Abstract

Purpose: In CLASSIC and MAGIC trials, microsatellite instability (MSI)-high status was a favorable prognostic and potential negative predictive factor for neoadjuvant/adjuvant chemotherapy in resectable gastric cancer (GC). Given the low prevalence of MSI-high in GC and its association with other positive prognostic variables, large datasets are needed to draw robust evidence of its prognostic/predictive value.

Patients and Methods: We performed a multinational individual patient data meta-analysis of the prognostic/predictive role of MSI in patients with resectable GC enrolled in MAGIC, CLASSIC, ARTIST, and ITACA-S trials. Prognostic analyses used multivariable Cox models (MVM). The predictive role of MSI was assessed both in an all-comer population and in MAGIC and CLASSIC trials by MVM testing of the interaction of treatment (chemotherapy plus surgery vs surgery) with MSI.

Results: MSI status was available for 1556 pts: 121 (7.8%) MSI-high, 576/980 European/Asian. In MSI-high vs MSI-low/MSS, 5-year DFS was 71.8% (95%CI: 63.8-80.7%) vs 52.3% (49.7-55.1%); 5-year OS was 77.5% (70.0-85.8%) vs 59.3% (56.6-62.1%). In MVM, MSI was associated with longer DFS (HR=1.88, 95%CI 1.28-2.76; p=0.0002) and OS (HR=1.78, 95%CI, 1.17-2.73; p=0.008), as were pT, pN, ethnicity and treatment. Patients with MSI-low/MSS GC benefitted from chemotherapy plus surgery: 5-year DFS was 57% vs 41% (HR=0.65, 95%CI 0.53-0.79), 5-year OS 62% vs 53% (HR=0.75, 95%CI 0.60-0.94), while MSI-high did not: 5-year DFS was 70% vs 77% (HR=1.27, 95%CI 0.53-3.04), 5-year OS 75% vs 83% (HR=1.50, 95%CI 0.55-4.12).
**Conclusions:** In patients with resectable primary GC, MSI is a robust prognostic marker that should be adopted as stratification factor by clinical trials. Chemotherapy omission and/or immune-checkpoint blockade should be prospectively investigated in MSI-high GCs according to clinically- and pathologically-defined risk of relapse.

**Keywords:**

Microsatellite Instability; gastric cancer; meta-analysis
**Introduction**

Gastric cancer (GC) is the third most common cause of cancer-related deaths globally; almost one million cases are diagnosed worldwide annually.\(^1\) Treatment of patients with locally advanced resectable GC displays significant geographic variation; in Asia surgery plus adjuvant chemotherapy is preferred, whereas outside of Asia perioperative chemo(radio)therapy is a more frequent choice. Adjuvant and perioperative chemotherapy are both evidence-based, guideline endorsed treatments for GC patients. However, more than half of patients still relapse and die from their disease.\(^2\)\(^-\)\(^7\) On the other hand, there are some patients who are cured by surgery alone and do not benefit from potentially toxic chemotherapy treatment. Current chemotherapy approaches are based on patient-related factors and clinicopathological staging, and there is only one recently validated biomarker to select GC patients for chemotherapy in the adjuvant setting. This prognostic and predictive tool, which is based on the expression levels of four candidate classifier genes (GZMB, WARS, SFRP4, and CDX1), was developed in Asian GC patients treated in routine practice with surgery alone or surgery followed by adjuvant fluorouracil-based chemotherapy, and validated on a cohort of patients enrolled in the CLASSIC trial, showing promising results.\(^8\)

Mismatch repair deficiency and microsatellite instability (MSI) has been associated with improved survival in colon cancer and may be predictive of lack of benefit from adjuvant chemotherapy in Stage II disease.\(^9\)\(^,\)\(^10\) As a result, assessment of mismatch repair deficiency by immunohistochemistry or microsatellite instability is recommended for patients with resected colorectal cancer in order to guide treatment decisions and screen for Lynch Syndrome.\(^11\)\(^,\)\(^12\) Results from two pivotal clinical trials have suggested a similar relationship between MSI and outcomes in patients with curatively resected GC. In the MAGIC trial, which established perioperative chemotherapy as a standard of care for non-Asian GC
patients,\textsuperscript{3} MSI-high patients, compared to microsatellite stable (MSS)/MSI-low subgroup, had an improved prognosis in the surgery alone treatment arm (HR=0.35; 95% CI, 0.11-1.11; p=0.08), and worse survival outcome in the chemotherapy plus surgery arm (HR=2.22; 95% CI, 1.02-4.85; p=0.04).\textsuperscript{13} In the CLASSIC trial, which established adjuvant capecitabine and oxaliplatin as a standard for Asian GC patients with resectable disease,\textsuperscript{2} patients with MSI GC did not derive any survival benefit from adjuvant chemotherapy (5-year disease free survival surgery versus chemotherapy groups: 83.9% versus 85.7%; p=0.93).\textsuperscript{14}

Despite these results from historically important randomised clinical trials (RCTs), a significant challenge for the adoption of MSI/MMR deficiency testing as a routine biomarker in patients with operable GC is the low MSI prevalence in GC (8-10%) which led to limited statistical power of the observations in individual trial datasets.\textsuperscript{13-15} With this in mind, we pooled individual patient data from four large multinational RCTs performed in patients with resectable GC (MAGIC\textsuperscript{3}, CLASSIC\textsuperscript{2}, ARTIST\textsuperscript{16} and ITACA-S\textsuperscript{17}), and evaluated the relationship between MSI status, overall survival (OS) and disease-free survival (DFS) and biomarker interaction with chemo(radio)therapy treatment.

Methods

Study Design and Trial Populations

We performed an IPD meta-analysis from four multicenter RCTs in patients with resectable GC: the MAGIC, CLASSIC, ITACA-S and ARTIST trials.

Two trials investigated whether the addition of a peri-operative or adjuvant chemotherapy to radical surgery could improve the survival in this patients’ population. In the MAGIC\textsuperscript{3} trial, patients were randomly allocated to peri-operative chemotherapy (epirubicin, cisplatin and 5-fluorouracil) plus surgery or to surgery alone, while in the CLASSIC\textsuperscript{2} trial, patients who
had received radical surgery were randomised to follow-up or adjuvant chemotherapy with capecitabine plus oxaliplatin. The ITACA-S\textsuperscript{17} trial patients were randomised to receive two different schedules of adjuvant chemotherapy, an intensified combination chemotherapy schedule (5-fluorouracil/leucovorin plus irinotecan followed by cisplatin plus docetaxel) versus single-agent chemotherapy (5-fluorouracil/leucovorin), whereas in the ARTIST\textsuperscript{16} trial, patients were randomised to adjuvant chemotherapy (capecitabine/cisplatin) versus chemotherapy (capecitabine/cisplatin) plus concurrent irradiation. Comprehensive eligibility criteria and complete results of each trial have been previously published.\textsuperscript{2, 3, 16, 17} In addition, post-hoc analyses of all trials aimed at investigating the interaction between MSI status and patients’ outcome have already been presented.\textsuperscript{13-15, 18} The purpose of our study was to assess the potential prognostic role of MSI status in patients with radically resected GC and its potential value to predict the outcome to systemic treatment in this patients’ population.

**Data gathering**

Our analysis was designed in 2018 and the members of all trial management committees gave their approval, according to a formal protocol. The requested data consisted of patients’ characteristics (including age, sex, ethnicity, ECOG performance status, tumour localization, T and N stage which were re-classified according to TNM 7\textsuperscript{th} edition, histology type according to Lauren Classification and treatment arm classified as multimodal treatment versus surgery-only), MSI status (MSI-low/MSS versus MSI-high) and outcome data (disease relapse and survival). Patients with unknown/not assessed MSI status were excluded. For detailed methodology on MSI assessment in each trial see Supplementary Methods.
A trial database was set up to include the information extrapolated by the four studies datasets in order to ensure the collection of appropriately comparable data, thus allowing the planned IPD pooled analysis.

All patients had given informed consent for trial participation and this study was approved by the ethical committee of Fondazione IRCCS Istituto Nazionale dei Tumori (Identifier: INT117/15).

**Statistical analysis**

Trial heterogeneity according to patient and disease characteristics was tested by using the Kruskal-Wallis test for numerical variables or Fisher-Freeman-Halton Fisher test for categorical variables, respectively.\(^\text{19}\) To investigate dishomogeneity between MSI status categories we used the Mann–Whitney–Wilcoxon test for numerical variables or Fisher-Freeman-Halton Fisher test for categorical variables, respectively.

DFS and OS curves were estimated with the Kaplan Meier method and between groups differences were tested with the log-rank test.

The prognostic role of MSI was assessed in the whole population. IPD multivariable analyses were performed using Cox models including trial as random variable. The multivariable models, together with MSI, included the following variables (covariates): patient characteristics (age, sex, ethnicity), ECOG performance status, tumour localization, T and N stage, histology and treatment arm. We also fitted random effect models with inverse variance weighting to pool the MSI hazard ratios estimated from trial-level univariable Cox models.

The predictive role of MSI was assessed in multivariable Cox models with trial as random variable, using IPD both from the 4 trials and from the 2 trials with a surgery alone arm (MAGIC+CLASSIC); the models included all the above covariates and the interaction treatment arm by MSI.
Age was modeled as a continuous variable using 3-knot restricted cubic spline, and the other covariates as categorical using dummy variables.

In the multivariable analyses patients with covariate missing data were excluded.

The analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC) and R software.

Results
Overall, 2648 patients were included in the initial trial database, 1092 patients were excluded from the study as per pre-specified criteria (did not have radical surgery for the primary tumour and lymph nodes or MSI status unknown/not assessed). Therefore, a total of 1556 patients were included in the final dataset of the pooled analysis (317 from MAGIC, 592 from CLASSIC, 259 from ITACA-S and 388 from ARTIST trial, respectively (Figure 1)). The selected population from each trial was not significantly different from the corresponding whole trial population, as reported previously.13-15,21

Baseline patients and disease characteristics are illustrated in Supplementary Table 1. Briefly, two trials (CLASSIC and ARTIST) were conducted in Asian countries, while the other two (MAGIC and ITACA-S) were performed on a European population. In the overall study population, median age was 59 (range 20-85), patients with Asian and European ethnicity were 63% and 37%, respectively. Most patients were male (70.1%), most tumours were located in the stomach compared to distal oesophageal/gastroesophageal junction (91.7% versus 8.3%). Intestinal histology represented 45.1%, while other histotypes were 54.9% of the whole trial population. Overall, 1101 (70.8%) patients received multimodal treatment, whereas 455 (29.2%) were treated with surgery only.
Supplementary Figure 1 shows the Kaplan-Meier curves of DFS and OS in the four trial populations, and Supplementary Table 2 shows the corresponding 5-year estimates.

The frequency of MSI-high GCs was well-balanced between the four trials and in the two treatment arms (multimodal treatment versus surgery only). MSI-high status, as compared to MSI-low/MSS, had a significant association with older median age (66 versus 58 years) and intestinal type histology compared to diffuse/other type (intestinal type: 67.5 versus 43.2%) (both p<0.0001). The incidence of MSI-H in gastric tumors was 8.7%; this was 3.3% for gastroesophageal junction tumors (p=0.056). (Table 1).

Comparing patients with MSI-high versus MSS/MSI-low GC, 5-year DFS was 71.8% (95% CI 63.8-80.7%) versus 52.3% (95% CI 49.7-55.1%) (32 versus 678 events; p=0.0001; Figure 2A), and 5-year OS was 77.5% (95% CI 70.0-85.8%) versus 59.3% (95% CI 56.6-62.1%) (26 versus 566 events; p=0.0004; Figure 2B).

In the multivariable prognostic IPD analyses (Supplementary Table 3), MSI was independently associated with DFS (HR MSS/MSI-low versus MSI-high = 1.88, 95% CI 1.28-2.76; p=0.001) and OS (HR=1.78, 95% CI, 1.17-2.73; p=0.008). Other significant factors for survival were ethnicity (worse prognosis associated with European), T and N stage (TNM 7th ed.) and treatment arm (worse prognosis associated with surgery only), whereas sex was significantly associated to OS only (worse prognosis associated with male). To compare the above MSI results with those from the “classical” meta-analytic approach based on aggregate data from each trial, we estimated the pooled HR from trial-level univariable Cox models (Supplementary Figure 2; p=0.010 for both DFS and OS). Due to the heterogeneity of MSI effect between the trials and the dishomogeneity of patients’ characteristics, we obtained more imprecise HR estimates (larger confidence intervals) compared with the IPD result. In addition, while the DFS HR resulted slightly lower in the
aggregate data analysis versus IPD analysis (1.80 versus 1.88), the OS HR was slightly higher (1.88 versus 1.78).

We also investigated whether the MSI prognostic effect could vary according to ethnicity, by fitting multivariable Cox model including the interaction MSI by ethnicity. The latter was statistically significant for both DFS (p=0.041) and OS (p=0.035), and this translates in a greater MSI prognostic effect in Asian (HR MSS/MSI-low versus MSI-high = 2.83, 95% CI 1.55-5.15 for DFS and 2.97, 95% CI 1.46-6.04 for OS) than European patients (HR=1.26, 95% CI 0.76-2.07 for DFS and 1.15, 95% CI 0.67-1.96 for OS) (other data not shown). Supplementary Figures 3 and 4 shows the Kaplan-Meier DFS and OS curves according to N and T stage.

In the whole population analyses of the predictive role of MSI (Table 2; Figures 3A and 4A), the interaction treatment by MSI was not statistically significant for either DFS and OS (p=0.133 and 0.180). However, only patients with MSS/MSI-low gastric cancers had a significant benefit for chemotherapy versus surgery only; 5-year DFS was 57% versus 41% (HR=0.65, 95% CI 0.53-0.79), and 5-year OS was 62% versus 53% (HR=0.75, 95% CI 0.60-0.94). Patients with MSI-high tumors had a 5-year DFS of 70% versus 77% in MSS/MSI-L (HR=1.27, 95% CI 0.53-3.04), and a 5-year OS 75% versus 83% respectively (HR=1.50, 95% CI 0.55-4.12). The corresponding results in the MAGIC and CLASSIC trials are shown in Table 2 and Figures 3B and 4B.

Finally, we performed a survival after recurrence (SAR) analysis in the whole trials population stratified according to MSI status. We did not find a significant difference in terms of SAR in MSI-high versus MSS/MSI-low patients, as 12-month SAR was 33.0% and 35.9%, respectively (HR=1.01; 95% CI, 0.68-1.50; p=0.947) (data not shown).
Discussion

The results of this meta-analysis confirm the positive prognostic role of MSI in surgically resected GC and suggest a potential lack of benefit of perioperative or adjuvant chemotherapy for MSI GC patients who undergo surgery. These findings are significant because they are consistent with the results of individual clinical trials which alone were underpowered.\textsuperscript{13, 14} Pooling the individual patient data from the four RCTs has allowed the possibility to provide clinically robust and more generalisable results on the prognostic and predictive value of MSI. Moreover, we show the limitations of meta-analyses based of summary data extracted from trial reports (Supplementary Figure 2) and that more precise estimates of MSI hazard ratios can be obtained by multivariable modeling of IPD data considering patients and trial heterogeneity. Finally, we were also able to perform additional subgroup analyses to provide more insights from IPD data. Based on these results, we suggest considering that, in patients with MSI-high GC which is resectable, careful multidisciplinary discussion should be adopted in light of the overall prognostic assessment and potential harm from systemic chemotherapy. As MSI-high GC patients represent up to 10% of operable GCs this has implications for many thousands of patients annually globally.\textsuperscript{1}

In our prognostic analyses, we evaluated the relationship between GC MSI status and outcome in patients treated in all four trials. Our meta-analysis demonstrated a significant benefit from chemotherapy in MSS/MSI-low patients with respect to DFS and OS, and suggests a lack of benefit from chemotherapy in MSI-high patients (HR estimates in Table 2). The interaction test for differential outcome according to biomarker status in chemotherapy and non-chemotherapy treated patients was not significant in the entire cohort, however given the small size of the MSI-high subgroup and the consequent low number of events, the interaction test could be quite underpowered. We also investigated
the predictive effect of MSI limited to the MAGIC and CLASSIC trials. The latter were the only trials contributing surgery only arms; in this group of patients, the p values for treatment by MSI interaction (0.147 for DFS and 0.070 for OS) became closer to conventional 5% significance level. However the caveats of small subgroup analyses also still apply.

Consistent with previous findings, elderly patients were diagnosed more frequently with MSI-high GC. There was no female patient preponderance. Our results also confirm that MSI-high tumours are more commonly found in the stomach rather than the GEJ or lower oesophagus (9% versus 3% respectively) and are more commonly of Lauren’s intestinal subtype. However, we did not find that MSI was enriched in early stage cancers, as has been suggested. This could be accounted for by stringent trial eligibility criteria requiring enrolment of predominantly more locally advanced cancers. However, the results of this multivariable analysis demonstrate that MSI is prognostic independent of T and N stage. This may imply that even in GCs with more advanced disease stage it might be possible to forego chemotherapy for patients who have operable MSI tumours. “Moreover, given the evidence supporting a poor prognostic effect of MSI-high status in advanced/metastatic colorectal cancer patients we investigated whether, in our pooled analysis in the GC setting, a similar result could be observed. No significant differences were reported in terms of survival after recurrence in MSI-high compared to MSS/MSI-low patients, even though the reliability of these data are limited by the small number of patients experiencing a disease progression in the MSI-high subgroup. Additionally, as all patients with mGC have a limited survival compared to patients with mCRC, differences in outcome may be more difficult to measure. Moving forward, in the era of immunotherapy, it will be challenging to perform further analyses on the topic of SAR, since most patients with MSI-high metastatic GC would receive an immune-checkpoint inhibitor, thus potentially changing the natural history of this disease subgroup.
The results of the multivariable analyses also highlight the superior survival of Asian compared to European GC patients (HR 4.38 European versus Asian, p=0.002). This is well recognised and has potentially confounded the results of international GC trials.26, 27 Unexpectedly, the positive prognostic effect of MSI status was more pronounced in Asian than European patients (HR for DFS/OS 2.83/2.97 versus 1.26/1.15, respectively). This could perhaps be explained by the negative results in chemotherapy treated patients in the MAGIC trial, however similar findings are noted in ITACA-S and ARTIST, neither of which have non-chemotherapy control groups. It is possible that either the driver state (tumour mutation burden) or the response to hypermutation is different between the two patient groups. It has been noted previously that the immune microenvironment in Asian GC differs from non-Asian cancers.28 Further research is required in order to clarify whether this is responsible for the difference in magnitude of effect of MSI in Asian GC patients.

The major challenge to the generalizability of our results is the heterogeneity of patients included in the meta-analysis which were of European and Asian origin, who had different surgical approaches (D1 versus D2 resection) and treatments (neoadjuvant versus adjuvant chemotherapy). These differences do indeed lead to different prognoses in each cohort. However, in each individual study (and CLASSIC and MAGIC which have control groups), MSI has an almost identical interaction with chemotherapy. What we aim to show is that the effect of MSI is universal although it may be different in magnitude in selected groups because of the afore-mentioned reasons. Furthermore, MSI status was assessed using a widely available standard, validated assay, implying that integrating MSI status into standard preoperative evaluation on biopsy material may be feasible.29 Another potential criticism is that we did not assess MSI in pretreatment biopsies or by using immunohistochemistry, although we are reassured that concordance between MSI and MMRD in gastric cancer was >95% in previous datasets.13 Because MSI is a fundamental characteristic of a tumour, there
are few concerns that this would change in a post-treatment specimen, as used in our study. Family history status was not available to evaluate any differential outcomes in Lynch versus sporadic MSI cancers, however in previous studies the >80% of MSI gastric cancers are MLH1 methylated and sporadic.\textsuperscript{30} Finally, although our dataset is large (1556 patients), the absolute number of MSI tumours is relatively low (n=121) which might be considered few when compared to comparable studies in colorectal cancer.\textsuperscript{31} However, this represented the totality of the trials in this setting with tissue available.

In conclusion, we present to our knowledge for the first time, the results of an IPD meta-analysis of the effect of MSI status on long-term oncological outcome for patients with resectable GC treated in clinical trials. Based on our findings, we believe that MSI-high GC patients treated with surgery alone could perform well even without adjunctive chemotherapy. In contrast, MSI patients treated with chemotherapy (perioperative or adjuvant) might not benefit from this treatment. We suggest that patients who have operable GC which is MSI and resectable should undergo critical multidisciplinary discussion if neoadjuvant or adjuvant chemotherapy is to be considered. As anti-PD-1 antibodies are associated with response rates of >50% in MSI-high advanced GC,\textsuperscript{25, 32} the next rational step is to design a clinical trial in which patients with operable MSI-high GC are treated with neoadjuvant or adjuvant immune checkpoint blockade in order to further improve survival for this biomarker selected group of patients.
Contributors

FP, RM, and ES were responsible for the initial concept of this analysis. FP and ES did the review of the individual patient data sent by the participating centres. RM did statistical analyses. FP, RM, AR and ES interpreted the statistical analysis results data and wrote the report. All authors had input into the data interpretation and preparation of the report for publication and approved the final version of the report.

Declaration of interests

FP declares honoraria for advisory role from Amgen, Bayer, Eli-Lilly, Merck Serono, Roche, Sanofi, and Servier.

FM declares honoraria for advisory role from Sanofi and Servier.

NV received honoraria from Merck Serono, Bayer and Eli-Lilly.

MdiB declares honoraria for advisory role from Amgen, Eli-Lilly, MSD, Roche and Servier.

DC declares research funding from 4SC (Inst); Amgen (Inst); AstraZeneca (Inst); Bayer (Inst); Celgene (Inst); Clovis Oncology (Inst); Janssen (Inst); Lilly (Inst); MedImmune (Inst); Merck (Inst); Merrimack (Inst); Sanofi (Inst)

ECS declares honoraria for advisory role from Astellas, BMS, Celgene, Gritstone Oncology, Five Prime Therapeutics, and Servier.

All other authors declare no conflicts.
Role of the funding source

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FP, RM, AR and ES had full access to the data and final responsibility to submit.

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REFERENCES


18. Choi YY, Cheong JH: To treat, or not to treat, that is the question: Biomarker-guided adjuvant chemotherapy for stage II and III gastric cancer. Ann Surg, 2018

19. FREEMAN GH, HALTON JH: Note on an exact treatment of contingency, goodness of fit and other problems of significance. Biometrika 38:141-149, 1951


Figure Legends

Figure 1. Flow diagram of patients selection for the meta-analysis.

Figure 2. Kaplan-Meier curves of disease-free survival (A) and overall survival (B) according to MSI status (MSS/MSI-low versus MSI-high).

Figure 3. Kaplan-Meier curves of disease-free survival according to treatment (surgery+chemotherapy versus surgery only) and MSI status (MSI-high versus MSS/MSI-low). Whole trials population (A) and MAGIC and CLASSIC trials only (B).

Figure 4. Kaplan-Meier curves of overall survival according to treatment (surgery+chemotherapy versus surgery only) and MSI status (MSI-high versus MSS/MSI-low). Whole trials population (A) and MAGIC and CLASSIC trials only (B).