At a Glance: Biomarkers of Response to Immune Checkpoint Inhibitor Therapy in Colorectal Cancer

Background and Epidemiology
The use of checkpoint inhibitors is changing the therapeutic paradigm in a variety of cancers and is rapidly infiltrating the treatment landscape for colorectal cancer (CRC) as well. As the gastrointestinal tract is an immune organ with its own antigen-presenting cells, it has been an obvious target for investigation of these agents. In early clinical trials, response to immune checkpoint inhibition was observed in a small proportion of patients with advanced CRC. Those patients who responded were noted to have mismatch repair deficiency (MMRD).[1] Since this initial observation, additional studies in patients with MMRD/microsatellite instability-high (MSI-H) CRC have noted robust antitumor response to immune checkpoint inhibitors such as nivolumab and ipilimumab.[2]

IHC Staining for PD-L1 Within the Tumor Immune Microenvironment
Approximately 15% of CRC tumors are MMRD/MSI-H.[3] MSI-H tumors tend to show high expression of CD8, FOXP3, PD-L1, and HLA in the tumor cells. In the images below, the brown staining represents the noted marker. The top left image shows tumor cells with membrane staining positive for PD-L1. The top right image shows a high concentration of CD8-positive immune cells infiltrating both the tumor and the interface between the tumor and normal tissue. In the bottom 2 images, HLAs tend to be overexpressed in MSI-H cancers because the neoantigens are generally coexpressed with HLA (bottom left). FOXP3 (bottom right) is an immune cell activity marker on inflammatory cells.
Testing for MSI in CRC

Both genetic and epigenetic defects in mismatch repair lead to MSI-H. Germline mutations lead to the development of Lynch syndrome, which is associated with MMRD and MSI-H tumors. Sporadic mutations in MMR-associated proteins can also occur leading to MSI-H status. PCR-based MSI testing is generally performed using 6-7 markers. At least two thirds of the markers tested must show allelic shift (see figure below) in order to characterize any tumor as MSI-H. In addition, MSI in sporadic CRC is most often associated with hypermethylation of the MLH1 gene promoter, which is correlated with the BRAF V600E mutation and has not been observed in patients with germline mutations in MMR.

<table>
<thead>
<tr>
<th>Test</th>
<th>Endpoints</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>HNPCC</th>
<th>Sporadic</th>
</tr>
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<tbody>
<tr>
<td>IHC</td>
<td>MMR protein expression</td>
<td>91%</td>
<td>87%</td>
<td>Loss of MLH1, MSH2, MSH6</td>
<td>Loss of MLH1, PMS2</td>
</tr>
<tr>
<td>PCR</td>
<td>Allelic shift/ MSI status</td>
<td>97%</td>
<td>95%</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>hMLH1 methylation</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>BRAF V600E mutation</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Germline mutation</td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>
in MMR genes

**Testing for MSI in CRC**

HNPCC, hereditary nonpolyposis colorectal cancer.

**MSI Analysis Using Microsatellite Markers**

Allelic shift in tumor demonstrated by presence of new peaks vs control normal tissue

**Loss of MLH1 by IHC**

Complete Loss of Protein in All Tumor Cells Required

**MLH1**

Tumor cells

Stromal cells

**MSH2**
MMRD/MSI-H Metastatic CRC: Response to Immune Checkpoint Inhibitors

Immune Checkpoint Inhibitors With Efficacy in MMRD/MSI-H Metastatic CRC

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<tr>
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<tbody>
<tr>
<td><strong>Dose in phase II</strong></td>
<td>10 mg/kg Q2W</td>
<td>3 mg/kg Q2W</td>
<td>Nivolumab 3 mg/kg + ipilimumab 1 mg/kg</td>
</tr>
<tr>
<td><strong>Targets</strong></td>
<td>PD-1</td>
<td>PD-1</td>
<td>PD-1, CTLA-4</td>
</tr>
<tr>
<td><strong>Response data</strong></td>
<td><strong>In MMRD CRC</strong></td>
<td><strong>In MMRD/MSI-H CRC</strong></td>
<td><strong>In MSI-H CRC</strong></td>
</tr>
<tr>
<td>ORR: 57%</td>
<td>ORR: 31%</td>
<td>ORR: 55%</td>
<td></td>
</tr>
<tr>
<td>Durable responses</td>
<td>Median DoR not reached</td>
<td>Median DoR not reached</td>
<td>Median DoR not reached</td>
</tr>
<tr>
<td>observed in &gt; 50% of</td>
<td>83% of responses ongoing</td>
<td>85% of responses ongoing</td>
<td></td>
</tr>
<tr>
<td>patients with MMRD</td>
<td>after a median follow-up</td>
<td>after a median follow-up</td>
<td></td>
</tr>
<tr>
<td>metastatic CRC</td>
<td>of 7.4 mos</td>
<td>of 8.4 mos</td>
<td></td>
</tr>
</tbody>
</table>

*Approved for patients with MSI-H or MMRD CRC that has progressed after treatment with fluoropyrimidine, oxaliplatin, and irinotecan.

DoR, duration of response.

Survival in Patients With MMRD/MSI-H Metastatic CRC Treated With Immune Checkpoint Blockade

Pembrolizumab in MMRD CRC: OS and PFS

OS

- MMRD (median OS: Not reached)
- MMRP (median OS: 5.98 mos)

PFS

- MMRD (median PFS: Not reached)
- MMRP (median PFS: 2.3 mos)
Nivolumab Monotherapy in MSI-H CRC: OS and PFS\textsuperscript{[2]}

![Graph showing OS and PFS for Nivolumab Monotherapy in MSI-H CRC]

- **Median OS, mos (95% CI):** Not reached (17.1-NE)
- **12-mo OS rate, % (95% CI):** 73.8 (59.8-83.5)
- **Median PFS, mos:** Not reached (NE-NE)
- **12-mo PFS rate, % (95% CI):** Not reached (4.3-NE)

NE, not estimable.

Nivolumab + Ipilimumab in MSI-H CRC: OS and PFS\textsuperscript{[7]}

![Graph showing OS and PFS for Nivolumab + Ipilimumab in MSI-H CRC]

- **Median OS, mos (95% CI):** 6 mos = 89 (80.2-94.2)
- **Median OS, mos (95% CI):** 9 mos = 88 (78.1-93.1)
- **12-mo OS rate, % (95% CI):** Not reached (NE-NE)
- **Median PFS, mos:** Not reached (NE-NE)
- **12-mo PFS rate, % (95% CI):** 77 (66.5-85.1)
- **Median PFS, mos:** Not reached (11.5-NE)

References