Mutational burden, tumor PDL-1 expression, and microsatellite instability in gynecologic malignancies: Implications for immune therapy

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Background

Immune checkpoint inhibitor therapies (ITs) have profoundly changed the treatment landscape of various cancers including NSCLC and melanoma, and data is emerging in gynecologic malignancies [1]. While MSI-H status qualifies patients for IT regardless of tumor type[2], increased expression of PD-L1 and elevated tumor mutational burden have been associated with higher likelihood of responding[3]. PD-L1 mediates tumor-induced immune suppression through T-cell downregulation, MSI and TMB levels likely create neoantigens which may increase likelihood of response to IO. Looking at these three signatures may result in focusing our treatment on those tumors that higher likelihood of responding to IO.

Methods

- 5588 tumors were retrospectively analyzed by multiplatform profiling: 3223 ovarian, 1989 uterine, 284 cervical, 49 vulvar and 19 vaginal
- NextGen sequencing (NGS) was performed on 592 genes (Illumina NextSeq platform).
- Mutational burden 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.

	N				%		
Cancer	High	Equivocal	Stable	Grand Total	High	Equivocal	Stable
Cervical Cancer	6		278	284	2.1%	0.0%	97.9%
Ovarian Cancer	35	5	3174	3214	1.1%	0.2%	98.8%
Uterine Cancer	323	15	1651	1989	16.2%	0.8%	83.0%
Vaginal Cancer		1	18	19	0.0%	5.3%	94.7%
Vulvar Cancer		1	48	49	0.0%	2.0%	98.0%
Other	1		23	24	4.2%	0.0%	95.8%

B.

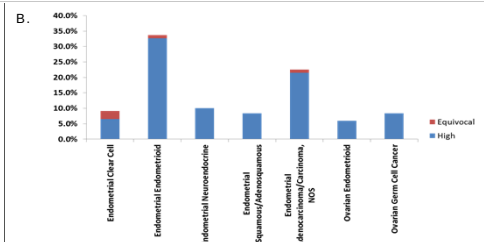

Figure 1. MSI in GYN Cancers. MSI high/equivocal and stable in various cancers (**A**). **B:** Selected cancer histologies with MSI >5%. MSI-High found in 16% of uterine cancers (33% endometrioid , 10% neuroendocrine, 8% squamous , 7% clear cell, 5% carcinosarcoma, 3% serous, 2% leimoyosarcoma, 2% stromal sarcoma); 1% of ovarian cancers (8% germ cell, 6% endometrioid, 3% low grade, 2% mucinous, 2% clear cell, 1.2% Carcinosarcoma, 0.7% serous), 2% cervical and 0% of vulvar/vaginal cancers.

A.

	N				%			
	TMB High	TMB Intermediate	TMB Low	Grand Total	TMB High	TMB Intermediate	TMB Low
Cervical Cancer	17	152	114	283	6.0%	53.7%	40.3%
Ovarian Cancer	59	1337	1796	3192	1.8%	41.9%	56.3%
Uterine Cancer	252	866	860	1978	12.7%	43.8%	43.5%
Vaginal Cancer	4	11	4	19	21.1%	57.9%	21.1%
Vulvar Cancer	3	22	24	49	6.1%	44.9%	49.0%
Other	2	12	10	24	8.3%	50.0%	41.7%

B.

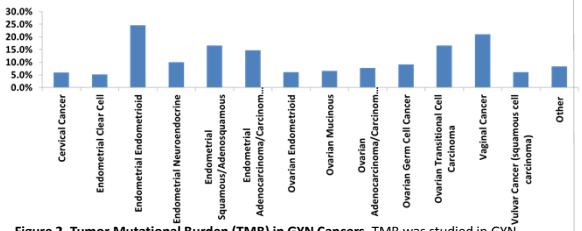

Figure 2. Tumor Mutational Burden (TMB) in GYN Cancers. TMB was studied in GYN cancers with overall levels noted in **A.** High TMB (TMB-H) was noted in 13% of uterine cancers (25% EM, 17% SQ, 10% NE, 5% serous, 5% CC, 5% SS, 4% CS, 3% LMS), 2% of ovarian cancers (9% germ cell, 6% EM, 3% LG, 7% mucinous, 4% CC, 3% CS, 1% serous), 6% cervical, 6% vulvar and 21% of vaginal cancers. **B:** Those GYN tumors histologies with >5% TMB-H.

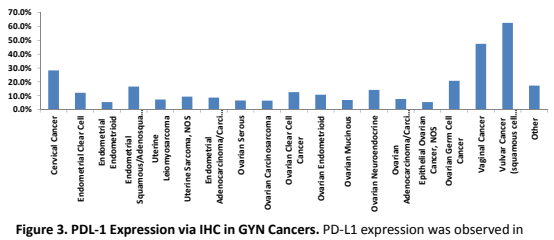

Figure 3. PDL-1 Expression via IHC in GYN Cancers. PD-L1 expression was observed in only 7% of uterine and ovarian tumors but in 28% cervical, 63% vulvar and 47% of vaginal cancers. This figure represents those tumors with >5% PDL-1 expression

A.

	Single +	Double +	Triple +	Triple -	Grand Total	Single +	Double +	Triple +	Triple -
Cervical Cancer	82	9	1	190	282	29%	3%	0%	67%
Ovarian Cancer	224	22	10	2802	3058	7%	1%	0%	92%
Uterine Cancer	242	186	23	1461	1912	13%	10%	1%	76%
Vaginal Cancer	5	4		10	19	26%	21%	0%	53%
Vulvar Cancer	27	3		18	48	56%	6%	0%	38%
Grand Total	585	225	34	4498	5342	11%	4%	1%	84%
Other	5	1		17	23	22%	4%	0%	74%

B.

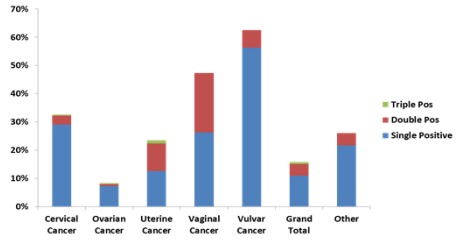

Figure 4. Evaluation of all 3 markers. Triple negative phenotype was identified in more than 85% of uterine serous, carcinosarcom, LMS, and stromal sarcoma and ovarian serous, carcinosarcom, LMS, and low grade. Single or double positive markers were ≥15% in the following cancers: cervical; uterine endometriod (with squamous differentiation had high rate of double and triple positive rate of , clear cell, mucinous; ovarian clear cell, endometriod, germ cell; vulvar and vaginal. Triple positive was <5% in all cancers studied.

	MSI and TMB	TMB and PD-L1	MSI-PD-L1
Cervical Cancer	0.016935409	0.220644243	0.807
Endometrial Adenocarcinoma NOS	**1.76531E-29**	0.023151858	0.020
Endometrial Carcinosarcoma	**1.04707E-29**	0.784542889	0.526
Endometrial Clear Cell	**5.95914E-10**	0.347246242	0.924
Endometrial Endometrioid	**7.95562E-44**	0.409598637	0.370
Endometrial Mucinous	0.196705602	n/a	n/a
Endometrial Neuroendocrine	0.001565402	n/a	n/a
Endometrial Serous	**8.3767E-31**	0.606687545	0.036
Endometrial Squamous/Adenosquamous	0.035673993	0.050214423	0.127
Endometrial Stromal Sarcoma	**4.59906E-10**	0.822673036	0.995
Epithelial Ovarian Cancer, NOS	**1.46611E-91**	0.121998317	0.001
Other	**8.32123E-05**	0.507795925	0.865
Ovarian Adenocarcinoma NOS	n/a	0.628583933	n/a
Ovarian Carcinosarcoma	**3.32924E-18**	0.279644997	0.033
Ovarian Clear Cell Cancer	**5.84404E-08**	0.014063825	0.491
Ovarian Endometrioid	**0.000639777**	0.956570162	0.590
Ovarian Germ Cell Cancer	0.081342153	0.655923157	0.449
Ovarian Low Grade/Borderline Epithelial Cancer	**9.60425E-18**	0.287331616	0.793
Ovarian Mucinous	0.000647433	0.142076399	0.005
Ovarian Neuroendocrine	n/a	0.449328964	n/a
Ovarian Serous	**0**	**1.63271E-08**	**0.000**
Ovarian Sex Cord Stromal Tumors	**1.08427E-11**	0.971811661	0.941
Ovarian Transitional Cell Carcinoma	n/a	n/a	n/a
Uterine Leiomyosarcoma	0.005619335	0.044040565	0.197
Uterine Sarcoma, NOS	**6.23625E-08**	0.490098444	0.743
Vulvar Cancer	**0.00046894**	0.30362833	0.727

Figure 5. Association of Markers. 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). There was NO significant association found for all three markers except in ovarian serous and clear cell carcinomas with p<0.05.

Conclusions

- MSI and TMB were highly correlated (p<0.0006)
- Limited correlation between TMB/PD-L1 (except in ovarian clear cell and serous and uterine leiomyosarcoma) and MSI/PD-L1 (except in uterine serous, ovarian mucinous and serous).
- No significant correlation for MSI/TMB/PD-L1 except in ovarian serous and Clear cell (p<0.05).
- >85% tumors uterine serous, carcinosarcoma, leiomyosarcoma and stromal sarcomas as well as ovarian serous, carcinosa
- Given this, certain histologies appear better suited for immunotherapy: cervical, vulvar, vaginal, uterine endometriod and clear cell, and ovarian endometriod, clear cell, mucinous, germ cell cancers.
- Given each cancer demonstrates a unique phenotype, panel results may be key in directing therapy.

References

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2. Le 2017 Science, 357, 409–413
3. Goodman 2017 Mol Cancer Ther 16(11):2598-2608