Immune infiltration in p53abn endometrial carcinomas

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Abstract

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## 1 Introduction

Endometrial cancer is the second most common type of gynecologic malignancy worldwide and the most common gynecologic malignancy in North America (Siegel et al. 2024). Molecular classification has separated endometrial cancers into 4 prognostically distinct subtypes: POLE (polymerase epsilon-mutated), MMRd (mismatch repair-deficient), p53abn (p53 abnormal) and NSMP (no specific molecular profile, (Getz et al. 2013; A. Talhouk et al. 2015; Aline Talhouk et al. 2017; Kommoss et al. 2018)). p53abn cancers, the worst prognostic subtype, comprise only 15% of all endometrial cancers but account for 50-70% of mortalities (A. Talhouk et al. 2015; On-Castillo et al. 2020; Jamieson et al. 2021; Siegenthaler et al. 2022), with extrauterine involvement in over 50% of cases (Kommoss et al. 2018; Momeni-Boroujeni et al. 2021). Most p53abn tumors recur within five years on standard-of-care carboplatin-paclitaxel chemotherapy with or without radiotherapy, highlighting the need for alternative therapeutic options (On-Castillo et al. 2020; Ott et al. 2017; Jamieson et al. 2021). While several studies have investigated associations between the immune response to endometrial carcinomas, to date there have been no large scale studies investigating the immune composition of p53abn tumors.

The immune system plays a key role of preventing carcinoma development and growth via multiple mechanisms. All tumors harbor tumor-specific mutations, and a subset of these may form mutated peptides that are presented on major histocompatibility molecules (MHC), recognized by T cells as neoantigens, and result in anti-tumor immunity. Several hurdles must be overcome for effective anti-tumor immunity: (1) The tumor must express muted neoantigens on MHC molecules. (2) T cells must recognize the neoantigens and become activated. (3) Activated T cells must extravasate and infiltrate the tumor microenvironment. (4) Intratumoral, activated T cells must recognize and kill tumor cells that harbor neoantigens. Beyond these core requirements, the interplay between different immune cell types within the tumor microenvironment helps determine the effectiveness of anti-tumor immunity. CD8+ cytotoxic T lymphocytes (CTL) are the main cells that recognize and kill tumor cells. CD4+ helper T cells (TH) recognize neoantigens in MHC class II molecules presented by antigen presenting cells (dendritic cells, macrophages, and B cells). These TH cells secrete cytokines that potentiate the activity of CTL and anti-tumor macrophages. CD68+ macrophages can either help activate CTL, by presenting neoantigens and co-stimulatory molecules, or inhibit CTL, by presenting neoantigens with inhibitory ligands. In addition, CD3+FOXP3+ regulatory T cells (Tregs) inhibit antitumor immunity by secreting cytokines that block CTL maturation and activity and induce macrophages to express immune inhibiting molecules (Chan et al. 2019). Finally, CD20+ B cells and CD79a+ plasma cells may either inhibit or potentiate antitumor immunity via multiple mechanisms. Given these functions, it is unsurprising that increased CTL and TH tumor infiltrating lymphocytes (TIL) are associated with longer survival in endometrial carcinoma (Jong et al. 2009), while immunosuppressive regulatory T cells are associated with shorter survival (Willvonseder et al. 2021).

Two key functional pathways that inhibit the immune system are the IDO1 cytokine and the PD-1/PD-L1 immune checkpoint pathways. Macrophages and tumor cells may express IDO1, which inhibits CTL proliferation and increases Treg differentiation. In addition, as a failsafe to prevent uncontrolled autoimmunity, activated CTL express programmed cell death-1 (PD-1), which, when bound to the PD-1 ligand (PD-L1), inhibit CTL mediated killing of target cells. Tumors co-opt this mechanism by upregulating PD-L1 on epithelial cells and macrophages, thereby preventing CTL mediated tumor destruction. The cytokines released when CTL kill tumor cells cause upregulation of both IDO1 and PD-L1; thus, expression of PD-L1 has been used as a marker for an active anti-tumor immune response in multiple different cancers. It is the mix of these intratumoral cell types and their relative functions within the tumor microenvironment that help to dictate whether anti-tumor immunity will successfully eradicate a tumor.

Treatment with immune checkpoint antibodies that disrupt the PD-1–PD-L1 pathway reactivates exhausted T cells and is particularly effective in tumors with elevated numbers of neoantigens due to high mutational burden (Chan et al. 2019). In endometrial cancer, POLE and MMRd tumors have over 10 times as many mutations as p53abn and NSMP tumors (Getz et al. 2013) and correspondingly higher TIL densities (Aline Talhouk et al. 2019). While systemic therapy is typically unnecessary in POLE cancers due to exceptionally favorable outcomes with hysterectomy alone, anti-PD1 immune-checkpoint inhibitors have demonstrated promising efficacy in advanced, recurrent, or persistent MMRd endometrial cancers, even after multiple lines of therapy (Konstantinopoulos et al. 2019; Antill et al. 2021). More recently, Mansoor R. Mirza et al. (2023) showed that the anti-PD1 antibody dostarlimab was associated with significantly improved outcomes not only in MMRd but also MMRp (MMR proficient, non-POLE) endometrial cancer when added to standard-of-care chemotherapy. While the benefit of dostarlimab in MMRp cancers was less than in MMRd cancers, subgroup analysis showed that the benefit in MMRp was driven by p53abn cases (M. R. Mirza et al. 2023). The factors underlying this promising response to immune checkpoint inhibitors in p53abn endometrial cancer remain poorly understood.

Additional classes of targeted therapies under investigation in p53abn endometrial cancer include PARP inhibitors and HER2-directed antibodies (Bosse et al. 2022). Spurred by remarkable clinical responses in HGSC (Moore et al. 2018), PARP inhibitors have become standard-of-care in *BRCA1*/*BRCA2*-mutated or homologous recombination deficient (HRD) cancers across multiple cancer types (Friedlander, Lee, and Tew 2023). Compared to HGSC, HRD is less common in p53abn endometrial cancer, with approximately 25% of cases showing evidence of HRD and fewer than 5% with *BRCA* mutations (Wallbillich, Morris, and Ali-Fehmi 2020). In HGSC, HRD tumors have higher immunogenicity than non-HRD tumors, and markers of adaptive immunity are associated with longer overall survival in HRD but not non-HRD tumors (Allen W. Zhang et al. 2018). Understanding the relationship between HRD and the immune microenvironment in p53abn endometrial cancers may help inform combination PARP inhibitor and immunotherapy clinical trials (Konstantinopoulos et al. 2022).

To understand the clinical relevance of the immune response to p53abn endometrial cancer, we systematically profiled the immune cell composition of 256 clinically annotated p53abn endometrial cancers with multiplex immunofluorescence for CTL, TH, Tregs, B cells, plasma cells and macrophages. Further, we evaluated the expression patterns of PD1, PDL1 and IDO1, three pharmacologically actionable immunosuppressive molecules with translational relevance to current clinical trials in endometrial cancer. Finally, we investigated the relationship between immune composition, homologous recombination deficiency, and HER2 in p53abn endometrial cancer.

## 2 Methods

### 2.1 Data acquisition

#### 2.1.1 Sample acquisition

Ethics approval for this study was obtained from the University of British Columbia (UBC) Research Ethics Board (REB NUMBERS). The cohort consisted of 256 treatment-naive p53-abnormal endometrial carcinomas collected between XXXX and XXXX in Vancouver and in the Cross Canada endometrial cancer cohort. CONSENT? Clinicopathologic and outcome data were collected by chart review. All cancers were classified as p53abn according to the ProMisE algorithm (A. Talhouk et al. 2015) by immunohistochemistry for p53 and MMR and next-generation sequencing for POLE and p53 mutations.

### 2.2 Experimental methods

#### 2.2.1 Tissue microarray construction

Representative samples of p53abn endometrial carcinomas from our Vancouver cohort were cored (0.6 mm) in duplicate and arrayed as described previously (A. Talhouk et al. (2015)). CROSS CANADA COHORT? TMAs were cut at WHAT thickness for immunofluorescence. At least two cores were sampled from each tumor.

#### 2.2.2 Multiplex immunofluorescence

Two multiplex immunofluorescence panels were constructed consisting of the following antibodies (1) (CD3, CD8, FOXP3, CD20, CD79a, and (2) (PD1, PDL1, IDO1, CD8, CD68). For each immunofluorescence panel, optimal staining parameters were determined as single stains and validated in the multiplex. Once stained, TMAs were imaged using (###).

### 2.3 Computational analysis

#### 2.3.1 Cell and region counting

Analysis was performed using HALO (Version number). Briefly, three tissue segmentation and cell phenotyping algorithms were trained in HALO using 10-30 representative images for each algorithm. Regions were classified as epithelial, stromal, glass, or other (including necrosis) for tissue segmentation. The mean and standard deviation of cell count and region area across all algorithms was calculated for each core and reviewed by a technologist. Any core with a standard deviation of >5 among the three algorithms, or with abnormal features as identified by the technologists, were flagged for review and manual annotation by a pathology resident and/or subspecialist gynecological pathologist. Sarcomatoid areas in carcinosarcomas were considered epithelial. Intraepithelial area that was negative for CD68 and CD8 was approximated as tumor cell area. All immune cells touching tumor cells were considered intraepithelial.

#### 2.3.2 Mixture modeling of cell counts

TIL count for a given core , cell type , and region (tumour/stroma) were described as follows:

where the mean follows:

where is the inferred cluster of core , is the area of region in core , and is the mean density (count divided by area) value across all cores . The Gamma distribution was parametrized in terms of a mean and scale parameter, with the scale parameter set to 100 to allow for a fairly uninformed prior.

To reduce the dimensionality of the parameter space, the dispersion parameter was formulated semi-parametrically in terms of a set of Gaussian radial-basis kernels with parameters and , for a specified number of centers uniformly distributed between 0 and the maximum number of counts (Kapourani and Sanguinetti 2016; A. W. Zhang et al. 2019), with the default number of centers set equal to 20:

where and follow relatively agnostic lognormal priors as above.

Inference was performed in pymc v5.9.1 (Abril-Pla et al. 2023). To determine the optimal number of clusters , a Dirichlet process prior was first fitted to a version of the model marginalized over cluster membership . At a minimum threshold of 0.05 for cluster proportion, two clusters with proportions of at least 0.05 were inferred (first cluster with mean proportion 0.588, 95% CI 0.512-0.656; second cluster with mean proportion 0.347, 95% CI 0.289-0.407). The lower bound of the 95% CI for the third most prevalent cluster was 0.004 (equivalent to 1 out of the 256 input samples), supporting the presence of only 2 clusters. Thus, the model was fitted with 2 clusters () to sample cluster membership .

#### 2.3.3 Shallow whole-genome copy number analysis

TODO. In short, sWGS signatures taken from CITE paper, and copy number results for HER2 derived from …

#### 2.3.4 Statistical analysis

All statistical analysis was performed in R (v4.3.2). The Mann-Whitney U test was used to evaluate significance in two-way comparisons. Comparisons for categorical count data were evaluated for significance with Fisher’s exact test. Multiple testing correction was performed with the Holm method. Statistically significant results were considered those with *P* < 0.05.

P values for Kaplan-Meier analyses were computed with log-rank tests. Cox proportional hazards analysis was performed in R with the survival package (v3.5-7). Proportional hazards assumptions were evaluated with weighted Schoenfeld residuals (Grambsch and Therneau 1994). Posterior distribution samples were used to calculate confidence intervals and *P* values.

#### 2.3.5 Code availability

Code associated with this project is publicly available at <https://github.com/Irrationone/tfri_halo>.

## 3 Results

### 3.1 Cohort

We assembled a cohort of 256 treatment-naive p53abn endometrial carcinomas diagnosed between XXXX and XXXX ([Table 1](#tbl-cohort-overview), OTHER REF) (Aline Talhouk et al. 2019). Histotypes of p53abn tumors included were serous (n=136), endometrioid (n=52), carcinosarcoma (n=31), clear cell (n=15), mixed (n=17), undifferentiated/dedifferentiated (n=2) and other (n=3). All patients received treatment in accordance with standard-of-care at the time of diagnosis, with most patients receiving adjuvant chemotherapy and a smaller proportion receiving adjuvant brachytherapy or radiotherapy ([Table 1](#tbl-cohort-overview)). No patients received immunotherapy or neoadjuvant chemotherapy.

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| Table 1: Cohort statistics   |  | | --- | | (a) | |

Source: [Cohort-level analysis](https://Irrationone.github.io/tfri_halo/notebooks/cohort_analysis-preview.html#cell-tbl-cohort-overview)

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| Figure 1: Cohort overview |

Source: [Cohort-level analysis](https://Irrationone.github.io/tfri_halo/notebooks/cohort_analysis-preview.html#cell-fig-cohort-track)

### 3.2 Clustering based on immune composition

We performed multiplex immunofluorescence and automated image analysis to segment tumors into epithelial, stromal and other regions and quantified lymphocyte subsets, including CTL (CD3+CD8+), TH (CD3+CD8-FOXP3-), Treg (CD3+CD8-FOXP3+), B cells (CD20+CD79a+) and plasma cells (CD20-CD79a+) ([Section 2](#sec-methods)). Cells within epithelial and stromal regions were counted separately, and automated counts were manually verified. We found that the density of each cell type within epithelial compared to stromal regions was highly correlated ([Figure S1](#suppfig-densities-correlation)).

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| Figure S1 |

Source: [Clustering results](https://Irrationone.github.io/tfri_halo/notebooks/clustering-preview.html#cell-suppfig-densities-correlation)

Next, we clustered tumors based on epithelial and stromal counts normalized by region area with a negative binomial mixture model. The optimal number of clusters, as determined by a Dirichlet process prior, was two: a TIL-rich and a TIL-poor cluster ([Section 2](#sec-methods)). T and B cell subsets, including CTL, TH, Treg, B cells, and plasma cells infiltrated both tumor stroma and epithelium in TIL-rich tumors. We found no evidence of a subgroup with stroma restricted lymphocytes, contrary to our findings of an immunologically and genomically distinct subgroup of tumors with stroma-restricted TIL in high-grade serous ovarian carcinoma (Allen W. Zhang et al. 2018).

### 3.3 TIL-rich tumors are associated with longer survival

Increased numbers of TIL have been associated with improved survival in endometrial carcinoma in general, but the different prognoses and levels of immune infiltration of the four subtypes of endometrial carcinomas may have confounded these results. Thus, we assessed TIL cluster, clinical parameters, and survival in our cohort of p53abn endometrial carcinoma. In multivariate analysis, significant associations were identified between overall survival and adjuvant chemotherapy (P = 0.041) and FIGO stage (P < 0.001 for stage III and IV compared to stage I), while increased age trended towards shorter overall survival (P = 0.14). TIL-high tumors significantly associated with prolonged overall (P = 0.031) and disease-specific survival (P = 0.037) in multivariate Cox proportional-hazards analysis accounting for age at diagnosis, FIGO stage (Creasman 2009), and adjuvant treatment ([Table 2](#tbl-cox-hazards)), and trended towards longer progression-free survival (P = 0.15). Surprisingly, univariate Kaplan-Meier analyses failed to identify significant association between TIL-high cases and overall, progression-free, or disease-specific survival (all *P >* 0.175) ([Figure 3](#fig-km-stage)). The discordance in the effect of TIL between univariate and multivariate analyses was at least partially explained by differences between early and advanced stage disease. Univariate Kaplan-Meier analyses stratified by stage highlighted that the association between TIL cluster and survival was most pronounced in patients with stage III disease ([Figure 3](#fig-km-stage)). The median 5-year overall survival in stage III disease was 24.9% (95% CI, 9.6%-64.7%) for TIL-poor cancers and 55.2% (95% CI, 38.3%-79.6%) for TIL-rich stage III cancers (n = 71). In contrast, the median 5-year overall survival in stage I disease was 74.9% (95% CI, 61.9%-90.6%) for TIL-poor cancers and 79.1% (95% CI, 67%-93.4%) for TIL-rich stage I cancers (n = 118). Thus, TILs are associated with longer survival in p53abn endometrial carcinoma, particularly in high stage disease. Thus we asked whether chemotherapy, typically offered for advanced stage disease, associated with TILs. Despite the higher rates of chemotherapy in stage III disease, TILs were associated with significantly longer disease-specific survival and trended towards longer progression-free survival and overall survival in patients who did not receive chemotherapy, but not in patients who did ([Figure S2](#suppfig-km-chemo)).

### 3.4 Association between TIL-high and histologic subtypes of p53abn endometrial carcinoma

p53abn endometrial carcinomas are comprised of a mixture serous and multiple different histotypes (including carcinosarcoma, endometrioid, clear cell, and others). Therefore, we assessed for associations between TIL cluster and histotype in our cohort and found TIL subgroup was significantly associated with histotype (P = 6.51e-03). Indeed, 74% (23/31) of carcinosarcomas were TIL-poor, compared to only 43% 97/224 of non-carcinosarcoma histotypes (adjusted P = 0.011) ([Figure 2](#fig-densities-tilclust)). No significant association was found between TIL cluster and other non-serous histotypes. In multivariate survival analysis accounting for carcinosarcoma histotype and TIL cluster, carcinosarcomas trended towards shorter overall, progression-free and disease-specific survival, consistent with prior findings (Chuyao Zhang et al. 2015; Bernardini et al. 2016; Raffone et al. 2022), but this was not statistically significant (all P > 0.067). The association between TIL subgroup and overall survival remained significant (HR 0.65, 95% CI 0.426-0.989, P = 0.044), suggesting that TIL-high classification is prognostic independently of histotype.

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| Figure 2: Clusters and association with survival |

Source: [Clustering results](https://Irrationone.github.io/tfri_halo/notebooks/clustering-preview.html#cell-fig-densities-tilclust)

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| Table 2: Cox hazards table   |  | | --- | | (a) | |

Source: [Clustering results](https://Irrationone.github.io/tfri_halo/notebooks/clustering-preview.html#cell-tbl-cox-hazards)

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| Figure 3 |

Source: [Clustering results](https://Irrationone.github.io/tfri_halo/notebooks/clustering-preview.html#cell-fig-km-stage)

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| Figure S2 |

Source: [Clustering results](https://Irrationone.github.io/tfri_halo/notebooks/clustering-preview.html#cell-suppfig-km-chemo)

### 3.5 Immune composition and inhibition is altered in TIL-rich samples

To further explore anti-tumor immunity and the tumor response to immunity, we assessed for CD8 T cell activation, immune subset composition, and macrophage and tumor expression of immune inhibiting molecules. Consistent with our previous findings (Aline Talhouk et al. 2019), TIL-rich tumors contained more of both CD8+PD1+ (activated CTL) and CD8+PD1- (Naïve T cells) ([Figure 4](#fig-tilclust-ardensities)). However, activated CTL made up a greater percentage of total CD8 T cells (averages and p value) ([Figure 4](#fig-tilclust-ardensities)). Furthermore, the CTL to Treg ratio (CTL:Treg) was significantly elevated within epithelium but not stroma of TIL-rich tumors, suggestive of increased anti-tumor intra-epithelial CTL activation in TIL-high tumors ([Figure 2](#fig-densities-tilclust)). In response to cytokines released by activated CTL, both tumor cells and macrophages can upregulate expression of PD-L1 and IDO1, thereby inhibiting anti-tumor immune attack (Tucci et al. 2019). While there was no significant difference in the number of PDL1-negative macrophages between TIL-rich and TIL-poor tumors, we found PDL1-positive macrophages were significantly enriched in TIL-rich tumors (P < 0.001) ([Figure 4](#fig-tilclust-ardensities)). Furthermore, TIL-rich tumors showed significantly higher levels of PDL1-positive and IDO1-positive macrophages (all P < 0.001) ([Figure 4](#fig-tilclust-ardensities)). These results highlight increased intraepithelial CTL invasion and corresponding upregulation of the PDL1 by tumor cells and macrophages in TIL-rich p53-abnormal endometrial carcinomas. which may be therapeutically exploitable with immune checkpoint blockade.

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| Figure 4 |

Source: [Adaptive response](https://Irrationone.github.io/tfri_halo/notebooks/adaptive_response-preview.html#cell-fig-tilclust-ardensities)

### 3.6 Mutational signatures and TILs in endometrial cancer

Finally, we evaluated the relationship between TIL subgroups and mutational processes. Using shallow whole genome sequencing data-derived copy number signatures that we previously described in endometrial cancer (Clinical Cancer Research, accepted), we correlated signature exposures to TIL subgroup for n=126 p53abn endometrial carcinomas. All of the copy number signatures, including the homologous repair deficient (HRD) signature VS3 (CHECK WITH DAWN), failed to significantly associate with TIL cluster in p53abn endometrial carcinoma ([Figure 5](#fig-mutational-signatures)). Moreover, densities of individual TIL types in epithelial and stromal regions failed to significantly correlate with any mutational signature, and all mutational signatures failed to significantly associate with survival in multivariate Cox analyses that include TIL cluster. Finally, the TIL clusters did not correlate with HER2 IHC status (P = 0.95) or HER2 amplification by shallow whole-genome sequencing (P = 0.11) ([Figure 5](#fig-mutational-signatures)). Thus, mutational signatures show different patterns of co-segregation with TILs and prognostic significance in p53abn endometrial carcinoma compared to HGSC (Allen W. Zhang et al. 2018).

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| Figure 5: Mutational signatures |

Source: [Mutational signatures](https://Irrationone.github.io/tfri_halo/notebooks/mutational_signatures-preview.html#cell-fig-mutational-signatures)

## 4 Discussion

Historically considered one of the less immunogenic subtypes of endometrial cancer, p53abn tumors have received minimal attention in immunotherapy research. Initial trials showed only modest response rates to pembrolizumab in advanced and recurrent MMRp endometrial cancer (Ott et al. 2017), mirroring similar results in epithelial ovarian cancer (Matulonis et al. 2019). More recently, dostarlimab in addition to standard-of-care chemotherapy demonstrated benefit in both MMRd and MMRp cancers (Mansoor R. Mirza et al. 2023), with p53abn cases responding substantially better than NSMP cases (M. R. Mirza et al. 2023). We systemically profiled the immune microenvironment in one of the largest cohorts of p53abn endometrial cancers to date, revealing 2 immunologically distinct subgroups defined by extensive and limited infiltration of T cells, B cells, and macrophages. Over half of p53abn cancers are highly infiltrated by TIL, challenging the belief that p53abn cancers are immune depleted (Mendiola et al. 2022). Cytotoxic T cells, but not immunosuppressive T regulatory cells, co-localized with tumor cells in TIL-rich tumors. TIL-rich tumors expressed high levels of PD1 and PDL1, providing mechanistic rationale for the effectiveness of anti-PD1 inhibitors. TIL-rich tumors were also associated with longer overall and disease-specific survival, in contrast to prior findings in smaller cohorts (Aline Talhouk et al. 2019). Notably, TILs were more strongly associated with survival in patients with advanced disease, who have the most presing treatment requirements. These findings may inform clinical trials of immunotherapies in early-stage endometrial cancer (NCT04634877, NCT04214067), where TILs were not significantly associated with longer survival.

The pervasiveness of TIL-rich p53abn endometrial cancers underscores potential shortcomings of molecular subtype-based strategies for selecting patients for immunotherapy (Ott et al. 2017; Yamashita et al. 2017). While TIL-rich tumors are more frequent in MMRd (78% (Aline Talhouk et al. 2019)) than other subtypes, 53% of p53abn tumors in our study were also TIL-rich. Histotype likely has limited importance, as apart from carcinosarcomas, which were mostly TIL-poor, histotype was not associated with TIL status. NSMP, the most TIL-poor molecular subtype (Aline Talhouk et al. 2019; Chong Zhang, Wang, and Wu 2023), derived correspondingly limited benefit from dostarlimab in the RUBY trial (M. R. Mirza et al. 2023). Thus, TIL response, not molecular subtype alone, may be more informative for stratifying endometrial cancer patients for immunotherapy.

In our study, IDO1 expression was not associated with TIL-rich tumors, contrasting with PD1 and PDL1 expression patterns. This may indicate a different mechanism for IDO1 induction in the tumor microenvironment. While IDO1+ macrophages were less prevalent than PDL1+ macrophages, their presence irrespective of overall TIL infiltration may make IDO1 a suitable target for immunotherapy in a subset of TIL-poor tumors.

Our data show for the first time the relationship between immune response and mutational processes in p53abn endometrial cancer. Despite genomic similarities between HGSC and p53abn endometrial cancer (Getz et al. 2013; Allen W. Zhang et al. 2018), TILs were not correlated with mutational signatures, including HRD, in p53abn endometrial cancer. In contrast to HGSC, p53abn endometrial cancer may elicit TIL responses through mechanisms independent of HRD. Other mutational processes generating widespread genomic instability in endometrial cancer [CITE DAWN] may elicit TIL responses through common cGAS-STING pathway activation (Deng et al. 2014; Woo et al. 2014). Additionally, TILs were not correlated with HER2 status by immunohistochemistry or whole-genome sequencing. Immunotherapies, anti-HER2 therapies, and PARP inhibitors targeting HRD tumors may represent orthogonal approaches effective in different groups of p53abn endometrial cancers, with some tumors susceptible to multiple agents.

As the use of immunotherapies extends to MMRp endometrial cancers, the immune microenvironment must be considered in addition to molecular subtype as a relevant factor. Our findings highlight properties of the immune microenvironment that may portend susceptibility to immune checkpoint susceptibility in p53abn endometrial cancers, and how these relate to other clinically actionable targets being explored in clinical trials.

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