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

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-L1 expression.

Methods: Tumors from various GI sites: right-sided and left-sided colon cancers (RT and LS), rectal cancer (RC), small bowel adenocarcinoma (SBA), gastric adenocarcinoma (GA), anal cancer (SCCA), hepatocellular carcinoma (HCC), esophageal adenocarcinoma (EAC), and esophageal squamous cell carcinoma (E-SCC), biliary cancer (BC), pancreatic adenocarcinoma (PA), and pancreatic neuroendocrine tumors (PNET) were analyzed by NextSeq sequencing. TMB was calculated by student’s t test. A custom SureSelect XT assay was used to enrich 592 whole megabase (MB) in the 12 cancer types.

Results: In total, 1174 tumors were examined. Among the different GI cancer types, RT and LT had the highest TMB (mean: 11.6 and 9.9 mutations/megabase [mut/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 MSI-L colon tumors with POLE mutations, but not in other MSI tumors (n = 32, p < 0.0001). Similarly, among the 6 tumors that tested positive for MSI, high TMB was seen in 2 MSI-H whereas low TMB was seen in the 4 MSI 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-L1 correlated with 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.

Abstract

- Immune checkpoint inhibitors have changed the treatment paradigm of various cancer types. The cutoff of TMB is 10%.
- Increased clinical activity of ICIs is 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.
- Gastrointestinal cancers are generally considered insensitive to immune checkpoint inhibitors; 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 mutualistic neoantigen could provide a reliable surrogate in colorectal cancer for MSI testing to identify potential responders to immune checkpoint inhibitors.
- Increased PD-L1 expression was also shown to predict response to ICIs in NSCLC, melanoma, and bladder cancer.
- We aim to investigate TMB across 12 subtypes in gastrointestinal tumors and its correlation with PD-L1/PD-1 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, CA).

- The cutoff of TMB-high vs. TMB-low was used as 17 mutations/megabase due to the high concordance observed between TMB-high and MSI-H in colorectal cancer.

- Tumor mutational load was measured for tumors sequenced using the NextSeq platform [MSI-H: 1.4–2.5 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 MSIa (Microsatellite Instability Analysis) fragment analysis panel (Promega).

- Correlation of PD-L1/PD-1 expression and MSI with TMB was calculated by student’s t test.

Results

1. Patient characteristics

- Cancer Types
- Primary vs. Metastasis
- Primary-only
- Metastasis-only
- Total

Table

<table>
<thead>
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<tr>
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</table>
| TMB vs. PD-L1 vs. MSI

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

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

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

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).

6. Results

   - 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.

Conclusions

- The median TMB across 12 GI cancer types is not significantly different; however, the percent tumors 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.