Gene-specific features (MLH1, MSH2, MSH6, PMS2) of mismatch repair (MMR) protein expression and somatic mutations, microsatellite instability (MSI), and tumor mutational burden (TMB) in MSI-H and MMR-mutated tumor genomic profiles

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BACKGROUND AND METHODS

BACKGROUND

MSI-H is a tumor phenotype associated with increased tumor mutational burden (TMB-H) and response to anti-PD1/PD-L1 immune checkpoint inhibition (ICI).

Loss of expression of the mismatch repair (MMR) heterodimers including the MutL homolog 1 (MLH1/PMS2) and MutS homolog 2 (MSH2/MSH6) is characteristic of many MSI-H tumors and is commonly assayed through immunohistochemistry (IHC).

Somatic mutations of the individual MMR genes are less clearly associated with MSI-H, TMB-H, PD-L1 expression, and response to ICI. These relationships are critical in understanding why immune therapies are effective and how resistance develops.

METHODS

The results from clinical somatic tumor profiles conducted by Caris Life Sciences were examined in two samples of histologically diverse tumors undergoing somatic tumor profiling: 1) all MSI-H tumors or 2) all tumors with somatic MMR mutations. The datasets overlap among tumors with somatic MMR mutations that are also MSI-H (~ 60-70% of tumors with somatic MMR mutations).

The relationship of relevant biomarkers of ICI to TMB-H, PD-L1 expression, IHC loss of expression of the MMR proteins were examined. Associations with the recurrent MSH6 F1088fs mutation, POLE, and HRD somatic mutations (e.g. BRCA1/2, ATM, PALB2, etc.) were also explored.

TABLE 1: Study samples

	MSI-H tumors (n=1057)		Tumors w/MMR mutations (n=470)			
Characteristic	**MLH1/PMS2 loss IHC N=544**	**MSH2/MSH6 loss IHC N=81**	**MLH1 mut N=135**	**MSH2 mut N=91**	**MSH6 mut N=255**	**PMS2 mut N=50**
All tumors %	77	12	29	19	54	11
CRC %	70	16	8	5	14	2
EC %	90	3	4	3	17	2
Other %	61	25	17	11	23	7
Column p	<0.0001	<0.0001	0.008	0.003	0.004	0.03
MeanTMB (mut/mb)						
All	24.8	47.0	36.8	49.9	37.1	23.1
CRC	32.8	56.1	42.2	61.5	49.4	37.5
EC	20.2	45.4	19.0	74.6	42.1	54.8
Other	25.4	36.5	29.8	36.5	31.4	11.9
Column p	<0.0001	0.36	0.2113	0.7507	0.0012	0.4841
PD-L1+ %	18	15	26	29	20	19
POLE mut %	0.6	3.7	7	14	8	4
MSI-H %	-	-	69	66	66	40
IHC loss						
MLH1/PMS2 %	-	-	45	1	59	4
MSH2/MSH6 %	-	-	5	50	30	3

FIGURE 1: Frequency and overlap of MSI, TMB, and MMR mutation

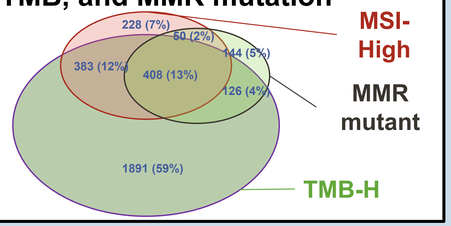
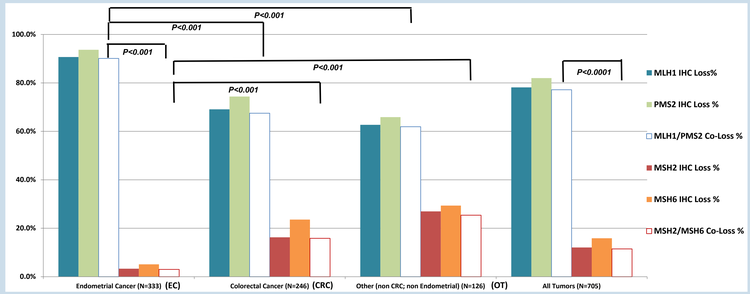


TABLE 2: Tumor histologies

Cancer type	1057 cohort	470 cohort
Endometrial Cancer	449	112
Colorectal Adenocarcinoma	283	113
Ovarian Surface Epithelial Carcinomas	47	41
Gastric Adenocarcinoma	45	13
Lung Non-small cell lung cancer	42	40
Cancer of Unknown Primary	26	11
Small Intestinal Malignancies	23	8
Pancreatic Adenocarcinoma	19	8
Breast Carcinoma	16	14
Prostatic Adenocarcinoma	16	8
Esophageal and Esophagogastric Junction Carcinoma	12	7
Cholangiocarcinoma	11	6
Neuroendocrine tumors	10	5
Cervical cancer	9	4
Melanoma	8	8
Glioma	7	17
Soft Tissue Tumors	6	3
Uterine Sarcoma	6	5
Bladder Cancer	5	8
Non-Melanoma Skin Cancers - Squamous Cell Skin Cancer	4	5
Female Genital Tract Malignancy-Other	3	5
Kidney Cancer	3	3
Other	3	14
Head and neck Squamous Carcinoma	2	2
Liver Hepatocellular Carcinoma	2	
Thymic Carcinoma	2	2
Bone Cancer	1	
Lung Small Cell Cancer	1	3
Lymphoma	1	1
Non Epithelial Ovarian Cancer	1	
Thyroid Carcinoma	1	2
Uveal Melanoma	1	2
Grand Total	1057	470

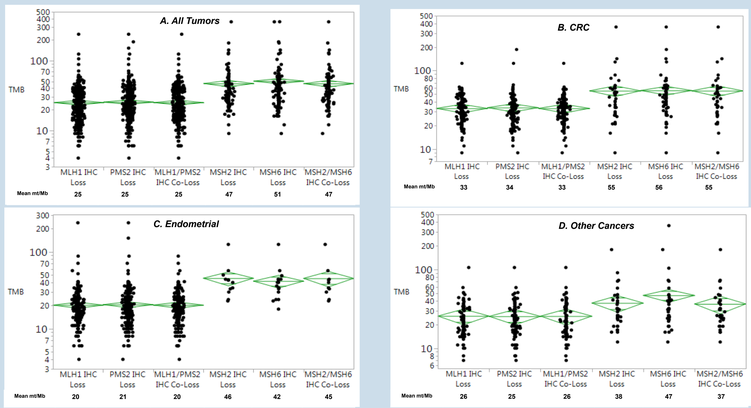
MSI-H TUMORS (n=1057)

Figure 2: MMR protein expression losses by IHC



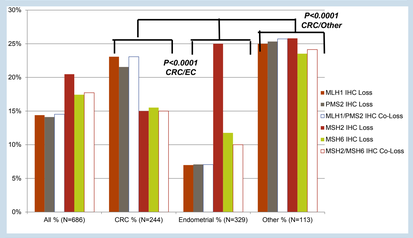
EC are more likely to show protein expression losses (by IHC) of MLH1/PMS2 than are CRC and OT. OT had the highest rates of loss of MSH2/MSH6.

Figure 2: TMB by MMR protein losses and histology



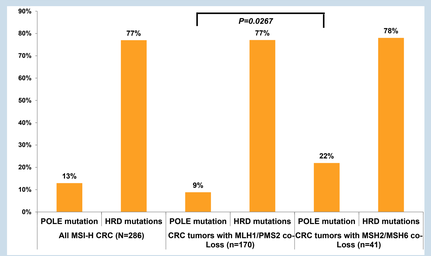
TMB is seen to vary by MMR protein loss and by tumor histology

Figure 3: PD-L1 expression



PD-L1 expression is variable by MMR gene and histology.

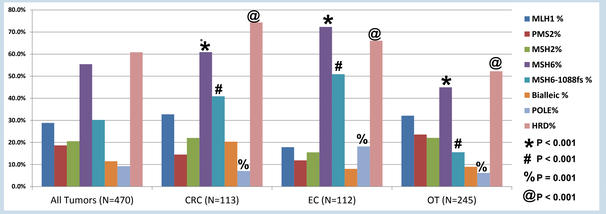
Figure 4: POLE and HRD mutations/VUS in MSI-H CRC



MSI-H CRC have a high rate of HRD mutations and VUS. POLE mut/VUS are more common in tumors with MSH2/MSH6 co-loss by IHC.

TUMORS WITH SOMATIC MMR MUTATIONS (N=470)

Figure 5: Somatic MMR mutations (mut) by tumor



Left: Variability in rate of MSH6 mut, POLE mut/VUS, and HRD mut by tumor histology can be seen

Figure 6: TMB variability and MSH6 somatic mutants

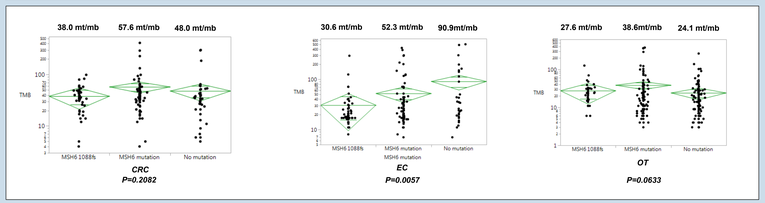
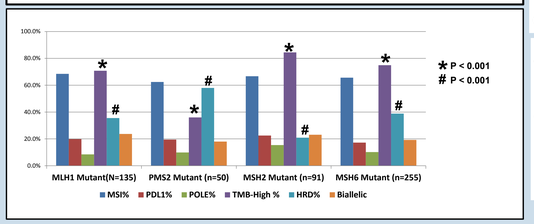


Figure 7: Rates of MSI-H, TMB-H, PD-L1 expression, HRD and POLE variants and biallelic MMR mutations



Above: Somatic muts in MSH6 are associated with variability in TMB by tumor type and mutation (F1088fs vs. other MSH6).

Left: Significant differences in the proportion of tumors that are TMB-H and HRD mutated by somatic MMR mutation were seen

LIMITATIONS AND CONCLUSIONS

LIMITATIONS

Heterogeneity of testing conducted was present in both sample groups: e.g., not all tumors had IHC for the MMR proteins conducted, particularly non-CRC non-EC.

Germ-line matched DNA samples were not collected, limiting the ability to distinguish rare germline variants from somatic mutations. Family history data were also uncollected.

CONCLUSIONS

MSI-H, TMB-H, and PD-L1 expression are related but non-identical phenotypes that are associated with response to ICI.

MMR gene- and histology-specific variability in MSI-H, TMB-H, and PD-L1 expression suggest that the underlying causes of increased tumor mutation and checkpoint evasion may be relevant to treatment efficacy and resistance.

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