Impact of MLH1, PMS2, MSH2 and MSH6 alterations on tumor mutation burden (TMB) and PD-L1 expression in 1,057 microsatellite instability-high (MSI-H) tumors

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Background

- Immune checkpoint inhibitors (ICI) have revolutionized cancer therapy, resulting in remarkable and long-lasting clinical responses, although in a small subsets of patients
- Response to ICIs has been shown to correlate with MSI-high status [1] , TMB, and PD-L1 overexpression [2,3].
- TMB-high and MSI-high rates, and PD-L1 overexpression vary significantly across solid tumors [4,5].
- Although the majority of MSI-H tumors exhibit high TMB and PD-L1 overexpression [6], the precise relationship between TMB level and individual MMR gene alterations remains to be elucidated.
- Herein, we aimed to assess the relationship between the level of TMB and the four MMR genes alterations in different types of cancers among 1057 MSI-H tumors.

Methodology

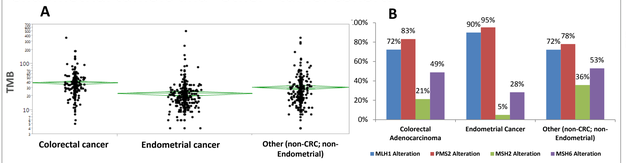
- **Patients:** MSI-H tumor samples profiled by Caris Life Sciences between 2015 and January of 2018 were analyzed.
- **Next generation sequencing** was performed using the NextSeq platform (Illumina, Inc., San Diego, CA) o a 592-gen panel.
- **MMR protein expression** was tested by IHC using antibody clones (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; PMS2, EPR3947 antibody [Ventana Medical Systems, Inc., Tucson, AZ, USA]). dMMRP was the complete loss of protein expression (0+ in 100% of cells)
- **Microsatellite instability (MSI)** was examined using over 7,000 target microsatellite loci and compared to the reference genome . The number of altered microsatellite loci was counted for each sample. MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional PCR-based methods. (sensitivity of > 95% and specificity of > 99%.
- **Tumor mutational burden (TMB)** was measured by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations (592 genes and 1.4 megabases [MB] sequenced per tumor). The threshold to define TMB-high was greater than or equal to 17 mutations/MB and was established by comparing TMB with MSI by fragment analysis in CRC cases
- **PD-L1 IHC** analysis was performed using the primary antibody SP142 (Spring Biosciences). The staining was regarded as positive if its intensity on the membrane of the tumor cells was ≥ 2+ and the percentage of positively stained cells was 5%.
- **Statistical Analysis** ANOVA and chi-square tests were used for comparisons.

Results

1. Cancer types included in the analysis

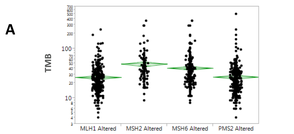
Cancer Type	N	Cancer Type	N
Endometrial Cancer	449	Soft Tissue Tumors	6
Colorectal Adenocarcinoma	283	Bladder Cancer	5
Ovarian Surface Epithelial Carcinomas	47	Non-Melanoma Skin Cancers - Squamous Cell Skin Cancer	4
Gastric Adenocarcinoma	45	Female Genital Tract Malignancy-Other	3
Lung Non-small cell lung cancer (NSCLC)	42	Kidney Cancer	3
Cancer of Unknown Primary	26	Other	3
Small Intestinal Malignancies	23	Head and neck Squamous Carcinoma	2
Pancreatic Adenocarcinoma	19	Thymic Carcinoma	2
Prostatic Adenocarcinoma	16	Liver Hepatocellular Carcinoma	2
Breast Carcinoma	16	Non Epithelial Ovarian Cancer (non-EOC)	1
Esophageal and Esophagogastric Junction Carcinoma	12	Bone Cancer	1
Cholangiocarcinoma	11	Uveal Melanoma	1
Neuroendocrine tumors	10	Thyroid Carcinoma	1
Cervical cancer	9	Lung Small Cell Cancer (SCLC)	1
Glioma	7	Lymphoma	1
Uterine Sarcoma	6	Total	1057

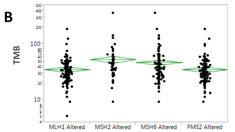
Figure 1: TMB (A) and MMR alteration frequencies (B) in CRC tumors, endometrial tumors and "other" tumor cohorts.



- MSI-H CRCs had the highest TMB (39 mt/MB) compared to MSI-H EC (23 mt/MB) and "all others" (31 mt/MB) (P < 0.0001)
- The most frequently altered (IHC loss or mutation) MMR genes were MLH1 and PMS2 across cancer types. MSH2 and MSH6 were more frequently altered in CRC than endometrial cancer (21% vs. 5% and 49% vs. 28%, respectively; p<0.0001).

Figure 2: TMB seen in MLH1, PMS2, MSH2 and MSH6-altered cohorts in all tumors (A); CRC (B)





All tumors P<.0001

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	616	26.140	1.192	23.802	28.478
MSH2 Altered	120	48.558	2.702	43.261	53.855
MSH6 Altered	314	40.118	1.670	36.843	43.392
PMS2 Altered	598	26.547	1.210	24.174	28.92

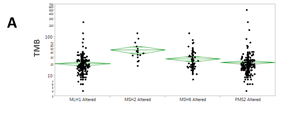
CRC P<.0001

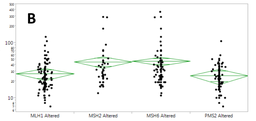
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	182	34.736	2.082	30.646	38.827
MSH2 Altered	53	52.528	3.859	44.949	60.108
MSH6 Altered	128	46.406	2.483	41.529	51.284
PMS2 Altered	187	34.583	2.054	30.548	38.618

Overall MSH2 or MSH6 alterations were associated with higher TMB (48.5 and 40.1 mt/MB, respectively) than MLH1 or PMS2 alterations (27 mt/MB for both)
Similar effects were seen in CRC, endometrial and other cancer types.

Results

Figure 3: TMB seen in MLH1, PMS2, MSH2 and MSH6-altered cohorts endometrial (A) and other tumors (B)





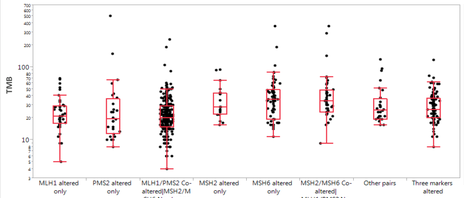
Endometrial tumors, p<0.0001

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	312	20.638	1.381	17.928	23.348
MSH2 Altered	17	46.941	5.914	35.331	58.552
MSH6 Altered	104	27.519	2.391	22.825	32.213
PMS2 Altered	319	22.229	1.365	19.550	24.909

All other tumors, p<0.0001

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	122	27.385	3.447	20.606	34.165
MSH2 Altered	50	44.9	5.38	34.31	55.49
MSH6 Altered	82	46.282	4.204	38.011	54.55
PMS2 Altered	92	25.185	3.969	17.378	32.992

Figure 4: TMB in cohorts with single, double or triple alterations in MMR genes

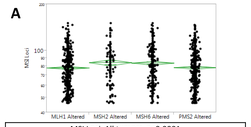


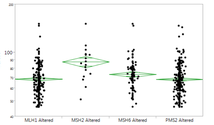
all tumors

Level	Mean	Lower 95%	Upper 95%
MLH1 altered only	26.2	16.6	35.8
PMS2 altered only	44.9	33.2	56.7
MLH1/PMS2 Co-altered| MSH2/MSH6 No change	24.0	20.9	27.0
MSH2 altered only	37.4	23.4	51.3
MSH6 altered only	43.5	36.4	50.7
MSH2/MSH6 Co-altered| MLH1/PMS2 No change	50.4	41.1	59.6
Other pairs	35.6	23.8	47.4
Three markers altered	31.1	25.5	36.7

Tumors with MSH2/6 co-alterations (4% of all tumors) had a higher TMB compared to those with MLH1/PMS2 (39% of all tumors) co-alteration (50 vs. 24 mt/MB; P <0.0001)

Figure 5: Altered MS loci in all tumors (A) endometrial tumors (B), CRC (C) and other tumors (D):



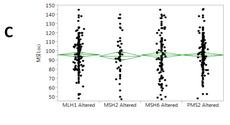


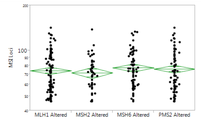
MSI Loci, All tumors, p<0.0001

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	616	77.682	0.862	75.99	79.373
MSH2 Altered	120	83.967	1.954	80.134	87.799
MSH6 Altered	314	83.446	1.208	81.077	85.815
PMS2 Altered	598	78.020	0.875	76.303	79.737

MSI Loci, Endometrial Cancer, p<0.0001

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
MLH1 Altered	312	68.465	0.863	66.77	70.16
MSH2 Altered	17	87.765	3.699	80.504	95.025
MSH6 Altered	104	73.279	1.495	70.343	76.214
PMS2 Altered	319	68.235	0.854	66.559	69.911

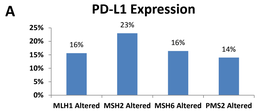




MSH2/6 alterations in EA were associated with more microsatellite alterations (MSH2: 88, MSH6: 73; MLH1: 68; PMS2: 68, p<0.0001), whereas in CRC, all MMR genes were relatively equal.

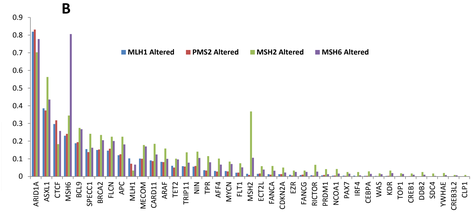
Results

Figure 6: PD-L1 expression seen in MLH1, PMS2, MSH2 and MSH6-altered cohorts (A) and frameshift (fs) mutations that are significantly different among the four groups (B)



	Positive	Negative	Total	PD-L1 Expression
MLH1 Altered	91	494	585	16%
MSH2 Altered	26	87	113	23%
MSH6 Altered	48	245	293	16%
PMS2 Altered	81	499	580	14%

P=0.0149



- PD-L1 overexpression was seen at a higher frequency in tumors with MSH2 (23%) compared to MSH6 (16%), MLH1 (16%), or PMS (14%); P = 0.01
- In CRC, MSH2 alteration is significantly associated with fs mutations in BRD4, ARF, PAX7, POLE, ARHGEF12, EBF1, SPECC1, SDC4, NFKBIA and MYCN
- In endometrial cancer, MSH2 is associated with higher fs mutations in 36 other genes.

Conclusions

- The prevalence of MMR genes alterations differs among different tumor types. For instance, MSH2 and MSH6 are more frequently altered in CRC than endometrial cancers.
- MSH2 and/or MSH6 alterations are associated with a significantly higher TMB than MLH1 and/or PMS2 across several cancer types.
- TMB varies significantly across MSI-H tumors. MSI-H CRCs carry the highest TMB compared to MSI-H endometrial cancers and others MSI-H solid tumors. Furthermore, left side CRC tumors exhibit higher TMB than right side tumors.
- There is a significant association between the altered microsatellite loci and the level of TMB. Furthermore, the association between MSI-H, TMB status and microsatellite loci is tumor type specific.
- PD-L1 overexpression was seen at a significantly higher frequency in tumors with MSH2 (23%) compared to MSH6 (16%), MMLH1 (16%), or PMS (14%) alterations in all tumor types.
- Our findings suggest that alteration in specific MMR gene may have different impact on MS indels, frameshift mutations or microsatellite loci length and TMB level.
- Further investigations into the biology, etiology and optimal treatment of MSI-H tumors are still appropriate.
- Additional analysis to assess the correlation between specific MMR gene alterations and response to checkpoint inhibitors is underway.

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