Mismatch Repair Deficiency, Microsatellite Instability, and Survival
An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial

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IMPORTANCE Mismatch repair (MMR) deficiency (MMRD) and microsatellite instability (MSI) are prognostic for survival in many cancers and for resistance to fluoropyrimidines in early colon cancer. However, the effect of MMRD and MSI in curatively resected gastric cancer treated with perioperative chemotherapy is unknown.

OBJECTIVE To examine the association among MMRD, MSI, and survival in patients with resectable gastroesophageal cancer randomized to surgery alone or perioperative epirubicin, cisplatin, and fluorouracil chemotherapy in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial.

DESIGN, SETTING, AND PARTICIPANTS This secondary post hoc analysis of the MAGIC trial included participants who were treated with surgery alone or perioperative chemotherapy plus surgery for operable gastroesophageal cancer from July 1, 1994, through April 30, 2002. Tumor sections were assessed for expression of the MMR proteins mutL homologue 1, mutS homologue 2, mutS homologue 6, and PMS1 homologue 2. The association among MSI, MMRD, and survival was assessed.

MAIN OUTCOMES AND MEASURES Interaction between MMRD and MSI status and overall survival (OS).

RESULTS Of the 503 study participants, MSI results were available for 303 patients (283 with microsatellite stability or low MSI [median age, 62 years; 219 males (77.4%)] and 20 with high MSI [median age, 66 years; 14 males (70.0%)]). A total of 254 patients had MSI and MMRD results available. Patients treated with surgery alone who had high MSI or MMRD had a median OS that was not reached (95% CI, 11.5 months to not reached) compared with a median OS among those who had neither high MSI nor MMRD of 20.5 months (95% CI, 16.7-27.8 months; hazard ratio, 0.42; 95% CI, 0.15-1.15; \( P = .09 \)). In contrast, patients treated with chemotherapy plus surgery who had either high MSI or MMRD had a median OS of 9.6 months (95% CI, 0.1-22.5 months) compared with a median OS among those who were neither high MSI nor MMRD of 19.5 months (95% CI, 15.4-35.2 months; hazard ratio, 2.18; 95% CI, 1.08-4.42; \( P = .03 \)).

CONCLUSIONS AND RELEVANCE In the MAGIC trial, MMRD and high MSI were associated with a positive prognostic effect in patients treated with surgery alone and a differentially negative prognostic effect in patients treated with chemotherapy. If independently validated, MSI or MMRD determined by preoperative biopsies could be used to select patients for perioperative chemotherapy.
Gastric cancer is the fifth most common cancer and the third most common cause of cancer-related death globally. In Western countries, patients with operable gastric or gastroesophageal adenocarcinoma frequently undergo neoadjuvant or perioperative chemotherapy before surgical resection. This adjunctive chemotherapy is associated with a modest benefit in terms of overall survival (OS) compared with surgery alone but also with toxic effects, including neutropenia and thromboembolic disease. Unfortunately, after optimal multimodality therapy, approximately half of patients undergoing resection will relapse and die of their cancer. There are no validated prognostic biomarkers for patients with gastroesophageal cancer who receive neoadjuvant treatment, and current patient selection is based purely on preoperative radiologic staging.

Mismatch repair deficiency (MMRD) is a pattern of cancer with a relative or absolute loss of function of proteins of the mismatch repair (MMR) system. MMRD is caused by mutations in genes encoding MMR proteins. The loss of MMR function is associated with the increased accumulation of spontaneous mutations in the tumor genome, often referred to as microsatellite instability (MSI).

The objective of this study was to investigate whether patients with operable gastroesophageal cancer and high MMRD have different survival compared with patients with microsatellite-stable gastroesophageal cancer when treated with surgery alone or surgery plus perioperative chemotherapy.

Methods

MSI Assessment
This secondary analysis of the MAGIC trial included participants who were treated with surgery alone or perioperative chemotherapy plus surgery for operable gastroesophageal cancer from July 1, 1994, through April 30, 2002. Genomic DNA was extracted from macrodissected cancer and noncancer tissue using the QiAamp DNA FFPE Tissue Kit (Qiagen). The MSI status was determined using the Promega MSI Analysis System (Promega Corp). A detailed description of the MSI assessment method is in the eMaterial in the Supplement.
Analyses were mainly performed within treatment arms because of the differences in timing of surgery to reduce potential bias in the estimates of effects. Interactions between treatment arm and biomarker status were used to highlight potential differences in prognostic effect and were assessed using a Cox proportional hazards regression model. Date of surgery could not be confirmed for 9 patients in the chemotherapy plus surgery arm, and these patients were excluded from the survival analyses. Differences in OS by MSI and MMR protein status were assessed using the Kaplan-Meier method and compared using Cox proportional hazards regression. The Cox proportional hazards regression model was univariate for MSI and MMRD status. All MMR proteins were assessed individually and as a group to include any absent MMR protein. P < .05 was considered statistically significant using 2-sided Cox proportional hazards regression. All analyses were conducted using STATA software, version 14 (StataCorp).

Results

**MSI Prevalence and Clinical Characteristics**

The MSI results were available for 303 patients (of 456 patients who had undergone resection). Because the data were obtained from resection specimens and analyses examine survival from the date of surgery, only patients who had undergone surgery (456 of 503 enrolled in the MAGIC trial) are potentially included (eFigure in the Supplement).

No difference was found in median survival between patients who had tissue available for MSI analysis and those who did not (20.7 [95% CI, 17.5-28.3] vs 17.9 [95% CI, 13.5-24.2]; HR, 0.91; P < .48). Twenty patients (6.6%) had MSI-H, and 2 (0.7%) did not (20.7 [95% CI, 17.5-28.3] vs 17.9 [95% CI, 13.5-24.2]; HR, 0.77; P = .42). A total of 20 patients (16.3%) had a TRG 1 or 2 response (vs 3-5) in the resection specimen. None of these differences were statistically significant using 2-sided Cox proportional hazards regression. All analyses were conducted using STATA software, version 14 (StataCorp).

**MMRD Prevalence and Clinical Characteristics**

Association with MMRD with clinicopathologic characteristics was similar to that for MSI (Table 2).

**MSI and Pathologic Response to Chemotherapy**

No patient with an MSI-H tumor treated with chemotherapy had a significant pathologic response as measured by a Standard TRG of 1 or 2 (vs 3-5) in the resection specimen. Of patients with MSS or MSI-L tumors treated with chemotherapy, 20 of 123 (16.3%) had a TRG 1 or 2 response (P = .22 for MSI-H vs MSS or MSI-L). The χ² between the 2 pathologists for TRG assessment was 0.64, which increased to 0.70 when the TRG was grouped as TRG 1 and 2 (responders) vs TRG 3 to 5 (nonresponders).

**MMRD and Pathologic Response to Chemotherapy**

No patient with MMRD cancer treated with chemotherapy had a good pathologic response to chemotherapy (defined as TRG 1 or TRG 2) compared with 14 of 100 patients (14.0%) with MMR proficiency (MMRP) (P = .36 for comparison of MMRP and MMRD).
Table 2. Clinicopathologic Characteristics of Patients With MMRD vs MMRP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MMRP (n = 246)</th>
<th>MMRD (n = 22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR) [range], y</td>
<td>61 (54-69) [23-79]</td>
<td>66 (61-68) [36-76]</td>
<td>.19</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>.79</td>
</tr>
<tr>
<td>Male</td>
<td>190 (77.2)</td>
<td>18 (81.8)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56 (22.8)</td>
<td>4 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Site of tumor</td>
<td></td>
<td></td>
<td>.02</td>
</tr>
<tr>
<td>Stomach</td>
<td>183 (74.4)</td>
<td>22 (100)</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>34 (13.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gastroesophageal junction</td>
<td>29 (11.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
<td></td>
<td>.07ab</td>
</tr>
<tr>
<td>Diffuse</td>
<td>67 (27.2)</td>
<td>2 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>138 (56.1)</td>
<td>17 (77.3)</td>
<td></td>
</tr>
<tr>
<td>Mixed or other</td>
<td>32 (13.0)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>9 (3.7)</td>
<td>2 (9.1)</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td>.18ab</td>
</tr>
<tr>
<td>T1</td>
<td>10 (4.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>72 (29.3)</td>
<td>11 (50.0)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>151 (61.4)</td>
<td>9 (40.9)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>5 (2.0)</td>
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</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td>1.00ab</td>
</tr>
<tr>
<td>N negative</td>
<td>51 (20.7)</td>
<td>3 (13.6)</td>
<td></td>
</tr>
<tr>
<td>N positive</td>
<td>135 (54.9)</td>
<td>9 (40.9)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>60 (24.4)</td>
<td>10 (45.5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; MMRD, mismatch repair deficiency; MMRP, mismatch repair proficiency.  
ab Excluding those with missing data.

Correlation of MMRD With MSI Status

A total of 254 patients had MSI and MMR results available. Of these, 15 of 17 MSI-H tumors had MMRD detected. Thirteen of 15 MLH1-negative tumors (86.7%) with available MSI results had MSI-H tumors compared with 4 of 239 MLH1-positive tumors (1.7%). This finding results in a sensitivity of MLH1 deficiency testing for MSI prognosis of 76.5% (95% CI, 50.1%-93.2%) and a specificity of 99.2% (95% CI, 97.0%-99.9%). All patients with absent MSH2 and MSH6 had MSI-H tumors. Twelve of 16 patients (75.0%) with absent PMS2 and MSI results had MSI-H tumors compared with 4 of 236 patients (1.7%) with PMS2-positive tumors. Overall concordance between MSI-H and MMRD status was 97.6% (eTable in the Supplement).

Survival Analysis

MSI and Survival

For patients treated with surgery alone, OS was better for patients with MSI-H than for patients with MSS or MSI-L because median OS was not reached for patients with MSI-H (95% CI, 4.4 months to not reached), whereas the median OS for patients with MSS and MSI-L was 20.3 months (95% CI, 16.7-27.7 months; HR, 0.35; 95% CI, 0.11-1.11; P = .08) (Figure 1). For patients treated with chemotherapy plus surgery, OS was better for patients with MSS or MSI-L (median OS, 22.5 months; 95% CI, 16.1-42.1 months), whereas median OS for patients with MSI-H was 9.6 months (95% CI, 0.1-21.9 months; HR, 2.22; 95% CI, 1.02-4.85; P = .04) (P = .007 for the interaction between MSI and treatment for OS) (Figure 1).

MMRD and Survival

Patients treated with surgery alone who had MMRD had a median OS that was not reached (95% CI, 4.4 months to not reached); for patients with MMRP tumors, the median OS was 20.7 months (95% CI, 17.5-28.6 months; HR, 0.40; 95% CI, 0.13-1.26; P = .12) (Figure 2). Patients treated with chemotherapy plus surgery who had MMRD had a median OS of 9.7 months (95% CI, 0.2-42.4 months); for patients with MMRP treated with chemotherapy, the median OS was 20.1 months (95% CI, 15.5-35.7 months; HR, 1.62; 95% CI, 0.81-3.26; P = .18) (P = .04 for the interaction between MMR protein status and survival).

MSI and/or MMRD and Survival

Patients treated with surgery alone who had either MSI-H or MMRD had better OS than did patients who had neither MSI-H nor MMRD; median survival was not reached (95% CI, 11.5 months to not reached) for the MSI-H or MMRD group compared with those who had MSS or MSI-L, who had a median OS of 20.5 months (95% CI, 16.7-27.8 months; HR, 0.42; 95% CI, 0.15-1.15; P = .09). After treatment with chemotherapy plus surgery, patients who had either MSI-H or MMRD had a median OS of 9.6 months (95% CI, 0.1-22.5 months) compared with those who had neither MSI-H nor MMRD, who had a median OS of 19.5 months (95% CI, 15.4-35.2 months; HR, 2.18; 95% CI, 1.08-4.42; P = .03).

Discussion

Our study is the first, to our knowledge, to report the differentially prognostic effects of MSI and MMR protein expression on survival in a randomized clinical trial with a nonchemotherapy control arm for perioperatively treated gastroesophageal cancer. We found that patients with MSI-H or MMRD tumors have superior survival compared with patients with MSS/MSI-L or MMRP tumors when treated with surgery alone and conversely have inferior survival to patients with MSS/MSI-L or MMRP tumors when treated with perioperative chemotherapy plus surgery. These findings are significant, because if validated, they suggest that patients with MSI-H or MMRD may not benefit (or may experience a detrimental effect) from perioperative chemotherapy and may be better served by a surgery-only approach. Because MSI or MMRD tumors comprise up to 10% to 20% of stomach cancers in some series, this finding has the potential to affect large numbers of patients.15

Our results are consistent with the results of similar previous Asian and Western retrospective studies9,10,11,13 that found a significant positive prognostic effect of MSI-H status for patients with resected gastric cancer. In our study, MSI-H and MMRD tumors were only detected in patients with gastric cancer; this finding is commensurate with previous studies14,16 that found a low prevalence of MSI and MMRD in gastroesophageal junction and esophageal tumors. The con-
Patients were dichotomized into 2 groups: high MSI (MSI-H) and microsatellite stable (MSS) or low MSI (MSI-L), which are analyzed separately in each treatment arm. Differences in overall survival were assessed using the Kaplan-Meier method and the log-rank test. The hazard ratios for MSI-H vs MSS or MSI-L were 0.35 (95% CI, 0.11-1.11) for surgery alone ($P = .08$) and 2.22 (95% CI, 1.02-4.85) for chemotherapy and surgery ($P = .04$) (interaction $P = .01$). $P < .05$ was considered to be statistically significant.

Figure 1. Overall Survival by Microsatellite Instability (MSI) Status and Treatment Arm in the Study Patients

![Image of Figure 1]

Figure 2. Overall Survival by Mismatch Repair (MMR) Protein Status in the Study Patients

![Image of Figure 2]

Consistent effect of MSI-H status on prognosis is supported by a pooled analysis\textsuperscript{17} of 17 studies that found an HR for OS of 0.76 (95% CI, 0.65-0.88; $P < .001$) and limited heterogeneity. In contrast, much fewer data are available on the interaction between MSI status and chemotherapy. In this regard, our results are comparable to the 2 largest retrospective Asian studies\textsuperscript{9,13} in which patients with resected gastric cancer were treated with postoperative fluoropyrimidine chemotherapy. In these retrospective series, patients with stage II and III MSS cancer derived a benefit from adjuvant fluorouracil-based chemotherapy, whereas patients with MSI-H cancer did not. Although our analysis is post hoc, our study is the first randomized clinical trial, to our knowledge, with a control group to validate these findings.

In colorectal cancer, the putative prognostic effect of MMR protein status on the benefit of adjuvant chemotherapy is limited to patients with stage II disease.\textsuperscript{5} This finding is hypothesized to be attributable to the relatively small benefit associated with adjuvant fluoropyrimidine therapy in patients with stage II colorectal cancer and to the postulated effects of MMRD on the DNA damage response to fluoropyrimidines.\textsuperscript{18} First, because the relative benefit of perioperative chemotherapy for gastroesophageal cancer is greater than the benefit of adjuvant chemotherapy in stage II colorectal cancer and second, because cisplatin and epirubicin were used in the MAGIC trial in addition to fluorouracil, our results are possibly unexpected (however, because data on complete nodal staging were absent in a substantial percentage of patients, we cannot definitively stage the disease of all patients). One potential explanation for this phenomenon is that the effect of MMRD on the DNA damage response to platinum compounds is differential based on the platinum analog used.\textsuperscript{19} The MLH1-deficient cell line models have been reported to be relatively resistant to cisplatin but not oxaliplatin, which in turn reflects the differences in platinum compounds used in the MAGIC trial and colorectal cancer. This circumvention of the DNA damage repair mechanism by oxaliplatin may have important clinical implications; since the MAGIC trial was presented, oxaliplatin has been determined to be clinically equivalent to cisplatin and has replaced it in many gastric can-
Mismatch Repair Deficiency, Microsatellite Instability, and Survival in the MAGIC Trial

Mismatch repair (MMR) proteins play a critical role in maintaining genome integrity by repairing DNA damage caused by environmental factors and during the cellular replication process.

### Limits and Considerations

- **Interobserver Variability**: Immunohistochemical analysis for MMRD can exhibit interobserver variability. This variability can be attributed to differences in the interpretation of immunohistochemical staining patterns among different observers.

### Conclusions

We report for the first time, to our knowledge, in a randomized clinical trial of patients with operable gastroesophageal cancer treated with chemotherapy with a surgery-only control group that the presence of MMRD is associated with a positive prognostic effect in patients treated with surgery alone and a differentially negative prognostic effect in patients treated with chemotherapy plus surgery. If validated, this finding has the potential to improve patient selection for perioperative chemotherapy and spare a significant proportion of patients with gastric cancer unnecessary treatment. We do not believe that these data justify a change in clinical practice; however, we recommend prospective trial validation to ascertain the optimal perioperative treatment for patients with MSI-H gastric cancer. In light of the remarkable success of anti-programmed cell death protein 1 therapies in MMRD colorectal cancer, alternative treatment strategies could be reasonably investigated for these patients.

### Author Contributions

- **Drs Smyth and Cunningham** had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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### Conflict of Interest Disclosures

- Dr Smyth reported receiving honoraria from Five Prime Therapeutics for advisory board participation and from Nestlé and Eli Lilly. Ms Peckett reported receiving honoraria for consulting or an advisory role from Sanofi. Mr Nanci and Dr Langley reported receiving research support from the Medical Research Council Clinical Trials Unit.
Mismatch Repair Deficiency, Microsatellite Instability, and Survival in the MAGIC Trial

**Original Investigation**

Research


Boothman DA. Role of the hMLH1 DNA mismatch repair system in platinum drug resistance.


