Reduced PD-1/PD-L1 expression in KRAS-mutant versus wild-type Microsatellite-Instability-High (MSI-H) Colorectal Cancer (CRC) and association of Wnt pathway corepressor TLE-3.

[1]Namrata Vijayvergia, [1]Julie Innocent, [1]Aarti Khushal Bhatia, [1]Raji Shameem, [2]Joanne Xiu, [2]Zoran Gatalica, [1]Sergei Grivennikov, [1]Wafik S. El-Deiry
[1]Department of Hematology/Oncology, Fox Chase Cancer Center, Philadelphia, PA; [2]Caris Life Sciences, Phoenix, AZ

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Abstract #3611

Background: MSI-H CRC comprises ~15% of CRC & has higher PD-1 & PD-L1 (P(+)) expression in CD4+ lymphocytes & tumor cells, respectively, compared to non-MSI-H CRC. Immune-checkpoint inhibitor therapy is being tested in clinical trials for MSI-H CRC (NCT02060188, NCT01876511). Human homologue of Groucho, Transducin-like-Enhancer of Split orthologue (TLE-3), inhibits Wnt signaling by competition with b-catenin for LEF/TCF binding. We investigated different biomarkers that may characterize P(+) MSI-H CRC.

Methods: 55 MSI-H (including 13 Lynch Syndrome cases) CRCs were profiled at Caris Life Sciences (Phoenix, AZ). Immunohistochemistry (IHC) & mutation analysis was performed to compare P(+) & P(-) MSI-H CRC. PD-1 expression was measured on Tumor Infiltrating Lymphocytes (TIL) with the cutoff of 1+, 1% (1/HPF); PD-L1 expression was measured on tumor cells with the cutoff of 2+, 5%. IHC assays underwent a stringent validation process meeting CLIA/CAP criteria. Fisher's exact test was used to compare categorical variables.

Results: Analysis of 55 MSI-H tumors revealed 39 (71%) were P(+) (39 PD-1(+); 1 also PD-L1(+)) & 16 (29%) were P(-). The P(+) group expressed TOPO-1/2 (43%/93%), EGF-R (88%), TS (90%), RRM1 (77%), PTEN (77%), c-MET (50%), and MGMT (53%) by IHC, and the expression was not significantly different compared to the P(-) group. TLE-3 protein expression, however, was 43% in P(+) and only 8% in P(-) MSI-H tumors (P = 0.04). Only 9 (27%) of P(+) tumors harbored a KRAS mutation, while 10 (62%) had KRAS mutation in P(-) tumors (P = 0.04).

Conclusions: PD-1/PD-L1 protein expression is inversely associated with KRAS mutation status in MSI-H CRC. This is relevant to clinical trials testing immune checkpoint inhibitors in MSI-H CRC. The signaling between KRAS mutation & low or absent PD-1/PD-L1 should be further investigated. TLE-3 expression may dampen b-catenin signaling in P(+) tumors.

Methods

The study included 55 MSI-H (including 20 confirmed lynch syndrome cases), 9 MSI-low (MSI-L) and 558 microsatellite stable (MSS) cases that were profiled at Caris Life Sciences (Phoenix, AZ) using immunohistochemistry (IHC) and sequencing (NextGen and Sanger).
MSI status was determined using a combination of IHC (MLH1, PMS2, MSH2, MSH6) and MIA (Microsatellite Instability Analysis) fragment analysis. MIA included fluorescently-labeled primers for co-amplification of seven markers including five mononucleotide repeat markers (BAT-25, BAT26, NR-21, NR24 and MONO-27) and two pentanucleotide repeat markers (Penta C and D). The mononucleotide markers were used for MSI determination while the pentanucleotide markers were used to detect either sample mix-ups or contamination. A sample was considered MSI-H if two or more mononucleotide repeats were abnormal while MSI-L if one mononucleotide repeat was abnormal. The tumors were considered MSS if mononucleotide repeats were identical between the tumor and adjacent normal tissue.
Other specific tests were performed per physician request. Biomarker associations were statistically analyzed by two-tailed Fisher Exact tests.

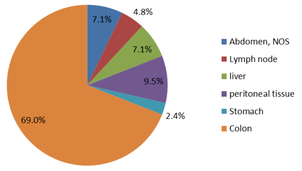
Results

Table 1:Patient characteristics among PD-1/PD-L1+ (P+) and negative (P-) MSI colorectal tumors

	Patient N	Average age	Age range	Gender
MSI-H Cohort	**55**	**59.6**	**35-98**	**M: 51%; F: 49%**
Confirmed Lynch syndrome	13	45.7	39-54	M: 73%; F: 27%
P+ MSI-H Cohort	**39**	**60.7**	**35-81**	**M: 47%; F: 53%**
P-MSI-H Cohort	**16**	**57.1**	**39-98**	**M: 60%; F: 40%**

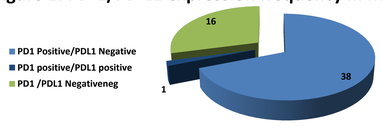
- Out of the 55 MSI-H patients, 20 had confirmed Lynch syndrome, while the other 35 carried the MSI-H phenotype as tested by IHC and MIA, but no further information on sporadic or germline status.
- Compared with the complete cohort of MSI-H CRC patients, those with confirmed Lynch syndrome are younger in age and are more likely to be male.
- Patient age and gender are not significantly different in P+ and P- MSI-H patients.

Figure 1: Tumor sites



Results-continued

Figure 2: PD-1/PD-L1 expression frequency in MSI-H CRC



- PD-1 expression on tumor-infiltrating-lymphocyte (TIL) is found in 73% of MSI-H CRC tumors while PD-L1 expression on tumor cells is rare.
- 1 case was found to be high in both PD-1 and PD-L1

Figure 3: Sporadic MSI-H CRC (BRAF V600E) **H&E** (**a:** 40x) **and PD-1 staining on TIL** (**b:** 40x, TIL count/HPF>5)

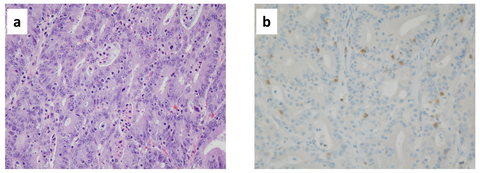
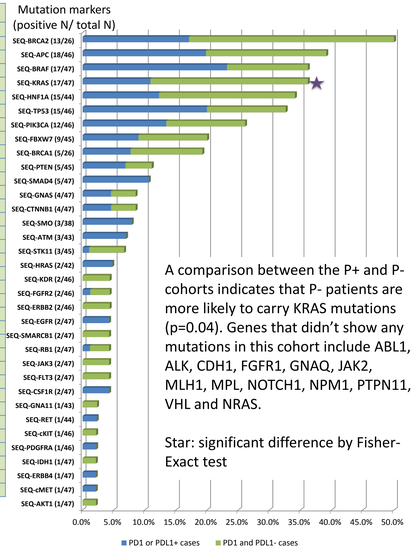


Figure 4: Mutation frequencies observed in MSI-H CRC cohort.

P+			P-		
MT	Total	%	MT	Total	%
7	18	39%	6	8	75%
13	33	39%	5	13	38%
14	34	41%	3	13	23%
9	34	26%	8	13	62%
9	32	28%	6	12	50%
12	33	36%	3	13	23%
9	34	26%	3	12	25%
6	32	19%	3	13	23%
3	18	17%	2	8	25%
4	32	13%	1	13	8%
5	34	15%	0	13	0%
3	34	9%	1	13	8%
3	34	9%	1	13	8%
3	28	11%	0	10	0%
3	30	10%	0	13	0%
1	33	3%	2	12	17%
2	32	6%	0	10	0%
0	33	0%	2	13	15%
1	33	3%	1	13	8%
0	33	0%	2	13	15%
2	34	6%	0	13	0%
0	34	0%	2	13	15%
1	34	3%	1	13	8%
0	34	0%	2	13	15%
0	34	0%	2	13	15%
2	34	6%	0	13	0%
0	31	0%	1	12	8%
1	32	3%	0	12	0%
0	33	0%	1	13	8%
1	33	3%	0	13	0%
0	34	0%	1	13	8%
1	34	3%	0	13	0%
1	34	3%	0	13	0%
0	34	0%	1	13	8%



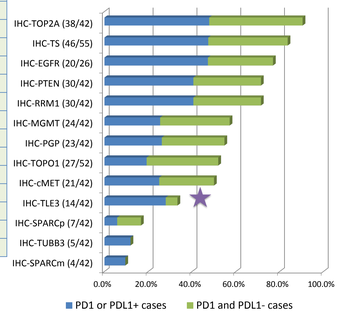
A comparison between the P+ and P- cohorts indicates that P- patients are more likely to carry KRAS mutations (p=0.04). Genes that didn't show any mutations in this cohort include ABL1, ALK, CDH1, FGFR1, GNAQ, JAK2, MLH1, MPL, NOTCH1, NPM1, PTPN11, VHL and NRAS.

Star: significant difference by Fisher-Exact test

Results - continued

Figure 5: Protein overexpression frequencies observed in MSI-H CRC.

P+			P-		
Pos	Total	%	Pos	Total	%
28	30	93%	10	12	83%
35	39	90%	11	16	69%
15	17	88%	5	9	56%
23	30	77%	7	12	58%
23	30	77%	7	12	58%
16	30	53%	8	12	67%
16	30	53%	7	12	58%
16	37	43%	11	15	73%
15	30	50%	6	12	50%
13	30	43%	1	12	8%
4	30	13%	3	12	25%
5	30	17%	0	12	0%
4	30	13%	0	12	0%



Overexpression of protein TLE3 is significantly higher in MSI-H CRC tumors that are infiltrated with PD-1 positive lymphocytes(p=0.04)
Star: significant difference by Fisher-Exact test

Conclusions

- Sequencing and IHC tests revealed molecular features of MSI-H CRC tumors, including high infiltration with PD-1 positive lymphocytes and low expression of PD-L1 on tumor cells.
- An inverse correlation of KRAS mutation with PD-1/PD-L1 expression may have implications in clinical trials using immune checkpoint inhibitors and their combination with EGFR antibodies, as the synergistic effect could be mediated by ADCC (antibody-dependent cell-mediated cytotoxicity).
- While the β-catenin signaling pathway has shown to be high in MSI-H tumors, we show that TLE-3 expression may dampen β-catenin signaling in P(+) tumors.
- Homologous recombination deficiency was observed in over 50% of MSI-H CRC tumors suggested by BRCA1/2 mutations suggesting some role of PARP inhibitors in this phenotype.
- Activation of PIK3CA /Akt/mTor pathway (PIK3CA, PTEN, STK11 mutations) and Wnt pathway (APC, CTNNB1) suggest opportunities for targeted therapies.

References

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