Tumoral programmed cell death 1 (PD1) expression in endometrial carcinoma is a prognostic marker for patient outcome

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ABSTRACT

Objective Immune checkpoint inhibitors have recently demonstrated benefit in patients with advanced and recurrent endometrial carcinoma. This retrospective study investigated immune checkpoint molecules in endometrial carcinoma as they pertain to the molecular subtypes, clinical outcomes, and predictive value.

Methods Tumoral RNA expression of genes controlling the immune checkpoint, programmed cell death 1 (PD1, encoded by *PDCD1*), its ligand (PDL1, encoded by *CD274*), and interferon gamma (*IFNG*) was determined in 239 endometrial carcinoma tissues by quantitative polymerase chain reaction (qPCR) and compared with endometrial tissue from 25 controls. A total of 81 endometrial carcinoma tissues were analyzed using the ProMiSe molecular classification, and patient trajectories were analyzed for the entire cohort. Findings were validated in an independent cohort from The Cancer Genome Atlas (TCGA; n=548).

Results *PD1*, *PDL1,* and *IFNG* expression was significantly higher in endometrial carcinoma when compared with non-malignant control tissue with a mean expression of 0.12, 0.05, and 0.05 in control tissue and 0.44, 0.31, and 0.35 in endometrial carcinoma, respectively. *POLE*-mutated and mismatch repair-deficient (MMRd) (immunologically hot) tumors showed the highest expression of *PD1* and *IFNG*. Increased expression of *PD1*, *PDL1,* and *IFNG* was associated with improved recurrence-free (HR 0.32, p<0.001; HR 0.30, p<0.001; HR 0.47, p=0.012, respectively), disease-specific (HR 0.38, p<0.001; HR 0.29, p<0.001; HR 0.45, p=0.017, respectively), and overall survival (HR 0.56, p=0.003; HR 0.38, p<0.001; HR 0.58, p=0.006, respectively). Cox regression confirmed the prognostic significance of *PD1* for recurrence-free survival (HR 0.39, p=0.009) and *PDL1* for overall survival (HR 0.55, p=0.037). The prognostic value of tumoral *PD1* on recurrence-free survival, disease-specific survival, and overall survival was confirmed in the TCGA cohort.

Conclusions Tumoral gene expression controlling the PD1 immune checkpoint, particularly expressed in "hot tumors", predicted recurrence-free, disease-specific, and overall survival in patients with endometrial carcinoma in two independent cohorts. Evaluation of these genes could be used to stratify patients who qualify for immune checkpoint inhibitors, which warrants prospective clinical trials.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Blocking immune checkpoints such as programmed cell death 1 (PD1) or its ligand PDL1 is a potential treatment option for patients with advanced endometrial carcinoma. While patients with mismatch repair-deficient (MMRd) tumors usually benefit from immune checkpoint inhibitor treatment, other predictors are not well understood.

WHAT THIS STUDY ADDS

⇒ Our study reveals that immune checkpoint molecules can serve as prognostic markers in endometrial carcinoma, particularly tumoral *PD1*, which predicts clinical outcomes of these patients. Furthermore, *PD1* is upregulated in immunologically hot tumors, which are known to respond well to immune checkpoint blockade.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ *PD1* expression could stratify patients for immune checkpoint inhibitor therapy in endometrial carcinoma; however, prospective clinical trials are needed to confirm this concept.

INTRODUCTION

Endometrial carcinoma is the most common gynecological tumor in Europe, with increasing incidence worldwide that may partly be explained by accumulating risk factors such as aging and obesity.[1] The prognosis for patients diagnosed with early-stage endometrial carcinoma remains favorable, whereas recurrent or metastatic disease is associated with poor outcome due to limited surgical and systemic (targeted) treatment options.[2] In recent years, introduction of molecular groups has allowed for implementation of precision medicine in endometrial carcinoma.[3] Such molecular classification categorizes four distinct molecular groups based on their transcriptional profile[4 5]: DNA polymerase epsilon (*POLE*)-ultramutated (*POLE*mut, ie, *POLE* EDM), mismatch-repair deficient (MMRd (ie, microsatellite instable (MSI)), no specific molecular profile (NSMP; ie, p53-wt), and p53 aberrant (ie, p53-abn, p53-mut).

Observing that patients with *POLE*mut endometrial carcinoma exhibit the best outcome and patients with p53-abn endometrial carcinoma have the poorest clinical survival has clarified the prognostic value of these parameters and may guide therapeutic decisions.[6]

Immune checkpoint inhibitors blocking CTLA-4 or programmed death 1 (PD-1) or its ligand PD-L1 have demonstrated robust therapeutic efficacy in various cancer entities even in advanced stages, and have revolutionized the practice of medical oncology.[7] The PD-1 axis is an immunosuppressive pathway that allows tumor cells to remain undetected by the immune system. In more detail, interferon-γ (IFN-γ) is common in the tumor microenvironment and body inflammation and induces the transcription of the *PDL1* gene, which encodes for the programmed death-ligand 1 (PD-L1).[8] When engaged to its receptor, PD-1 strongly interferes with T-cell receptor signal transduction allowing the tumor cell to escape immune-induced apoptosis. Interfering with PD-1 signal transduction either by antibody blockade or any other means enhances T-cell functions by potentiating signal transduction from the T-cell receptor (TCR) signalosome and inducing programmed cell death.[9] However, not every tumor entity responds to immune checkpoint inhibitors and efficacy varies between tumor types and patients. To improve success of therapy (and diminish potentially unnecessary toxicity of these compounds) predictive markers of response to immune checkpoint inhibition reflect an unmet clinical need for most tumor types.[10]

In 2017, pembrolizumab was approved by the US Food and Drug Administration (FDA) for patients with mismatch repair deficient (MMRd) or high microsatellite instable (MSI-H) tumors.[2 11] Two years later, the FDA provided breakthrough therapy designation to lenvatinib combined with pembrolizumab for the treatment of patients with advanced endometrial carcinoma that has progressed after at least one previous systemic therapy.[2 12] Dostarlimab, a PD-1 inhibitor, has recently also been approved for patients with advanced MSI-H/MMRd endometrial carcinoma.[3 13 14] Ongoing studies are investigating immune checkpoint inhibition combined with other targeted agents such as poly (ADP-ribose) polymerase inhibitors (PARPis).[15] In endometrial carcinoma, MSI-H or MMRd are predictors for immune checkpoint inhibitors; however, predictors for other genomic endometrial carcinoma subtypes are warranted.[2]

We hypothesized that the expression profile of genes involved in immunosuppressive pathways are of prognostic value in endometrial carcinoma. Here, we have investigated the expression of the immune checkpoint genes *PD1* and *PDL1* and their regulator *IFNG* in 239 endometrial carcinoma patients. We further analyzed the association with clinicopathological features and molecular subtypes and validated their predictive value in a second independent cohort comprising 548 patients (The Cancer Genome Atlas (TCGA) dataset).

METHODS

Patients and Samples

Endometrial tissue specimens from 239 endometrial carcinoma patients obtained at primary surgery and control tissue from 25 patients undergoing hysterectomy for non-malignant conditions such as fibroids were collected and processed by the Department of Obstetrics and Gynecology of the Medical University of Innsbruck between 1989 and 2015 as described recently.[16]

The Ethics Committee of the Medical University of Innsbruck (Ref. No.: 1210/2021) approved the study, which was conducted in accordance with the Declaration of Helsinki. Bokhman's type I and II classification was used to assess carcinoma risk. Patient characteristics are listed in Online Supplemental Table 1.

RNA Isolation and Reverse Transcription

Total cellular RNA extraction from endometrial tissue specimens and transcription were performed as described previously.[16]

Quantitative Real-time Polymerase Chain Reaction (qPCR)

Assays on demand for checkpoint genes PD1 (PDCD1; Hs01550088_m1), PDL1 (CD274; Hs00204257_m1), and interferon gamma (IFNG) (Hs00174143_m1) were purchased from Thermo Fisher Scientific (Waltham, MA, USA) as well as assays for the endogenous controls TATA box-binding protein (Hs99999910_m1)[16].

ProMiSe Molecular Subtypes

In a cohort of 81 patients, molecular subtypes were defined and assigned according to the ProMisE criteria.[17] Expression of MMR-proteins (MLH1, MSH2, MSH6, and PMS2) and p53 expression was assessed by immunohistochemistry. *POLE* mutation status was assessed by SNP mutation analysis for five known hotspots: P286R, V411L, S459F, S297F, and A456P. Patient characteristics are listed in Online Supplemental Table 2.

TGCA Cohort

To validate our results all analyses were performed on the TCGA publicly available dataset retrieved via firebrowse.org and from previous TGCA analysis.[18] Qualified patients were those with endometrial carcinoma and comprehensive data on age at diagnosis, tumor grade, International Federation of Gynecology and Obstetrics (FIGO) stage, survival, and gene expression analyses. Patient characteristics are listed in Online Supplemental Table 3.

Statistical Analysis

The non-parametric Mann–Whitney U test or Kruskal–Wallis test were applied to determine statistical significance between two or more groups, respectively. The Shapiro–Wilk test was used to test for normal distribution of data. For normal distributed data the Student's two-tailed *t*-test was used to test for statistical significance between two groups. Correlations between *PD1*, *PDL1*, and *IFNG* mRNA expression were analyzed using Spearman's rank correlation analysis. Univariate Kaplan–Meier analyses were conducted to explore the association of checkpoint gene expression with recurrence-free, disease-specific, and overall survival. The parameters that demonstrated an influence on the outcome in the univariate analysis ($p<0.05$) were subjected to multivariate Cox regression analyses.

For survival analyses patients were divided into low and high mRNA expression level groups by the optimal cut-off expression value calculated by Youden's index as previously described.[16] We chose the Youden index because it integrates sensitivity and specificity for each transcript with a value that ranges from 0 to 1. The index measures the effectiveness of a diagnostic marker and at the same time proposes an optimal threshold (cut-off) for the biomarker of interest.[19] We used the proposed cut-off to segregate

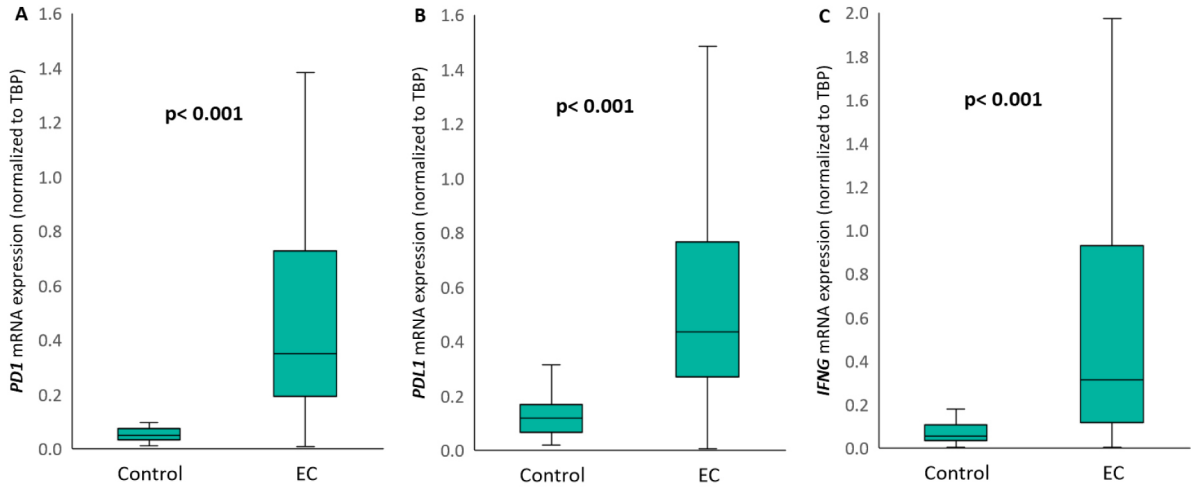

Figure 1 Programmed cell death 1 (PD1), its ligand (PDL1), and interferon gamma (IFNG) are increasingly expressed in endometrial carcinoma. Transcriptional levels of PD1 (A), PDL1 (B), and IFNG (C) in endometrial carcinoma (EC, n=239) compared with non-malignant control tissue (n=25). mRNA expression was normalized to TATA box-binding protein (TBP).

groups into patients with "high" and "low" expression. Statistical analysis was performed using the SPSS statistical software (version 29.0.0; SPSS, Chicago, IL, USA).

RESULTS

Immune Checkpoint Regulators are Overexpressed in Endometrial Carcinoma

We investigated *PD1*, *PDL1,* and *IFNG* expression in endometrial carcinoma patients and compared the expression to that of non-malignant control endometrial tissue by qPCR as previously described for ovarian cancer.[20] Notably, the expression of *PD1*, *PDL1,* and its inducer *IFNG* were increasingly expressed in endometrial carcinoma when compared with non-malignant endometrial tissue (Figure 1). More specifically, *PD1* expression was seven-fold (p<0.001; Figure 1A), *PDL1* expression was three-fold (p<0.001; Figure 1B), and *IFNG* expression was five-fold (p<0.001; Figure 1C) increased when compared with control tissue. Next, we sought to define the impact of clinical subtypes of endometrial carcinoma to the expression of *PD1*, *PDL1,* and *IFNG*. Most notably, *PD1* and *PDL1* were increasingly expressed in low FIGO stages (FIGO Stages I and II) compared with advanced stages (p=0.007 and p=0.003, respectively; Online Supplemental Figure 1A,B). Furthermore, *PD1* expression was increased in younger patients (≤68.8 years; p=0.043) as demonstrated in Online Supplemental Figure 1C.

The highest levels of *IFNG* were detected in high-grade endometrial carcinoma (p=0.010), which was the most frequent histological subtype in our cohort (Online Supplemental Figure 1D). Furthermore, we observed a strong correlation between the expression of *PD1*, *PDL1,* and *IFNG* in endometrial carcinoma tissue (Online Supplemental Table 4). In more detail, *PD1* correlated with *PDL1* (p<0.001, r_S=0.685), *IFNG* with *PD1* expression (p<0.001, r_S=0.804), and *IFNG* with *PDL1* (p<0.001, r_S=0.718) expression.

High Expression of Immune Checkpoints is Associated with Improved Survival

To evaluate the impact of intratumoral checkpoint molecule expression on clinical outcome we followed 239 patients for a median observation period of 5.8 years. Applying the Youden index to define a cut-off for *PD1*, *PDL1,* and *IFNG* transcripts, patients were stratified into a group with high expression of each transcript and a group with low expression, respectively. The univariate survival analysis revealed that high intratumoral expression of *PD1*, *PDL1,* and *IFNG* was associated with better outcome (Online Supplemental Table 5). As demonstrated in Figure 2, high expression of *PD1*, *PDL1,* and *IFNG* was associated with better recurrence-free survival (Figure 2A–C) (HR 0.32, 95% CI 0.19 to 0.53, p<0.001; HR 0.30, 95% CI 0.17 to 0.52, p<0.001; HR 0.47, 95% CI 0.26 to 0.86, p=0.012, respectively), disease-specific survival (Figure 2D–F) (HR 0.38, 95% CI 0.22 to 0.67, p<0.001; HR 0.29, 95% CI 0.15 to 0.55, p<0.001; HR 0.45, 95% CI 0.23 to 0.88, p=0.017, respectively), and overall survival (Figure 2G–I) (HR 0.56, 95% CI 0.38 to 0.83, p=0.003; HR 0.38, 95% CI 0.24 to 0.62, p<0.001; HR 0.58, 95% CI 0.39 to 0.86, p=0.006, respectively).

Considering the long study period (ranging from 1989 to 2015), we next assessed whether adjuvant therapy posed a potential bias. In our cohort, 49 of 239 patients received adjuvant chemotherapy (22.1%) and 206 patients received adjuvant radiation therapy (86.2%). In more detail, 45 patients received adjuvant polychemotherapy, 4 received adjuvant platinum monotherapy, 131 received vaginal brachytherapy, and 75 received brachytherapy combined with external body radiation therapy. Notably, subgroups analysis on recurrence-free survival confirmed the prognostic value of *PD1* in patients with polychemotherapy (HR 0.28, 95% CI 0.11 to 0.72, p=0.005; Online Supplemental Figure 2A) and without chemotherapy (HR 0.30, 95% CI 0.17 to 0.55, p<0.001; Online supplemental figure 2B). A similar trend was demonstrable in patients receiving vaginal brachytherapy (HR 0.49, 95% CI 0.17 to 1.42, p=0.181, Online Supplemental Figure 2C) or brachytherapy combined with external body radiation therapy (HR 0.15, 95% CI 0.23 to 1.26, p=0.15, Online Supplemental Figure 2D); however, this did not reach statistical significance. As such, subgroup analyses suggested that adjuvant therapy is unlikely to be a confounder in our study.

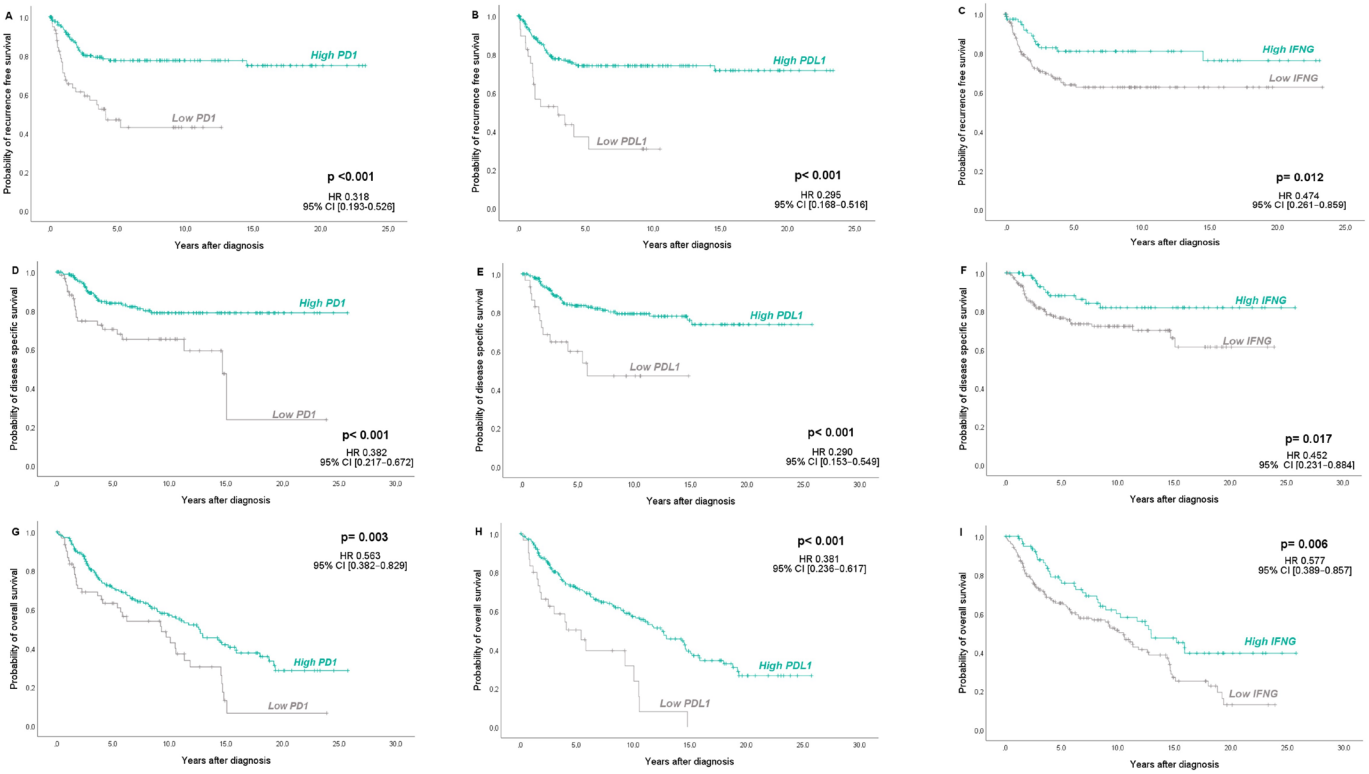

Figure 2 High expression of programmed cell death 1 (PD1), its ligand (PDL1), and interferon gamma (IFNG) is associated with improved clinical outcome in endometrial carcinoma (n=239). PD1 mRNA expression in endometrial carcinoma patients and (A) recurrence-free survival, (D) disease-specific survival, and (G) overall survival. PDL1 mRNA expression and (B) recurrence-free survival, (E) disease-specific survival, and (H) overall survival. IFNG mRNA expression and (C) recurrence-free survival, (F) disease-specific survival, and (I) overall survival. mRNA expression was normalized to TATA box-binding protein (TBP). CI, confidence interval; HR, hazard ratio.

PD1 and PDL1 Predict Endometrial Carcinoma Survival

By performing a multivariate analysis (Table 1) we identified that high FIGO stages, tumor grade 3, and age >68.8 years were evaluated as independent factors negatively predicting clinical outcome in our cohort. Notably, also high expression of *PD1* and *PDL1* were identified as independent prognostic factors for clinical outcome: High expression of *PD1* was predictive for recurrence-free survival (HR 0.39, 95% CI 0.19 to 7.93, p=0.009) in our cohort, while high *PDL1* expression was predictive for overall survival (HR 0.55, 95% CI 0.32 to 0.97, p=0.037).

Validation of the Prognostic Value in the TCGA Cohort

To validate these findings in an independent cohort, we applied the previous cut-offs of *PD1*, *PDL1,* and *IFNG* expression to the TCGA dataset (n=548; demographic features shown in Online Supplemental Table 3). As similarly observed in our cohort, high *PD1* expression (but not *PDL1* expression) was associated with improved clinical outcome (recurrence-free and overall survival p<0.001 and disease-specific survival p=0.004; Online Supplemental Table 6 and Online Supplemental Figure 3). By performing a multivariate analysis in the TCGA cohort, *PD1* was identified to be predictive for recurrence-free survival (HR 0.55, 95% CI 0.39 to 0.78, p<0.001), disease-specific survival (HR 0.51, 95% CI 0.30 to 0.87, p<0.012), and overall survival (HR 0.49, 95% CI 0.32 to 0.75, p<0.001), underlining the value of our inception cohort (Table 2).

PD1 and IFNG are Elevated in "Hot Tumors"

To evaluate the expression of checkpoint molecules and *IFNG* in the four prognostically distinguishable molecular subtypes, that is, *POLE*mut, MMRd, NSMP, and p53-mut, 81 samples of our cohort were analyzed according to the ProMisE classification. ProMisE molecular classification yielded 35 (43.2%) MMRd, 8 (9.9%) *POLE*mut, 32 (39.5%) NSMP, and 6 (7.4%) p53-mut. Applying this classification, we found an almost three-fold (2.9) induction of *PD1* (p=0.019) and a five-fold (5.3) induction of *IFNG* (p<0.001) in *POLE*mut endometrial carcinoma compared with other subgroups (Online Supplemental Figure 4). We further divided molecular subtypes into immunologically "hot tumors" (*POLE*mut and MMRd) and immunologically "cold tumors" (NSMP and p53-mut). Notably, "hot tumors" showed higher expression of *PD1* (p=0.015; Figure 3A) and *IFNG* (p<0.001; Figure 3B) compared with "cold tumors".

DISCUSSION

Summary of Main Results

We investigated *PD1*, *PDL1,* and *IFNG* (as regulator of PDL1) in endometrial carcinoma. Tumor tissue depicted increased expression of these genes when compared with non-malignant control tissue. More importantly, high expression of *PD1* and *PDL1* was associated with improved clinical outcome, (longer recurrence-free, disease-specific, and overall survival). Notably, *PD1* is prognostic

Table 1 Multivariable Cox regression analysis in the Innsbruck cohort (n=239)

		Recurrence-free survival		Disease-specific survival		Overall survival	
Variable	Discriminator	HR of recurrence (95% CI)	P value	HR of death (95% CI)	P value	HR of death (95% CI)	P value
Age	Low vs high (≤ or > median age)	1.95 (1.06 to 3.60)	**0.033**	2.51 (1.21 to 5.19)	**0.013**	3.02 (2.02 to 4.52)	**<0.001**
FIGO stage	I/II vs III/IV	2.14 (1.11 to 4.13)	**0.023**	2.60 (1.25 to 5.43)	**0.011**	1.74 (1.17 to 2.59)	**0.006**
Assessed risk	Low vs intermediate/ high	0.69 (0.35 to 1.36)	0.287	1.06 (0.62 to 1.79)	0.838	–	–
Grading	Grade 1/2 vs Grade 3	1.67 (1.15 to 2.43)	**0.007**	1.65 (1.06 to 2.59)	**0.028**	–	–
Histology	Non-endometrioid vs endometrioid	–	–	1.36 (0.52 to 3.57)	0.538	–	–
Myometrial invasion	<50% vs ≥50%	2.40 (0.87 to 6.66)	0.092	–	–	–	–
Lymphadenectomy	No vs yes	–	–	–	–	–	–
PD1 mRNA expression	Low vs high (< / > 25.9th percentile)	0.39 (0.19 to 7.93)	**0.009**	0.61 (0.27 to 1.36)	0.224	1.09 (0.68 to 1.76)	0.72
PDL1 mRNA expression	Low vs high (< / > 13th percentile)	0.70 (0.32 to 1.57)	0.388	0.69 (0.27 to 1.79)	0.443	0.55 (0.32 to 0.97)	**0.037**
IFNG mRNA expression	Low vs high (< / > 66.1th percentile)	0.65 (0.29 to 1.47)	0.301	0.51 (0.20 to 1.28)	0.150	0.76 (0.49 to 1.18)	0.22

Recurrence-free survival, disease-specific survival, and overall survival in 239 endometrial carcinoma patients. The optimal cut-off points were calculated by Youden's index. The significance level (P) was determined by Cox regression. Bold type denotes statistical significance.
–, not included; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio.

for recurrence-free, disease-specific, and overall survival independent of other clinicopathological characteristics such as age, FIGO stage, or tumor grading. Furthermore, *PD1* expression was associated with better clinical outcome in an independent validation cohort.

Results in the Context of Published Literature

Previous data on the association of checkpoint molecules with clinical outcome in endometrial carcinoma are conflicting. Yamashita et al demonstrated that immunohistochemically high PD-L1 is associated with better recurrence-free survival; however, there was no association with overall survival or PD-1 and endometrial carcinoma outcome, respectively.[21] Zong et al showed that PD-L1 positivity in tumor cells is associated with a favorable prognosis in patients with high-risk endometrial carcinoma.[22] High expression of stromal PD-1 in early endometrial carcinoma was demonstrated to reduce risk of relapse.[23] Other studies did not observe any associations of these checkpoint molecules with survival.[24–26]

A recent meta-analysis including four studies providing information on immunohistochemical PD-L1 expression and overall survival[21 26 27] indicated that PD-L1 overexpression had a non-significant association with overall survival.[28] Our results are in line with the data of Mendiola et al[23] and strengthen the prognostic significance of checkpoint molecules, especially of PD1, in

Table 2 Multivariable Cox regression analysis in The Cancer Genome Atlas (TCGA) cohort (n=548)

		Recurrence-free survival		Disease-specific survival		Overall survival	
Variable	Discriminator	HR of recurrence (95% CI)	P value	HR of death (95% CI)	P value	HR of death (95% CI)	P value
Age	Low vs high (≤ or > median age)	–	–	–	–	1.38 (0.90 to 2.12)	0.14
FIGO stage	I/II vs III/IV	3.06 (2.13 to 4.41)	**<0.001**	7.34 (4.02 to 13.40)	**<0.001**	3.51 (2.28 to 5.40)	**<0.001**
Grading	Grade 1/2 vs Grade 3	1.67 (1.10 to 2.53)	**0.016**	4.72 (1.85 to 12.00)	**<0.001**	2.40 (1.37 to 4.21)	**0.002**
Histology	Non-endometrioid vs endometrioid	0.78 (0.51 to 1.18)	0.241	0.77 (0.43 to 1.38)	0.377	0.78 (0.48 to 1.25)	0.3
Lymphadenectomy	No vs yes	–	–	0.64 (0.34 to 1.22)	0.175	–	–
PD1 mRNA expression	Low vs high (< / > 25.9th percentile)	0.55 (0.39 to 0.78)	**<0.001**	0.51 (0.30 to 0.87)	**0.012**	0.49 (0.32 to 0.75)	**<0.001**

Recurrence-free survival, disease-specific survival, and overall survival in 548 patients. The optimal cut-off points were calculated by Youden's index. The significance level (P) was determined by Cox regression. Bold type denotes statistical significance.
–, not included; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio.

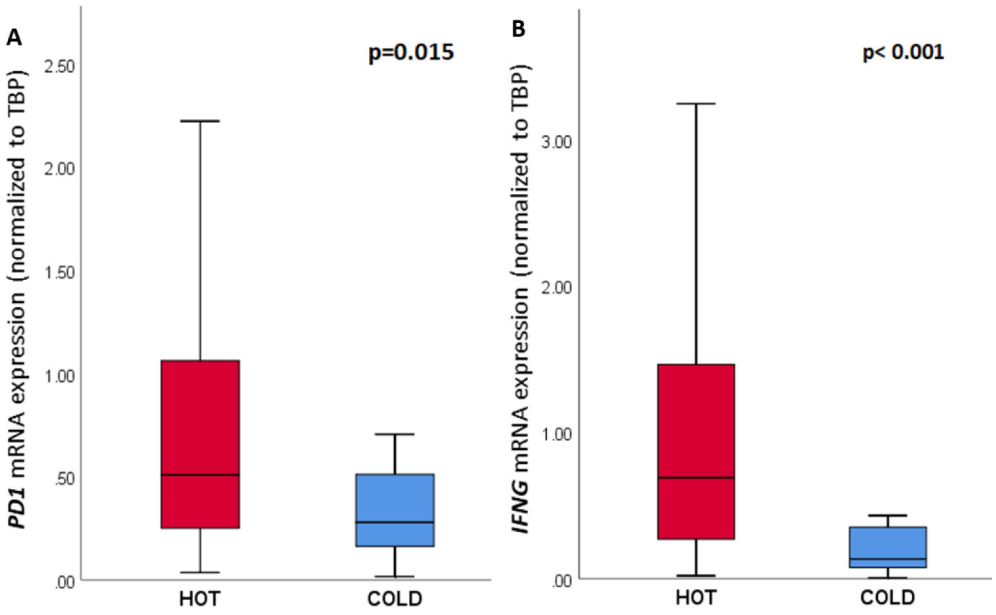

Figure 3 Programmed cell death 1 (PD1) (A) and interferon gamma (IFNG) (B) are highly expressed in immunologically "hot tumors" (n=43) compared with "cold tumors" (n=38). mRNA expression was normalized to TATA box-binding protein (TBP). "Hot tumors" comprise POLE-mutated and mismatch repair-deficient (MMRd) molecular subtypes (HOT) and "cold tumors" comprise no specific molecular profile (NSMP) and p53-mut molecular subtypes (COLD).

endometrial carcinoma. Nonetheless, further studies with large sample sizes are needed to clarify the clinical utility of these biomarkers.

Controversial results regarding PD-1 or PD-L1 expression and patients' outcome extend to other tumor entities like lung and colorectal cancer or melanoma, which can be explained by different antibodies used for immunohistochemistry quantification, different cut-off values, and observer interpretation of staining positivity and heterogeneous expression in the tumor.[29]

Strengths and Weaknesses

In contrast to previous studies on endometrial carcinoma, which used immunohistochemistry for the quantification of PD-1 and PD-L1, our findings are based on mRNA expression. By doing so, we may avoid the abovementioned concerns about immunohistochemistry quantification, namely interobserver disagreement. Furthermore, this may also compensate intra-tumor heterogeneity, which can only poorly be controlled in immunohistochemistry. The significance of our results can be depicted by the validation in the TCGA cohort. TCGA sample collection was performed in a similar fashion as in our discovery cohort (eg, primary, untreated tumor), and our discovery cohort (Innsbruck) and validation cohort (TCGA) demonstrated similar clinicopathological patient characteristics (Online Supplemental Table 7), thus supporting the validity of our validation approach.

In our study, transcriptional analysis differs between these cohorts, which reflects a limitation of our study. More specifically, RNA expression was analyzed by qPCR in the discovery cohort (Innsbruck), while expression in the TCGA was analyzed by RNA sequencing. Publicly available data on the human protein atlas providing a dataset of 548 patients with endometrial carcinoma also demonstrate a favorable outcome for patients with high intratumor *PD1* RNA expression (data not shown). Another limitation of our study is its reliance on retrospective data; prospective data and larger cohort sizes (considering the broad 95% confidence intervals of *PD1* and *PDL1* as prognostic markers) are needed to validate *PD1* and *PDL1* expression as predictive markers for immune checkpoint inhibitor treatment response and patient outcome in endometrial carcinoma. Tumoral *PD1* serves as a robust prognostic marker for recurrence-free, disease-specific, and overall survival, which we confirmed in an independent TCGA cohort. By contrast, the prognostic value of tumoral *PDL1* expression on clinical outcome appeared less consistent in our study. More specifically, increased *PDL1* expression was associated with improved recurrence-free, disease-specific, and overall survival in the univariate survival analyses, while the prognostic value was not confirmed by Cox regression in both study cohorts. A subgroup analysis by FIGO stage, histology, age, or ProMisE classification did not reveal a more consistent prognostic value of *PDL1* (data not shown). As such, our approach could not confirm *PDL1* as a reliable biomarker, which has been similarly described for other tumor entities.[30]

Implications for Practice and Future Research

Patients with MMRd tumors exhibit response rates that top response rates of other molecular endometrial carcinoma subtypes.[11] It is well established that MMRd tumors are immunologically "hot" due to high tumor mutational burden and consequent increased lymphocyte infiltration, a feature of immune response.[11] Therefore, blocking inhibitory signals such as PD-1 and PD-L1 in the tumor microenvironment in "hot tumors" enables the immune system to fight cancer, thereby achieving long-term response rates. Investigating expression of checkpoints in molecular subtypes according to ProMisE molecular classifiers[5] we found the highest levels of *PD1*, *PDL1,* and *IFNG* in *POLE*mut and MMRd tumors. This is in line with previous results, namely that PD-L1 expression was more frequent in *POLE*mut and MMRd subtypes than in p53-mutant and NSMP subtypes.[22] *POLE*mut and MMRd endometrial carcinomas are associated with high neoantigen loads and number of TILs,

which is counterbalanced by overexpression of PD-1 and PD-L1.[31] Based on our results we hypothesize that patients with high intratumor PD1 expression, which was especially found in POLEmut and MMRd endometrial carcinoma, may demonstrate remarkable response rates to immune checkpoint inhibitors. While it has already been clearly established that immune checkpoint inhibitor is greatly effective in patients with MMRd tumors,[11] emerging clinical evidence indicates that *POLE*mut tumors (ie, cases of colorectal and endometrial cancers) may also respond extraordinarily well to immune checkpoint inhibitors.[32 33]

CONCLUSIONS

Our data demonstrate that expression of tumoral immune checkpoint transcripts, especially *PD1*, predicts clinical outcome in endometrial carcinoma. *PD1* is upregulated in immunologically hot tumors, which are known to demonstrate good response rates to immune checkpoint blockade. Therefore, *PD1* expression could be used to stratify patients qualifying for immune checkpoint inhibitor therapy in endometrial carcinoma, but considering the retrospective nature of our findings, establishing this concept warrants controlled prospective clinical trials.

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