The differential response to immune checkpoint inhibitors in colorectal and endometrial cancer patients according to different mismatch repair alterations

Moh'd Khushman[1]; Michael Toboni[2]; Jia Zeng[3]; Joanne Xiu[3]; Alex Farrell[3]; Upender Manne[1]; Bassel El-Rayes[1], Emil Lou[4]; Anthony Shield[5]; Philip Agop Philip[5]; Mohamed Salem[6]; Jim Abraham[3]; David Spetzler[3]; John Marshall[7]; Priya Jayachandran[8]; Michael J Hall[9]; Heinz-Josef Lenz[8]; W. Michael Korn[2]; Mathew A. Powell[2]

1: The University of Alabama at Birmingham; 2: Barnes Jewish Hospital/Washington University ; 3: CARIS life science; 4: The University of Minnesota; 5:Wayne State University;6:Levine cancer institute; 7:Georgetown University ; 8:USC; 9: Fox Chase cancer center

Background

- The main mismatch-binding factor in humans is hMutSa, consisting of MSH2 and MSH6, which recognizes single-base mispairs. Upon mismatch binding, the hMutS complex undergoes conformational change into a sliding clamp and a hMutL heterodimer is recruited.
- The main hMutL complex is hMutLa, consisting of MLH1 and PMS2 and participating in the repair of single-base mismatches. When the hMutS-hMutL complex encounters a strand discontinuity, an excision machinery is recruited, the mismatch containing fragment is degraded, and a new strand synthesized

Figure 1: The different hMutS and hMutL complexes in human MMR.

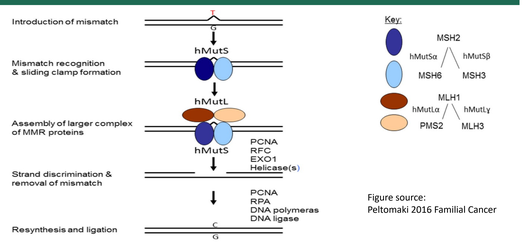


Figure source: Peltomaki 2016 Familial Cancer

- Salem et al. reported that in CRC and EC, loss of co-expression of MLH1/PMS2 was more common than loss of MSH2/MSH6 (P < .0001). Loss of co-expression of MLH1/PMS2 was associated with lower mean TMB (MLH1/PMS2: 25.03 mut/Mb vs MSH2/MSH6 46.83 mut/Mb; P < .0001).
- In colorectal cancer (CRC) and endometrial cancer (EC) patients (pts), preliminary data suggest a differential response to immune checkpoint inhibitors (ICIs) according to different MMR alterations.
- The drivers of this difference remain unknown and no reliable predictive biomarker has been found.
- We explored the genomic alterations, tumor mutation burden (TMB), immune-related gene expressions and signatures, tumor microenvironment (TME), neoantigen load and median overall survival (mOS) in CRC and EC pts treated with ICIs with different MMR alterations.

Materials and Methods

- CRC **(N= 14,949)** and EC **(N=3,574)** specimens were tested at Caris Life Sciences (Phoenix, AZ) with Next Gen Sequencing (NGS) of DNA (592-gene or whole exome sequencing) and RNA (whole transcriptome sequencing).
- MMR/MSI status was determined by IHC of MMR protein and/or NGS.
- Immune cell abundance was quantified using quanTIseq.
- Gene expression profiles were analyzed for T cell-inflamed signature (TIS) and IFN-gamma scores.
- Immune epitope prediction was performed using the NetMHCpan v4.0 method in the Immune Epitope Database.
- Real-world mOS was obtained from insurance claims data and calculated from tissue collection or ICIs start to last contact.
- Statistical significance was determined using chi- square/Fisher-Exact and adjusted for multiple comparisons (adjusted p < 0.05).

Results

Figure 2: Colorectal (CRC) and Endometrial (EC) Patients

Colorectal Cancer (N = 14949)

PMS2	MSH2	MSH6	MLH1 pos / MSH2 pos / MSH6 pos	MLH1 pos / MSH2 pos / MSH6 neg	MLH1 neg / MSH2 pos / MSH6 pos	MLH1 neg / MSH2 pos / MSH6 neg
pos	pos	pos	12852	37	6	84
pos	pos	neg	0	1	0	0
pos	neg	pos	59	0	0	1
pos	neg	neg	648	9	0	4
neg	pos	pos	0	0	0	0
neg	pos	neg	0	0	0	0
neg	neg	pos	0	0	0	0
neg	neg	neg	0	0	0	0

84 (0.6%) patients had MutS co-loss
648 (4.7%) patients had MutL co-loss
117 (0.9%) patients had other MMR IHC loss.

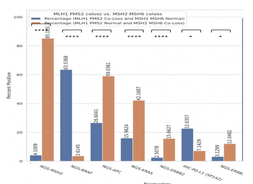
Endometrial Cancer (N = 3574)

PMS2	MSH2	MSH6	MLH1 pos / MSH2 pos / MSH6 pos	MLH1 pos / MSH2 pos / MSH6 neg	MLH1 neg / MSH2 pos / MSH6 pos	MLH1 neg / MSH2 pos / MSH6 neg
pos	pos	pos	2271	28	2	48
pos	pos	neg	1	0	0	0
pos	neg	pos	44	3	0	0
pos	neg	neg	915	2	0	1
neg	pos	pos	0	0	0	0
neg	pos	neg	0	0	0	0
neg	neg	pos	0	0	0	0
neg	neg	neg	0	0	0	0

48 (1.4%) patients had MutS co-loss
915 (27.6%) patients had MutL co-loss
81 (2.4%) patients had other MMR IHC loss.

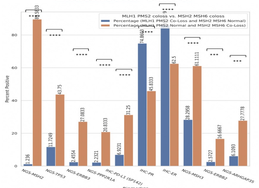
Figure 3: Genomic alterations

Colorectal Cancer



- **Mutation frequencies of APC, KRAS, ERBB2, ERBB3 are significantly higher in MutS.**
- **IHC PD-L1 positive rate, mutation frequency of BRAF is lower in MutS.**

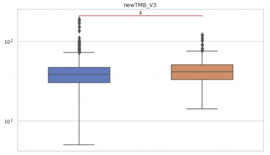
Endometrial Cancer



- **MSH2, TP53, ERBB3, PPP2R1A, IHC-PD-L1, MSH3, ERBB2 and ARHGAP35 positive rates are significantly higher in MutS.**
- **IHC PR and IHC ER loss positive rates are significantly lower in MutS**

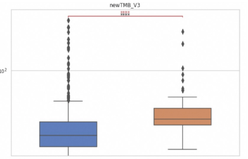
Figure 4: Tumor Mutation Burden

Colorectal Cancer



TMB values are significantly higher in MutS co-loss

Endometrial Cancer



TMB values are significantly higher in MutS co-loss

Figure 5: Immune related gene expression

Colorectal Cancer

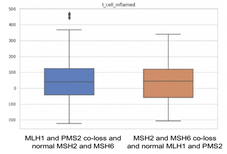
Expression of CD274, IFNG, IDO1 and LAG3 are significantly lower in MutS co-loss

Endometrial Cancer

Expression of CD80, CD274, CTLA4, HAVCR2, IFNG, IDO1, LAG3, PDCD1, and PDCD1LG2 are significantly higher in MutS co-loss

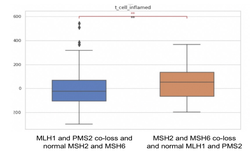
Figure 6: Immune signature (T-cell inflamed score)

Colorectal Cancer



No difference in T-cell inflamed score between the two groups

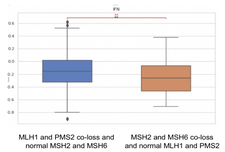
Endometrial Cancer



T-cell inflamed score is significantly higher in MutS co-loss

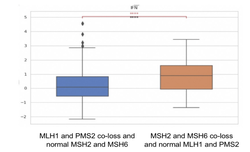
Figure 7: Immune signature (IFN-gamma score)

Colorectal Cancer



IFN-gamma scores are significantly lower in MutS co-loss group.

Endometrial Cancer



INF-gamma score is significantly higher in MutS co-loss

Figure 8: Tumor Microenvironment

Colorectal Cancer

Cell Type	fold change
B cell	0.9299854
Macrophage M1	1.0353868
Macrophage M2	0.7504589
Monocyte	inf
Neutrophil	1.0647099
NK cell	0.8898786
T cell CD4+ (non-regulatory)	1.0405909
T cell CD8+	1.4589834
T cell regulatory (Tregs)	1.0091382
Myeloid dendritic cell	0.97253186

- **B cells, Macrophage M2 (cold) and NK cells are significantly higher in MutS co-loss**

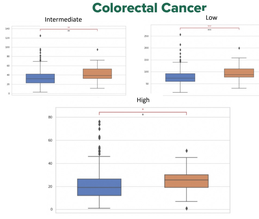
Endometrial Cancer

Cell Type	fold change
B cell	1.0926403
Macrophage M1	0.61074114
Macrophage M2	1.1284298
Monocyte	3.4750133
Neutrophil	1.0382272
NK cell	1.0406753
T cell CD4+ (non-regulatory)	0.8124716
T cell CD8+	0.32283562
T cell regulatory (Tregs)	0.8363445
Myeloid dendritic cell	1.2676436

- **Macrophage M1 and CD8+ cell are significantly higher in MutS co-loss**
- **Myeloid dendritic cell is significantly lower in MutS co-loss**

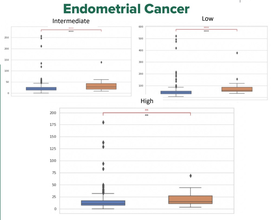
Figure 9: Neoantigen load (number of neoepitopes)

Colorectal Cancer



The numbers of high, intermediate, and low affinity neoepitopes are significantly higher in MutS co-loss

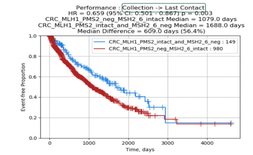
Endometrial Cancer



The numbers of high, intermediate, low affinity epitopes are significantly higher in MutS co-loss

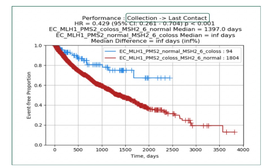
Figure 10: Median Overall Survival (collection to last contact)

Colorectal Cancer



- **The mOS in MutS co-loss (N = 149) vs. MutL co-loss (N = 980) was 56 months (m) vs. 36 m (p = 0.003).**

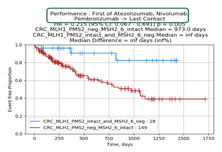
Endometrial Cancer



- **The mOS in MutS co-loss (N = 94) vs. MutL co-loss (N = 1804) was NR vs. 47 m (p < 0.001)**

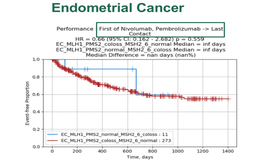
Figure 11: Median Overall Survival (ICIs treatment to last contact)

Colorectal Cancer



- **In ICI-treated pts, the mOS in MutS co-loss (N = 28) vs. MutL co-loss (N = 149) was not reached (NR) vs. 32 m (p = 0.005).**
- **BRAF mutation didn't impact survival in MutL co-loss**

Endometrial Cancer



- **In ICI-treated pts, the mOS in MutS co-loss (N = 11) vs. MutL co-loss (N = 273) was NR vs. NR (p = 0.559)**

Summary and Conclusion

Features	Colorectal Cancer (MutS co-loss vs. MutL co-loss)	Endometrial Cancer (MutS co-loss vs. MutL co-loss)
Incidence	0.6 vs 4.7	1.4 vs 27.6
Genomic alterations	High APC, KRAS, ERBB2, ERBB3 vs. PD-L1 and BRAF	MSH2, TP53, ERBB3, PPP2R1A, IHC-PD-L1, MSH3, ERBB2 and ARHGAP35 vs. ER and PR
TMB	**Higher in MutS co-loss**	**Higher in MutS co-loss**
T-cell inflamed score	No difference	Higher in MutS co-loss
IFN-gamma score	Lower in MutS co-loss	Higher in MutS co-loss
Immune related gene expression	CD274, IFNG, IDO1 and LAG3 are lower in MutS co-loss	CD80, CD274, CTLA4, HAVCR2, IFNG, IDO1, LAG3, PDCD1, and PDCD1LG2 are higher in MutS co-loss
Tumor microenvironment	B cells, Macrophage M2 (cold) and NK cells are higher in MutS co-loss.	Macrophages M1 and CD8+ cell are higher in MutS. Myeloid dendritic cell is lower in MutS co-loss.
Neoantigen Load	**High, intermediate, and low affinity neoepitopes are higher in MutS co-loss**	**High, intermediate, and low affinity neoepitopes are higher in MutS co-loss**
mOS (collection to last contact)	56 months (m) vs. 36 m (p = 0.003).	NR vs. 47 m (p < 0.001)
mOS(ICIs treatment to last contact)	NR vs. 32 m (p = 0.005)	NR vs. NR (p = 0.559)

- This is the largest study to explore differential response to ICIs in CRC and EC pts with different MMR alterations.
- In pts with CRC and EC, the mOS was longer in MutS co-loss compared to MutL co-loss.
- In ICI-treated pts, the mOS was longer in MutS co-loss compared to MutL co-loss in CRC but not in EC.
- Apart from TMB, among the explored biomarkers, neoantigen load was higher in MutS co-loss compared to MutL co-loss in both CRC and EC and maybe the driving factor for differential response to ICIs.

Corresponding author: Moh'd Khushman, MD. mkhushman@uabmc.edu