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

Elizabeth C. Smyth, MB, BCh, MSc; Andrew Wotherspoon, MD; Clare Peckitt, MSc; David Gonzalez, PhD; Sanna Huliki-Wilson, BSc, MSc; Zakaria Eltahir, PhD; Matteo Fassan, MD, PhD; Massimo Rugge, MD, FACG; Nicola Valeri, MD, PhD; Alicia Okines, MD; Madeleine Hewish, MD, PhD; William Allum, MD; Sally Sterling, MSc; Matthew Nankivell, MSc; Ruth Langley, MD, PhD; David Cunningham, MD, FMedSci

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 MMR 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, 1.1-2.2 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 positively prognostic for survival in patients with stage II colon cancer and may be negatively prognostic for the efficacy of fluoropyrimidine adjuvant chemotherapy in the same patient group. As a consequence, MMR protein status assessment is recommended by the National Comprehensive Cancer Network and the European Society for Medical Oncology guidelines for patients with resected stage II colorectal cancer before adjuvant chemotherapy. For patients with gastric cancer, the prognostic effect of MSI has been suggested in several studies. However, these studies are all retrospective, and each lacked a control group.

The United Kingdom Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial was an open-label, multicenter, phase 3 randomized clinical trial that compared the effect of 6 cycles of perioperative epirubicin, cisplatin, and infused fluorouracil chemotherapy (3 cycles before and 3 cycles after resection) plus surgery with surgery alone in patients with resectable gastroesophageal cancer. Patients treated with perioperative chemotherapy had improved OS compared with patients treated with surgery alone (5-year OS, 36% vs 23%; hazard ratio [HR], 0.75; 95% CI, 0.60-0.93; P = .009). As a result, perioperative epirubicin, cisplatin, and fluorouracil chemotherapy became one standard treatment regimen for patients with resectable gastroesophageal adenocarcinoma. The objectives of this work were to establish the proportion of patients with high MSI (MSI-H) or MMRD cancer in the MAGIC cohort and to evaluate whether the presence or absence of these biomarkers had a prognostic effect on survival in patients treated with surgery alone or chemotherapy plus surgery.

**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.

**Key Points**

**Question** Do patients with operable gastroesophageal cancers with high microsatellite instability have different survival compared with patients with microsatellite-stable gastroesophageal cancer when treated with surgery alone or surgery plus perioperative chemotherapy?

**Findings** Patients with operable gastroesophageal cancer with high microsatellite instability have superior survival compared with patients with gastroesophageal cancer with low microsatellite instability or microsatellite stable tumors when treated with surgery alone. However, patients with operable gastroesophageal cancer with low microsatellite instability or microsatellite stable tumors have superior survival compared with patients with gastroesophageal cancer with high microsatellite instability when treated with perioperative chemotherapy plus surgery.

**Meaning** Patients with operable gastroesophageal cancer with high microsatellite instability did not benefit from perioperative chemotherapy. Alternative treatment approaches should be investigated for these patients.

Tumors were classified as microsatellite stable (MSS) when all markers were stable, as having low MSI (MSI-L) when only 1 marker was unstable, and as MSI-H with minimum instability in ≥2 markers. The term instability in this context refers to the presence of an increased number of nucleotide repeats in tumor than in the nontumor control DNA for each sample. The MSI-L and MSS tumors were combined for analysis as per previous analyses in gastric cancer.

**Mismatch Repair Deficiency, Microsatellite Instability, and Survival in the MAGIC Trial**

**MMR Protein Assessment**

For MMR protein immunohistochemical analysis, 3- to 4-μm sections were prepared from the tissue microarray blocks and stained for the mutL homologue 1 (MLH1), mutS homologue 2 (MSH2), mutS homologue 6 (MSH6), and PMS homologue 2 (PMS2) proteins. The eMaterial in the Supplement provides a detailed description of the immunohistochemical analysis method. Loss of MMR protein expression (MMRD) was designated when none of the neoplastic epithelial cells had nuclear staining while positive internal control nuclei (lymphocytes and stromal cells) were present in the immediate vicinity of the tumor infiltrate. Normal expression was defined as the presence of nuclear staining of tumor cells irrespective of the proportion or intensity.

**Tumor Regression Grading Assessment**

Two pathologists (M.F., M.R.), who were masked to the treatment arm, reviewed the slides from all cases and graded the pathologic response using the Mandard tumor regression grading (TRG) system. Differences in opinion were resolved by discussion.

**Statistical Analysis**

Overall survival was calculated from surgery to death from any cause or the last date of follow-up. Progression-free survival was calculated from surgery to the first event (ie, local recurrence or progression, distant recurrence, or death from any cause). Date of surgery was selected as the baseline for biomarker analysis to reduce potential bias because only patients with a surgical specimen were available for inclusion.
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%) had MSI-L. The rate of D2 resection in patients with MSI-H was 55% (vs 41% in the entire MAGIC trial population), and proportions of D2 resections for patients with MSI-H were similar in both arms. Resections were considered by the surgeon to be curative in comparable numbers of patients with MSI-H treated with surgery and surgery plus chemotherapy.

All MSI-H tumors were located in the stomach vs the gastroesophageal junction and esophagus (20 stomach cancers vs 0 gastroesophageal or esophageal tumors, P = .04). A total of 20 of the 234 stomach cancers (8.5%) had MSI-H (Table 1). The site of the tumor was not prognostic for survival. Patients with MSI-H tumors compared with MSS or MSI-L tumors were more frequently female and had an older median age. The MSI-H tumors were more frequently of Lauren intestinal histologic subtype and less commonly had metastatic lymph nodes in the resection specimen. None of these differences were statistically significant. A total of 4 (44.4%) of the 9 patients with MSI-H were treated with postoperative chemotherapy, consistent with the proportion of patients in the total trial population.

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 κ 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 Prevalence and Clinical Characteristics
Assessment of the MMR protein was performed in 288 MLHI cases, 282 MSH2 cases, 281 MSH6 cases, and 273 PMS2 cases. The different numbers of cases assessable for each protein reflect exhaustion of tumor material in selected tissue microarrays and resection blocks. All 4 MMR proteins were assessable in 268 cases. In 15 of 288 cases (5.2%), MLH1 was absent; PMS2 was absent in 17 of 273 cases (6.2%); MSH2 was absent in 3 of 282 cases (1.1%); and MSH6 was absent in 2 (0.7%) of 281 cases. Association with MMRD with clinicopathologic characteristics was similar to that for MSI (Table 2).

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 1. Clinicopathologic Characteristics of Patients With MSS or MSI-L vs MSI-H

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MSS or MSI-L (n = 283)</th>
<th>MSI-H (n = 20)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR) [range], y</td>
<td>62 (54-69) [23-79]</td>
<td>66 (60-69) [36-76]</td>
<td>.18</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>219 (77.4)</td>
<td>14 (70.0)</td>
<td>.42</td>
</tr>
<tr>
<td>Female</td>
<td>64 (22.6)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Site of tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>214 (75.6)</td>
<td>20 (100)</td>
<td>.04</td>
</tr>
<tr>
<td>Esophagus</td>
<td>37 (13.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gastroesophageal junction</td>
<td>32 (11.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>75 (26.5)</td>
<td>2 (10.0)</td>
<td>.25b</td>
</tr>
<tr>
<td>Intestinal</td>
<td>163 (57.6)</td>
<td>15 (75.0)</td>
<td></td>
</tr>
<tr>
<td>Mixed or other</td>
<td>35 (12.4)</td>
<td>2 (10.0)</td>
<td>.18b</td>
</tr>
<tr>
<td>Missing</td>
<td>10 (3.5)</td>
<td>1 (5.0)</td>
<td>.21b</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>12 (4.2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>88 (31.1)</td>
<td>11 (55.0)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>169 (59.7)</td>
<td>8 (40.0)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>5 (1.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>8 (2.8)</td>
<td>1 (5.0)</td>
<td></td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N negative</td>
<td>54 (19.1)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
<tr>
<td>N positive</td>
<td>156 (55.1)</td>
<td>8 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>73 (25.8)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; MSI-H, high microsatellite stability; MSI-L, low microsatellite stability; MSS, microsatellite stable.

* Data are presented as number (percentage) of patients unless otherwise indicated.

b Excluding those with missing data.
for patients with MSS or MSI-L (median OS, 22.5 months; HR, 0.35; 95% CI, 0.11-1.11; 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 MMR Deficiency 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 studies10,11,13 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 compared using 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).

Difficulties in reliably staging the disease of all patients. One potential explanation for this phenomenon is that the effect of MMRD on the DNA damage response to fluoropyrimidines. 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. 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

Research Original Investigation

Mismatch Repair Deficiency, Microsatellite Instability, and Survival in the MAGIC Trial

Although our overall concordance is high, other studies\(^29,30\) in gastric cancer have found lower sensitivities of MMR protein immunohistochemical analysis for detection of MSI-H MMRD. For these reasons, a genomic rather than an immunohistochemical approach may be preferred for patients with gastric cancer. Finally, an alternative hypothesis is that the MSI-H status might be associated with other molecular changes that predispose patients to chemotherapy resistance. In preclinical gastric cancer models, epigenetic changes, such as methylation of bone morphogenetic protein 4 (BMP4) (OMIM 112262), are associated with platinum resistance.\(^31\) Clinical data reveal that, in neoadjuvantly treated patients with gastric cancer, those with lower levels of promoter gene methylation have improved survival compared with those with more frequent methylation.\(^32\) Promoter methylation of MLH1 has also been associated with inferior survival of patients with resected gastric cancer treated with oxaliplatin-based adjuvant chemotherapy.\(^33\) However, because MSI status is not reported in either of these series, the independent contribution of epigenetic changes remains unclear.

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.\(^24\)
Mismatch Repair Deficiency, Microsatellite Instability, and Survival in the MAGIC Trial

ORIGINAL INVESTIGATION  Research

Trials Unit, University College London. Dr Cunningham reported receiving research funding from Armen, Astrazeneca, Celgene, Medimmune, Merck Serono, Merimack, and Sanofi. No other disclosures were reported.

Funding/Support: This study was supported by the Royal Marsden Hospital/Institute of Cancer Research National Institute for Health Research Biomedical Research Centre (Drs Smyth, Wouterspoon, Gonzalez, Eltaih, Hulliki, Valeri, Allum, and Cunningham and Ms Peckitt). The Translational Work on the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (TransMAGiC) trial was funded by grant C20023/A7217 from Cancer Research UK.

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Meeting Presentations: The results relating to microsatellite instability in this article were previously presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium; January 15, 2015; San Francisco, California. The results relating to mismatch repair deficiency in the article were previously presented at the American Society of Clinical Oncology General Meeting, June 3, 2016; Chicago, Illinois.

REFERENCES