

Identification of an Immune Cell Infiltration-Related Prognostic Model to Guide Immunotherapy in Endometrial Carcinoma

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Abstract

Background: Uterine corpus endometrial carcinoma (UCEC) is a prevalent gynaecologic malignancy. It has been demonstrated that the immune cell infiltration (ICI) pattern plays a critical role in the tumour progression of UCEC. **Methods**: To further investigate the immune microenvironment landscape of UCEC, we analysed the gene expression data of 539 UCEC patients from The Cancer Genome Atlas (TCGA) database using CIBERSORT and ESTIMATE for consensus clustering of immune cells. We used the limma package to compare differentially expressed genes (DEGs) among ICI patterns and constructed a prognostic model using Cox regression to calculate the risk score of UCEC patients. The immunophenoscore was downloaded to explore the immunotherapeutic effect between low- and high-risk score patients. Finally, the tumour mutation burden (TMB) was calculated using the somatic mutation data. **Results**: We identified two different immune infiltration patterns in 539 UCEC samples, the immune-desert and immune-inflamed phenotypes, which had distinct prognostic and biological features. We obtained 29 DEGs to construct the ICI-related prognostic model and established a four ICI-related gene-based prognostic model comprising *LINC01871*, *CXCL13*, *IGKJ5*, and *LINC01281*. The risk score was associated with distinct clinical outcomes, ICI, and immunotherapeutic effects. Patients with a low risk score had a significantly higher immunophenoscore, suggesting a better immunotherapeutic outcome. Finally, TMB was confirmed to be associated with prognosis, which was synergistic with the risk score. **Conclusions**: This study comprehensively analysed the ICI pattern in UCEC patients and established a four ICI-related gene-based prognostic model to predict prognosis and guide precise immunotherapy strategies.

Keywords: uterine corpus endometrial carcinoma; immune cell infiltration; prognostic model; immunotherapy

1. Introduction

Uterine corpus endometrial cancer (UCEC) is a prevalent type of malignant tumour in gynaecology. According to global cancer statistics in 2020, the incidence of endometrial cancer was 4.5%, ranking sixth among women's cancers, and its morbidity and mortality increase annually [1]. In 2020, it was estimated that there would be 65,620 new cases and 12,590 deaths [2]. In recent years, the incidence and mortality of UCEC have risen rapidly, particularly in association with obesity and diabetes [3]. The poor prognosis of UCEC is related to both the molecular subtype and clinical stage. In 2013, The Cancer Genome Atlas (TCGA) Research Network classified UCEC into four molecular subtypes based on genomic and transcriptomic data: polymerase epsilob (POLE) ultramutated, microsatellite instability hypermutated (MSI-H), copy-number low (CNL) and copy-number high (CNH). The POLE ultramutated subtype was found to have a better prognosis, whereas the CNH subtype had the poorest outcome [4]. Patients diagnosed at later stages are more likely to experience recurrence and

metastasis and have a poor prognosis. The 5-year survival rate for relapsed patients is 68%, while that for metastatic patients is only 17% [5]. Although traditional treatments, such as surgical resection, chemotherapy, and radiotherapy, have been greatly improved, overall survival (OS) has been not significantly improved. Therefore, a novel prognosis prediction and therapeutic response assessment method are urgently needed to facilitate precision and individualized treatment.

The tumour microenvironment (TME) consists of an extracellular matrix secreted by tumour cells, stromal cells, endothelial cells, immune cells, and tumour-associated cells. The high heterogeneity of TME phenotypes and cancer cell genotypes is one of the factors contributing to clinical treatment sensitivities and outcomes among tumour patients [6]. TME components interact with tumour cells and regulate their growth and development. In recent years, increasing evidence has shown that the occurrence, development, and metastasis of malignant tumours are associated with the TME [7–9]. The infiltration of immune cells and

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Fig. 1. The workflow of the study. TCGA, The Cancer Genome Atlas; UCEC, uterine corpus endometrial carcinoma; ICI, immune cell infiltration; DEGs, differentially expressed genes; ICB, immune checkpoint blockade; IPS, immunophenoscore; TMB, tumour mutation burden; ROC, receiver operating characteristic.

their interaction with cancer cells form a unique tumour immune microenvironment (TIME) [10]. The immune cell infiltration (ICI) in the TIME plays a decisive role in patient prognosis. Many studies have shown that there is significant ICI in UCEC tissue, and these immune cells are involved in tumour development [11]. It has been demonstrated that patients with high infiltration of CD8+ T cells and CD45RO+ T cells have a better prognosis in UCEC, whereas patients with high infiltration of tumour-associated macrophages have a worse prognosis [12]. Moreover, ICI has also been reported to play an important role in immunotherapy for UCEC [13,14]. Therefore, a deeper understanding of the landscape of ICI in UCEC may improve the precision of prognosis prediction and reveal optimal treatment strategies.

In this study, we explored the ICI landscape of 539 UCEC samples from the TCGA database. We aimed to identify immune-associated subtypes by unsupervised clustering of 22 infiltrating immune cells in UCEC and constructed a prognostic risk model based on the differentially expressed genes (DEGs) among the ICI subgroups. By analysing the correlation between risk score and molecular subtype, clinical stage, immune score, immune checkpoint, and tumour mutation burden (TMB), we aim to predict which UCEC patients may benefit from immunotherapy. In summary, our results imply that ICI is of great help for tumour immunotherapy, and our ICI-related prognostic model can provide a basis for selecting precision immunotherapy strategies in UCEC patients. The design and procedures are shown in Fig. 1.

2. Materials and Methods

2.1 Endometrial Carcinoma Data Collection

We obtained the transcriptomic data and corresponding clinical characteristics of UCECs from the TCGA database, consisting of a total of 583 UCEC samples. We excluded normal samples, duplicate samples, and samples with missing survival times, leaving us with 539 samples for analysis. The transcriptomic profiles of TCGA-UCEC in the fragments per kilobase per million (FPKM) were transformed into transcripts per kilobase million (TPMs).

2.2 Evaluation of Tumour Immune Cell Infiltration

We applied the CIBERSORT package (http://cibersor t.stanford.edu/) to obtain a fraction matrix of ICI using the TPM data, which estimated the abundances of 22 tumour infiltration immune cells (TIICs) [15]. The ESTIMATE algorithm was used to infer the tumour purity and calculate the immune score and stromal score. Additionally, singlesample gene set enrichment analysis (ssGSEA) was performed on UCEC samples using the GSVA package (http: //www.bioconductor.org/) based on the expression levels of immune-related markers. We then used the "ConsensusClusterPlus" package (http://www.bioconductor.org/) to divide the UCEC samples into two subgroups. To ensure the stability of the classification, the analysis process was repeated 1000 times using the unsupervised clustering "Pam" method. We used the "pheatmap" R package (https://cran.rstudio.com) to draw the heatmap.



2.3 Identification of Immune Cell Infiltration-Related Differentially Expressed Genes

We divided UCEC samples into two ICI clusters based on the consensus clustering algorithm. Differentially expressed genes (DEGs) between the different ICI subgroups were identified by using the "limma" package. The significance cut-off was set as an adjusted p value < 0.05 and absolute fold-change >1. Then, gene ontology (GO) annotation was performed by using the "ClusterProfiler" package (http://www.bioconductor.org/).

2.4 Establishment of the Immune Cell Infiltration-Related Prognostic Risk Model

The "survival" package was used to perform univariate Cox regression analysis to identify DEGs related to the OS of UCEC patients. Then, we conducted multivariate Cox regression to construct an ICI-related prognostic risk model, and the risk score was calculated by the following formula for each UCEC patient. Risk score = \sum_{i}^{n} coef (genei) × expr (genei). Coef (genei) was defined as the coefficient of gene i and expr (genei) was defined as the expression of gene i.

2.5 Association between the Risk Score and Molecular Subtypes and Clinical Phenotypes

According to the molecular classification method based on TCGA [16], we divided the 539 UCEC patients into four molecular subtypes: POLE, MSI-H, CNL and CNH. To investigate the correlation between the risk score and molecular subtypes, we generated box plots using GraphPad Prism v8.0.2 software (GraphPad Software, Inc., San Diego, CA, USA) and performed unpaired t tests to determine statistical significance. We used the same method to examine the relationship between risk score and clinical stage, survival status, and age groups.

2.6 Association between the Risk Score and Tumour Immune Environment

We divided the 539 UCEC patients into two groups based on the median risk score. To explore the correlation between the risk score and TIME, we used the Wilcox test and drew box plots to demonstrate the difference in TIME between the low- and high-risk groups.

2.7 Association between the Risk Score and Immunotherapy

Research has demonstrated that the expression levels of certain key genes related to immune checkpoint blockade (ICB) may be correlated with the clinical outcomes of immune checkpoint inhibitor blockade therapy. To investigate the potential role of the risk score in immunotherapy, we analysed the correlation between the risk score and the expression levels of ICB-related genes. Here, we focus on the six key ICB genes, namely, *PD-L1 (CD274)*, *PD-1 (PDCD1)* [17], *PD-L2 (PDCD1LG2)*, *TIM-3 (HAVCR2)*, *CTLA-4* [18], and *IDO1* [19], that have been reported in previous studies. Additionally, we further compared the correlation between nine inflammation-related genes (*TNF*, *TBX2*, *PRF1*, *IFNG*, *CD8A*, *CLCL9*, *CXCL10*, *GZMA*, and *GZMB*) [20] and the risk score. To evaluate the potential impact of immunotherapy predicted by the risk score, we also analysed the correlation between the immunophenoscore (IPS) and the risk score of UCEC patients. The IPS data for UCEC patients are available for download from The Cancer Immunome Atlas (TCIA).

2.8 Construction of the Prognostic Nomogram

To enhance the interpretability of the prognostic risk model and facilitate patient evaluation, we developed a nomogram using the "rms" R package, which integrates the risk score and age to estimate OS at 1, 3, and 5 years. The nomogram assigns a score to each factor based on their respective contribution to the outcome variable and sums them to obtain the total score. To validate the prognostic value of the nomogram, we used calibration and receiver operating characteristic (ROC) curves.

2.9 Tumour Burden Mutation Analysis

To reveal the intrinsic link between the TIME and somatic mutations, we conducted an analysis to assess the correlation between TMB and risk score. Somatic mutation data were obtained from the TCGA database, and the "maftools" package (http://www.bioconductor.org /) was utilized to calculate the number of somatic nonsynonymous point mutations in each sample, which corresponded to the TMB. Furthermore, we generated separate waterfall plots for the low- and high-risk groups to visually display the distribution of gene mutations among patients in each risk score group.

2.10 Statistical Analysis

All statistical analyses were conducted using R version 4.1.0 (University of Auckland, Auckland, New Zealand). The fraction of 22 TIICs was calculated using the "CIBERSORT" package in R. The Wilcoxon test was used to compare differences between two groups. DEGs were identified using the "limma" package in R. Survival analysis was performed by the Kaplan-Meier (K-M) method, and comparisons between groups were made using the log-rank test. The "survivalROC" and "timeROC" packages were used to generate the ROC curve. GSEA was performed using GSEA software version 4.1.0 (Broad Institute, Inc., Cambridge, MA, USA).

3. Results

3.1 Identification of Two Immune Cell Infiltration Patterns

To further analyse the TIME in UCEC, we calculated the level of 22 immune cells in a total of 539 samples. Based on the ICI profile, the 539 samples were classified into different subtypes using the "ConsensusClusterPlus" package. To ensure sample equilibrium and clustering stability, we chose k = 2 (**Supplementary Fig. 1**), resulting in the division of samples into two ICI patterns, ICI Cluster A (329 samples) and ICI Cluster B (210 samples). We drew a comprehensive heatmap to further describe and explore the relationship between ICI patterns and clinical phenotypes, including age and survival status (Fig. 2A). Furthermore, we conducted Kaplan–Meier survival analysis to compare the prognosis of the two ICI subgroups and found that ICI Cluster B had a better prognosis than ICI Cluster A, although the difference was not statistically significant (p = 0.31, Fig. 2B).

To further investigate the association between immune scores and infiltrating immune cells, we compared the TIME between the two clusters. Our analysis revealed that ICI Cluster B displayed higher levels of plasma cells, CD8+T cells, activated state CD4+T cells, follicular helper T cells, regulatory T cells, and M1 macrophages, as well as high stromal and immune scores. These findings are consistent with previous studies showing that the abundance of these immune cells significantly correlates with antitumour immune status [21]. Furthermore, we compared the correlations between immune cells and the immune score, stromal score, and ESTIMATE score (Fig. 2C,D). Among these immune cells, plasma cells, CD8+ T cells, activatedstate CD4+ T cells, regulatory T cells, and M1 macrophages were significantly and positively correlated with immune scores. Therefore, we defined ICI Cluster B as immunoinflammatory. In contrast, most immune cell infiltrates were significantly lower in ICI Cluster A, except for restingstate CD4+ T cells, M0 macrophages, activated-state dendritic cells, higher levels of neutrophils, and lower stromal and immune scores. Resting-state CD4+ T cells, M0 macrophages, and activated-state dendritic cells were significantly and negatively correlated with immune scores. Therefore, ICI Cluster A can be considered the immune desert type.

To further assess the value of ICI subgroups in predicting prognosis, we conducted Kaplan–Meier survival analysis based on the different ICI subgroups. The results showed that regulatory T cells and CD8+ T cells enriched in ICI Cluster B were associated with a longer survival time and a good prognosis (Fig. 2E,F). In contrast, a high level of monocytes, which were enriched in ICI Cluster A, showed a poor prognosis (Fig. 2G).

Furthermore, we compared the expression levels of six key immune checkpoint blocking genes (*PD-1*, *CTLA-4*, *TIM-3*, *IDO1*, *PD-L1*, and *PD-L2*) in the two ICI clusters (Fig. 3). Our analysis revealed that the expression levels of ICB-related genes were significantly higher in ICI Cluster B than in ICI Cluster A, suggesting that ICI cluster B may be more suitable for immunotherapy.

3.2 Establishment of the Immune Cell Infiltration-Related Prognostic Signature

Using the limma package, we identified 29 DEGs between the two ICI clusters, which were defined as ICI- related genes (Table 1). We performed GO enrichment analysis with the ICI-related genes and found that most biological functions were related to the immune response, such as immunoglobulin production, humoral immune response, immunoglobulin complex, and immunoglobulin receptor binding (**Supplementary Fig. 2**). We then performed univariate Cox and multivariate Cox regression analyses to identify the ICI-related genes associated with OS and to establish a prognostic risk model. Finally, an optimal prognostic model was obtained: risk score = $(0.9842 \times expr$ (*LINC01871*)) + $(1.0121 \times expr$ (*LINC01281*)) + $(0.9983 \times$ expr (*CXCL13*)) + $(0.9990 \times expr$ (*IGKJ5*)). Kaplan–Meier analysis revealed that a high level of the four genes was significantly associated with longer survival time (Fig. 4).

To further validate the prognostic role of risk scores, risk curves and scatter plots were constructed according to the survival status of each sample (Fig. 5A,B). The risk curves and scatter plots visualized the risk scores and survival status of TCGA-UCEC patients, illustrating that patient death was closely associated with high-risk scores. Patients were divided into high- and low-risk groups with the median risk score as the cut-off. The Kaplan-Meier survival curve showed that the low-risk group had a longer survival time (p < 0.001, Fig. 5C). Additionally, we analysed the correlation between the risk score and molecular subtypes and clinical stages. We found that the POLE and MSI-H subtypes had a significantly lower risk score, while the CNH subtype had a significantly higher risk score (Fig. 5D). These findings suggest that the POLE and MSI-H subtypes had a better prognosis, and the CNH subtype had a worse prognosis, which is consistent with a previous report [16]. The stage III and stage IV groups had a higher risk score than the stage I and stage II groups, implying that early diagnosis is beneficial to prognosis (Fig. 5E). Moreover, we analysed the correlation between the risk score and clinical phenotypes (survival status and age). We found that the deceased group had a significantly higher risk score (Fig. 5F), but there were no significant differences between age groups (Fig. 5G).

3.3 Association between the Risk Score and Tumour Immune Environment

As the risk score was calculated by the ICI-related prognostic model, we further explored the differences in the tumour immune environment between the high- and low-risk groups. Our results showed that the risk score was negatively correlated with the level of various immune cells closely related to the antitumour immune response, including plasma cells, CD8+ T cells, activated state CD4+ T cells, follicular helper T cells, regulatory T cells, and M1 macrophages (Fig. 6A). Patients with low risk scores had higher stromal scores, immune scores, and ESTIMATE scores than patients with high risk scores (Fig. 6B). The ssGSEA results showed that both ICI and immune signal enrichment were significantly higher in the low-risk group





Fig. 2. The landscape of ICI in UCEC. (A) Heatmap of immune cells in UCEC patients. (B) K-M curves for the OS of UCEC patients in two ICI clusters. (C) The levels of immune cells, immune scores, and stromal scores in two ICI clusters. (D) Correlation of immune cells and immune scores. (E–G) OS analysis between patients with different infiltration levels of immune cells. (E) CD8+ T cells. (F) Regulatory T cells. (G) Monocytes. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant. OS, overall survival; NK, natural killer; K-M, Kaplan-Meier.



Fig. 3. The expression levels of six ICB genes in two ICI clusters. (A) *PD-1*. (B) *PD-L1*. (C) *PD-L2*. (D) *CTLA-4*. (E) *TIM-3*. (F) *IDO1*. **p < 0.01; ****p < 0.001. ICB, immune checkpoint blockade.

| Gene | logFC | AveExpr | t | p value | adj.p.Val | В |
|-----------|----------|----------|----------|--------------------------|-----------------------|----------|
| IFNG | 0.690138 | 4.619117 | 13.77466 | $3.43 	imes 10^{-37}$ | 3.16×10^{-33} | 73.68758 |
| LINC02446 | 0.642649 | 4.549493 | 12.79338 | $6.62 	imes 10^{-33}$ | 2.16×10^{-29} | 63.93433 |
| LINC01871 | 0.536552 | 4.811053 | 12.56105 | 6.49×10^{-32} | 1.63×10^{-28} | 61.68009 |
| RTP5 | 0.510671 | 4.106001 | 12.02844 | 1.11×10^{-29} | 1.81×10^{-26} | 56.59841 |
| CXCL13 | 0.564699 | 5.388636 | 11.63992 | 4.38×10^{-28} | 5.64×10^{-25} | 52.97128 |
| LINC01281 | 0.51517 | 4.183521 | 11.33138 | 7.71×10^{-27} | 8.70×10^{-24} | 50.14149 |
| IGLC6 | 0.555786 | 4.302542 | 10.47021 | 1.79×10^{-23} | 1.31×10^{-20} | 42.49629 |
| IGKV1.8 | 0.612746 | 4.640868 | 9.63965 | 2.16×10^{-20} | 1.11×10^{-17} | 35.50488 |
| IGKV1D.12 | 0.537981 | 4.131287 | 9.47581 | 8.36×10^{-20} | 3.86×10^{-17} | 34.17319 |
| IGLV3.1 | 0.547847 | 5.174836 | 9.304659 | 3.38×10^{-19} | 1.38×10^{-16} | 32.79948 |
| IGLC7 | 0.587786 | 4.819901 | 9.109792 | $1.62{\times}\;10^{-18}$ | 6.01×10^{-16} | 31.25746 |
| IGHV1.24 | 0.552303 | 5.045554 | 8.980839 | 4.51×10^{-18} | 1.55×10^{-15} | 30.25016 |
| IGKV1.12 | 0.512237 | 4.152039 | 8.842155 | 1.34×10^{-17} | 4.24×10^{-15} | 29.17869 |
| IGKV1.9 | 0.529306 | 5.143034 | 8.767901 | 2.39×10^{-17} | 7.12×10^{-15} | 28.6101 |
| IGKV1D.16 | 0.517931 | 4.363243 | 8.593826 | 9.17×10^{-17} | 2.40×10^{-14} | 27.29129 |
| IGHV3.20 | 0.550608 | 4.550816 | 8.549534 | 1.29×10^{-16} | $3.22 	imes 10^{-14}$ | 26.95891 |
| IGLV1.36 | 0.535547 | 4.607999 | 8.54105 | 1.37×10^{-16} | 3.40×10^{-14} | 26.89539 |
| IGKJ5 | 0.520335 | 4.383708 | 8.474194 | 2.28×10^{-16} | $5.53 	imes 10^{-14}$ | 26.39655 |
| IGHV2.70 | 0.571032 | 4.700231 | 8.465907 | 2.43×10^{-16} | $5.84 	imes 10^{-14}$ | 26.33492 |
| IGLV7.43 | 0.517652 | 4.898322 | 8.364099 | 5.23×10^{-16} | $1.23 	imes 10^{-13}$ | 25.58161 |
| IGHV1.69D | 0.527484 | 5.041317 | 8.31834 | 7.37×10^{-16} | 1.66×10^{-13} | 25.2453 |
| IGLV3.27 | 0.539226 | 4.720764 | 8.213847 | 1.60×10^{-15} | 3.38×10^{-13} | 24.48266 |
| IGKV1.17 | 0.511528 | 5.01979 | 8.088337 | 4.04×10^{-15} | 8.00×10^{-13} | 23.57654 |
| IGKV1.16 | 0.503234 | 5.066696 | 8.022742 | 6.51×10^{-15} | 1.26×10^{-12} | 23.10732 |
| IGHV1.3 | 0.515697 | 4.404484 | 7.997288 | 7.84×10^{-15} | 1.48×10^{-12} | 22.92604 |
| IGLV9.49 | 0.531905 | 4.728355 | 7.870241 | 1.96×10^{-14} | 3.51×10^{-12} | 22.02805 |
| IGHV1.69 | 0.511032 | 4.838856 | 7.788804 | 3.50×10^{-14} | 5.95×10^{-12} | 21.45843 |
| IGLV4.60 | 0.520134 | 4.652707 | 7.764731 | 4.16×10^{-14} | 6.93×10^{-12} | 21.29095 |
| IGKV2D.29 | 0.510528 | 4.673165 | 7.564385 | 1.70×10^{-13} | 2.51×10^{-11} | 19.91324 |

| Table 1. | . Differentially | expressed | genes | between | the tw | o ICI clusters. |
|----------|------------------|-----------|-------|---------|--------|-----------------|
|----------|------------------|-----------|-------|---------|--------|-----------------|

logFC, log(fold change).





Fig. 4. Kaplan–Meier OS curves of four ICI-related genes. (A) *LINC01871*. (B) *LINC01281*. (C) *CXCL13*. (D) *IGKJ5*. Patients with high expression of ICI-related genes showed a good prognosis (red curves), while patients with low expression of ICI-related genes showed a poor prognosis (blue curves).

than in the high-risk group (Fig. 6C). The heatmap exhibited the enrichment of the immune features of the patients in the high-risk and low-risk groups correspondingly (Fig. 6D). Based on these results, the high-risk and low-risk groups exhibited significantly different ICI phenotypes. Patients with low risk scores showed an immune-inflamed phenotype with a large number of immune cell infiltrates, while patients with high risk scores showed an immune desert phenotype with less immune infiltration. These findings indicated that the risk score was closely related to the TIME and may play an important role in immunotherapy.

3.4 Association between the Risk Score and Immunotherapy

In this study, we analysed ICB-related genes and inflammation-related genes to assess tolerance and immunoreactivity in patients with different risk scores (Fig. 7A). Almost all genes were significantly upregulated in patients with a low risk score, except for *TBX2* and *TNF*. These results imply that patients with a low risk score have better tolerability and immunoreactivity. To further reveal the potential role of the risk score in the ICB treatment of UCEC patients, we examined the correlation between the six key ICB genes and the risk score and drew pie charts (Fig. 7B). We found that the expression of all six genes was significantly negatively correlated with the risk score (Fig. 7C–H). These results suggest that the risk score may play an important role in predicting the response to ICB treatment in UCEC patients.

Two subtypes of IPS, IPS-PD1-positive and IPS-CTLA4-positive, were used as proxies for immunotherapy response in UCEC patients to assess the effect of immunotherapy predicted by risk scores (Fig. 7I–L). Our results showed that the IPS-CTLA4 and PD1 blockade score, IPS-CTLA4 blockade score, IPS-PD1 blockade score, and IPS score were significantly higher in the low-risk group. This indicates that patients with low risk scores are more suitable for immunotherapy.

3.5 Construction of the Prognostic Nomogram

We constructed a nomogram to predict the 1-, 3-, and 5-year OS of UCEC samples using the risk score and age



Fig. 5. Evaluation of the ICI-related prognostic model. (A) Distribution of the risk score for each patient. (B) Survival status for each patient. Red points correspond to surviving patients, and blue points correspond to deceased patients. (C) Survival analysis between patients with different risk scores. (D) Risk score in different molecular subgroups. (E) Risk score in different clinical stages. (F) Risk score in the surviving and deceased groups. (G) Risk score in different age groups. *p < 0.05; **p < 0.01; ****p < 0.0001; ns, not significant.

(Fig. 8A). To validate the performance of the prognostic nomogram, we plotted calibration curves (Fig. 8B–D). Furthermore, we drew an ROC curve to further evaluate the constructed prognostic model, and the area under curve (AUC) values of 1-, 3-, and 5-year OS were 0.642, 0.658, and 0.716, respectively (Fig. 8E). These results indicate that the model had high sensitivity and specificity.

3.6 Potential Association between the Risk Scores and Tumour Mutation Burden

It has been reported that high levels of TMB are associated with high infiltration of CD8+ T cells [22]. CD8+ T cells recognize tumour neoantigens and induce an immune response to destroy tumour cells. Therefore, TMB may serve as a prognostic factor for the effectiveness of the response to antitumour immunotherapy. In our study, we assessed the TMB levels in patients with both low and high risk scores and found that patients with a low risk score had a higher TMB (Fig. 9A). Additionally, our correlation analysis revealed a significant negative correlation between TMB and the risk score (Fig. 9B), indicating that a lower risk score may be associated with higher TMB and thus with a better response to antitumour immunotherapy.

Then, we divided the patients into two groups based on their TMB levels. Kaplan–Meier survival analysis showed that patients with high TMB had a greater probability of survival (Fig. 9C). To further assess the prognostic value of the risk score and TMB, we evaluated the synergistic effect of these two factors in predicting the survival of patients with UCEC (Fig. 9D). Patients with both a high TMB and low risk score exhibited the best survival probability, while patients with a low TMB and high risk score had the worst survival probability. These findings suggest that the risk score may serve as an independent prognostic factor and may be utilized to predict the clinical outcome of antitumour immunotherapy.

We also analysed the distribution of mutations between the low- and high-risk groups. The integrated so-



Fig. 6. Association between the risk score and TIME characteristics. (A) Immune cell infiltrating abundances in the low- and high-risk score groups. (B) Differences in stromal score, immune score, and ESTIMATE score between the low- and high-risk score groups. (C) Difference in immune functions between the low- and high-risk score groups. (D) Heatmap of immune cell infiltrating abundances between the low- and high-risk score groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant; TIME, tumour immune microenvironment.

matic mutation landscape was used to visualize the mutation patterns of the top 20 most frequently mutated driver genes (Fig. 9E,F). The mutation profile of significantly mutated genes showed a higher mutation rate of TP53 (41% vs. 33%) in the high-risk group and a higher mutation rate of TTN (49% vs. 27%) and KMT2D (35% vs. 18%) in the low-risk group. These findings provide deeper insight into the intrinsic link between the risk score and somatic mutations in UCEC immunotherapy.

4. Discussion

Endometrial cancer is a prevalent morbidity and malignant tumour in females. Its occurrence and development are regulated by various mechanisms, many of which are related to the TME [23]. The high heterogeneity of tumour microenvironmental phenotypes and cancer cell genotypes leads to different clinical treatment sensitivities and distinct clinical outcomes. Therefore, we need to seek a new treatment strategy to increase host antitumour immunity during esophageal cancer (EC) treatment. Immune-based antitumour therapy has made significant breakthroughs in a variety of cancers [24], and studies have demonstrated that infiltrating immune cells have an important impact on tumour progression and antitumour immunity [25,26]. This study aimed to analyse the immune microenvironment of UCEC through omics data and construct the first ICI-related prognostic model to monitor the prognosis and response to immunotherapy in UCEC patients.

Previous studies have shown that the level of immune cells associated with immune activation affects the clinical outcome of immunotherapy [27,28]. This study verified that the prognosis of UCEC is related to the immune microenvironment and found that patients with high levels of CD8+ T cells and regulatory T cells have a better survival. Furthermore, the immune microenvironment of UCEC can be divided into two different types: immune-inflamed phenotype and immune-desert phenotype. We can calculate the immune scores of different phenotypes to predict the prognosis of UCEC. This is the first study to establish immune microenvironment typing of UCEC, which is expected to be used in immunotherapy.

This study divided the 539 samples into two groups with distinct immune phenotypes. The K-M curves showed that ICI Cluster B had better OS than ICI Cluster A, although this difference was not statistically significant. This may be due to the limitation of the sample size. ICI Clus-



Fig. 7. Correlation of risk score with immunotherapy. (A) Differences in the expression levels of ICB-relevant genes (*CD274*, *PDCD1LG2*, *PDCD1*, *CTLA4*, *IDO1*, and *HAVCR2*) and inflammation-related genes (*CD8A*, *CXCL10*, *CXCL9*, *GZMA*, *GZMB*, *IFNG*, *PRF1*, *TBX2*, and *TNF*) between the low- and high-risk score groups. (B–H) Correlation between the risk score and immune check inhibitors. (I–L) Difference in the IPS score between the low- and high-risk score groups. *p < 0.05; **p < 0.01; ****p < 0.001; ns, not significant.

ter B had high levels of multiple immune cells, including CD8+ T cells, regulatory T cells, activated state CD4+ T cells, follicular helper T cells, and M1 macrophages, with high stromal and immune scores. These scores have been reported to be significantly correlated with antitumour immune status [18]. Therefore, we defined ICI Cluster B as an immune-inflamed phenotype. However, we defined ICI Cluster A as an immune-desert phenotype, with low stromal and immune scores.

In addition, we calculated the DEGs between ICI Cluster B and ICI Cluster A to obtain ICI-related genes. The results of the GO enrichment analysis based on the DEGs were mainly related to the immune response, demonstrating that the DEGs are associated with immunotherapy. We then constructed a prognostic model to calculate the risk score of UCEC patients based on four ICI-related genes (*LINC01871*, *LINC01281*, *CXCL13*, and *IGKJ5*). K-M survival analysis showed that the expression of the four genes was significantly associated with a longer survival time. *LINC01871* has been used to predict the prognosis of various cancers, including breast cancer, cervical can-

cer, and endometrial cancer [29–31]. Guihai Zhang *et al.* [32] identified 4 lncRNAs, including *LINC01281*, to predict the prognosis of laryngeal cancer. Jing Ye *et al.* [33] reported that cervical cancer patients with high levels of *LINC01281* had better survival. Many studies have shown that *CXCL13* is related to prognosis. For example, Liangliang Liao *et al.* [34] identified three prognostic markers, including *CXCL13*, in colon adenocarcinoma (COAD). Sidsel C Lindgaard *et al.* [35] reported that *CXCL13*, together with *IL-6*, *PDCD1*, and *TNFRSF12A*, can be used to discriminate patients with OS <90 days from those with OS >2 years in advanced pancreatic ductal adenocarcinoma (AUC = 0.97). *IGKJ5* had not been previously reported, suggesting that it may be a new prognostic marker for UCEC.

For the first time, we constructed an ICI-related prognostic model for UCEC and calculated the risk score using the gene expression levels. The risk score is not only related to survival time but also closely related to the immune microenvironment, which may guide the efficacy of immunotherapy. To explore the role of the risk score, we



Fig. 8. Evaluation of the risk score in prognosis. (A) A nomogram was constructed using age and risk score to predict the OS of UCEC patients. (B) 1-year nomogram calibration curves. (C) 3-year nomogram calibration curves. (D) 5-year nomogram calibration curves. (E) ROC curves for 1-, 3-, and 5-year survival. OS, overall survival; UCEC, uterine corpus endometrial carcinoma; ROC, receiver operating characteristic; AUC, area under curve.

divided the patients into low- and high-risk groups using the median score as the cut-off. We observed that the lowrisk group had better survival, indicating that the risk score may serve as a prognostic marker. We also compared the ICI patterns between the two groups, and the results showed that the low-risk group had a high stromal score, immune score and almost all immune-relevant signatures. These findings suggest that the low-risk group may be an immuneinflamed phenotype, and the risk score may serve as an immunosuppressive indicator. Moreover, we discovered that the risk score was significantly and negatively correlated with the expression of six ICB-related genes, indicating that patients with a low risk score might be more sensitive to immunotherapy. The immunophenoscore analysis showed

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that the low-risk score group had statistically higher IPS-CTLA4 and PD1 scores, suggesting that patients with a low risk score had a better response to CTLA4 and PD1 therapy. These findings signify that the risk score might serve as a powerful prognostic tool for UCEC patients.

Endometrial cancer can be effectively cured when diagnosed at an early stage. However, the treatment options become limited once the cancer reaches an advanced or metastatic stage. Tumours with high mutation rates and mismatch repair deficiency (dMMR) are more responsive to immunotherapy likely due to the increased expression of neoantigens that can be recognized by the immune system. Recent clinical studies have shown that immune checkpoint inhibitors, such as PD-1/PD-L1 inhibitors, have a signifi-



Fig. 9. Correlation between the risk score and TMB. (A) Difference in TMB between the low- and high-risk score groups. (B) Scatterplots depicting the negative correlation between risk score and TMB. (C) Survival analyses for patients with low and high TMB using K-M curves. (D) Survival analyses for patients stratified by both the risk score and TMB using K-M curves. The waterfall plot of tumour somatic mutations was constructed using the high-risk score (E) and low-risk score (F). ****p < 0.0001. TMB, tumour mutation burden.

cant effect on chemotherapy-resistant metastatic EC, especially in tumours with dMMR [36–38]. However, the relationship between tumour cell mutations and the immune microenvironment in immunotherapy is not yet clear. In this study, we aimed to establish an appropriate evaluation system for the TIME and develop a prognostic model. Our results showed that the POLE and MSI-H subtypes had lower risk scores, indicating that patients with the POLE and MSI-H subtypes may be more suitable for immunotherapy, while those with the CNH type had the highest risk score. These patients may require combination therapy for better treatment outcomes. These results were consistent with the molecular typing of endometrial cancer, where tumours with high mutation rates had the lowest risk score for ICI, indicating the potential for effective immunotherapy [38]. Our findings may aid in the clinical decision-making process for immunotherapy, but further experimental and clinical validation is needed.

Tumour mutation burden has been reported to be a predictive indicator of sensitivity to antitumour immunotherapy in various studies [39,40]. We calculated the TMB using somatic mutation data, and the results showed that TMB was significantly and negatively correlated with the risk score. Patients with a high TMB had better survival in UCEC, which was consistent with previous reports [41]. The synergistic effect of the TMB and risk score showed that patients with low risk scores and a high TMB had the best survival. All the above results demonstrate that the prognostic model may predict the benefit of immunotherapy in UCEC.

5. Conclusions

In conclusion, 539 UCEC samples were classified into two ICI patterns. Different ICI patterns had a distinct immune microenvironment, so we defined them as "immuneinflamed" and "immune-desert" phenotypes. Moreover, we established an ICI-related prognostic model to predict the prognosis of UCEC patients based on the DEGs between the two ICI patterns. The correlation between the risk score and expression of ICB genes was used to estimate the treatment effect of UCEC patients. Finally, we demonstrated the synergistic effect between the risk score and TMB in predicting prognosis. The ICI-related prognostic model needs further experimental verification and is expected to be used to direct precision immunotherapeutic strategies.

Availability of Data and Materials

All data used in this study were acquired from The Cancer Genome Atlas (TCGA) portal: TCGA-UCEC (http s://portal.gdc.cancer.gov).

Author Contributions

HZ, LS, XN, and QW designed the research study. HZ and FL performed the data analysis. FL, WH, SZ, and JC constructed the figures. FL and HZ wrote the manuscript. LS, XN, and QW reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.All authors contributed to the article and approved the final version.



Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10. 31083/j.ceog5010204.

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