Landscape of Tumor Mutation Load, Mismatch Repair Deficiency, and PD-L1 Expression in a Large Patient Cohort of Gastrointestinal Cancers


Abstract

The efficacy of immunotherapy varies widely among different gastrointestinal cancers. Response to immune checkpoint inhibitors is shown to correlate with tumor mutation load (TML), mismatch repair deficiency (dMMR) status, and programmed cell death-ligand 1 (PD-L1) expression. Herein, we quantify TML, dMMR, and PD-L1 expression and determine their interrelationship in gastrointestinal cancers. Here, a total of 4,125 tumors from 14 different gastrointestinal cancer sites were studied using validated assays. Next-generation sequencing was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor specimens using the NextSeq platform. TML was calculated using only somatic nonsynonymous missense mutations sequenced with a 592-gene panel.

Microsatellite instability (MSI) was assessed using direct analysis of altered known MSI loci in the target regions of the sequenced genes. PD-L1 expression was analyzed by IHC. Interestingly, right-sided colon and small-bowel adenocarcinomas had the highest prevalence of TML-high tumors (14.6% and 10.2%, respectively). Pancreatic neuroendocrine tumors and gastrointestinal stromal tumors had the lowest rates of TML-high (1.3% and 0%, respectively). TML-high was strongly associated with MSI-H (P < 0.0001). However, all TML-high anal cancers (6.3%) were microsatellite stable (MSS). Higher PD-L1 expression was more likely to be seen in MSI compared with MSS tumors (20.6% vs. 7.8%, P < 0.0001).

Implications: TML-high rate varied widely among gastrointestinal cancers. Although MSI is conceivably the main driver for TML-high, other factors may be involved. Future clinical trials are needed to evaluate whether the integration of TML, MSI, and PD-L1 could better identify potential responders to immunotherapy.

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antitumor immune response by increasing immune cell infiltration and thereby improving sensitivity to ICIs (7, 8). As tumor mutation load (TML) measures the total number of nonsynonymous, somatic mutations identified per megabase (MB) of the genome coding area, tumors with MSI-H are characterized by a higher TML. Furthermore, current evidence suggests that tumors with a TML-high status are associated with improved sensitivity to ICIs (9–11). Tumor cells also express several immunoregulatory molecules, such as PD-L1. PD-L1 binds to the immunoinhibitory receptor PD-1 on T cells, downregulates T-cell activation, and disrupts the local antitumor immune response. Increased PD-L1 expression by IHC has been shown to predict response to ICI in NSCLC (12), melanoma (13), gastric cancer and GEJ adenocarcinoma (6), and bladder cancer (14). For these reasons, TML, MSI, and PD-L1 seem to be biologically intertwined; however, the extent of this relationship and the mechanisms that regulate it are unclear.

dMMR/MSI-H phenotype can be detected by directly enumerating known MSI loci using targeted deep sequencing [MSI next-generation sequencing (MSI-NGS); ref. 15]. The Cancer Genome Atlas (TCGA) showed that three quarters of hypermutated colorectal cancers (defined as tumors harboring a mutation rate of >12 × 10^6) were MSI-H, whereas the rest had somatic MMR gene and polymerase ε (POLE) mutations, indicating the strong association between MSI-H and TML-high (16). Stadler and colleagues (17) recently used next-generation sequencing (NGS) analysis and suggest that, in colorectal cancer, TML is a highly accurate measure of screening for MSI. Defects in DNA polymerase ζ, which usually have proofreading capabilities (18, 19), and the DNA MMR system have been shown to cause hypermutation in tumors (20). Nonetheless, our understanding of the processes that cause hypermutational status in most cancer types is limited (21).

In the current study, we characterize the prevalence of MSI, TML, and PD-L1 in 4,125 gastrointestinal tumors and attempt to understand the relationship between these three biomarkers. To our knowledge, this is the largest study to examine the prevalence and correlation of MSI, TML, and PD-L1 in gastrointestinal cancers.

Materials and Methods

Patients
Tumors from patients with various gastrointestinal cancers were molecularly profiled using a single platform between 2009 and July of 2017, deidentified, and retrospectively analyzed. Gastrointestinal tumors tested were consecutive samples submitted for molecular profiling. Histologic and clinical diagnoses were obtained from submitted pathology reports that were confirmed by board-certified pathologists. For colorectal cancers, only tumors that were documented to arise from a specified location in the colon or rectum were included as primary tumors. Samples taken from locations other than that of the primary cancer were classified as metastases.

NGS
NGS was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina, Inc.). Matched normal tissue was not sequenced. A custom-designed SureSelect XT assay was used to enrich 592 whole-genome targets (Agilent Technologies). All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of >500 and an analytic sensitivity of 3%. Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques.

TML
TML was measured by counting all nonsynonymous missense mutations found per tumor that had not been previously described as germline alterations (592 genes and 1.4 MB sequenced/tumor). The threshold to define TML-high was greater than or equal to 17 mutations/MB and was established by comparing TML with MSI by fragment analysis in colorectal cancer cases, based on reports of TML having high concordance with MSI-H in colorectal cancer (22).

MSI
MSI was examined using over 7,000 target microsatellite loci and compared with the reference genome hg19 from the University of California, Santa Cruz (Santa Cruz, CA) Genome Browser database. The number of microsatellite loci that were altered by somatic insertion or deletion was counted for each sample. Only insertions or deletions that increased or decreased the number of repeats were considered. Genomic variants in the microsatellite loci were detected using the same depth and frequency criteria as used for mutation detection. MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional PCR-based methods. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of >95% and specificity of >99%.

PD-L1 expression
PD-L1 IHC analysis was performed on full slides of FFPE tumor samples using automated staining techniques. The primary antibody used was SP142 (Spring Biosciences). The staining was regarded as positive if its intensity on the membrane of the tumor cells was 2+ (on a semiquantitative scale of 0–3: 0 for no staining, 1+ for weak staining, 2+ for moderate staining, and 3+ for strong staining) and the percentage of positively stained cells was 5%.

Ethics statement
Human subjects had been deidentified prior to analysis, with this research being exempt per the Western Institutional Review Board.

Results

Tumor characteristics
In total, 4,125 tumor specimens obtained from patients with 14 different gastrointestinal cancer types were analyzed (Table 1). Overall, 56% of patients were male and 44% were female. The mean age was 61 years (range, 12–90). Most specimens were from primary tumor sites (59%) compared with the metastatic sites (37%). The origin of the remaining 4% of tumors was unclear.

TML distribution
Right-sided colon cancers and small-bowel adenocarcinomas exhibited the highest average TML (13 and 10.2 mutations/MB, respectively) and had the greatest prevalence of TML-high tumors (14.6% and 10.2%, respectively; Fig. 1). Both anal and gastric
cancers had a rate of TML-high of 8.3%, whereas pancreatic adenocarcinomas and pancreatic neuroendocrine tumors (pancreatic NET) had a TML-high rate of 1.5%. None of the gastrointestinal stromal tumors (GIST) demonstrated TML-high (0%). In addition, a considerable difference in the prevalence of TML-high between gastric cancers and GEJ adenocarcinomas was observed (8.3% vs. 3.1%, respectively). We then analyzed the relative TML distribution between primary and metastatic sites (Table 2). Overall, primary tumors had a greater TML-high rate than metastatic sites (5.7% vs. 3.0%, P < 0.001). However, significant differences were seen in right-sided colon cancers (16.9% vs. 6.9%, P = 0.0064) and small-bowel adenocarcinomas (14.4% vs. 3.7%, P = 0.0495) only. The lower frequency of TML-high in metastatic tumors may be related to the finding that primary tumors with TML-high are less likely to develop metastatic disease than TML-low primary tumors.

Association between TML and MSI

We examined the association between TML and MSI status. In most gastrointestinal cancers, TML-high was strongly associated with MSI-H (P < 0.0001), suggesting that MSI is the main cause of TML-high (Fig. 2). However, it is noteworthy that all cancers had a rate of TML-high of 8.3%, whereas pancreatic adenocarcinomas and pancreatic neuroendocrine tumors (pancreatic NET) had a TML-high rate of <1.5%. None of the gastrointestinal stromal tumors (GIST) demonstrated TML-high (0%).

Figure 1.
TML distribution across 14 gastrointestinal cancer types. Top, percentage of tumors with TML-high (≥17 mutations/MB) in descending order; bottom, boxplots of TML distributions. The green lines indicate the average TML in each cancer type, and the dotted line indicates the cutoff of TML = 17 mut/MB.

Table 1. Tumor characteristics of 4,125 patients divided by tumor location

<table>
<thead>
<tr>
<th>Cancer types</th>
<th>Total (N)</th>
<th>Female n (%)</th>
<th>Male n (%)</th>
<th>Local/primary n (%)</th>
<th>Metast/distant n (%)</th>
<th>Unclear n (%)</th>
<th>Average age</th>
<th>Age range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anal cancer</td>
<td>72</td>
<td>55 (76)</td>
<td>17 (23)</td>
<td>29 (27)</td>
<td>35 (48)</td>
<td>17 (23)</td>
<td>62.1</td>
<td>38–82</td>
</tr>
<tr>
<td>Biliary tract cancer</td>
<td>402</td>
<td>214 (53)</td>
<td>189 (46)</td>
<td>304 (77)</td>
<td>84 (20)</td>
<td>0 (0)</td>
<td>62.7</td>
<td>28–88</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>211</td>
<td>24 (11)</td>
<td>187 (88)</td>
<td>134 (63)</td>
<td>76 (36)</td>
<td>1 (0)</td>
<td>63.8</td>
<td>24–90</td>
</tr>
<tr>
<td>Esophageal squamous cancer</td>
<td>57</td>
<td>21 (36)</td>
<td>36 (63)</td>
<td>37 (64)</td>
<td>20 (35)</td>
<td>0 (0)</td>
<td>63.1</td>
<td>31–86</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>373</td>
<td>148 (39)</td>
<td>225 (60)</td>
<td>238 (63)</td>
<td>125 (33)</td>
<td>10 (2)</td>
<td>60.7</td>
<td>14–90</td>
</tr>
<tr>
<td>GEJ adenocarcinoma</td>
<td>97</td>
<td>26 (26)</td>
<td>71 (73)</td>
<td>53 (54)</td>
<td>23 (23)</td>
<td>21 (21)</td>
<td>61.7</td>
<td>26–84</td>
</tr>
<tr>
<td>GIST</td>
<td>130</td>
<td>57 (43)</td>
<td>73 (56)</td>
<td>72 (55)</td>
<td>39 (30)</td>
<td>19 (14)</td>
<td>58.8</td>
<td>14–90</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>135</td>
<td>38 (28)</td>
<td>97 (71)</td>
<td>86 (63)</td>
<td>46 (34)</td>
<td>3 (2)</td>
<td>62.4</td>
<td>12–89</td>
</tr>
<tr>
<td>Left-sided colon cancer</td>
<td>515</td>
<td>251 (48)</td>
<td>264 (51)</td>
<td>372 (72)</td>
<td>141 (27)</td>
<td>2 (0)</td>
<td>58.2</td>
<td>22–90</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>870</td>
<td>400 (45)</td>
<td>470 (54)</td>
<td>371 (42)</td>
<td>499 (57)</td>
<td>0 (0)</td>
<td>63.5</td>
<td>24–90</td>
</tr>
<tr>
<td>Pancreatic NET</td>
<td>75</td>
<td>31 (41)</td>
<td>44 (58)</td>
<td>24 (32)</td>
<td>51 (68)</td>
<td>0 (0)</td>
<td>55.7</td>
<td>29–82</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>541</td>
<td>212 (39)</td>
<td>329 (60)</td>
<td>259 (47)</td>
<td>232 (42)</td>
<td>50 (9)</td>
<td>58.7</td>
<td>26–90</td>
</tr>
<tr>
<td>Right-sided colon cancer</td>
<td>500</td>
<td>249 (49)</td>
<td>251 (50)</td>
<td>375 (74)</td>
<td>116 (23)</td>
<td>11 (2)</td>
<td>62.9</td>
<td>17–90</td>
</tr>
<tr>
<td>Small bowel adenocarcinoma</td>
<td>147</td>
<td>79 (53)</td>
<td>68 (46)</td>
<td>91 (61)</td>
<td>54 (36)</td>
<td>2 (1)</td>
<td>61.7</td>
<td>25–84</td>
</tr>
<tr>
<td>Total</td>
<td>4,125</td>
<td>1,805 (44)</td>
<td>2,320 (56)</td>
<td>2,449 (59)</td>
<td>1,540 (37)</td>
<td>136 (4)</td>
<td>61.4</td>
<td>12–90</td>
</tr>
</tbody>
</table>

20.0% 15.0% 10.0% 5.0% 0.0%
TML-High %

Right-sided colon cancer Small bowel adenocarcinoma Anal cancer Gastric cancer Biliary tract cancer Esophageal adenocarcinoma Lethalized colon cancer GEJ adenocarcinoma Rectal cancer Hepatocellular cancer PanNET GIST
TML-high anal cancers were MSS. Interestingly, among MSS tumors, the highest TML-high rate was seen in squamous-cell cancers, namely anal and esophageal cancers (8.3% and 3.5%, respectively). In addition, MSI-H was not seen in anal cancers, esophageal squamous cancers, pancreatic NETs, and GISTs; however, in these tumors, TML-high frequencies of 8.3%, 3.5%, 1.3%, and 0%, respectively, were observed, indicating that additional causes exist for TML-high that could result in increased neoantigen production. As expected, in all cancer types when MSI-H was seen, a significant correlation between MSI and TML was observed.

The distribution of PD-L1 tumor expression was variable. GISTs, anal cancers, and esophageal squamous cancer types showed low MSI/TML but high PD-L1 expression, whereas others such as right-sided colon cancers and small-bowel adenocarcinomas showed the reverse profile. We further assessed the

### Table 2. TML distribution in 14 gastrointestinal cancer types highlighting the difference between primary and metastatic sites.

<table>
<thead>
<tr>
<th>Cancer types</th>
<th>All tumors</th>
<th>Primary tumors</th>
<th>Distant/mets tumors</th>
<th>Unclear origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average TML</td>
<td>% TML-high</td>
<td>Average TML</td>
<td>% TML-high</td>
</tr>
<tr>
<td>Right-sided colon cancer</td>
<td>13.0</td>
<td>16.9</td>
<td>14.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Small-bowel adenocarcinoma</td>
<td>10.2</td>
<td>14.4</td>
<td>10.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>9.3</td>
<td>9.7</td>
<td>9.7</td>
<td>0.5</td>
</tr>
<tr>
<td>GEJ adenocarcinoma</td>
<td>7.8</td>
<td>5.8</td>
<td>8.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Anal cancer</td>
<td>8.8</td>
<td>4.8</td>
<td>8.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Pancreatic NET</td>
<td>5.8</td>
<td>4.2</td>
<td>6.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Left-sided colon cancer</td>
<td>10.1</td>
<td>4.0</td>
<td>10.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>7.6</td>
<td>3.5</td>
<td>8.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Biliary tract cancer</td>
<td>6.9</td>
<td>3.1</td>
<td>6.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>8.4</td>
<td>3.1</td>
<td>8.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Esophageal squamous cancer</td>
<td>7.5</td>
<td>2.7</td>
<td>7.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>8.1</td>
<td>1.5</td>
<td>7.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>6.1</td>
<td>1.3</td>
<td>5.7</td>
<td>0.1</td>
</tr>
<tr>
<td>GIST</td>
<td>5.1</td>
<td>0.0</td>
<td>5.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Grand total</td>
<td>8.5</td>
<td>5.7</td>
<td>8.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**NOTE:** Tumors are placed in descending order according to the TML-high% in primary tumors. In bold, % of TML-high tumors that were compared between primary and metastatic sites.

![Figure 2.](https://example.com/figure2.png)

**Figure 2.** TML and MSI status in 14 gastrointestinal cancer types. Top, percentage of TML-high in MSS tumors; bottom, TML in MSS (red) and MSI-H (blue) tumors. The blue line indicates the threshold of TML-high (>17 mutations/MB).
Prevalence of MSI, TML, and PD-L1 expression in 14 types of gastrointestinal cancers.

Figure 4.

Finally, we compared PD-L1 expression frequency between MSI/TML-negative tumors and in MSI or TML-positive tumors. PD-L1 expression was significantly higher in MSI-H/TML-high primary tumor samples from right-sided colon cancers (18% vs. 5%, \( P = 0.0002 \)), left-sided colon cancers (17% vs. 3%, \( P = 0.003 \)), gastric cancers (27% vs. 8%, \( P = 0.0004 \)), biliary tract cancers (31% vs. 8%, \( P = 0.0009 \)), and esophageal adenocarcinomas (67% vs. 8%, \( P = 0.0003 \)).

Discussion

The introduction of immune checkpoint (PD-1/PD-L1/CTLA4) inhibitors represents a major step forward in cancer therapy, resulting in a dramatic paradigm shift for the standard-of-care in many solid tumors. However, currently ICIs have shown activity in only a select subset of patients with gastrointestinal neoplasms, identified using MSI, TML, and PD-L1 expression separately in individual cancers. Whether the integration of these predictive biomarkers could better select patients who would benefit from these drugs is still unclear. The three biomarkers that were studied have been associated with the prediction of patient response to immune checkpoint inhibitors in gastrointestinal and nongastrointestinal malignances. However, the prevalence of these biomarkers and their interrelationship is still poorly understood. Describing the relative prevalence of these biomarkers at different disease sites and their association with each other can shed light on the biology of these diseases and provide a better understanding of the subsets of patients who may benefit from immunotherapy.

Higher levels of mutations are believed to induce the expression of immunogenic and cancer-specific neoantigens, leading to a robust antitumor immune response. Even though TML is tightly associated with MSI status, it is possible to observe TML-high in MSS tumors (23). In the current study, a cutoff of \( \geq 17 \) mutations per Mb was used to define TML-high tumors based on an established concordance (\( >99\% \)) with MSI-H in colorectal cancers. However, different thresholds to define TML-high were used in other studies, making it harder to compare data.

In the current study, right-sided colon cancers and small-bowel adenocarcinomas had the highest TML-high rate. Recent genomic
profiling of small-bowel adenocarcinomas demonstrated that 9.5% were TML-high (24). In addition, 7.6% of small-bowel adenocarcinomas were MSI-H, and all MSI-H tumors had an intermediate to high TML, which is consistent with other reports (25). A multicenter phase II study in patients with advanced small-bowel adenocarcinomas is ongoing to examine the efficacy of pembrolizumab within this patient population, even though patient selection or stratification according to the MSI or TML status is not planned (NCT02949219; ref. 26).

Here, we showed that TML-high is strongly associated with MSI-H status in most gastrointestinal cancers, except for anal cancers, esophageal squamous cell cancers, and pancreatic NETs. These tumors did not show MSI-H, even though they exhibited TML-high rates of 8.3%, 3.5%, and 1.3%, respectively. Therefore, it is possible that additional factors are responsible for increased neoantigen production leading to TML-high (Fig. 2). Anal cancers and esophageal squamous cancers have two risk factors in common: human papillomavirus (HPV; refs. 27, 28) and smoking. HPV+ esophageal squamous cancers have two risk factors in common: HPV infection and smoking. HPV-negative and HPV-positive smoking-related tumors have been shown to harbor higher mutation rates in comparison with their HPV-negative and nonsmoking counterparts (29, 30); thus, it is possible that HPV infection and smoking have a role in causing a high TML in squamous gastrointestinal cancers. In addition, HPV infection is present in >80% of anal cancers and is recognized as the main cause of anal squamous-cell carcinoma (28). Moreover, HPV-negative and HPV-positive cancers have been shown to have different mutation profiles (31, 32). It has been demonstrated that HPV-induced master regulators play crucial roles with regard to mutation and neoantigen load in cervical cancer (30). However, as we found that only 8.3% of anal cancers are TML-high, other factors may be involved in driving mutations. This knowledge will help us to better select patients and identify the subgroup that will benefit best from ICIs. This is especially important considering the recent demonstration of nivolumab efficacy in patients with anal cancers (5).

We also observed a considerable difference between the percentage of TML-high tumors in gastric cancers and GEJ adenocarcinomas (8.3% vs. 3.1%). Generally, gastric cancers and GEJ adenocarcinomas are grouped together, and many trials examining PD-L1/PD-L1 blockade in gastroesophageal cancers enroll patients with gastric cancers and GEJ cancers without distinction between the two sites (33). However, given these results, patient stratification into two groups is probably needed in future clinical trials of anti–PD-1/PD-L1 agents.

Another important observation was the difference between primary and metastatic sites. Overall, primary tumors carried a greater TML-high frequency than metastatic tumors. Nevertheless, considering each type of tumor separately, only right-sided colon cancers and small-bowel adenocarcinomas reached a significant difference (Table 2). Primary MSI-H colorectal cancers are less likely to metastasize to a distant organ, a finding that likely explains the differences in TML observed here. In addition, TML-high has been related to better prognosis (34). As mentioned above, an increased number of mutations increases the probability of having more immunogenic proteins that can be recognized as neoantigens by our immune system. This phenomenon may prevent cancer cells from metastasizing and could be the reason why we saw a greater TML-high rate in primary than in metastatic sites.

Recently, PD-L1 expression has been extensively studied as a prognostic and predictive biomarker in different tumor types. However, many clinical studies have reported controversial results (35), likely due to lack of consistency. Various PD-L1 antibody clones have been used, as well as different thresholds to define positive and negative staining. For a meaningful comparison among all gastrointestinal cancer types investigated here, a single clone and a uniform threshold is needed. We adopted SP142, a widely used clone that has generated highly concordant results in various cancer types with other commonly used antibodies (36). The threshold adopted in this study for PD-L1 positive is 5%, higher than the 1% required for entry into many clinical studies in gastrointestinal cancers. In various gastrointestinal cancers, including gastroesophageal cancer (3, 37) and HCC (4), further analysis of the clinical results showed that higher expression of PD-L1 is associated with improved survival in patients treated with immune checkpoint inhibitors. The most recent approval of pembrolizumab in gastroesophageal cancer adopts a threshold of 1% of combined positive score and takes into account staining on tumor cells, lymphocytes, and macrophages (6). For completeness of results, when we set the threshold for PD-L1 positivity at 1%, the overall prevalence of tumors with PD-L1 overexpression increased in all tumor types, as expected, maintaining the same distribution (Supplementary Table S2).

Although further analysis among patients with different PD-L1 expression levels is not yet available, it is not unreasonable to speculate that high PD-L1 expressers may have an increased likelihood to respond to the agent based on previously published studies. Here we found that PD-L1 expression on tumor cells

Figure 5.

PD-L1 expression frequency in the MSI-negative cohort (green) and MSI-high cohort (blue). A connective line indicates that PD-L1 expression is significantly higher in the MSI-H cohort.
than 40% of PD-L1
instance, squamous esophageal cancers showed promising RR to
ICI responses are likely driven by different biomarkers. For
several clinical trials (Supplementary Table S3), suggesting that
always co-occur with MSI-H/TML-high. In fact, as highlighted
expression (Fig. 4). More interestingly, PD-L1 expression does not
al squamous and anal cancers showing the highest rate of PD-L1
expression in gastrointestinal cancers. TML-high rate varied wide-
ly among gastrointestinal cancers. MSI-H is conceivably the main
driver for TML, even though other factors may be involved, such as
HPV infection, and deserve future study. PD-L1 expression does
not always associate with either MSI or TML-high, yet could
potentially identify additional responders to ICI treatment,
regardless of MSI status or mutation load. Our findings provide
baseline data for the prevalence of molecular and histologic
parameters potentially associated with responsiveness to ICI in
gastrointestinal malignancies. Our results also suggest that testing
for all three markers (TML, MSI, and PD-L1) may be necessary to
broaden the identification of responders to ICI treatment and
better stratify patients. Future investigations in prospective trials
are needed to evaluate the integration of these biomarkers with
other potential factors (e.g., CD8+ tumor-infiltrating lymphocytes
and IFNγ gene signature). Finally, standardized methodologies
and thresholds are needed, as well as the exploration and stan-
dardization of new methods to measure TML, such as liquid
biopsy-based assays (41).

Disclosure of Potential Conflicts of Interest

D. Raghavan is a consultant/advisory board member for Caris Life Sciences
Medical Advisory Board. R.M. Goldberg is a consultant/advisory board member for
Caris Life Sciences. W.M. Korn has ownership interest (including patents) in
OncoCyte and is a consultant/advisory board member for Eli Lilly and Merck.
B.A. Weinberg reports receiving a commercial research grant from Novartis, has
received speakers bureau honoraria from Eli Lilly, and is a consulting/advisory
board member for Caris Life Sciences. A.F. Shields reports receiving commercial
research support from Caris Life Sciences. J.L. Marshall is the director (POA)
at Caris Life Sciences. No potential conflicts of interest were disclosed by
the other authors.

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Conclusions

This is the first comprehensive analysis of TML, MSI, and PD-L1
expression in gastrointestinal cancers. TML-high rate varied wide-

References

1. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HI, Morse MA,
et al. Nivolumab in patients with metastatic DNA mismatch repair-
deficient or microsatellite instability-high colorectal cancer (CheckMate
142): an open-label, multicentre, phase 2 study. Lancet Oncol 2017;
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Nivolumab treatment for oesophageal squamous-cell carcinoma: an

Pembrolizumab for patients with PD-L1-positive advanced gastric cancer

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