with PD-L1 expression seen here is considerably higher than was observed in a prior study from this institution, which showed tumor cell staining in only 8% of cancers,[27] and in a prior study from Webb and colleagues,[26] which showed tumor staining in 13% of tumors. Importantly, the prior study consisted almost exclusively of women of European descent and the methodology for both the original UVA study and the current UVA-ACCES study was identical, including the utilization of CD68 in attempt to ensure that tumor-associated macrophages were not erroneously scored as tumor cells. Further studies including a comparison group of women of European descent are needed to ascertain whether PD-L1 expression is indeed more common in tumors from AA women, thereby suggesting that there may be increased vulnerability to immunotherapy targeting the PD-1/PD-L1 axis within the population.

The proportion of cases with PD-L1-positive immune cells (74%) was identical to what was observed in the prior UVA study.[27] The presence of prominent PD-L1 staining within the immune compartment is intriguing, particularly since most of this staining centered on macrophages. Evidence from murine models has shown that tumor-associated macrophage expression of PD-L1 correlates negatively with tumor phagocytosis, and that blocking PD-1/PD-L1 *in vivo* reduces tumor growth in a macrophage-dependent fashion.[32] This

suggests that PD-1/PD-L1 blockades may be of clinical utility even when tumor cell staining is not identified. However, preliminary evidence on response to anti-PD-1/PD-L1 therapy has been relatively lackluster in HGSOC despite high rates of PD-L1-positive macrophages. In the KEYNOTE-028 trial, a nonrandomized, multicohort phase Ib trial of pembrolizumab in patients with PD-L1-positive solid tumors, 11% of ovarian carcinomas showed complete or partial response (ClinicalTrials.gov identifier: NCT02054806).[33] Similarly, in a phase II trial of nivolumab in platinum-resistant cancers showed a best overall response rate of 15% (UMIN Clinical Trials Registry identifier: UMIN000005714).[21,34] Given that PD-L1 positive tumor-associated macrophages are so common in serous ovarian cancer—but that treatment anti-PD-1/PD-L1 response is relatively uncommon—the predictive utility of PD-L1-positive macrophages remains dubious in this tumor type. That said, response rates are equivalent to what is seen with other chemotherapeutic agents in the platinum-resistant/refractory setting, and given their improved tolerability relative to existing options anti-PD-1/PD-L1 drugs may still play an important role in this context.

IDO expression has not been well characterized in ovarian carcinomas, and no previous studies have focused on AA women. IDO impairs T cell function and survival through two mechanisms: starvation (via tryptophan depletion), and toxicity (through the generation of the toxic metabolite kynurenine. IDO has also been implicated in the peritoneal dissemination of ovarian cancer cells[30] and mouse models of ovarian carcinoma have shown that IDO inhibition with the small molecule IDO inhibitor 1-methyl-tryptophan (1-MT) can augment the efficacy to cytotoxic chemotherapies.[28] IDO is of particular interest in HGSOC given limited responses to PD-1/PD-L1 inhibitors. We found tumoral IDO expression in 58% of HGOSC from AA women, including 78% of all PD-L1-positive cancers. The high rates of PD-L1 and IDO co-expression suggest that IDO may represent a mechanism of anti-PD-1/anti-PD-L1 resistance, and that dual antagonistic therapy may be of benefit in this setting. As with PD-L1, tumoral IDO expression was typically highest at the infiltrative margin of the tumor, particularly in areas of heightened lymphocyte infiltration.

The observed rates of IDO positivity parallel prior work in this tumor type, which have demonstrated IDO expression in more than half of ovarian cancers.[28] However, ascertaining whether prior reports specifically refer to tumor cell vs. immune cell staining is difficult in the absence of an associated CD68 stain given the ease with which macrophages can be mistaken for tumor cells.[27] It may be that true tumor cell staining is indeed elevated in AA patients once potential false positive staining in tumor-associated macrophages is taken into account. Studies using identical methodologies are therefore needed to determine whether racial differences exist in tumoral IDO expression. Furthermore, the significance of IDO positivity within the immune compartment is unclear. Presumably, IDO produced by either the tumor or by associated lymphocytes and macrophages should have the same effect on dampening the immune response, but this has not been demonstrated. Investigations detailing role of immune cell IDO expression in predicting response to anti-IDO therapy will therefore also be of interest. At present, there are limited data on the role of therapies targeting IDO in this tumor type. The KEYNOTE-037 clinical trial (ClinicalTrials.gov identifier: NCT02178722) is currently enrolling subjects to assess the safety, tolerability, and efficacy of pembrolizumab in combination with the IDO inhibitor epacodostat in patients

with ovarian cancer as well as a variety of other malignancies, however IDO expression is not a criterion for enrollment and preliminary response data are pending.

CD8-positive cytotoxic T cell infiltrates and associated FOX3p-positive regulatory T cells demonstrated a range of densities among HGSOCs in the AA population. Prior studies enumerating CD8-positive cells in racially unselected HGSOCs have shown that higher numbers (mean: 5.75/HPF) correlates with a good outcome, whereas lower numbers (mean: 0.12/HPF) correlate with poor outcome.[35] It is notable that average CD8-positive cells per HPF were higher in our study at 20.5/HPF, suggesting increased tumor-associated lymphocytes in the AA population. However, drawing direct comparisons is difficult given variabilities in methodologies across studies.

Cytotoxic CD8-positive lymphocyte counts provided some prognostic information in the context of PD-L1 positivity, with a significantly reduced rate of mortality among women with PD-L1 staining and high CD8 counts; however, these findings were based on a relatively small sample size and further confirmation of these findings in a large sample is warranted. PD-L1 and IDO expression did not independently correlate with outcome in this study population. The absence of prognostic significance to these variables is not entirely surprising given the complexity of the immune interplay and the heterogeneity of data from other tumor types. Although, for instance, cytotoxic T cell infiltrates and PD-L1 expression sometimes correlate with better outcome, they also often correlate with higher tumor grade and other negative prognostic variables.[25,36–38] Furthermore, the examined markers are just a subset of many immune modulatory molecules in play in the tumor microenvironment. It is possible that a more complete characterization of the immune milieu including analysis of other checkpoint inhibitors and lymphocyte activators/suppressors might have prognostic power. Largest studies comparing patients with and without neoadjuvant therapy would also be warranted, as preliminary data here suggests that prior therapy does not increase immune modulatory molecule expression.

Another limitation to note is the absence of multiplex staining, which makes it challenging to definitively determine whether immune modulatory molecule expression is centered on tumor cells or associated immune cells. This can be particularly problematic as tumor-associated macrophages may be quiet common in HGSOC and frequently approach the size of tumor cells. While the inclusion of a separately stained CD68 slide helped prevent egregious misinterpretation macrophages as tumor cells, this doesn't entirely eliminate this problem as it requires switching slides and is subject to cutting in/out artifacts. The problem is compounded by the fact that true tumoral staining seems to concentrate at the infiltrating edge of tumor nests and this area also often shows a high density of macrophages. Furthermore, IHC interpretation was performed by a single pathologist so this study is not powered to address interpretive reproducibility.

A final limitation worth emphasizing is the lack of information on *BRCA* mutation status. Existing evidence from Strickland et al. has shown that *BRCA*-mutated serous ovarian carcinomas have a higher neoantigen load than *BRCA*-wildtype cancers, and that these tumors increased CD8+ lymphocytic infiltrates and elevated PD-L1 positivity.[25] Indeed, CD8+ counts approached 40/HPF in *BRCA*-mutated cancers from that series, indicating that

mutational status is a significant contributor to immunogenicity. Data from our prior series also showed increased PD-L1 expression in *BRCA*-mutated cases (50% vs. 11.8% of cases with no mutation) however statistical significance was limited by the small number of patients with *BRCA* mutation testing results. [27] However, AA women have not been shown to have significantly higher rates of deleterious *BRCA* mutations relative to other racial groups[39] therefore *BRCA* status is unlikely to disproportionally account for immune activation in tumors from this population.

In summary, we herein characterize expression of the targetable immune regulatory molecules PD-L1 and IDO in HGSOCs from AA women. We demonstrate that PD-L1 and IDO are seen in a subset of these cancers, with higher tumoral PD-L1 expression relative to existing data from racially unselected cohorts. The majority of PD-L1-positive cases also express IDO, suggesting a role for dual immunotherapy. However, diffuse expression of PD-L1 and IDO remains rare, invoking some caution regarding the potential for immunotherapy in this tumor type should high expression levels prove necessary for treatment response. Clinical trials are needed to determine biomarker thresholds predictive of response to immune modulatory molecule antagonism in HGSOCs, as are comparison studies with European Americans to assess racial differences in immune modulatory molecule expression and in the overarching tumoral microenvironment.

Supplementary Material

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Acknowledgments

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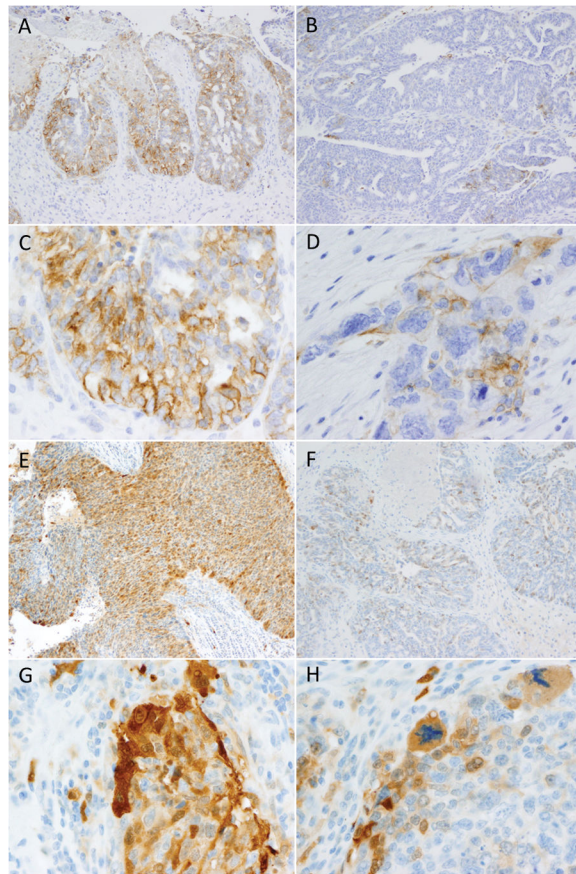
Figure 1.
Examples of PD-L1 and IDO tumoral expression patterns in high grade serous ovarian carcinomas. Tumoral PD-L1 expression was seen in a 29% of high-grade serous ovarian carcinomas and never exceeded 10% overall positivity. The case illustrated in plate A had 10% positivity which was concentrated in the focus pictured here; the remainder of the tumor was predominantly PD-L1-negative. In contrast, most positive cases showed a pattern similar to the case illustrated in plate B, with only scattered patches of expression. Crisp membranous staining was required for positivity, as is illustrated in C-D, as was the exclusion of macrophage staining based on comparison with the CD68 stain. IDO expression was more common with 58% of cases showing tumoral staining. Occasional cases showed up to 25% positivity, as pictured in plate E; however, as was observed with PD-L1, such strongly positive swathes of tumor were admixed with larger negative areas. The most common pattern of expression was patchy positivity as pictured in plat e F. Clear cytoplasmic staining as depicted in G–H was required for diagnosis, as was exclusion of macrophages using the CD68 comparison slide.
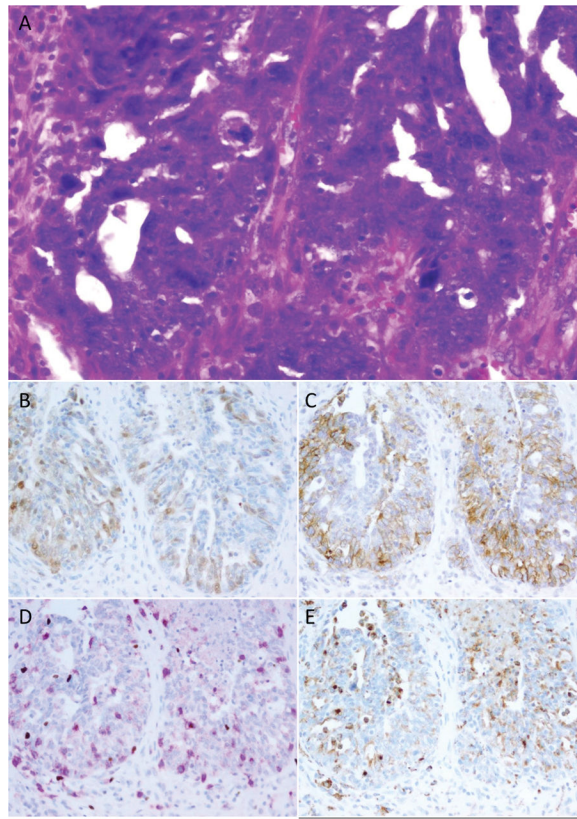
Figure 2. Relationship between PD-L1, IDO, and tumor-associated lymphocytes and macrophages (Example 1)

High-grade serous ovarian carcinomas often showed immune modulatory molecule expression in areas of increased lymphocytic infiltration. This case (A) shows patchy IDO (B) and stronger PD-L1 (C) expression in an area of high density tumor-infiltrating lymphocytes (D; pink=CD8, brown=FOX3p). Some IDO and PD-L1-positive cells correlated with the distribution of CD68-positive tumor-associated macrophages (E) however some tumoral staining was also present for both markers.
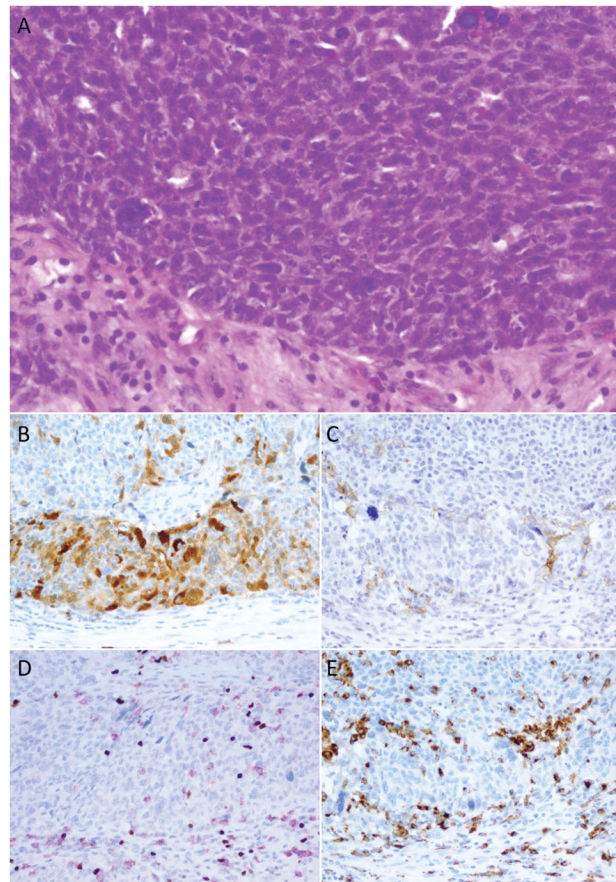
Figure 3. Relationship between PD-L1, IDO, and tumor-associated lymphocytes and macrophages (Example 2)

Some high-grade serous ovarian carcinomas showed bands of immune modulatory molecule expression at the tumor periphery. This case (A) showed a dense border of tumoral positivity for IDO (A) at the tumor's infiltrating edge, where both cytotoxic and regulatory T cells were congregated (C). In contrast, PD-L1 staining (B) was limited to scattered intratumoral cells favored to be macrophages based on their overlap with CD68 (D) positivity.
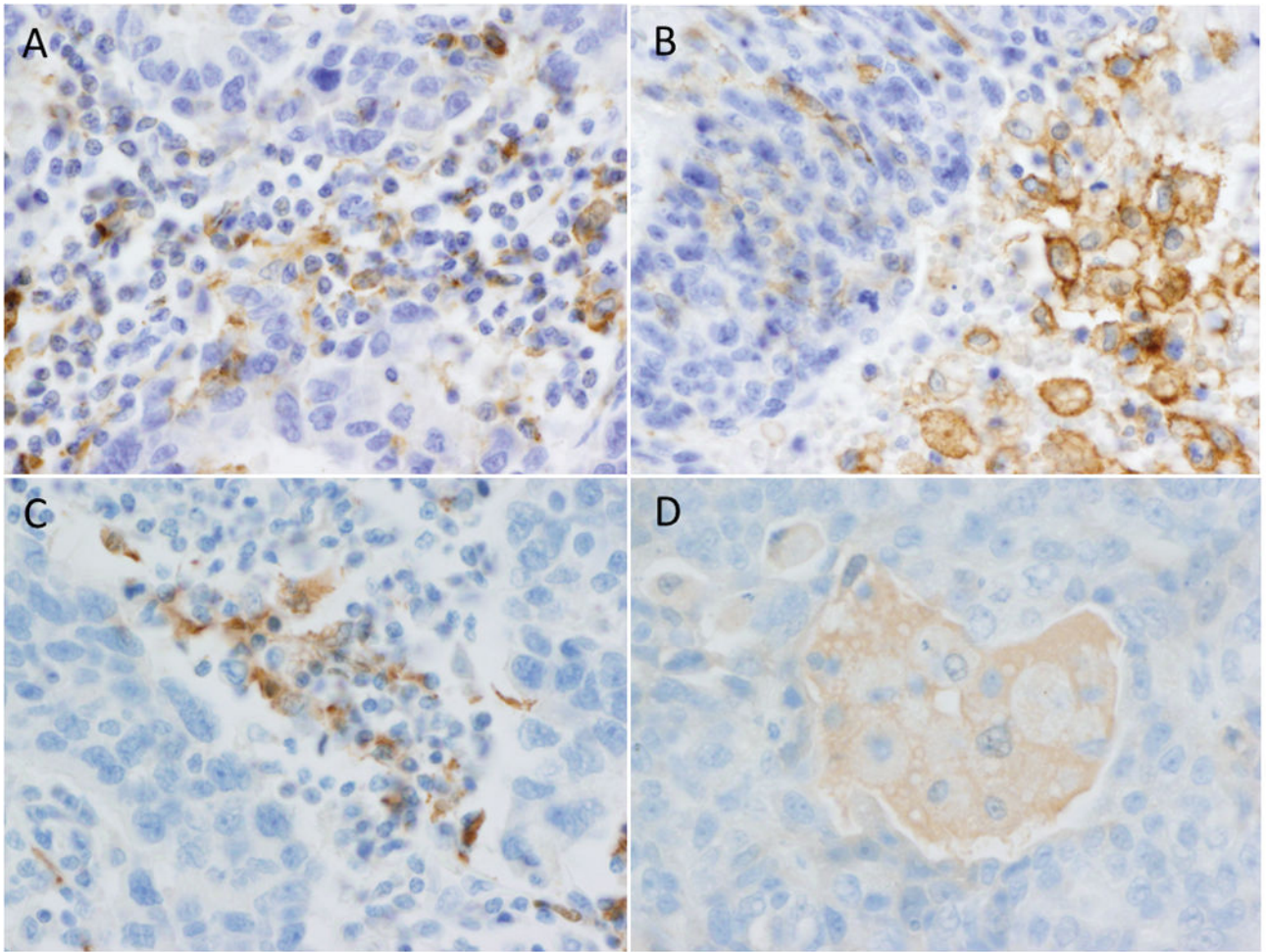
Figure 4.
Examples of PD-L1 and IDO expression in tumor-associated immune cells. PD-L1 was frequently positive in tumor-associated immune cells with 73% showing some reactivity in tumor-associated lymphocytes (A) and/or macrophages (B). Macrophage staining was seen in cells peppered throughout the tumor, in bands at the infiltrating edge of the tumor, and in pools of necrosis adjacent to tumor (as pictured in B). Immune cell staining was less common for IDO with 54% of cases showing some staining and only rare cases showing diffuse staining. Lymphocyte staining was often identified in clusters located at the tumor periphery (C). When macrophage expression was present it was typically very faint and blush-like (D).
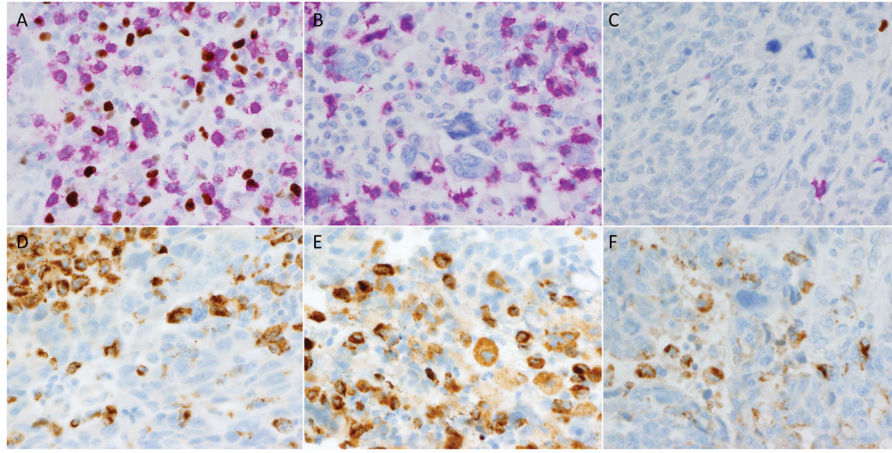
Figure 5.

Examples of tumor-associated lymphocytes and macrophages. Some tumors showed robust infiltrates of both CD8+ (pink) and FOX3p+ (brown) cells (A), while others showed predominantly CD8+ infiltrates (B). Still others showed only sparse lymphocytic inflammation (C). CD68 immunostaining was performed to highlight macrophages in order to facilitate mapping of immune modulatory molecule expression to either tumor cells or macrophages. Plates D–F demonstrate the extent to which CD68-positive macrophages can be subtly admixed among tumor cells, which can complicate assessment of tumoral expression for immune modulatory molecule markers.

Table 1

Patient Characteristics (N=112)

Patient Characteristics	n (%)
Age at diagnosis, mean (SD)	59.7 (10.0)
Region of residence[a]	
Northeast	28 (25)
Southeast	77 (69)
South	7 (6)
Stage	
I–II	19 (17)
III–IV	92 (83)
Unknown	1
Neoadjuvant chemotherapy	
Yes	15 (16)
No	79 (84)
Unknown	18
Adjuvant chemotherapy	
Yes	81 (93)
No	6 (7)
Unknown	25
Residual disease[b]	
Optimal debulking	57 (61)
Suboptimal debulking	36 (39)
Unknown	19
Survival duration (years), mean (SD)	2.9 (1.5)
Vital Status	
Deceased	47 (42)
Alive	65 (58)

SD: standard deviation.

[a] The Northeast region includes Michigan, Illinois, New Jersey and Ohio. The Southeast region includes Georgia, Tennessee, North Carolina and South Carolina. The South region includes Alabama, Texas and Louisiana.

[b] Residual disease is defined as optimal if <1cm tumor diameter after cytoreductive surgery or a CA-125 <35 after the last cycle of the first round of adjuvant chemotherapy.

Table 2

Distribution of Immune Markers (N=112)

Immune Markers	n (%)
PD-L1 Tumor	
0%	80 (71)
1–5%	22 (20)
6–10%	10 (9)
11–25%	0 (0)
26–50%	0 (0)
>50%	0 (0)
PD-L1 Immune	
0%	30 (27)
1–5%	31 (28)
6–10%	24 (21)
11–25%	18 (16)
26–50%	9 (8)
>50%	0 (0)
IDO Tumor	
0%	47 (42)
1–5%	43 (38)
6–10%	15 (13)
11–25%	7 (6)
26–50%	0 (0)
>50%	0 (0)
IDO Immune	
0%	51 (46)
1–5%	53 (47)
6–10%	6 (5)
11–25%	1 (1)
26–50%	1 (1)
>50%	0 (0)

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Table 3

Co-Expression of CD8 and Fox3p with PD-L1 and IDO (N=112)

	PD-L1 Tumor Staining			PD-L1 Immune Staining			IDO Tumor Staining			IDO Immune Staining		
	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
CD8												
Low (<12.95)	45 (56)	11 (34)	0.04	23 (77)	33 (40)	0.0006	30 (64)	26 (40)	0.01	35 (69)	21 (34)	0.0003
High (12.95)	35 (44)	21 (66)		7 (23)	49 (60)		17 (36)	39 (60)		16 (31)	40 (66)	
Fox3p												
Low (<3.2)	44 (55)	12 (38)	0.09	21 (70)	35 (43)	0.01	28 (60)	28 (43)	0.08	30 (59)	26 (43)	0.09
High (3.2)	36 (45)	20 (63)		9 (30)	47 (57)		19 (40)	37 (57)		21 (41)	35 (57)	

Table 4

Estimated hazard ratios and 95% confidence intervals for the relationship between PD-L1 and IDO and overall survival (N=93)

	Total		Low CD8		High CD8		Low Fox3p		High Fox3p	
	Cases (Deaths)	HR (95% CI)[a]	Cases (Deaths)	HR (95% CI)[a]	Cases (Deaths)	HR (95% CI)[a]	Cases (Deaths)	HR (95% CI)[a]	Cases (Deaths)	HR (95% CI)[a]
PD-L1 Tumor										
Negative	66 (33)	1.00 (Referent)	36 (16)	1.00 (Referent)	30 (17)	1.00 (Referent)	35 (16)	1.00 (Referent)	31 (17)	1.00 (Referent)
Positive	27 (7)	0.61 (0.26–1.41)	9 (4)	0.81 (0.26–2.51)	18 (3)	**0.19 (0.04–0.94)**	10 (3)	0.45 (0.12–1.60)	17 (4)	0.89 (0.26–3.02)
PD-L1 Immune										
Negative	23 (11)	1.00 (Referent)	17 (8)	1.00 (Referent)	6 (3)	1.00 (Referent)	15 (7)	1.00 (Referent)	8 (4)	1.00 (Referent)
Positive	70 (29)	1.43 (0.68–3.00)	28 (12)	1.33 (0.50–3.53)	42 (17)	0.90 (0.23–3.44)	30 (12)	1.42 (0.49–4.14)	40 (17)	1.22 (0.40–3.67)
IDO Tumor										
Negative	36 (20)	1.00 (Referent)	23 (12)	1.00 (Referent)	13 (8)	1.00 (Referent)	20 (11)	1.00 (Referent)	16 (9)	1.00 (Referent)
Positive	57 (20)	0.79 (0.41–1.51)	22 (8)	0.83 (0.32–2.16)	35 (12)	0.56 (0.21–1.48)	25 (8)	0.70 (0.25–1.94)	32 (12)	0.67 (0.26–1.74)
IDO Immune										
Negative	41 (19)	1.00 (Referent)	27 (14)	1.00 (Referent)	14 (5)	1.00 (Referent)	23 (11)	1.00 (Referent)	18 (8)	1.00 (Referent)
Positive	52 (21)	1.11 (0.57–2.17)	18 (6)	0.86 (0.32–2.32)	34 (15)	1.03 (0.34–3.13)	22 (8)	0.94 (0.35–2.50)	30 (13)	0.99 (0.36–2.70)

[a] Adjusted for age at diagnosis, stage, region of residence, and residual disease.