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Immune Network Construction and Prognostic Evaluation of Checkpoint Genes in Endometrial Cancer Using STRING, MCODE, and GEPIA2

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Abstract

Background/Aim: Endometrial cancer (EC) is an important health issue among women, with immunotherapy emerging as a promising option for advanced cases. Tumor-infiltrating lymphocytes (TILs) and immune checkpoints, including *PDCD1*, *CD274*, and *PDCD1LG2*, are increasingly recognized as prognostic markers. This study aimed to construct an *in silico* immune network and assess the prognostic impact of checkpoint genes in EC using STRING, MCODE, and GEPIA2.

Materials and Methods: Retrospective analysis used TCGA-UCEC (The Cancer Genome Atlas – Uterine Corpus Endometrial Carcinoma) and GTEx datasets and reported immune-related genes. Genes were analyzed in STRING v12.0 (confidence ≥0.7; up to 10 neighbors per node) to generate a protein–protein interaction (PPI) network, exported to Cytoscape v3.10.2, and processed with MCODE to identify functional clusters. Hub genes were evaluated for expression and overall survival (OS) in GEPIA2 using median-based stratification and log-rank tests (p<0.05). Six immune signatures were assessed in TIMER2.0. *PDCD1* and *CD274* showed strong interactions with other immune effectors.

Results: CD40 and *LGALS9* were down- and upregulated, respectively, without affecting OS. Combined overexpression of *CTLA4*, *PDCD1*, *TIGIT*, *CD8A*, *CD8B*, *GZMB*, *PRF1*, *TBX21*, *FOXP3*, *CXCL9*, *CD28*, and *ICOS* correlated with improved OS, suggesting direct immune effects and enhanced responses to targeted therapies.

Conclusion: This *in silico* immune network highlights checkpoint centered hubs and coordinated immune programs with prognostic relevance in endometrial cancer, providing a rationale for biomarker guided immunotherapy development and patient stratification. Validation in independent cohorts and correlation with clinicopathologic and treatment response data are needed to support clinical translation.

Keywords: Immune system phenomena, regulator genes, endometrial neoplasms, prognosis, computer simulation, medical informatics applications.

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Introduction

Endometrial cancer (EC) is the sixth most common malignancy among women worldwide and represents the most frequent gynecological tumor in developed regions (1). Overall, uterine corpus tumors show a rising incidence, mainly due to aging, obesity, and sedentary lifestyle (1, 2). EC is usually diagnosed at early stages and has a favorable prognosis; however, about one-third of cases progress unfavorably (3). Histologically, tumors are classically divided into type I, including endometrioid carcinomas grade 1 and 2, associated with hyperestrogenism and less aggressive behavior, and type II, which comprises endometrioid grade 3 and non-endometrioid carcinomas with worse prognosis, such as serous carcinoma, undifferentiated carcinoma, carcinosarcoma, and other high-grade subtypes (3-6).

Most patients present with early-stage disease (stages 1 and 2), for which surgery is the main treatment. However, 10-15% of cases are diagnosed at advanced stages (3 and 4), with a 5-year survival rate of only 16.3% in metastatic disease (7). In advanced or recurrent EC, platinum-based chemotherapy remains the first-line treatment, though it is limited by adverse effects and resistance (7). Therefore, no widely accepted standard regimen exists, making clinical management of EC a current challenge. Immunotherapy has emerged as a promising alternative, given its potential to modulate the immune microenvironment and enhance cytotoxic antitumor responses (7).

Growing interest has been directed toward molecular and genetic markers of the tumor immune response, particularly tumor-infiltrating lymphocytes (TILs) and immune checkpoint molecules such as PD-1 (*PDCD1*), PD-L1 (*CD274*), and PD-L2 (*PDCD1LG2*) (8, 9). $CD8^+$ and $CD4^+$ T cells are associated with improved antitumor activity, whereas $FOXP3^+$ T regulatory cells (Tregs) indicate an immunosuppressive, pro-tumor phenotype. Notably, PD-L1 expression has been linked to both immune evasion and therapeutic response in microsatellite instability-high (MSI-H) tumors (10). In this context, bioinformatics tools have become increasingly useful for integrated exploration of prognostic molecular mechanisms regulating tumor–immune interactions at the microenvironment level (11). Construction of protein–protein interaction (PPI) networks allows identification of hub genes, functional modules, and biological pathways associated with tumor immunoregulation, supporting survival analyses (12).

Thus, this study aimed to construct an *in silico* immune network to evaluate the prognostic impact (overall survival, OS) of checkpoint genes involved in tumor immunoregulation in EC patients using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins), the MCODE extension (Molecular Complex Detection), and the GEPIA2 platform (Gene Expression Profiling Interactive Analysis 2).

Materials and Methods

Study design. This is a retrospective *in silico* analysis based on publicly available gene expression data.

PPI network construction. To increase disease specificity, STRING-derived interactions were contextualized using TCGA-UCEC (The Cancer Genome Atlas – Uterine Corpus Endometrial Carcinoma) transcriptomic data. Differential expression analysis (tumor *vs.* normal endometrium) and tumor-only expression levels were assessed in GEPIA2. Additionally, pairwise Spearman correlation coefficients were estimated across TCGA-UCEC tumor samples for each gene pair connected by a STRING edge. Only interactions supported both by STRING evidence and meaningful tumor co-expression ($|\rho| \geq 0.30$) in TCGA-UCEC were considered biologically supported within the endometrial cancer context. The proportion of STRING edges corroborated by TCGA-UCEC co-expression was calculated and reported (13).

Network visualization. The generated file was exported in XGMML format and processed using Cytoscape v3.10.2, an open-source software used for visualization and analysis of biological networks (14). Within this environment, the MCODE plugin (Molecular Complex Detection) was

Table I. *Seeds input into STRING.*

Gene	Main immune function (16)
PDCD1	Encodes the PD-1 receptor, acting as an inhibitory immune checkpoint in T lymphocytes.
CD274	Encodes PD-L1, which inhibits T cell activation and promotes tumor immune evasion.
PDCD1LG2	Encodes PD-L2, with a function similar to PD-L1.
CD8A	α-chain of the CD8 coreceptor; marker of cytotoxic T lymphocytes ($CD8^+$ T cells).
CD8B	β-chain of CD8; forms a functional heterodimer with CD8A in cytotoxic T lymphocytes.
CD4	Coreceptor of helper T lymphocytes (T helper); essential for adaptive immune activation.
FOXP3	Transcription factor marker of regulatory T cells (Tregs).
GZMB	Granzyme B – cytotoxic enzyme secreted by CD8+ T cells and NK cells; induces apoptosis.
PRF1	Perforin, forms pores in target cell membranes, facilitating granzyme entry.
LAG3	Inhibitory immune checkpoint; negatively regulates activated T cells.
HAVCR2	Encodes TIM-3 receptor, an immune checkpoint with T cell suppressive function.
CTLA4	Inhibitory receptor; competes with CD28 for CD80/CD86 binding, blocking T cell activation.
TIGIT	Alternative immune checkpoint; expressed on T and NK cells with suppressive effects.
CD40	Costimulatory molecule on APCs (*e.g.*, DCs); activates $CD4^+$ T and B cells.
CD68	Classical marker of macrophages and mononuclear phagocyte lineage cells.
ITGAE	Encodes integrin αE (CD103); marker of tissue-resident TILs in epithelial tissue.
TNFRSF9	Encodes CD137 (4-1BB); coactivation receptor on activated T and NK cells.

NK: Natural killer; APCs: antigen-presenting cells; DCs: dendritic cells; TILs: tumor-infiltrating lymphocytes.

applied to identify densely connected modules or clusters within the network. The parameters used were degree cutoff=2, node score cutoff=0.2, k-core=2, and max depth=100. The MCODE algorithm assigns a score to each cluster, reflecting the density of connections among the genes/proteins within the module. Higher MCODE scores indicate greater topological centrality and a higher likelihood that the set represents a functional complex or a relevant biological pathway. Genes with high scores were therefore considered central hubs in the constructed immune network.

Definition of central (Hub) genes. Central genes (hubs) were defined *a priori* based on a combination of quantitative and topological criteria: (i) high node degree within the STRING-expanded network, (ii) participation in at least one high-density MCODE cluster, and (iii) functional relevance to immune regulation supported by Gene Ontology enrichment. Degree centrality was computed on the expanded PPI network generated from the initial immune seed list. The final set of 17 central genes represents nodes consistently meeting all three criteria, rather than solely reflecting MCODE-derived metrics.

Differential gene expression analysis. Subsequently, the main identified genes were subjected to differential expression and prognostic impact analyses using the GEPIA2 platform (Gene Expression Profiling Interactive Analysis 2), an open-access website integrating transcriptomic data from The Cancer Genome Atlas – Uterine Corpus Endometrial Carcinoma (TCGA-UCEC) and the Genotype-Tissue Expression (GTEx) Project (15). Expression analysis compared tumor and normal samples, while OS associations were explored using Kaplan-Meier curves stratified by the median gene expression (high *vs*. low expression). Significance was determined by the log-rank test, considering $p<0.05$. The seeds listed in Table I were input into STRING for analysis (16).

Additionally, six functional immune signatures described in the literature – namely inhibitory checkpoints, lymphocyte cytotoxicity, regulatory TILs, tumor-associated macrophages, "hot tumor" inflammatory profiles, and costimulatory molecules –were evaluated using the publicly accessible TIMER2.0 platform (17). The signatures were analyzed for their impact on OS, estimating hazard ratios (HR) with 95% confidence intervals.

PPI network construction and identification of central immune genes. A PPI network was constructed using the STRING v12.0 database, a validated platform for inferring protein interactions based on experimental evidence, co-expression, genomic co-occurrence, curated database information, and text-mining predictions (13). Technical criteria for analysis included a minimum interaction confidence level of 0.7 (high confidence) and a limit of 10 neighbors per node.

To analyze the functional organization of the PPI network generated in STRING, the network file was imported in XGMML format into Cytoscape v3.10.2, a platform used for visualization and analysis of biological networks (14).

To identify interconnected protein clusters representing functional complexes or immune-related signaling pathways, the MCODE algorithm, a Cytoscape plugin specialized in detecting dense modules within PPI networks, was applied. This allowed identification of clusters and analysis of topological centrality and functions performed by each gene. MCODE parameters were set as follows: Degree Cutoff=2, establishing the minimum number of connections for a node to be included; Node Score Cutoff=0.2, defining the minimum score threshold for local node density; K-Core=2; and Max Depth =100, limiting cluster exploration depth. This step aimed to isolate functional protein subsets, *i.e*., clusters with a high degree of internal connectivity, whose co-expression or functional occurrence suggests joint participation in relevant immune processes, such as lymphocyte activation, immune checkpoint inhibition, or tumor immune evasion pathways. Based on the topological analysis of the PPI network in Cytoscape, key central genes (hubs) were selected according to quantitative criteria of connectivity (degree) and participation in multiple functional modules identified by MCODE. Selection prioritized genes with a high density of interactions and involvement in critical immune processes, such as lymphocyte activation and antitumor response regulation (18-20).

These genes were then subjected to expression and prognostic analysis using the GEPIA2 platform, integrating transcriptomic data from TCGA-UCEC and GTEx. The "*n*" represents the number of RNA sequencing (RNA-seq) samples available in TCGA (tumor) and GTEx (normal) for each gene and endometrial cancer.

Seeds, enrichment, and GEPIA2 settings. The complete list of immune-related seed genes input into STRING is provided in Table I. These seeds were included in both network expansion and hub ranking analyses. Gene Ontology enrichment was performed using ClueGO within Cytoscape, focusing on immune system processes and lymphocyte activation pathways. GEPIA2 analyses were conducted using TCGA-UCEC tumor samples and GTEx normal endometrial tissue, applying default filtering parameters without matched-normal restriction, as matched samples are not uniformly available for UCEC.

MCODE analysis. MCODE was used to identify densely interconnected clusters (modules) within the PPI network. Importantly, MCODE generates a score at the cluster level, reflecting internal connection density, rather than a unique score per gene. Genes presented in Table II are listed according to the MCODE score of the cluster to which they belong, explaining the presence of identical values across multiple genes. Individual node properties (*e.g*., degree) were analyzed separately.

Survival analysis. Survival analysis was performed using the "Survival Analysis" feature, generating Kaplan-Meier curves based on patient stratification into high and low expression groups according to median transcriptional levels. Associations between gene expression and OS were evaluated. Statistical significance was determined using the log-rank test, and results were expressed as HR and *p*-values. Values of $p<0.05$ were considered statistically significant.

Statistical analysis. To evaluate the prognostic impact of functional sets of immune-related genes in endometrial cancer, six immune signatures previously described in the literature (9, 10, 21-24) were selected and grouped based on their predominant biological function. Analysis

Table II. *Molecular Complex Detection (MCODE) scores generated in Cytoscape.*

Gene	MCODE Score
CD274	15.69
PDCD1	15.69
CD86	15.69
TIGIT	15.69
HAVCR2	15.69
LAG3	15.69
PDCD1LG2	14.78
FOXP3	14.78
TNFRSF18	14.78
IDO1	14.78
TNFRSF4	14.10
CD27	14.03
TNFRSF9	13.98
CD4	13.91
CCR7	13.48
CD8A	13.29
CD40	13.28
CD80	12.78
CD28	12.78
ICOS	12.78
CTLA4	12.78
CD69	12.67
GZMB	12.67
BTLA	12.00
LOC102723996	11.98
LGALS9	11.87
CD276	11.63
TNFSF4	11.63
ICOSLG	10.74
TNFSF9	10.64
PRF1	9.90
KLRG1	9.49
ITGAE	9.00
LGALS9B	8.84
LGALS9C	8.84
CD68	8.00
CD8B	3.00

was conducted using TIMER2.0, Gene_Outcome module, which allows investigation of associations between combined gene expression and patient OS based on TCGA-UCEC data. These signatures were input into TIMER2.0 as gene sets and analyzed for association with OS in endometrial cancer patients. Stratification was based on the median combined expression of genes in each group, and Kaplan-Meier curves were automatically generated by the platform, accompanied by HR and *p*-values (log-rank test). The significance level adopted in this analysis was 5% (15, 17, 25-28).

Results

The network obtained in STRING (Figure 1) revealed an interconnected set of proteins, with *PDCD1* and *CD274* highlighted as central nodes, suggesting high connectivity with effector lymphocyte subtypes (CD8A and CD40), which supports their role in tumor immune evasion axes (16). Table II below summarizes the MCODE scores, representing the connection density of genes within functional modules identified in Cytoscape. Higher values indicate greater centrality and topological relevance of the gene in the immune network context.

Figure 2 depicts the functional network of immunoregulatory genes generated in Cytoscape based on functional enrichment analysis (GO terms) of the PPI network. The nodes, shaped as ellipses, represent genes related to tumor immune response, with colored sectors indicating participation in multiple biological processes simultaneously. Central genes in the network include *TNFSF4*, *TNFRSF9*, *TNFRSF4*, *TIGIT*, *PDCD1LG2*, *LGALS9*, *ICOS*, *HAVCR2*, *CTLA4*, *CD8A*, *CD86*, *CD80*, *CD40*, *CD28*, *CD276*, *CD274*, and *CD27*, which are key in immune response activation and regulation, as well as tumor progression/suppression.

Supplementary Figure 1 highlights the comparative expression analysis of each of the 17 central genes between endometrial tumor samples (*n*=174) and healthy tissue samples (*n*=91). Additionally, OS analysis was performed in endometrial tumor patients according to low or high gene expression. The analysis for *CD40* and *LGALS9* genes is shown in Figure 3. The full analysis for all 17 genes is available in the supplementary data.

The analysis shown in the figure above demonstrated that endometrial tumor samples exhibited decreased *CD40* expression and increased *LGALS9* expression compared to healthy tissue samples, with statistical significance, which was not observed for the other 15 analyzed genes, as shown in the complete supplementary figure.

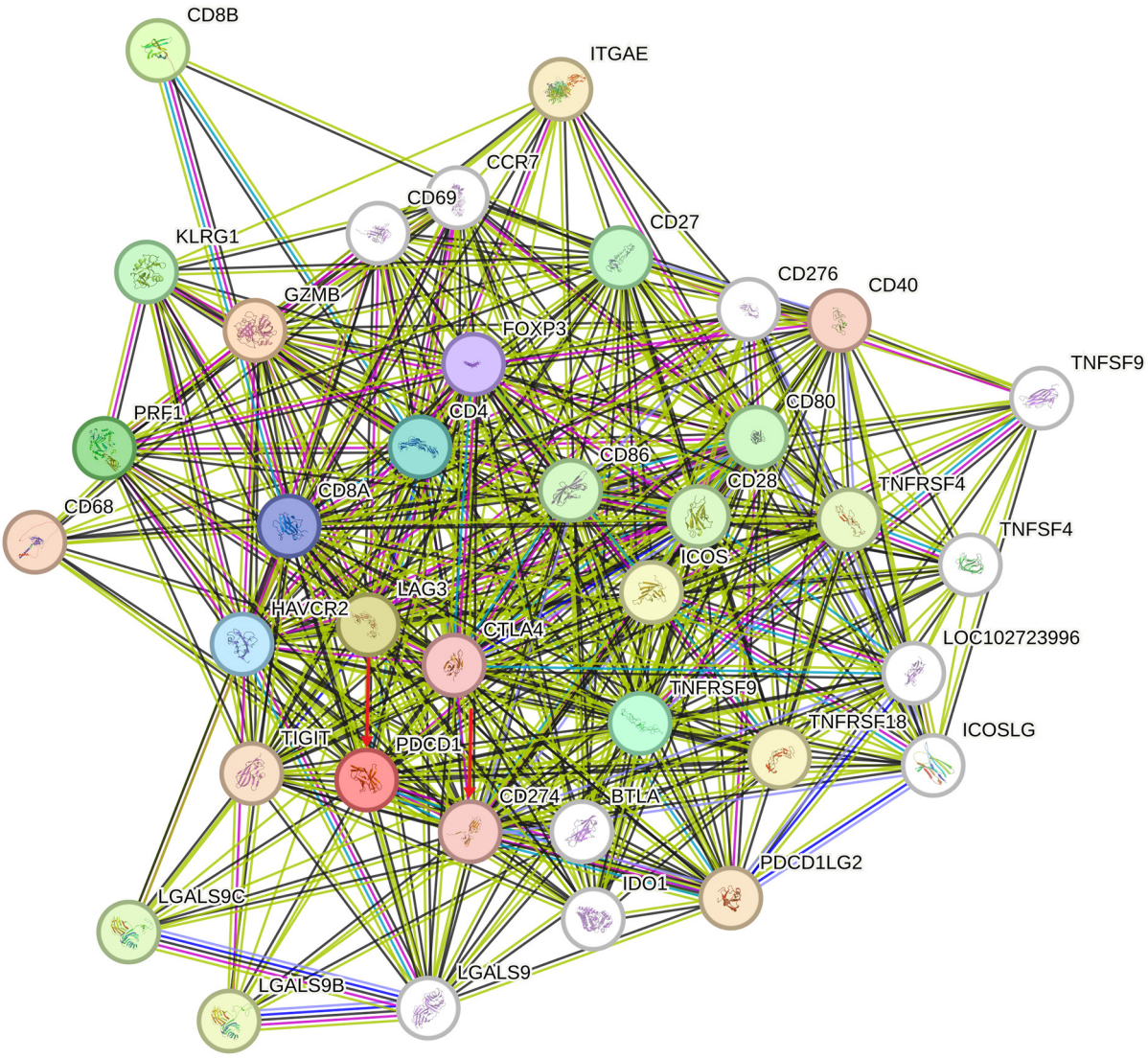

Figure 1. *Protein–protein interaction (PPI) network of immune-related genes constructed using the STRING v12.0 database. Red arrows highlight the central genes of the network (PDCD1 and CD274).*

Table III summarizes the OS analysis in this study. No significant differences in OS were observed between the low and high expression groups for each of the 17 genes analyzed. It is important to consider that after 5 years of follow-up, not all deaths can be directly attributed to the tumor, as they may result from sequelae, other causes, or new neoplasms.

Among the six immune signatures analyzed for a cohort of 545 endometrial cancer patients (Figure 4), Signature 1 corresponds to immunoinhibitory checkpoints, reflecting tumor immune evasion mechanisms. It included *PDCD1* (PD-1), *CD274* (PD-L1), *PDCD1LG2* (PD-L2), *CTLA4*, *LAG3*, *HAVCR2* (TIM-3), and *TIGIT*. Combined overexpression of these genes may indicate an exhausted immune microenvironment with functionally inactive lymphocytes (21). Signature 2 comprised genes associated with cytotoxic T lymphocyte activity, indicating effective antitumor immune response, including *CD8A*, *CD8B*, granzyme B (*GZMB*), perforin (*PRF1*),

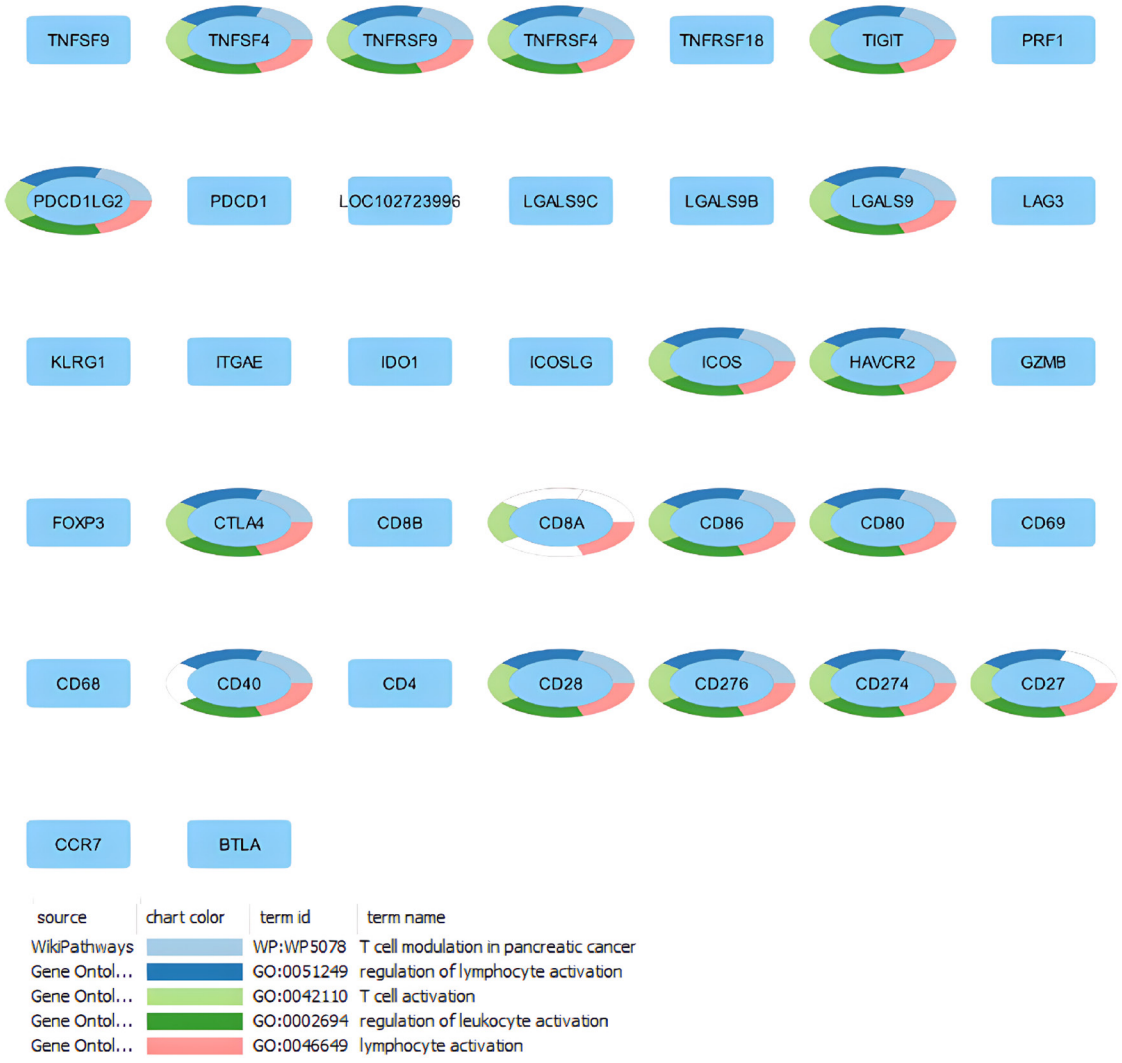

Figure 2. *Functional organization of central immune genes identified through Cytoscape-based network and Gene Ontology (GO) enrichment analysis. Each node corresponds to a gene involved in the tumor immune response, and connections indicate functional co-occurrence or joint participation in biological pathways. Genes with multiple connections were highlighted (elliptical shape) as potential regulatory hubs, suggesting a role in lymphocyte activation, immune checkpoint inhibition, or tumor evasion mechanisms.*

interferon-gamma (*IFNG*), and T-bet transcription factor (*TBX21*). This signature represents tumors with active lymphocyte infiltration and potentially better prognosis (22). Signature 3 grouped markers of regulatory T cells (Tregs), with immunosuppressive function. Included genes were *FOXP3*, *IL2RA* (CD25), *IKZF2*, *CTLA4*, and *TGFB1*, often associated with suppression of effector immune responses and promotion of tumor escape (9).

Signature 4 involved markers of tumor-associated macrophages (TAMs), with pro-tumoral M2 phenotype, including *CD68*, *CD163*, and *IL10*, whose high expression has been linked to immunosuppressive microenvironment induction and tumor progression (23). Signature 5, termed "hot tumor," included genes indicative of high immune inflammation, such as *CD8A*, *GZMB*, *IFNG*, *CXCL9*, *CXCL10*, *CD274*, and *PDCD1*, reflecting

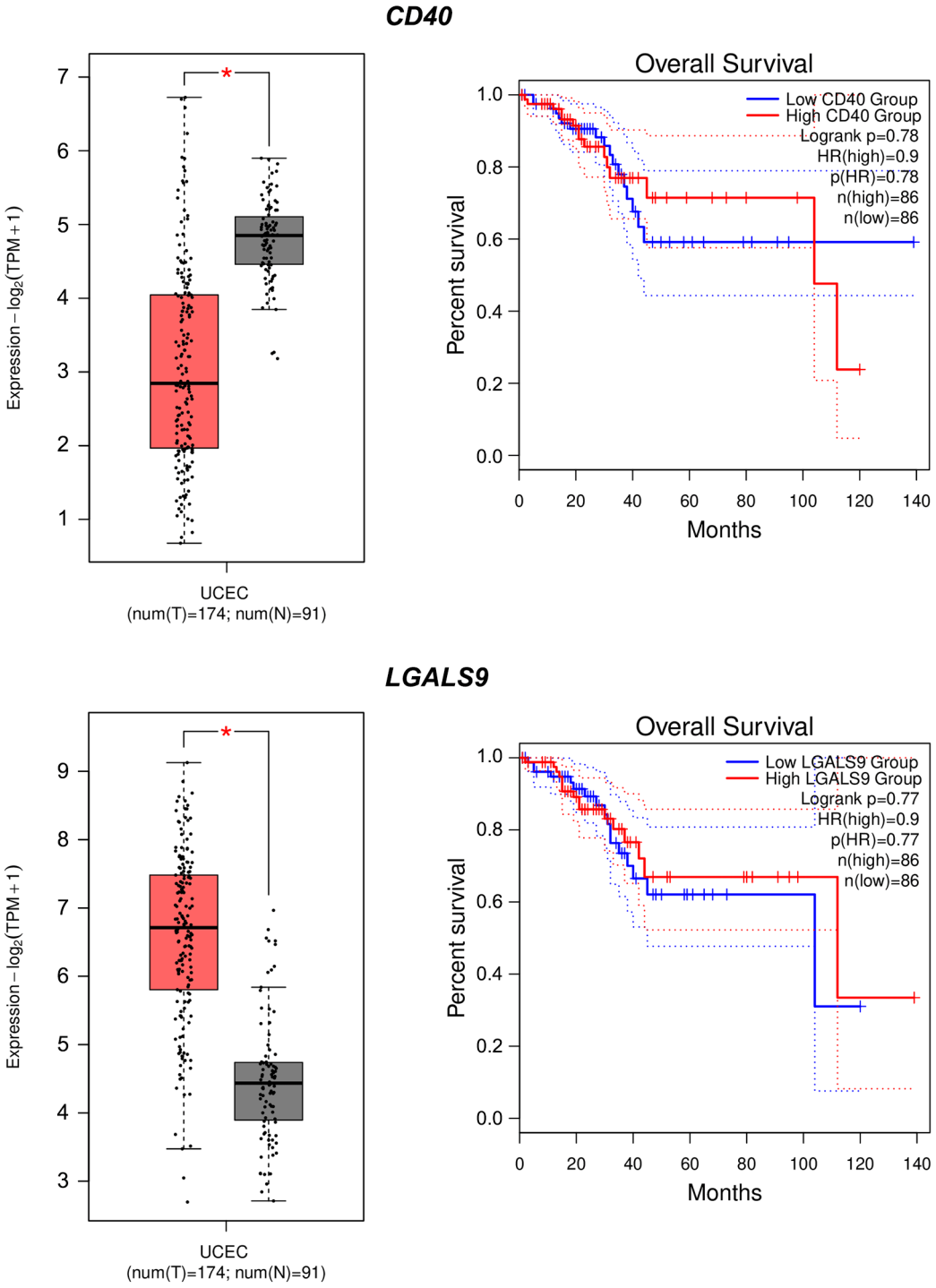

Figure 3. *Differential expression of CD40 and LGALS9 and overall survival (OS) according to CD40 and LGALS9 expression in endometrial cancer using GEPIA2. Expression of CD40 and LGALS9 was compared between endometrial tumor samples (T; red bar) and healthy tissue samples (N; gray bar). Additionally, OS analysis was performed in endometrial tumor patients according to CD40 and LGALS9 gene expression levels (low vs. high). TPM: Transcripts per million; UCEC: Uterine Corpus Endometrial Carcinoma. *p<0.05.*

Table III. *Overall survival analysis among the patients analyzed.*

Gene	n (high)	n (low)	HR (high *vs.* low)	*p*-Value*
CD8A	86	86	0.59	0.14
CD80	86	84	0.96	0.91
CD86	86	86	0.86	0.69
CTLA4	85	86	0.62	0.20
HAVCR2	86	86	0.76	0.44
ICOS	84	86	0.90	0.76
CD40	86	86	0.90	0.78
CD28	85	86	1.40	0.35
CD276	86	86	0.78	0.49
CD274	86	84	1.20	0.55
CD27	86	86	0.73	0.38
LGALS9	86	86	0.79	0.77
PDCD1LG2	86	83	1.3	0.5
TIGIT	86	85	0.50	0.074
TNFRSF9	86	84	0.47	0.059
TNFRS4	86	86	1.1	0.86
TNFSF4	84	86	1.2	0.55

high: High gene expression; low: low gene expression; HR: hazard ratio. *From log-rank test.

intense inflammatory infiltrate and often associated with positive immunotherapy response (10). Finally, Signature 6 comprised co-stimulatory markers related to T cell activation *via* antigen-presenting cells (APCs), including *CD40*, *TNFRSF9* (4-1BB), *CD28*, *ICOS*, *CD80*, and *CD86*, whose combined expression may reflect a microenvironment permissive to adaptive immune activation (24).

The analysis showed that specific combined overexpression in each of the six analyzed signatures of genes *CTLA4*, *PDCD1*, and *TIGIT*; *CD8A*, *CD8B*, *GZMB*, *PRF1*, and *TBX21*; *FOXP3*; *CXCL9*; *CD28*; and *ICOS* in endometrial cancer patients was significantly associated with longer overall survival (better prognosis).

Overlaying TCGA-UCEC expression data onto the STRING-derived PPI demonstrated that a substantial proportion of interactions were supported by tumor-specific co-expression patterns. Notably, edges involving PDCD1, CD274, CTLA4, TIGIT, CD8A, and CD40 showed consistent positive correlations across tumor samples, reinforcing the biological relevance of these immune checkpoints and effector interactions in endometrial cancer.

Discussion

PD-1 (*PDCD1*) is a protein expressed on macrophages, B lymphocytes, dendritic cells (DCs), monocytes, activated T cells, myeloid cells, and natural killer (NK) cells (28-30). PD-L1 (*CD274*), in turn, is expressed on macrophages, activated T cells, B cells, DCs, and some epithelial cells (particularly under inflammatory stimuli) (28-30). Under normal conditions, PD-1 inhibits autoimmunity, reduces tissue damage caused by infections, and promotes immune self-tolerance. However, in cancer, high PD-1 expression, especially on T cells, and the interaction between PD-1 and PD-L1 inhibit immune system activation and promote tumor progression through various immune-mediated mechanisms (9, 28-30). Recent evidence has further highlighted the clinical relevance of PD-L1 expression in endometrial cancer beyond its role as a membrane-bound immune checkpoint. Tissue-based analyses combined with plasma biomarkers have demonstrated that both tumoral PD-L1 expression and circulating soluble PD-L1 levels are associated with disease characteristics and immune modulation in endometrial cancer patients. These findings support the concept that PD-L1 reflects not only local immune evasion mechanisms within the tumor microenvironment but also systemic immune alterations, reinforcing its value as a prognostic and potentially predictive biomarker in endometrial cancer (31).

This knowledge has led to the development of several immunotherapies, such as pembrolizumab, nivolumab, durvalumab, and dostarlimab. Beyond monotherapy approaches, increasing evidence supports the integration of immunotherapy with conventional chemotherapy in advanced or recurrent endometrial cancer. Combination strategies have shown potential to enhance antitumor immune responses, overcome resistance mechanisms, and improve clinical outcomes. In this context, immune checkpoint gene expression profiles, such as those identified in the present immune network, may serve as valuable tools for identifying patients most likely to benefit from combined therapeutic regimens (32). In EC, PD-1 and PD-L1 expression also play a central role in tumorigenesis and in identifying patients likely to

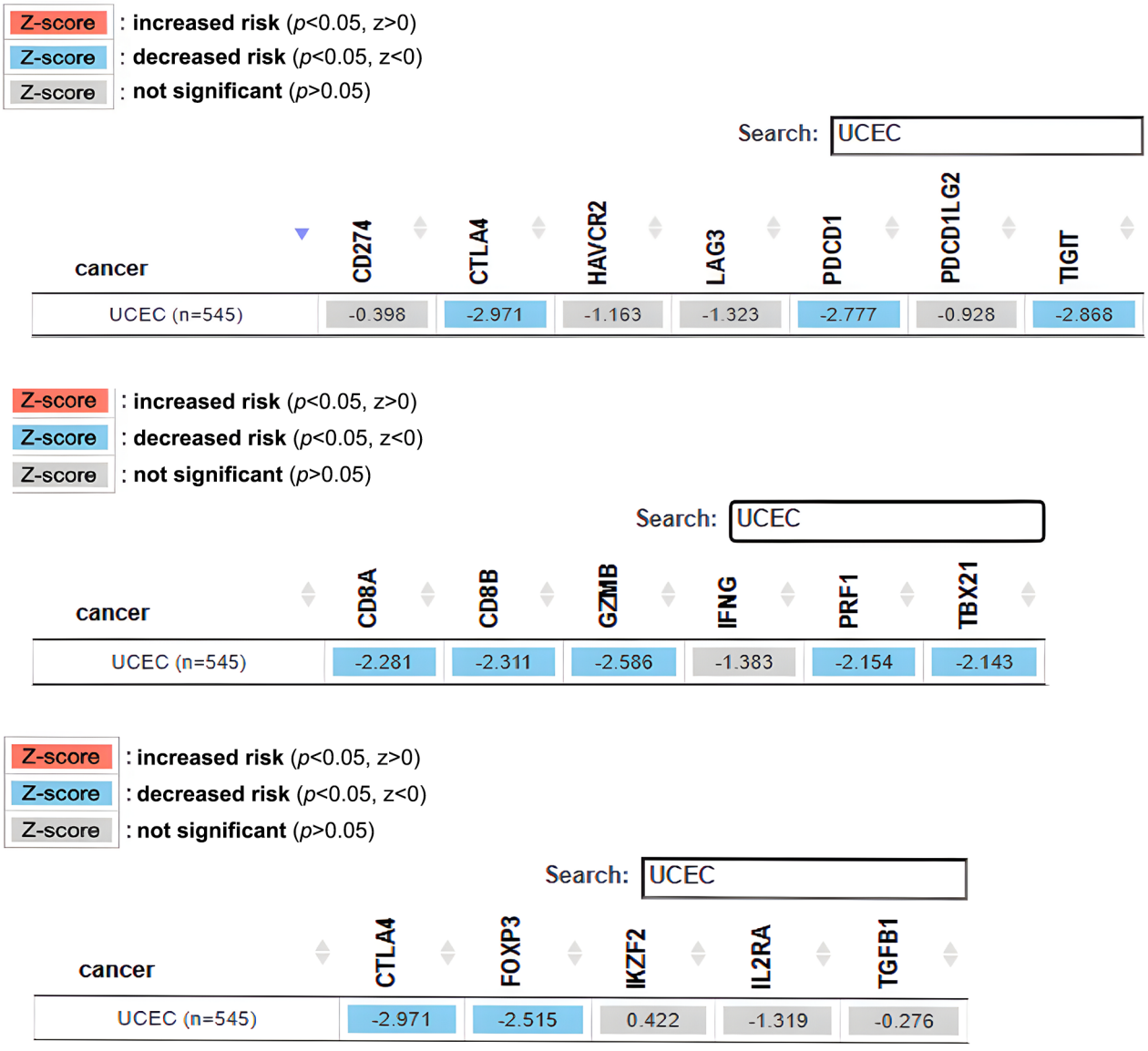

Figure 4. *Continued*

benefit most from immunotherapy (24). Therefore, the PPI network generated in STRING in this study aligns with this emerging understanding, showing the central impact that PD-1 and PD-L1 encoding genes (*PDCD1* and *CD274*, respectively) and their interactions with other genes expressing various innate and adaptive immune effectors, such as CD8A and CD40, have on the prognostic evolution of EC patients. Importantly, the prognostic and biological significance of PD-1 and PD-L1 expression appears to extend to early-stage endometrial carcinoma. Studies evaluating uterine endometrioid tumors at initial stages have reported detectable PD-1 and PD-L1 expression within the tumor microenvironment, suggesting that immune checkpoint activation is an early event in endometrial tumorigenesis. This observation aligns with our findings and supports the rationale for immune-based

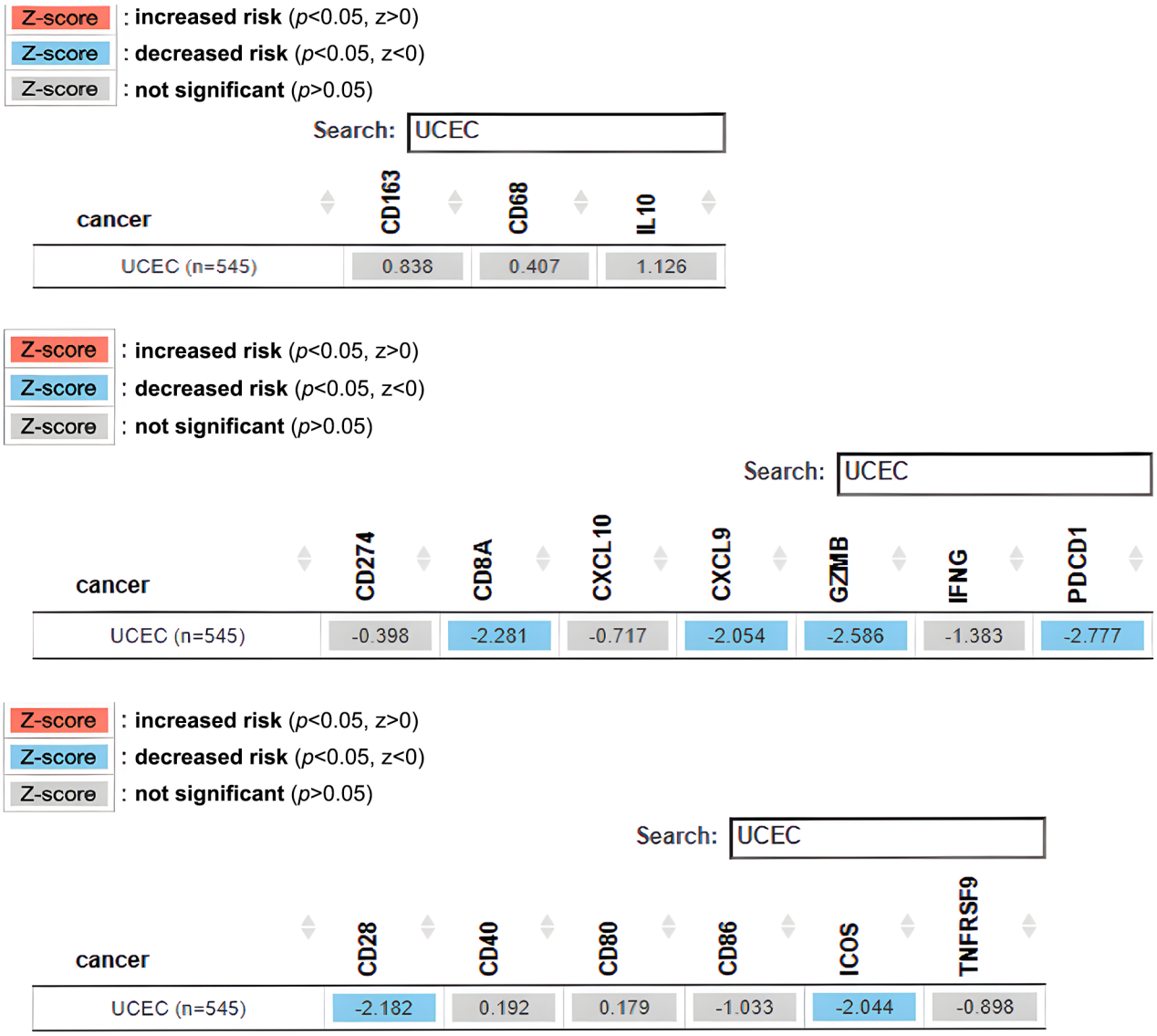

Figure 4. *Prognostic impact of six immune-related gene signatures (gene-by-gene associations within each signature) on overall survival in endometrial cancer. UCEC: Uterine Corpus Endometrial Carcinoma.*

stratification even in patients with ostensibly favorable-stage disease (33).

TNFRSF4, also known as OX40 or CD134, is a molecule that exerts co-stimulatory functions on T cells when bound to TNFSF4, mainly expressed on $CD4^+$ and $CD8^+$ T cells. High TNFRSF4 expression tends to enhance the presence of $CD4^+$ and $CD8^+$ T cells in the tumor microenvironment and improve endometrial cancer prognosis. Consequently, this biomarker is currently being studied as a potential molecular target for immunotherapies aiming to stimulate T cell-mediated antitumor activity (34, 35). *TNFRSF9* expression is associated with PD-1 (*PDCD1*) expression. Recent evidence indicates that activation of $CD103^-$, $CD39^+$, and $CD8^+$ T cells in mismatch repair-deficient endometrial tumors is also associated with TNFRSF9 expression (36).

TIGIT is frequently co-expressed with PD-1 in tumor-infiltrating lymphocytes (TILs) and is linked to prognosis and immunotherapy response in endometrial cancer. Ongoing clinical trials are testing anti-TIGIT antibodies (vibostolimab) as monotherapy or in combination with pembrolizumab in advanced tumors (37). A study of 94 patients with serous endometrial carcinoma by Chen *et al.* demonstrated that high *TIGIT* expression was associated with better prognosis and longer overall survival (38). PD-L1 (*CD274*) and PD-L2 (*PDCD1LG2*) are ligands of PD-1 with critical roles in T cell activity and are important prognostic biomarkers in EC. Unlike PD-L1, PD-L2 expression is highly restricted, predominantly on DCs and macrophages. However, its expression can be induced in immune and non-immune cells; for example, cytokines produced by T helper 2 (Th2) cells stimulate PD-L2 production, which also participates in Th2 response regulation (39, 40).

LGALS9 encodes galectin-9 (Gal-9), which exhibits, among other biological effects, anti-metastatic and pro-apoptotic tumor activity (41). Sun and Dai conducted a study with 51 endometrial cancer patients to evaluate the prognostic impact of galectin-1 and galectin-9, showing that Gal-9 expression (*LGALS9*) was associated with early pathological changes (42). *ICOS* encodes the inducible T cell co-stimulator, associated with improved prognosis in endometrial cancer patients (43, 44). Significant expression of *HAVCR2* and *CTLA-4* has also been reported in endometrial tumors, suggesting potential roles in EC tumorigenesis (45). The prognostic value of TILs in endometrial cancer is an expanding field, and the roles of their various subpopulations remain poorly understood. Current literature indicates that expression of *CD8A*, *CD86*, *CD80*, *CD40*, *CD28*, *CD276*, and *CD27* may favor EC prognosis through activation of innate and adaptive immune effectors, particularly $CD4^+$ and $CD8^+$ T cells and B cells (23, 46-48).

Thus, the Cytoscape analysis results support this emerging knowledge and suggest central roles for *TNFSF4*, *TNFRSF9*, *TNFRSF4*, *TIGIT*, *PDCD1LG2*, *LGALS9*, *ICOS*, *HAVCR2*, *CTLA4*, *CD8A*, *CD86*, *CD80*, *CD40*, *CD28*, *CD276*, *CD274*, and *CD27* in multiple processes involved in tumor immunity in EC. Notably, *CD40* appears protective prognostically, as endometrial tumor samples showed significantly reduced expression, potentially leading to impaired $CD4^+$ T and B cell activation in the tumor microenvironment (23). In agreement with the literature, *LGALS9* expression was significantly increased, potentially contributing to early tumor development (23, 42). However, no significant differences in OS were observed for these genes among analyzed patients, which may relate, in part, to the available sample size.

OS analysis performed using TIMER2.0 based on TCGA-UCEC data enabled a more robust evaluation with a larger sample (n=545), revealing the significant prognostic impact of the combined overexpression of *CTLA4*, *PDCD1*, and *TIGIT* (Signature 1); *CD8A*, *CD8B*, *GZMB*, *PRF1*, and *TBX21* (Signature 2); *CTLA4* and *FOXP3* (Signature 3); *CD8A*, *CXCL9*, *GZMB*, and *PDCD1* (Signature 5); and *CD28* and *ICOS* (Signature 6) in EC patients, resulting in improved OS. These results should be interpreted in light of the fact that combined gene overexpression may confer better OS both through direct activation of immune effectors in the tumor microenvironment and by enhancing response to targeted therapies (9, 10, 21-24).

In parallel with network-based and immune signature approaches, machine learning models incorporating molecular and clinical variables have emerged as powerful tools for survival prediction in endometrial cancer. Population-based studies have demonstrated that integrative models can improve prognostic accuracy compared to clinicopathological factors alone. The immune network and gene signatures identified in this study may represent biologically meaningful features that could be incorporated into future predictive algorithms to refine individualized risk stratification (49).

This study is limited by its reliance on the quantity and quality of publicly available data due to its secondary nature. Nevertheless, a robust analysis of a data set still underexplored in the literature was conducted. This represents emerging knowledge with potential to enhance prognostic and therapeutic understanding of EC, guiding future studies toward targeted therapy development

for advanced and recurrent cases. By integrating TCGA-UCEC expression and co-expression data, the immune network constructed in this study moves beyond a generic immune interactome and reflects tumor-specific immune organization in endometrial cancer. This approach strengthens the biological plausibility of identified hubs and supports their relevance as prognostic and therapeutic biomarkers within this disease context.

Conclusion

The constructed immune network showed that *PDCD1* and *CD274* genes have a critical impact on immune system organization in the tumor microenvironment and on EC prognosis, interacting with various immune effectors. *CD40* and *LGALS9* showed significantly decreased and increased expression, respectively, in endometrial tumor patients, suggesting potential prognostic roles. OS analysis via six immune signatures demonstrated that combined overexpression of *CTLA4*, *PDCD1*, and *TIGIT*; *CD8A*, *CD8B*, *GZMB*, *PRF1*, and *TBX21*; *FOXP3*; *CXCL9*; *CD28*; and *ICOS* in their respective signatures was associated with better prognosis (longer OS), either through direct effects on innate or adaptive immune effectors or by enabling better efficacy of molecularly targeted therapies.

Supplementary Material

Supplementary data can be found at: https://doi.org/10.6084/m9.figshare.31004155

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author, REARC, on reasonable request.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

REARC: design of the work; interpretation of the data; draft of the work; revision of the work. JCT: draft of the work; revision of the work. LCZ: draft of the work; revision of the work. All authors have approved the submitted version.

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