Immune checkpoint expression, microsatellite instability, and mutational burden: Identifying immune biomarker phenotypes in uterine cancer

N.L. Jones[1], J. Xiu[2], T. Herzog[3], and I. S. Winer[4]
[1]Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA, [2]Caris Life Sciences, Irving, TX, USA, [3]UC Health Barrett Cancer Center, Cincinnati, OH, USA, [4]Wayne State University, Detroit, MI, USA,

Background

Endometrioid endometrial cancer (EEC) is categorized on a histologic continuum from Grade 1 to 3 (G1 or low grade (LG), G2, G3 or high-grade (HG)). Increasing grade is associated with aggressive behavior and poor prognosis. Treatment options for advanced/recurrent disease are limited. Emerging data has shown promise of immune checkpoint therapy (IT) in gynecologic malignancies [1]. MSI-H status, tumor mutation burden, and high PD-L1 expression have been associated with higher response rates [2]. Herein we identify distinct immune "biomarker phenotypes" to identify patients who may benefit from immune therapy (IT).

Methods

- 621 endometrioid endometrial tumors were retrospectively analyzed for immune biomarker phenotype by multiplatform profiling: 156 grade 3, 172 grade 2, 113 grade 1, 180 unknown.
- NextGen sequencing (NGS) was performed on 592 genes (Illumina NextSeq platform).
- Mutational burden was calculated based on somatic nonsynonymous missense mutations; TMB-high was defined as ≥17 mutations/megabase.
- Microsatellite Instability (MSI) was determined by examining altered microsatellite loci using NGS (≥46 loci).
- Antibody used for PD-L1 was SP142 and positivity was defined as ≥2+, >5% staining on tumor cells.
- Data were compared using chi-square tests.

Results

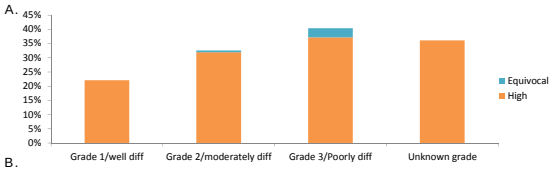
A.

B.

	MSI			TMB			PD-L1		
	N		%	N		%	N		%
	High	Total	High	High	Total	High	Pos	Total	Pos
Grade 1/well diff	25	113	22%	15	113	13%	1	107	1%
Grade 2/moderately diff	55	172	32%	39	171	23%	5	169	3%
Grade 3/Poorly Diff	58	156	37%	53	156	34%	18	153	12%

Figure 1. Overview of Immune Biomarker Phenotypes in EECs.

A.

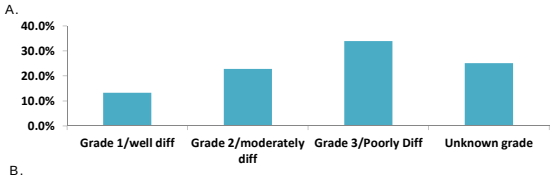


B.

	N				%		
EEC Grade	High	Equivocal	Stable	Total	High	Equivocal	Stable
Grade 1/well diff	25		88	113	22%	0%	78%
Grade 2/moderately diff	55	1	116	172	32%	1%	67%
Grade 3/Poorly diff	58	5	93	156	37%	3%	60%
Unknown grade	65		115	180	36%	0%	64%

Figure 2. MSI in EECs. A. MSI high (MSI-H), equivocal and stable in histologic grades 1, 2, and 3 ECCs. **B:** Overall, MSI-H was found in 33% (203/621) of EECs: 22% in G1/well-differentiated tumors, 32% in G2/moderately differentiated tumors, and 37% in G3/poorly-differentiated tumors (p=0.007).

A.

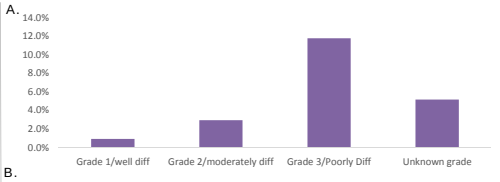


B.

	N				%		
EEC Grade	TMB High	TMB Intermediate	TMB Low	Total	TMB High	TMB Intermediate	TMB Low
Grade 1/well diff	15	49	49	113	13.3%	43.4%	43.4%
Grade 2/moderately diff	39	72	60	171	22.8%	42.1%	35.1%
Grade 3/Poorly Diff	53	57	46	156	34.0%	36.5%	29.5%
Unknown grade	45	81	53	179	25.1%	45.3%	29.6%

Figure 3. Tumor Mutational Burden (TMB) in EECs. A. High TMB (TMB-H) levels in histologic grades 1, 2, and 3 ECCs. **B.** Overall, TMB-H was identified in 25% (152/619) of EECs: 13% in G1/well-differentiated tumors, 23% in G2/moderately differentiated tumors, and 34% in G3/poorly-differentiated tumors (p=0.006).

A.

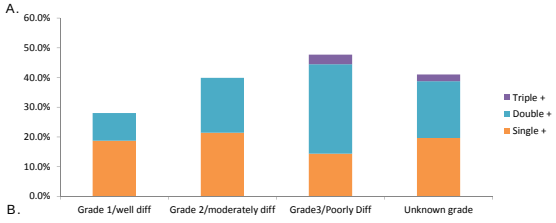


B.

	N			%
EEC Grade	Positive	Negative	Total	PDL1
Grade 1/well diff	1	106	107	0.9%
Grade 2/moderately diff	5	164	169	3.0%
Grade 3/Poorly Diff	18	135	153	11.8%
Unknown grade	9	165	174	5.2%

Figure 4. PDL-1 Expression via IHC in EECs. A. PD-L1 expression in histologic grades 1, 2, and 3 ECCs. **B:** Overall, PD-L1 expression was found in 5.5% (33/603) of EECs: 1% in G1/well-differentiated tumors, 3% in G2/moderately differentiated tumors, and 12% in G3/poorly-differentiated tumors (p<0.0001).

A.



B.

	N					%			
EEC Grade	Single +	Double +	Triple +	Triple -	Total	Single +	Double +	Triple +	Triple -
Grade 1/well diff	20	10		77	107	18.7%	9.3%	0.0%	72.0%
Grade 2/moderately diff	36	31		101	168	21.4%	18.5%	0.0%	60.1%
Grade3/Poorly Diff	22	46	5	80	153	14.4%	30.1%	3.3%	52.3%
Unknown grade	34	33	4	102	173	19.7%	19.1%	2.3%	59.0%

Figure 5. Evaluation of all 3 markers in EECs. A. Combined markers in histologic grades 1, 2, and 3 ECCs. **B**. Triple negative phenotype was identified in 60% of all EECs: 72% in G1, 60% in G2, 52% in G3. Triple positive phenotype was not present in G1 or G2, and in only 3.3% of G3. Double positive markers were present in 9% of G1, 19% of G2, and 30% or G3.

	MSI and TMB	TMB and PD-L1	MSI and PD-L1
All EE tumors	**3.08E-44**	0.015	0.213
Grade 1/well-diff	**0.000009**	0.685	0.589
Grade 2/moderately diff	**7.57E-08**	0.887	0.417
Grade 3/Poorly Diff	**1.25E-18**	0.352	0.832

Figure 6. Dual and triple analysis performed using chi-square analysis with adjustments made for multiple comparisons. Green: p<0.05; bold: p<0.00064 (after correct for multiple tests). MSI and TMB were highly correlated in all ECCs. There was NO significant association found for all three markers in EECs.

Conclusions

- We evaluated TMB, MSI and PD-L1 expression in over 600 EECs.
- MSI and TMB are highly correlated in low grade , intermediate and high grade tumors while the correlation of PD-L1 expression with MSI or TMB is not seen.
- High grade tumors appear to be more immunogenic than low grade tumors, and may preferentially benefit from IT providing a potentially powerful treatment option. Conversely, LG tumors are less likely to benefit from IT.

References

1. *Varga 2017, Journal of Clinical Oncology* 35, no. 15_suppl (May 2017) 5513-5513
2. Goodman 2017 Mol Cancer Ther 16(11):2598-2608