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Cancer-Immunity Marker RNA Expression Levels across Gynecologic Cancers: Implications for Immunotherapy

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Abstract

Our objective was to characterize cancer-immunity marker expression in gynecologic cancers and compare immune landscapes between gynecologic tumor subtypes and with nongynecologic solid tumors. RNA expression levels of 51 cancer-immunity markers were analyzed in patients with gynecologic cancers versus nongynecologic cancers, and normalized to a reference population of 735 control cancers, ranked from 0 to 100, and categorized as low (0–24), moderate (25–74), or high (75–100) percentile rank. Of the 72 patients studied, 43 (60%) had ovarian, 24 (33%) uterine, and 5 (7%) cervical cancer. No two immune profiles were identical according to expression rank (0–100) or rank level (low, moderate, or high). Patients with cervical cancer had significantly higher expression level ranks of immune activating, proinflammatory, tumor-infiltrating lymphocyte markers, and checkpoints than patients with uterine or ovarian cancer ($P < 0.001$ for all comparisons). However, there were no significant differences in immune marker expression between uterine and ovarian cancers. Tumors with PD-L1 tumor proportional score (TPS) ≥1% versus 0% had significantly higher expression levels of proinflammatory markers (58 vs. 49%, $P = 0.0004$). Compared to patients with nongynecologic cancers, more patients with gynecologic cancers express high levels of IDO-1 (44 vs. 13%, $P < 0.001$), LAG3 (35 vs. 21%, $P = 0.008$), and IL10 (31 vs. 15%, $P = 0.002$.) Patients with gynecologic cancers have complex and heterogeneous immune landscapes that are distinct from patient to patient and from other solid tumors. High levels of IDO1 and LAG3 suggest that clinical trials with IDO1 inhibitors or LAG3 inhibitors, respectively, may be warranted in gynecologic cancers.

Introduction

Immunotherapies have revolutionized the treatment of solid tumors, with efficacy, even in patients with metastatic disease and multiple lines of prior therapy (1). Importantly, there is a growing body of literature to support the role of the immune system in the development, response to treatment, and behavior of gynecologic cancers and, hence, immunotherapy has emerged as an area of special focus for these malignancies.

Recent data suggest endometrial cancers can be classified into subtypes that may inform precision genomic and immunotherapies (2). Endometrial cancers have been found to have one of the highest programmed cell death ligand 1 (PD-L1) expression levels (3) and prevalence of microsatellite instability (MSI) compared with other cancer types (4). High tumor mutational burden (TMB) and neoantigen load from polymerase epsilon (POLE) mutant and MSI-high (MSI-H) malignancies, each of which can correlate with subgroups of endometrial cancer, associate with increased tumor-infiltrating lymphocytes (TIL; ref. 5) and improved survival (6). For these reasons, perhaps, immunotherapy strategies have seen clinical efficacy in endometrial cancers. In 2017, the FDA issued its first tissueagnostic approval for pembrolizumab, a programmed cell death protein-1 (PD-1) signal pathway inhibitor, in solid tumors with MSI-H or mismatch repair (MMR) deficiency (7–9),

and eventually to all tumors with high TMB (≥10 mutations/megabase; ref. 10). The combination of pembrolizumab and a multikinase inhibitor lenvatinib was next approved in 2019 for patients with MMR-proficient endometrial tumors (11). In April 2021, the FDA granted accelerated approval for dostarlimab (anti–PD-1) for the treatment of adult patients with deficient MMR recurrent or advanced endometrial cancer (12). Ultimately, in March 2022, the FDA also granted approval of pembrolizumab for patients with MSI-H/dMMR advanced endometrial cancer, who have disease progression following prior systemic therapy based on the updated results of KEYNOTE-158 (13).

Persistent human papillomavirus (HPV) infection is believed to cause 99.7% of invasive cervical cancers, making cervical cancer another disease site anticipated to be responsive to immunotherapy, because viral neoantigens may be immunogenic (14). Moreover, HPV infections have been found to increase PD-L1 expression (15), thereby creating an immune-privileged environment (16). In the phase III, KEYNOTE 826 trial, pembrolizumab given upfront with chemotherapy improved both overall survival (OS) and progression-free survival (PFS) in patients with PD-L1–positive cervical cancer (17), supporting the FDA approval of pembrolizumab in these patients. For patients with PD-L1–negative cervical cancer, the combination of ipilimumab (anti–CTLA-4) and nivolumab (anti–PD-1) demonstrated an objective response rate of 31.6% (18).

In patients with ovarian cancer, the benefit of immunotherapy is less clear. Given patients with ovarian cancer with increased TILs have longer survival (19), it would seem logical for immunotherapy to have a successful role. However, the response rate to single-agent nivolumab in platinum-resistant patients was only 15%, with no correlation between clinical response and PD-L1 expression level (20). The incorporation of avelumab, an anti–PD-L1 mAb, into first-line chemotherapy in patients with ovarian cancer also failed to show efficacy (21). While a higher objective response rate (ORR) has been observed in ovarian tumors with PD-L1 IHC combined positive score (CPS) ≥10% compared with patients with CPS <10% (17.5 vs. 8%; ref. 22), the ORRs for both are low. Furthermore, in the placebo controlled, randomized, phase III IMagyn050/GOG 3015/ENGOT-OV39 trial, the addition of atezolizumab to platinum-based combination chemotherapy and bevacizumab failed to show an improvement in oncologic outcomes (23). Overall, there is an impression that the immunosuppressive tumor microenvironment in patients with ovarian cancer is difficult to overcome with a single-agent approach.

There is a strong rationale for using immunotherapy in patients with gynecologic tumors, but the efficacy of this approach is limited in part by our understanding of the biologic/immune underpinning of these cancers. In our study, we utilized RNA-sequencing (RNA-seq) immune gene expression to begin describing the immune pressures in ovarian, uterine, and cervical tumors. Our findings may begin to explain the differential responses to immunotherapies between gynecologic disease sites and compared with nongynecologic malignancies.

Materials and Methods

Patients

Cancer-immunity markers among 72 eligible, consecutive patients with gynecologic solid cancers seen at the University of California San Diego Moores Cancer Center for Personalized Therapy were evaluated at a Clinical Laboratory Improvement Amendments (CLIA)-licensed and College of American Pathologist (CAP)-accredited clinical laboratory, OmniSeq (https://www.omniseq.com/). All investigations followed the University of California San Diego Institutional Review Board protocol for data collection (Profile Related Evidence Determining Individualized Cancer Therapy, NCT02478931) and for any investigational interventions for which the patients provided written, informed consent.

Tissue samples and analysis of cancer-immunity markers

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were evaluated with RNA-seq by OmniSeq laboratory. Total RNA was extracted utilizing the truXTRAC FFPE extraction kit (Covaris, Inc.), following the manufacturer's instructions with modifications as needed. After purification, RNA was eluted in 50 μL water and yield was assessed by the Quant-iT RNA HS Assay (Thermo Fisher Scientific), as per manufacturer's recommendation. A predefined yield of 10 ng RNA was considered acceptable to ensure library preparation. RNA-seq absolute reads were generated using the Torrent Suite's plugin immuneResponseRNA (v5.2.0.0). The RNA expression of 51 targeted immune response genes were assessed as follows: nine checkpoints (PD-1, PD-L1, PD-L2, BTLA,CTLA-4, LAG3,TNFRSF14, TIM-3, and VISTA); three metabolic immune escape markers (ADORA2A, IDO1, and CD39); two anti-inflammatory response markers (IL10 and TGFB1); five macrophage-associated markers (CCL2, CCR2, CSF1R, CD163, and CD68); 15 T-cell priming markers (GITR, CD137, ICOS, OX40, CD27, CD28, CD80, CD86, CD40, CD40 ligand, ICOS ligand, OX40 ligand, GZMB, IFNG, and TBX21); seven proinflammatory response markers (IL1B, MX-1, STAT1, TNF, DDX58, CXCL10, CXCR6); eight tumor infiltrating lymphocyte markers (CD4, CD8, FOXP3, CD2, CD3, KLRD1, SLAMF4, and CD20); and other immunotherapy markers [CD38 and GATA3; Supplementary Table S1 for list and function information (24)]. Transcript abundance was normalized and compared with an internal reference population (735 patients with a range of solid tumors). This platform method has been previously validated (25). Specifically, the platform utilizes custom normalization procedure after obtaining raw read counts from immune response RNA. Raw reads counts are first background subtracted and then normalized to housekeeping profile of each sample. After housekeeping normalization, reads are further normalized to per million bases, giving a value of normalized reads per million. These normalized reads per minute (nRPM) values are further ranked to a reference population of >700 clinical tested reference population across multiple histologies. This allows us to interpret the high, moderate, and low expression of immune genes across a wide natural distribution. Batch effect correction is also achieved with this ranking procedure. The assay enables quantitative evaluation of the expression of markers associated with different leukocyte subsets, antigen presentation, checkpoint pathways, and tumor progression and can measure the expression of genes involved in tumor–immune interactions, including the low-expressing genes involved in inflammatory signaling. Rank values were set on a scale

of 0 to 100 and percentile rank levels were categorized as “high” (75–100), “moderate” (25–74), “low” (0–24). Checkpoint markers, macrophage-associated markers, anti-inflammatory markers, and metabolic immune escape markers were considered “immune-suppressive markers.” T cell–primed markers and proinflammatory response markers were considered “immune-activating markers.”

Frequency of patients with high RNA expression level ranks (RNA expression levels ≥75th percentile rank) and low RNA expression level ranks (RNA expression levels <25th percentile rank) were calculated and shown on bar graphs (Figs. 1A and 2A).

PD-L1 protein expression was measured using the tumor proportional score (TPS; percentage of viable tumor cells showing partial or complete membrane IHC staining of any intensity). The expression of PD-L1 on the surface of tumor cells was assessed via the Dako Omnis platform (Agilent) using the anti–PD-L1 22C3 pharmDx antibody (Agilent), and expression levels were scored as per manufacturer guidelines.

Immunoprints refer to an overview of each individual patient’s immune profile. Each column represents a unique patient and each row represents the immune markers investigated. The corresponding cell is colored according to RNA expression level rank: high, moderate, or low.

An immunogram is an overview of immune profiles across patient cohorts. Immune markers were categorized according to their function, to one of six categories: immune checkpoints, immune escape/anti-inflammatory markers or macrophage-associated markers, tumor-infiltrating lymphocyte markers, proinflammatory markers, and T-cell primed markers (26). Each immune marker category makes up each “spoke” of the radar plot. The length of each spoke corresponds to the average RNA transcript rank expression level (0–100). The mean RNA expression level rank of immune factors in each immune marker category was plotted on each spoke and connected to visually compare the “immune pressures” between disease sites and cohorts (27–30).

Endpoints and statistical methods

Patient characteristics and the pattern of cancer-immunity markers were summarized by descriptive statistics. Proportions were compared using Fisher exact test. Means were compared using Student *t* test. The correlation between the disease sites and comprehensive expression patterns was evaluated through principal component analysis (PCA) and quantified by calculating silhouette scores. Statistical analyses were performed using SPSS version 28.0, Microsoft Excel version 16.49, and R 3.6.1 (R Foundation for Statistics Computing). A P value ≤ 0.05 was considered significant.

Data availability

Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online Supplementary Data information.

Patient consent for publication

All investigations followed the Institutional Review Board protocol for data collection (Profile Related Evidence Determining Individualized Cancer Therapy, NCT02478931) and for any investigational interventions for which the patients consented.

Ethics approval

This study involves human subjects, and all samples were obtained following individual informed consent and ethical approval by the Institutional Review Board.

Results

Patient and tumor characteristics

Clinicopathologic features of our cohort of 72 patients with gynecologic cancers are depicted in Table 1. Forty-three patients (60%) had ovarian cancer, 24 (33%) uterine cancer, and 5 (7%) cervical cancer. Of the cohort of patients with ovarian cancer, 52% were less than 65 years of age, no tumors had TMB ≥10 mutations/megabase, and 49% had tumors with a PD-L1 TPS of ≥1%. Of the cohort of patients with uterine cancer, 46% were less than 65 years of age, 8% ($n = 2$) had tumors with TMB ≥10 mutations/megabase (both of which were MSI-High), and 37% had tumors with a with a PD-L1 TPS of ≥1%. Of the small cohort of patients with cervical cancer ($n = 5$), the majority ($n = 4$) were less than 65 years of age, no patient had a tumor with TMB ≥10 mutations/megabase and 2 patients had a PD-L1 TPS score that was positive.

The immune profiles of the patients varied between patients and disease and are shown in Figs. 1–5, Supplementary Fig. S1, and Tables 1 and 2.

Multiple potentially actionable immune-inhibitory and immune-stimulatory markers were expressed in gynecologic malignancies

Both immune-suppressive markers and immune-activating markers were examined. High levels of immune-suppressive markers are relevant as they can be counteracted by agents that inhibit them. Low levels of immune-activating markers are relevant as they would need to be augmented in a therapeutic setting. Figure 1A summarizes the percentage of patients with gynecologic cancers that had high RNA expression of each immune marker. Among immune-suppressive markers, patients with gynecologic cancers were most likely to have high expression of IDO1 (44%), LAG3 (35%), IL10 (29%), VISTA (24%), CCR2 (21%), CCL2 (21%), PD-1 (19%). Among immune-activating markers, patients with gynecologic cancers were most likely to have high expression of MX1 (42%), DDX58 (39%), ICOSL (33%), TNF (32%), STAT1 (32%), CXCL10 (29%), CD40 (29%), and OX40 L (28%). Among tumor-infiltrating lymphocyte markers, patients with gynecologic cancers were most likely to have high expression of CD8 (22%) and KLRD1 (21%). Figure 2A depicts the percentage of patients with gynecologic cancers that had low RNA expression of each immune marker. For example, among immune-activating markers, patients with gynecologic cancers were most likely to have low expression of IFNG (43%), ICOS (40%), CD40LG (40%), CD28 (40%), and TBX21 (38%). Among immune-suppressive markers, patients with

gynecologic cancers were most likely to have low expression of CD68 (53%), ADODRA2A (40%), TIM3 (39%), and CCR2 (38%).

The percentage of patients with high RNA expression of immune-suppressive markers within gynecologic disease sites is further depicted in Fig. 1B–D. The most common highly expressed immune-suppressive markers in patients with ovarian cancer were IDO1 in 37% of patients, LAG3 (30%), and IL10 (30%; Fig. 1B). In patients with uterine cancer, high expression levels of IDO1 were again seen in 50% of patients and high expression levels of LAG3 were seen in 42% of patients (Fig. 1C). Four of five patients with cervical cancer expressed high levels of CTLA4, BTLA, and IDO1 and three of five patients expressed high levels of PD-L1 (Fig. 1D).

The percentage of patients with low RNA expression of immune-activating markers within gynecologic disease sites is further depicted in Fig. 2A–D. In patients with ovarian cancer, low levels of IFNG are seen in 51% of patients, CD28 (47%), CD40LG (42%), and CD27 (42%; Fig. 2B). In patients with uterine cancer, 54% of patients had low expression levels of CD86, IFNG, and ICOS (Fig. 2C). In patients with cervical cancer, three of five patients had low RNA expression levels of OX40L, and one of five had low expression of CD40LG, CD40 CD28, and DDX58 (Fig. 2D).

IDO1, an immune-suppressive marker, is the transcript most frequently highly expressed in gynecologic cancers

Figure 1A demonstrates that 44.4% of gynecologic cancers had high expression of the immune-suppressive marker IDO1 RNA. IDO1 was highly expressed in 37% of ovarian cancers, 50% of uterine cancers, and 4 of 5 patients with cervical cancer (Figs. 1B–D).

LAG3, an immune-suppressive marker, is highly expressed in ovarian and uterine cancers

LAG3 was the second most frequently expressed immune-suppressive marker in ovarian (30% of cases) and in uterine cancer (42% of cases). In cervical cancer, it was highly expressed in 2 of 5 patients (Figs. 1A–3D).

A significantly higher proportion of patients with gynecologic cancers express high levels of IDO-1, LAG3, and IL10 compared with nongynecologic solid tumors

For immune-inhibitory factors, the proportion of patients with high expression in gynecologic malignancies ($N = 72$) was compared with those with high expression in nongynecologic malignancies ($N = 442$ patients profiled at UCSD). Potential drugs would be those agents that suppress high expression of immune inhibition.

Thirty-two of 72 (44%) patients with gynecologic cancers expressed high levels of IDO-1 compared with 13% of patients with nongynecologic cancers ($P < 0.0001$; Table 2). When IDO-1 is high, it can potentially be targeted with IDO1 inhibitors such as epacadostat, indoximod, etc.

Patients with gynecologic cancers also have high expression levels of LAG3 (35% vs. 21%, $P = 0.008$), compared with patients with nongynecologic solid tumors. When LAG3 is high,

it is potentially targetable with LAG3 inhibitors such as relatlimab, BI 754111, LAG525, and MK-4280.

Patients with gynecologic cancers also have high expression levels of IL10 compared with those with nongynecologic cancers (31% vs. 15%, $P = 0.002$). When IL10 levels are high, they are potentially targetable with IL10 inhibitors such as MK-1966 (see Supplementary Table S1).

A significantly smaller proportion of patients with gynecologic cancers express high levels of ADORA2A as compared with other cancers (6.9% vs. 23.1%; $P = 0.002$), which may mean less success with using drugs that target this protein in this patient population.

For immune-stimulatory factors, we similarly compared the proportion of patients with low expression in those with gynecologic malignancies to those with nongynecologic malignancies. Potential drugs would be agents that stimulate the specific immune-stimulatory function. There were no statistically significant differences in proportion of patients with low expression of immune stimulatory factors (i.e., IFNG, ICOS, CD40LG, IL1B, CD137, CITR, TNF, OX40L, OX40, ICOSLG) between those with gynecologic cancers and those with nongynecologic cancers.

Immune marker RNA expression level differed from patient to patient among 72 individuals with gynecologic cancer

Fifty-one immune markers were investigated. Figure 3A depicts an immunoprint of RNA expression levels (low ≤25%, moderate 25–74%, or high ≥75%) across immune markers for each individual patient studied. Figure 3B depicts an immunoprint of only high RNA expression levels (≥75%) across immune markers. There were no two gynecologic cancers with identical immune portfolios according to immune RNA expression rank numbers (1–100) or according to immune expression rank levels (low, moderate, or high).

Immune marker RNA expression level could not be clustered on the basis of uterine or ovarian histology

A cluster plot with principal component analysis (Fig. 4) that summarized 51-dimensional data corresponding to the 51 different cancer-immunity markers on a two-dimensional field demonstrated that the distribution cancer-immunity marker RNA expression for patients with uterine and ovarian cancer were largely overlapping, which suggests that the pattern of RNA expression of 51 markers was not associated with the disease site (because there were only 5 patients with cervical cancer, these tumors were not mapped on this plot). The finding was validated by the calculation of silhouette score, which represents the variation within clusters compared with the variation between clusters. The silhouette score was 0.011, while the silhouette score of one million times of randomization of cancer sites in the same cohort was calculated as 0.00 ± 0.012 (mean and error). This suggests that the comprehensive expression patterns of 51 genes did not correlate with disease site.

Patients with cervical cancer, but not those with ovarian or uterine cancer, have higher RNA expression level ranks of immune checkpoints, TILs, proinflammatory, and T cell–primed markers than approximately 70% of patients with other cancer types

An immunogram was generated by plotting the average RNA expression level rank of immune markers for each corresponding immune marker category on a radar plot (Fig. 5A). Although the sample size was small, cervical cancers had a mean RNA expression rank level of immune checkpoints, TILs, proinflammatory, and T cell–primed markers higher than ovarian and uterine cancers ($P < 0.0001$ for all comparisons) and higher than approximately 70% of all other cancer types. Cervical cancers have an average RNA expression rank level of immune escape/anti-inflammatory markers (51% rank), and slightly less than average (47%) rank level of macrophage-associated markers compared with other cancer types.

Ovarian and uterine cancers have lower than average (<50% rank level) expression of almost all immune categories including immune checkpoints, TIL, T-cell primed, and macrophage-associated markers compared with other cancer types. Ovarian cancers have a slightly higher than average RNA expression level (52%) of proinflammatory markers compared with other cancer types. There were no significant differences in mean expression levels of any immune marker categories between uterine and ovarian cancers.

Patients with PD-L1 TPS ≥ 1% have significantly higher mean RNA expression rank levels of proinflammatory markers than those with PD-L1 TPS of 0%

An immunogram was next generated to depict mean RNA expression levels of each immune category by PD-L1 IHC status (Fig. 5B). Patients with PD-L1 IHC TPS ≥ 1% had significantly higher expression levels of proinflammatory markers compared with those with PD-L1 IHC TPS 0% tumors (58 vs. 49%, $P = 0.0004$). Immunograms of each disease site by PD-L1 status are shown in Supplementary Fig. S1A–S1C.

Discussion

In our report, we characterize the immune profiles of patients with gynecologic cancers using 51 RNA transcript levels associated with the cancer-immunity cycle. Notably, we found that no two tumors have an identical immune profile. This finding highlights the complexities of immune interactions, as well as the need to interrogate each tumor in the context of choosing precision immunotherapeutics. Contemporary work with The Cancer Genome Atlas (TCGA) and other large databases, integrating immunogenomics using powerful computational science have started to describe six distinct clusters of immune subtypes with potential implications for cancer treatment, although the prior work also demonstrated significant interpatient immune landscape heterogeneity (31, 32). These unique immune profiles again highlight the opposing immune pressures that affect the tumor microenvironment. Moreover, patient and tumor heterogeneity have implications for treatment choice. It remains challenging to design clinical trials in the context of big data and next-generation sequencing (NGS), the latter which also suggests that the molecular genomic profile of metastatic tumors differ from patient to patient; however, emerging observations, at least in the precision genomics space, suggest that treatment regimens based on analyzing each patient’s cancer genomic landscape using NGS may improve patient

outcomes (33–35). A similar paradigm of individual tumor–immune profile interrogation and matching cancers with the right immunotherapy may be required.

We used an immunogram as the framework to describe the interacting immune pressures mentioned previously (27). Of interest, patients with cervical cancer (although the numbers of patients were small) have higher RNA expression levels of immune-activating factors than immune-inhibitory factors, which may signify a generally “hotter” tumor. Moreover, patients with cervical cancer have higher RNA expression levels of immune-activating factors compared with many other types of solid tumors including uterine and ovarian cancers, consistent with larger studies utilizing the TCGA databases of diverse solid tumors (30). Patients with uterine and ovarian cancers have a slightly lower than average (<50% expression rank level) RNA level of both immune-activating and immune-inhibitory markers. These findings may begin to explain the success of incorporating pembrolizumab (anti–PD-1) in the treatment of metastatic cervical cancer, and the comparatively lackluster response with the incorporation of immune checkpoints for patients with ovarian cancer (17, 36).

Upregulation of alternative checkpoints may also explain why some patients do not respond to anti–PD-L1/anti–PD-1 agents (24). An analysis of immune gene expression in patients with cervical cancer using the TCGA and Gene Expression Omnibus (GEO) databases was able to delineate high- versus low-risk immune profiles that reliably predicted survival. The high-risk group was characterized by overexpression of macrophages and mast cells, probably due to their ability to promote lymphangiogenesis and angiogenesis (37). In our study, we found higher expression levels of proinflammatory markers in patients with PD-L1 IHC–positive tumors across all three disease sites, but lower levels of other immune markers. This may begin to explain why PD-L1 IHC serves as a limited therapeutic biomarker as it likely captures only one aspect of the immune microenvironment. The IHC 22C3 antibody only measures tumor PD-L1, whereas IHC SP142 measures tumoral and immune cell PD-L1. In patients with cervical cancer, those with PD-L1^{+} tumors had higher levels of TIL markers as well. However, in patients with ovarian and uterine cancers, PD-L1 positivity did not correlate with higher TIL markers. Perhaps this again explains, at least in part, the unique success of using checkpoint inhibitors in patients with cervical cancer, but not in ovarian cancers. On the other hand, uterine cancers are also sensitive to anti–PD-1 checkpoint blockade, and this might be due to other factors, such as the presence of MSI-High, high TMB, and POLE mutations (2, 38, 39). An interesting observation was that uterine and ovarian cancers had a relatively similar immune landscape (Fig. 5A) despite the fact that these types of cancers respond differently to immune checkpoint inhibition. It is conceivable that the differences in response are due to immune or genomic factors not examined in this study.

We also investigated overexpression of immune-inhibitory factors and underexpression of immune-activating factors in gynecologic versus nongynecologic tumors with the hypothesis that drugs that block inhibition and or stimulate activation factors may be good candidates for therapeutics. Of interest, patients with gynecologic cancers had higher IDO1 RNA expression levels compared with other cancer types, with about 44% of gynecologic cancers expressing high IDO1 compared with less than 13% of other cancer types (*P*

< 0.001). IDO1 is the first and rate-limiting enzyme in the degradation of tryptophan, which is expressed in cancer cells or draining lymph nodes (40). When IDO1 is high, it can potentially be targeted with IDO1 inhibitors such as epacadostat, indoximod, etc. Previously, bioinformatics analysis of IDO1 immune function in gynecologic cancers using databases such as Oncomine, GEPIA, etc., also found overexpression of IDO1 RNA as well as protein expression in gynecologic tumors (41). Although phase I studies showed promising tolerance and response rates to the IDO inhibitor epacadostat (42), phase II studies comparing the IDO inhibitor epacadostat against tamoxifen in ovarian cancer types did not show significantly improved efficacy (43). IDO1 inhibitors have also failed in other tumor types (44). However, none of these clinical trials were designed to select patients with specific biomarkers (such high IDO RNA expression levels) for response. Because only a minority of patients (13% in our series) with nongynecologic cancers have high IDO transcript levels, these results suggest that selection of patients with higher IDO1 levels for IDO1 inhibitor trials may be warranted. Moreover, because almost half of gynecologic cancers express high IDO1 levels, gynecologic malignancies may be a worthwhile target for IDO1 inhibitor studies. The clinical utility of the IDO inhibitor epacadostat in combination with pembrolizumab (NRG GY016) showed a promising ORR in a small cohort of pretreated clear cell ovarian cancer patients (ORR 21%), although the study was terminated prematurely due to lack of drug (Gien and colleagues IGCS 2022)

We also found higher expression of the checkpoint LAG3 in gynecologic cancers as compared with nongynecologic cancers (34.7% vs. 20.6%; $P = 0.008$); similar findings were previously shown in endometrial cancers (45). The anti-LAG3 relatlimab was recently FDA approved for melanoma (46) and other LAG3 inhibitors are in clinical trials. It is plausible that LAG3 may be an alternative checkpoint upregulated in some of the tumors resistant to anti–PD-1/PD-L1 agents, and LAG3 inhibitors merit investigation in patients with gynecologic cancers, especially those with high LAG3 expression. It may be counterintuitive that we observed increased proinflammatory markers in gynecologic tumors that stain positive for PD-L1. However, prior reports suggest that it is possible that PD-L1 expression occurs in reaction to inflammatory cytokines (47).

There were several limitations to our study. First, our sample size was relatively small, especially in regard to cervical cancer, with uneven distribution among gynecologic disease sites. Second, because the database was not clinically annotated, we were not able to associate immune biomarker expression levels with immunotherapeutic responses or other clinical oncologic endpoints. Although these data were obtained from a CLIA-licensed laboratory, immune marker RNA from normal tissues were not delineated. Finally, while we were able to delineate differences in immune landscape between PD-L1–positive versus -negative cancers, the small number of neoplasms with high TMB or MSI-high disease precluded analysis of these subsets. Another limitation to the study is this relatively small number gynecologic versus nongynecologic cancers and even smaller cohorts of individual types of gynecologic cancers. Further studies could also examine the relationship between specific immune-modularity molecules and the T-cell repertoire. Despite these limitations, this study provides comprehensive insight into the complexities of the immune landscape in gynecologic malignancies.

Proteomic and genomic-based biomarkers such as MMR, MSI, PD-L1, and TMB have demonstrated efficacy in predicting treatment response to immunotherapies (38, 48, 49). RNA-seq may be an opportunity for discovering a broader cadre of biomarkers to improve the precision and limit the toxicities of this drug class (50, 51). RNA-seq provides valuable information such as transcript abundance, molecular alterations, and alternative promoter/splice sites that may be used to predict response or resistance to immune therapies (52, 50). In fact, one study found that almost 90% of patients with (classic) biomarker-negative tumors (MMR proficient, MSI stable, PD-L1 < 1%, TMB <10 mutations/mb), had high levels of other immune marker RNA that were potentially targetable with drugs under active investigation (53). Overall, our study indicates that patients with gynecologic cancers have complex immune landscapes that differ from patient to patient even within the same histology, but that certain pharmacologically tractable immune markers, such as high levels of IDO1 and LAG3 are present in these cancers. Our data also suggest that immunomic testing of tumors is needed to optimize our understanding of individual tumor biology. Further study is warranted in order to ascertain the correlation of RNA-seq with therapeutic impact.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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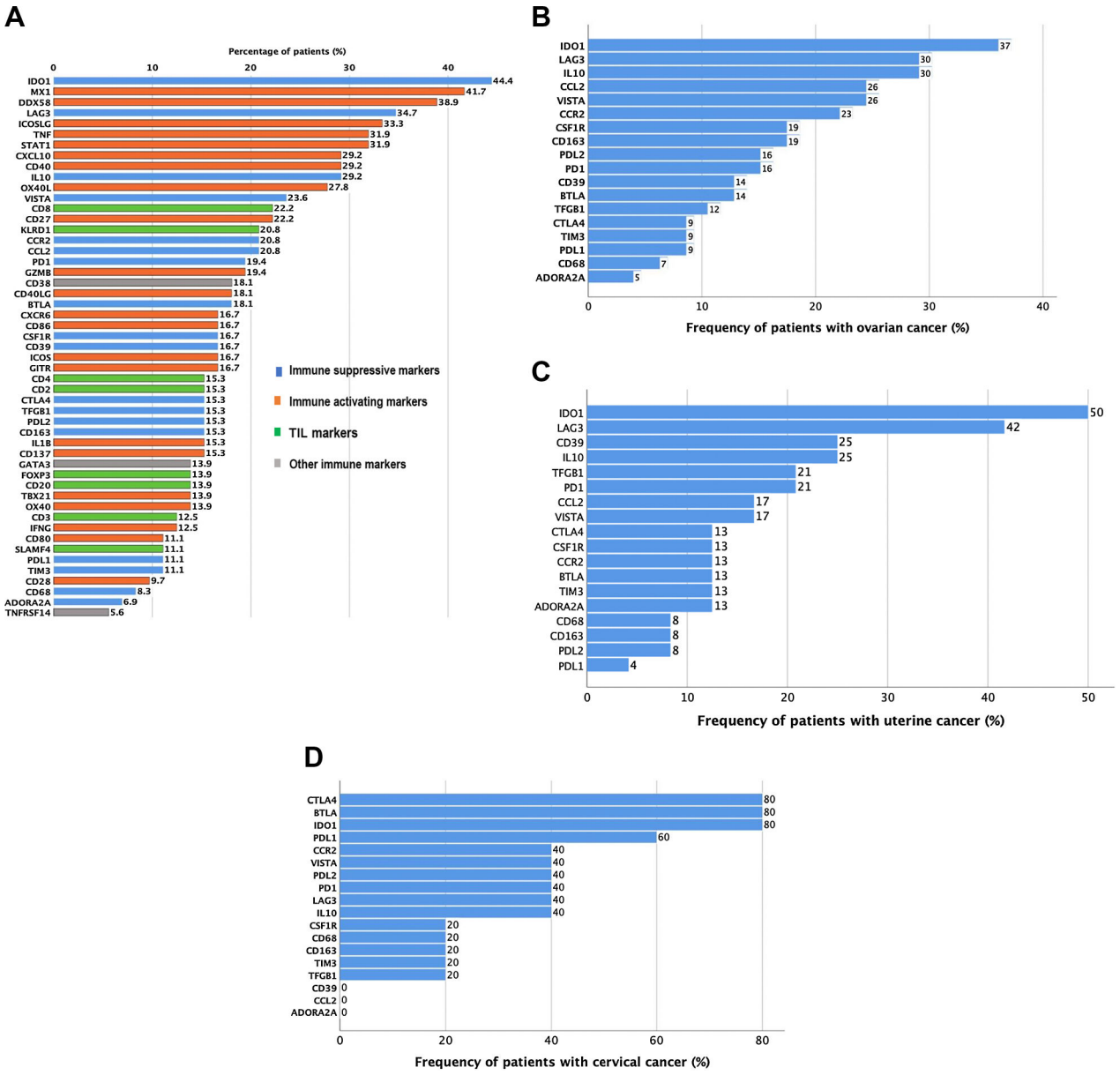

Figure 1.

A, Frequency of high RNA expression (≥75 percentile rank) among cancer-immunity markers across gynecologic disease sites (see also Supplementary Table S1 and Materials and Methods section; $N = 72$). **B,** Frequency of high RNA expression (≥75 percentile rank) among immune suppressive markers in patients with ovarian cancer (see also Supplementary Table S1 and Materials and Methods section; $N = 43$). **C,** Frequency of high RNA expression (≥75 percentile rank) among immune-suppressive markers in patients with uterine cancer (see also Supplementary Table S1 and Materials and Methods section; $N = 24$). **D,** Frequency of high RNA expression (≥75 percentile rank) among immune-suppressive markers in patients with cervical cancer (see also Supplementary Fig. S1 and Materials and Methods section; $N = 5$).

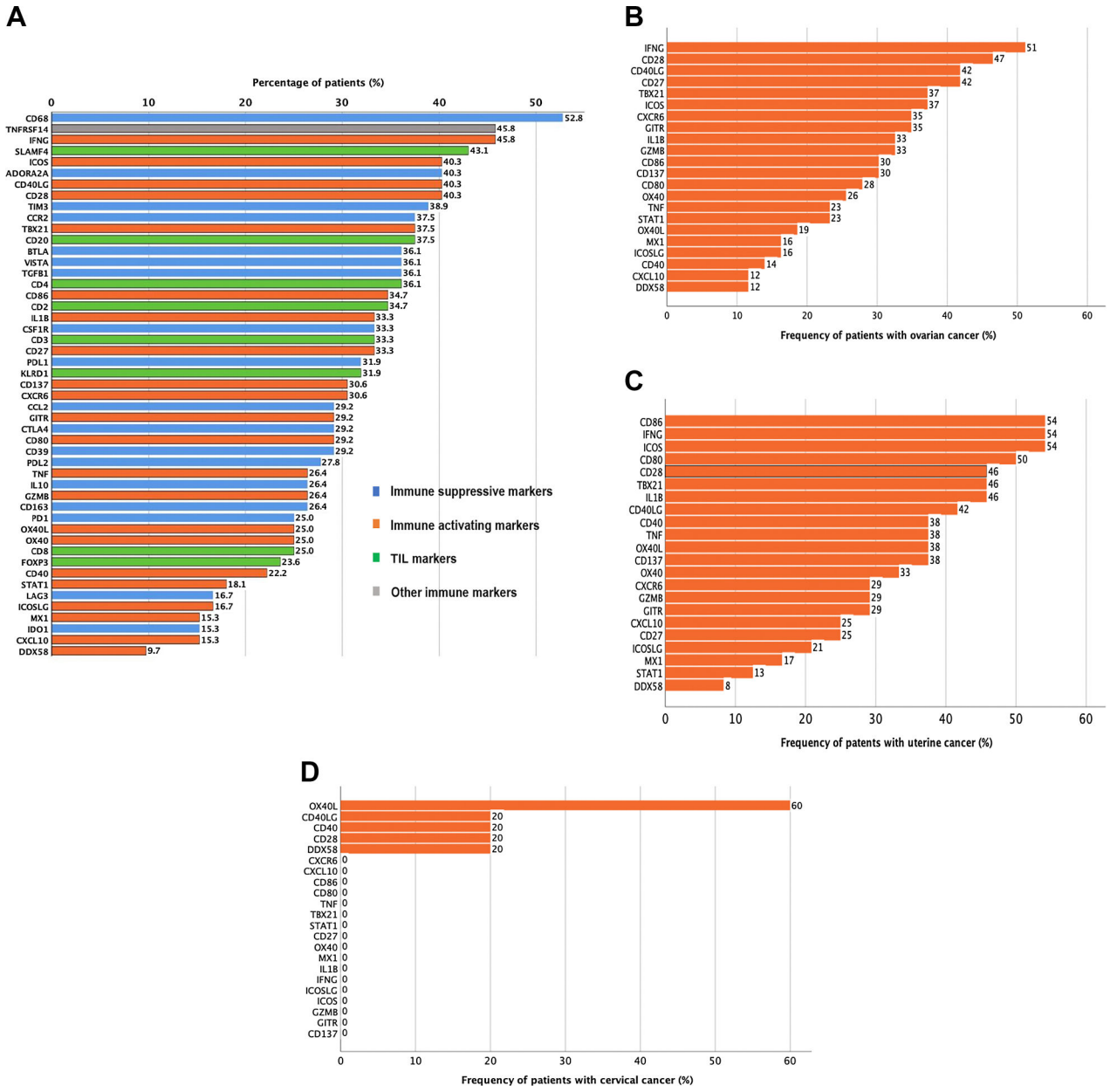

Figure 2.

A, Frequency of low RNA expression (<25 percentile rank) among cancer-immunity markers across gynecologic disease sites (see also Supplementary Table S1 and Materials Methods section; $N = 72$). **B,** Frequency of low RNA expression (<25 percentile rank) among immune-activating markers in patients with ovarian cancer (see also Supplementary Table S1 and Materials and Methods section; $N = 43$). **C,** Frequency of low RNA expression (<25 percentile rank) among immune-activating markers in patients with uterine cancer (see also Supplementary Table S1 and Materials and Methods section; $N = 24$).

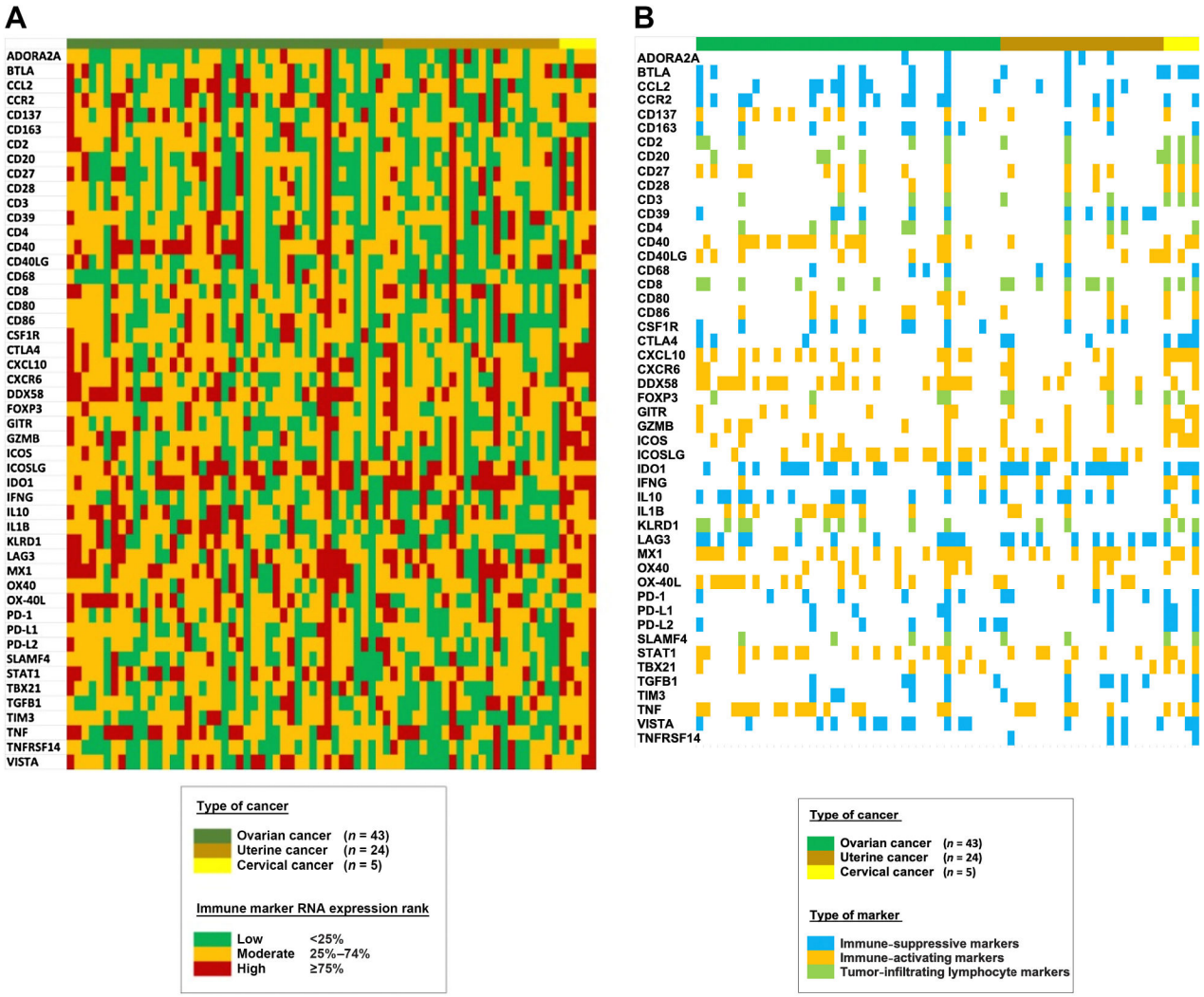

Figure 3.

A, Immunoprint showing on overview of RNA expression level of multiple immune markers for each individual case ($N = 72$). Percentile rank of transcriptomic expression was determined by normalizing to RNA of 735 control patients with diverse solid tumors. **B,** Immunoprint showing an overview of high (≥75 percentile rank) RNA expression level of multiple immune markers for each individual case ($N = 72$). Each column indicates an individual patients' case. See also Supplementary Table S1 and Materials and Methods section for classification of immune markers as immune-suppressive, immune-activating, or tumor-infiltrating lymphocyte markers. This figure shows that no two patients had the same pattern of high RNA expression. Each column indicates individual patients' case (see also Supplementary Table S1 and Materials and Methods section). This figure shows that no two patients had the same immune-marker expression pattern.

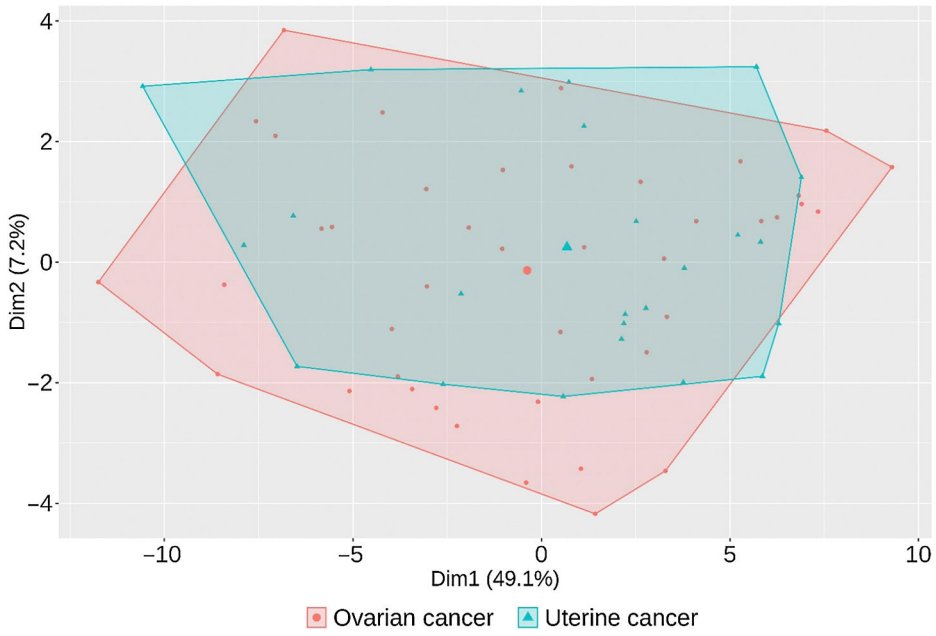

Figure 4.

Cluster plot of principal component analysis for 51 cancer-immunity marker RNA expression ranks in patients with ovarian and uterine cancer ($n = 72$) (see also Supplementary Fig. S1 and Materials and Methods section). Largely overlapping clusters suggest that expression patterns of cancer-immunity markers were not associated with disease site.

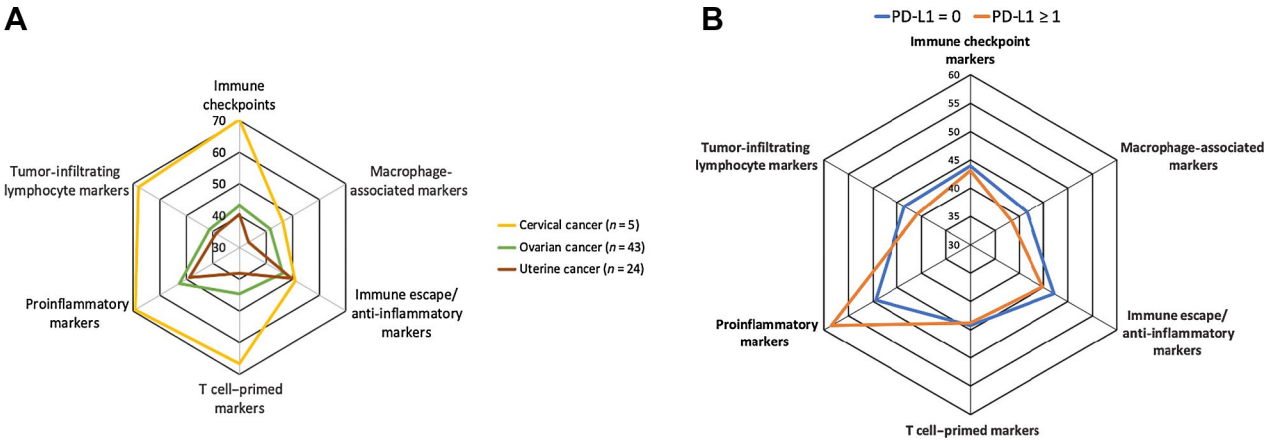

Figure 5.

A, Sample immunogram of mean cancer-immunity marker RNA expression rank in patients with gynecologic cancers ($N = 72$) relative to control cancer types ($n = 735$; see also Supplementary Table S1). **B,** Sample immunogram of mean cancer-immunity marker RNA expression rank in gynecologic cancers by PD-L1 status IHC (tumor proportion score; see also Supplementary Table S1; $N = 72$). Data is relative to control cancer types ($n = 735$).

Table 1.

Clinicopathologic variables among gynecologic disease sites ($N = 72$).

	Ovarian ($n = 43$; %)	Uterine ($n = 24$; %)	Cervix ($n = 5$; %)
Age (years)			
<65	22 (51.2)	11 (45.8)	4 (80.0)
≥65	21 (48.8)	13 (54.2)	1 (20.0)
TMB (mutations/megabase)			
<10	41 (95.3)	19 (79.2)	4 (80.0)
≥10	0 (0)	2 (8.3)	0(0)
Unknown	2 (4.7)	3 (12.5)	1 (20.0)
MSI status[a]			
Low/Stable	35 (81.4)	19 (79.2)	3 (60.0)
High	0 (0)	2 (8.3)	0 (0)
Unknown	8 (18.6)	3 (12.5)	2 (40.0)
PD-L1 IHC (%)[b]			
0	22 (51.2)	15 (62.5)	3 (60.0)
1	8 (18.6)	5 (20.8)	1 (20.0)
2–9	9 (20.9)	2 (8.3)	0 (0)
10–50	4 (9.3)	1 (4.2)	1 (20.0)
>50	0 (0)	1 (4.2)	0 (0)

[a] MSI-H defined as instability in at least 2 of 5 tested microsatellites.

[b] PD-L1 testing by IHC antibody 22C3 testing (percentage by tissue proportion score).

Table 2.

Proportion of high (≥75 percentile rank) RNA expression of immune-inhibitory immune markers among gynecologic cancers (n = 72) compared with nongynecologic solid tumors (n = 442)[a].

	Gynecologic cancers (n = 72; %)	Nongynecologic cancers (n = 442; %)[a]	*P*	Potential drugs
			Immune-inhibitory factors[1]	
IDO1	32 (44.4)	58 (13.1)	**<0.001**	When IDO1 is high, it is potentially targetable with IDO1 inhibitors. Examples include: Epacadostat Indoximod BMS-986205 KHK2455 LY3381916
LAG3	25 (34.7)	91 (20.6)	**0.008**	When LAG3 is high, it is potentially targetable with LAG3 inhibitors. Examples of LAG3 inhibitors include: Relatlimab BI 754111 LAG525 MK-4280
IL10	22 (30.6)	68 (15.4)	**0.002**	When IL10 is considered as an immunosuppressive cytokine, it is potentially targetable with IL10 inhibitors such as: MK-1966
CSF1R	12 (16.7)	103 (23.3)	0.213	
VISTA	17 (23.6)	148 (33.5)	0.096	
CCR2	15 (20.8)	102 (23.1)	0.186	
PD1	14 (19.4)	79 (17.9)	0.094	
CTLA4	11 (15.3)	76 (17.2)	0.691	
PDL2	11 (15.3)	89 (20.1)	0.910	
PDL1	8 (11.1)	59 (13.3)	0.265	
TIM3	8 (11.1)	81 (18.3)	0.134	
ADORA2A	5 (6.9)	102 (23.1)	**0.002**	

Note: Potential drugs would be those agents that suppress the specific immune-inhibitory function.

[a] N = 442 nongynecologic cancers profiled at UCSD.