Characterization of Tumor Mutation Burden (TMB) in Gastrointestinal (GI) Cancers.

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Abstract

Background: GI cancers are generally insensitive to immune checkpoint inhibitors (ICIs). Response to ICIs has been shown to correlate with TMB. Herein, we attempt to quantify TMB in GI cancers and its correlation with PD-1/PD-L1 expression.

Methods: Tumors from various GI sites: right-sided and left-sided colon cancers (RT and LT), rectal cancer (RC), small bowel adenocarcinoma (SBA), gastric adenocarcinoma (GA), anal cancer (SCCA), hepatocellular carcinoma (HCC), esophageal adenocarcinoma (EA) and esophageal squamous cell carcinoma (E-SCC), biliary cancer (BC), pancreatic adenocarcinoma (PA), and pancreatic neuroendocrine tumors (PNET) were analyzed by NextGen sequencing. TMB was calculated using only somatic nonsynonymous missense mutations sequenced with a 592-gene panel. Microsatellite instability (MSI) was assessed by fragment analysis. Correlation of PD-1/PD-L1 expression with TMB was calculated by student's t test.

Results: In total, 1374 tumors were examined. Among the different GI cancer types, RT and LT had the highest TMB (mean: 11.6 and 9.9 mutations [mut]/megabase [MB]), whereas BC and PA had the lowest levels (mean: 5.7 and 4.9 mut/MB) (Table). Overall, primary tumors had higher TMB than metastases (mean: 8.3 vs. 6.5 mut/MB, p = 0.037). Using a cut-off of 17 mut/MB to define high vs. low TMB, high TMB was seen in all 24 MSI-H and 2 MSS colon tumors with POLE mutations, but not in other MSS colon tumors (n = 325, p < 0.0001). Similarly, among 6 GA tumors tested for MSI, high TMB was seen in 2 MSI-H whereas low TMB was seen in the 4 MSS tumors. Overall high TMB was seen most frequently in RT (12%), GA (11%), and SCCA (8%), and least frequently in PA (1.3%) and E-SCC (0%). PD-1 correlated with TMB in some tumor types (RT and RC), as did PD-L1 (RT and HCC).

Conclusions: TMB varies among GI cancers. Forthcoming prognostic analysis to assess the correlation between TMB and response to ICIs in GI cancers is underway.

Cancer	N	%High TMB	Mean TMB (mut/MB)	P value for TMB correlation with PD-1	P value for TMB correlation with PD-L1
Right-sided colon	197	12	11.6	0.03	0.03
Gastric adeno	113	11	9.0	0.7	0.2
Anal cancer	25	8	8.3	0.5	0.1
Small bowel adeno	62	6.5	9.2	0.5	0.4
Rectal cancer	168	4.2	7.4	0.04	0.5
Hepatocellular	62	3.2	7.2	0.4	0.01*
Left-sided colon	163	3	9.9	0.3	0.1
Pancreatic neuroendocrine tumors (PNET)	33	3	5.4	0.5	0.7
Biliary cancer	136	2.9	5.7	0.05	0.1
Esophageal adeno	82	2.4	6.7	0.4	0.4
Pancreatic adeno	317	1.3	5.0	0.05	0.1
Esophageal squamous	17	0	6.4	0.9	0.1

* inverse correlation

Background

- Immune checkpoint inhibitors have changed the treatment paradigm of various cancer types including melanoma, and NSCLC.
- Increased clinical activity of ICIs are seen in tumors carrying high tumor mutational burden including bladder cancer, NSCLC, and melanoma, likely due to the increased presence of mutation-associated tumor neoantigens.
- Gastroesophageal tumors are generally considered insensitive to immune checkpoint inhibition; however, a phase II clinical trial showed an impressive response rate of 40% in colorectal cancers with mismatch repair deficiency when treated with ICIs, making treating MSI-H CRC patients with ICIs part of standard therapy.
- A study done at Memorial Sloan Kettering shows that using NextGen sequencing and mutational load evaluation could provide a reliable surrogate in colorectal cancer for MSI testing to identify potential responders to immune checkpoint inhibitors
- Increased PD-L1 expression has also been shown to predict response to ICI's in NSCLC, melanoma and bladder cancer.
- We aim to investigate TMB across 12 subtypes in gastroesophageal tumors and its correlation with PD-1/PD-L1 expression.

Methods

- Next-generation sequencing (NGS) was performed on genomic DNA isolated from FFPE tumor samples using NextSeq platform (Illumina, Inc., San Diego, CA). A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA).
- The cutoff of TMB-high vs. TMB-low used was 17 mutations/megabase due to the high concordance observed between TMB-high and MSI-high in colorectal cancer.
- Tumor mutational load was measured for tumors sequenced using the NextSeq platform (592 genes and 1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that have not been previously described as germline alterations
- Microsatellite instability was tested with MIA (Microsatellite Instability Analysis) fragment analysis (Promega)
- Correlation of PD-1/PD-L1 expression and MSI with TMB was calculated by student's t test.

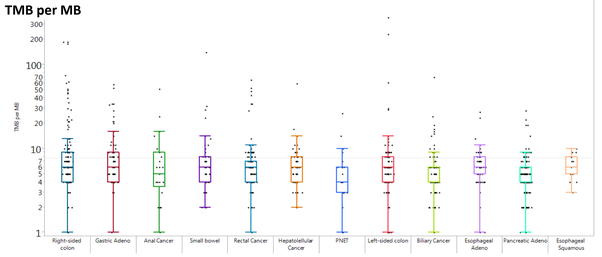
Results

1. Patient characteristics

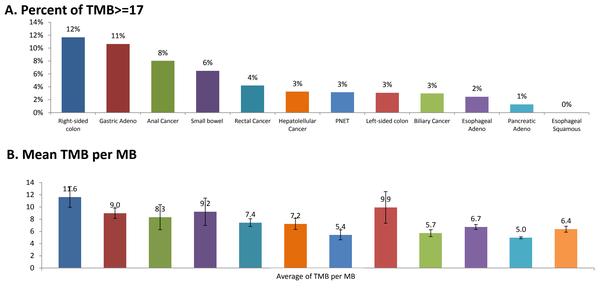
Cancer Types	Gender		Average Age	Primary vs. Mets			Total
	female	male		Primary	Mets	Unclear	
Right-sided colon	107	90	61.1	116	47	34	**197**
Gastric Adeno	51	62	60.5	84	27	2	**113**
Anal Cancer	16	9	60.8	9	10	6	**25**
Small bowel	34	28	62.2	32	24	6	**62**
Rectal Cancer	75	93	57.8	82	72	14	**168**
Hepatocellular Cancer	16	46	61.6	40	22		**62**
Left-sided colon	78	85	57.1	132	31		**163**
PNET	16	16	56.3	11	21		**32**
Biliary Cancer	65	71	61.6	83	29	24	**136**
Esophageal Adeno	10	72	61.4	53	23	6	**82**
Pancreatic Adeno	160	157	63.2	144	172	1	**317**
Esophageal Squamous	7	10	64.9	7	9	1	**17**
Total	635	739	60.7	793	487	94	1374

Results

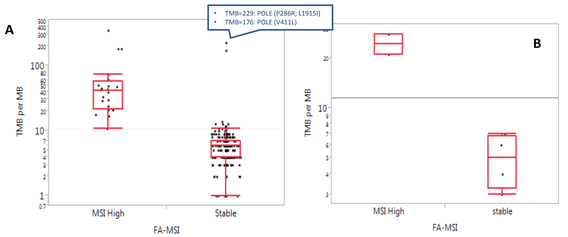
2. Box plot of tumor mutational burden in the 12 gastrointestinal cancer types. The line within the box represents the median value, while the ends of the box represent the 1st and 3rd quartile.



3. (A) Percent of cases carrying a TMB of >= 17 and (B) Mean TMB per megabase (MB) in the 12 cancer types. Error bars on (B) are standard errors.



4. TMB and MSI are highly correlated in (A) colorectal and (B) gastric tumors. Two MSI-stable CRC tumors with high TMB carried POLE mutations.

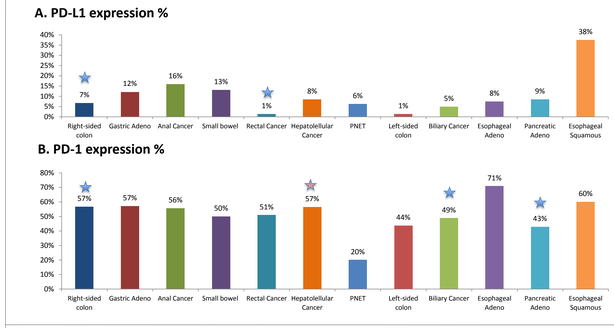


P < 0.0001, t Test

P = 0.05, t Test

Results

5. (A) Tumor cell PD-L1 expression and (B) PD-1 expression on tumor-infiltration lymphocytes on the 12 GI cancer types . A star indicates a correlation of PD-L1 or PD-1 expression with TMB (p < 0.05)



Conclusions

- The median TMB across 12 GI cancer types is not significantly different; however, the percentage of TMB-high (using a cutoff of 17 mutations/megabase) varies from 0 to 12%.
- Right-sided colon, gastric adenocarcinoma, and anal tumors carry the highest percent TMB-high, while esophageal adenocarcinoma, pancreatic adenocarcinoma and, esophageal squamous cell tumors carry the lowest percent of TMB-high cases.
- In colorectal tumors and gastric adenocarcinoma, MSI-high is highly correlated with TMB-high.
- Clinical activities of ICIs observed in cancer types with low TMB but high PD-L1 expression (e.g., esophageal squamous cell carcinoma) suggest that a combination of TMB and PD-L1 as well as other predictive markers may be needed to identify responders to ICIs in GI cancers.

References

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