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An Important Feature of Gastric Adenocarcinoma: Heterogeneous Microsatellite Status

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Mismatch repair (MMR) genes play a central role in correcting errors that arise during DNA replication. Gastric adenocarcinomas (GA) with high microsatellite instability (MSI-H) are characterized by deficient MMR (dMMR) machinery, resulting in a hypermutator phenotype. MSI-H cancers exhibit up to a 1000-fold increase in missense mutations compared with microsatellite-stable (MSS) malignancies.[1]

While the combination of fluorouracil (FU), leucovorin, oxaliplatin, and docetaxel (FLOT) is the preferred neoadjuvant chemotherapy regimen for MSS GA, accumulating evidence suggests that MSI-H GA is less likely to respond to perioperative or postoperative FU-based chemotherapy.[2] Given the high response rates and survival benefits of immune checkpoint inhibitors (ICIs) in advanced GA, neoadjuvant ICI therapy appears promising for localized MSI-H GA.[3] However, the impact of intratumoral MSI heterogeneity on response to ICI monotherapy has not yet been evaluated in clinical trials.

Real-world data on the management of GA with ICIs remain limited, owing to the absence of large cohorts or phase III studies focused exclusively on MSI-H GA patients. Here, we present two cases of MSI-H GA that progressed as MSS disease during treatment with ICI monotherapy.

A patient in her 60s presented with dysphagia and a 12-kg weight loss. Imaging revealed a 3.5 × 2.5 cm hypermetabolic mass resembling a gastrointestinal stromal tumor arising from the lesser curvature of the stomach, along with celiac lymphadenopathy. Endoscopic ultrasound (EUS)-guided biopsy confirmed poorly differentiated adenocarcinoma. Diagnostic laparoscopy showed no macroscopic implants. While awaiting molecular testing, neoadjuvant FLOT chemotherapy was initiated. Immunohistochemistry (IHC) and polymerase chain reaction confirmed MSI-H status with loss of MLH1 and PMS2, HER2-negative disease, and a PDL-1 combined positive score (CPS) of 40. Treatment was switched to nivolumab plus ipilimumab according to the GERCOR NEONIPIGA phase II study.[3] After four cycles, imaging demonstrated partial regression (PR), which persisted on positron emission tomography/computed tomography (PET/CT) after six cycles, prompting referral for surgery. The patient underwent robotic total gastrectomy with D2 lymphadenectomy. Pathology revealed ypT4aN2 diffuse-infiltrative poorly cohesive gastric carcinoma (signet ring cell type). MSI analysis of the surgical specimen was performed to investigate resistance to neoadjuvant immunotherapy. IHC indicated proficient MMR (pMMR), and fragment analysis of five short tandem repeat (5-STR) loci revealed a low-level MSI (MSI-L) phenotype, with instability at only one locus, ultimately interpreted as MSS. Given the signet ring cell component with MSS features in the residual tumor, adjuvant therapy was administered consisting of six cycles of folinic acid, FU, and oxaliplatin (FOLFOX) combined with nivolumab, with nivolumab planned for up to 1 year. Follow-up PET/CT after the sixth cycle of adjuvant chemoimmunotherapy (CIT) showed no recurrence or distant metastasis.

A 77-year-old woman presented with melena and anemia. Esophagoduodenoscopy (EGD) revealed a corpus ulcer with irregular mucosa, and biopsy confirmed moderately differentiated intestinal-type adenocarcinoma. EUS indicated cT4N0 disease, while imaging and peritoneal lavage cytology excluded metastasis. Considering the patient's advanced age, neoadjuvant therapy was initiated with the FOLFOX regimen. IHC demonstrated dMMR with loss of MLH1 and PMS2, and STR fragment analysis confirmed MSI-H status. HER2 and PD-L1 were negative. Based on prior experience with intratumoral heterogeneity in dMMR/MSI-H tumors, FOLFOX was continued and nivolumab was added concurrently. After four cycles of FOLFOX and two cycles of nivolumab, imaging showed PR, prompting two additional cycles. Due to worsening neuropathy, the sixth cycle was administered as FUFA plus nivolumab. Subsequent imaging demonstrated a complete response. EGD and EUS confirmed no residual primary tumor, and biopsies were negative for malignancy. The patient underwent total gastrectomy with D2 lymphadenectomy,

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These findings were presented as an oral presentation titled "Heterogenous Nature of the Microsatellite Status Should Be Considered in the Treatment of Gastric Cancer: Beware of Immunotherapy Alone?" at the 13th International Gastrointestinal Cancers Conference, Antalya, Türkiye, December 1-3, 2023. Presenter: Dr. Mirac Ajredini.

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and intraoperative peritoneal lavage was negative for malignancy. Final pathology revealed ypT3N3a adenocarcinoma with a mixed histology: 60% tubular, 30% cribriform, and 10% signet ring cell components. MSI analysis of the surgical specimen, including IHC and 5-STR fragment testing, indicated pMMR and MSI-L with instability at one locus (NR21), ultimately interpreted as MSS. Adjuvant therapy included two additional cycles of FUFA plus nivolumab, followed by 1 year of maintenance nivolumab. On the most recent follow-up imaging, there was no evidence of recurrence or metastasis.

Our cases highlight the heterogeneity of microsatellite status and the challenges it poses in treating localized and advanced GA. Both patients were initially diagnosed with MSI-H GA and responded to immunotherapy; however, histopathological analysis of the residual or unresponsive tumor components revealed MSS disease.

While multiple studies have evaluated immunotherapy alone in MSI-H/dMMR advanced GA,[4-6] there is a paucity of large-scale studies assessing the efficacy of ICIs as neoadjuvant therapy in localized GA or directly comparing ICI monotherapy with CIT in MSI-H tumors.

The GERCOR NEONIPIGA phase II study[3] demonstrated that neoadjuvant nivolumab plus ipilimumab followed by adjuvant nivolumab is feasible in localized dMMR/MSI-H GA, with a pathologic complete response (pCR) rate of 58.6%. Moreover, four patients achieved less than 10% residual viable tumor cells, yielding practice-changing outcomes, and the overall major pCR rate reached 72%. Nonetheless, the NCCN Guidelines Version 2.2025, published on April 4, 2025, recommend neoadjuvant therapy with nivolumab plus ipilimumab, pembrolizumab, or the tremelimumab-durvalumab combination for MSI-H/dMMR GA.[7] The INFINITY trial evaluated the combination of the anti-CTLA-4 antibody tremelimumab and the anti-PD-L1 antibody durvalumab as neoadjuvant therapy for patients with resectable MSI-H/dMMR GA and gastroesophageal junction (GEJ) cancer.[8] The results were promising, with a pCR rate of 60% and a major pCR rate of 80%, consistent with findings from the GERCOR NEONIPIGA trial. However, information on the MSI status of progressive or residual tumor tissue was not reported for 1 of the 15 patients who experienced disease progression in the INFINITY study, nor for patients with partial responses in the INFINITY or NEONIPIGA trials. Based on our center's experience, the NCCN-recommended tailored approach may risk undertreating the concurrent MSS component of GA, which is known for its histologic and molecular heterogeneity, potentially leading to disease progression, as illustrated in our cases. Phase III KEYNOTE-585 and Matterhorn trials, which evaluated the efficacy of CIT in the neoadjuvant setting, included locally advanced gastric or GEJ adenocarcinoma patients regardless of MSI status and reported higher pCR rates with CIT compared to chemotherapy alone.[9,10] However, the proportion of MSI-H GA patients enrolled in these trials was limited (7%-9%), and the comparator arm consisted of chemotherapy rather than ICI monotherapy. Consequently, whether neoadjuvant immunotherapy alone represents the optimal approach for MSI-H GA remains uncertain, though it continues to be recommended in current NCCN guidelines, informed primarily by phase II study findings.

Cercek et al.[11] reported a 100% complete clinical response with single-agent PD-1 blockade in dMMR/MSI-H locally advanced rectal cancer, marking a milestone in immuno-oncology.[12] However, with a 56% pCR rate observed in the NEONIPIGA study, caution is warranted when extrapolating recommendations for neoadjuvant ICI monotherapy from rectal cancer to GA, as clinical contexts and outcomes differ significantly.

Current evidence for ICI monotherapy and chemotherapy in MSI-H advanced GA is primarily derived from subgroup analyses of MSI-H patients enrolled in phase II/III trials comparing chemotherapy alone to chemotherapy plus ICI in non-selected HER2-negative metastatic GA. In the CheckMate 649 trial, improved overall survival (OS) was observed with nivolumab plus chemotherapy in advanced GA patients with a PD-L1 CPS score ≥ 5.[12] Similarly, the KEYNOTE-859 trial demonstrated that first-line pembrolizumab combined with chemotherapy improved OS, progression-free survival, and overall response rate in metastatic HER2-negative gastric and GEJ adenocarcinoma.[13] Subgroup analyses further indicated that MSI-H patients derived superior benefit from ICI plus chemotherapy compared with chemotherapy alone.

In conclusion, ICI combined with chemotherapy has a clear role in the treatment of MSI-H GA and should be considered in the management of tumors with heterogeneous microsatellite status.

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