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ORIGINAL RESEARCH

Predictive role of microsatellite instability for of PD-1 blockade in patients with advanced gastric cancer: a meta-analysis of randomized clinical trials

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Background: Several *post hoc* analyses of randomized controlled trials (RCTs) suggested the importance of microsatellite instability (MSI) as a positive predictive factor to immunotherapy in patients with advanced gastric cancer (GC); however, individually these have low statistical power.

Methods: RCTs investigating treatment with or without an anti-programmed cell death protein 1 (PD-1) agent for advanced GC and providing outcome according to MSI status were selected. The hazard ratio (HR) and the odds ratio were used to compare the treatment effect on survival outcomes and tumor response, respectively, for anti-PD-1-based therapy compared with standard therapy. Evidence for treatment effect by MSI status was evaluated by a test of interaction.

Results: The phase III KEYNOTE-062, CheckMate-649, JAVELIN Gastric 100 and KEYNOTE-061 trials were included. A total of 2545 patients with evaluable MSI status were included and 123 (4.8%) had MSI-high cancers. The HR for overall survival benefit with anti-PD-1-based regimens was 0.34 (95% CI: 0.21-0.54) for MSI-high cancers versus 0.85 [95% confidence interval (CI): 0.71-1.00] for microsatellite stable. The treatment effect was significantly different in the two subgroups (*P* for interaction 0.003). In the MSI-high subgroup, the HR for progression-free survival was 0.57 (95% CI: 0.33-0.97; $P = 0.04$) and the odds ratio for response was 1.76 (95% CI: 1.10-2.83; $P = 0.02$).

Conclusions: Patients with MSI-high GC should be regarded as a specific and highly immunosensitive population worthy of dedicated clinical trials.

Key words: advanced gastric cancer, immune checkpoint inhibitors, microsatellite instability, randomized clinical trials, meta-analysis

INTRODUCTION

Microsatellite instability (MSI) is a positive predictive biomarker for the efficacy of immunotherapy, independent from the tumor site of origin.[1] Although MSI-high status is typically associated with gastrointestinal cancers,[2] its prevalence is only <5% in patients with advanced disease. In patients with MSI-high metastatic colorectal cancer (mCRC) and non-colorectal cancers, non-randomized studies demonstrated unprecedented and durable responses to anti-programmed cell death protein 1 (PD-1) +/− anti-CTLA-4 agents.[3-6] However, despite the relatively low chemosensitivity of MSI-high cancers[7] and the favorable safety profile of anti-PD-1 agents, the latter options were not approved in several non-US countries due to a lack of randomized controlled trials (RCTs). More recently, in treatment-naive mCRC, initial results of the KEYNOTE-177 phase III trial demonstrate superiority of pembrolizumab over first-line chemotherapy with or without biological agents in terms of progression-free survival (PFS), overall response rate (ORR) and safety.[8] On the contrary, given the relatively lower incidence of gastric cancer (GC) compared with CRC, no RCTs with PD-1 blockade have specifically focused on the small subgroup of patients with MSI-high advanced GC. However, *post hoc* analyses of RCTs suggest the superior efficacy of anti-PD-1-based regimens compared with chemotherapy in MSI-high subgroups,[9-13] even for those trials with negative results in the overall population.[9,11] The main limitations of such analyses are their retrospective nature, the lack of stratification for MSI status and, above all, the small number of patients with MSI-high GC—varying from 5 to 19 subjects per study arm.

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Herein we conducted a meta-analysis of RCTs investigating anti-PD-1-based regimens versus chemotherapy, with the aim of providing a more accurate estimate of the activity and efficacy of anti-PD-1-based regimens in patients with MSI-high advanced GC.

METHODS

Search strategy and inclusion criteria

Embase, Medline and The Cochrane Library were searched until 1 October 2020 for the following terms: (gastric cancer or gastric adenocarcinoma) and (MSI or microsatellite instability) and (pembrolizumab or nivolumab or avelumab or durvalumab or atezolizumab or ipilimumab). Also, we reviewed abstracts and presentations from major conference proceedings such as American Society of Clinical Oncology (ASCO), World Congress on Gastrointestinal Cancer (WCGI) and European Society for Medical Oncology (ESMO) from 2010 to 25 November 2020 to identify unpublished studies/data. Studies were eligible if they were RCTs, published in the English language, in which treatment with an immune checkpoint inhibitor, either alone or combined with standard therapy, had been compared with the same standard therapy for patients with advanced GC. In addition, studies had to provide data on overall survival (OS) or PFS outcomes. Studies were excluded if they did not provide sufficient quantitative data about the outcome [hazard ratios (HRs) or survival curves] and if they were retrospective, phase I or single-arm phase II studies.

Potentially relevant studies were retrieved in full text (or abstract form) and assessed to determine whether they matched the study eligibility criteria. Hand searches of the reference lists of the relevant reports were carried out to identify any relevant studies that were missed with the search strategy. If multiple reports referred to the same data, the report containing the (largest and) most recent data were included in the review, and these data were cross-checked against the other reports. Review of papers for inclusion was undertaken independently by two investigators (FPe and FPi) with any discrepancies resolved by the other investigators (GR).

Statistical analysis

The HR was used to compare the treatment effect on survival outcomes for anti-PD-1-based regimens versus standard therapy. Included studies usually reported HRs derived from Cox proportional-hazards models. Summary estimates of anti-PD-1-based regimens effect, expressed as HRs for MSI and microsatellite stable (MSS) cancers, were pooled separately using a random- or fixed-effect model based on the inverse variance method. Evidence for treatment effect by MSI status was evaluated by a test of interaction (reported as *P* for subgroup difference).

Heterogeneity between studies was assessed using the Cochrane's Q statistic and I^2 statistic. Risk of publication bias was assessed by visual inspection of funnel plots and Egger's linear regression test. All reported *P* values are two-sided.

RevMan software (ver. 5.3; Cochrane Collaboration, Copenhagen, Denmark) was used for all pooled analyses.

RESULTS

As shown in Table 1, a total of four phase III studies were included, although KEYNOTE-062 had two evaluable experimental anti-PD-1-based treatment arms (overall: $n = 5$ experimental arms; $n = 4$ control arms).[9-14] Two studies were conducted in first-line (KEYNOTE-062; CheckMate-649), one in maintenance (JAVELIN Gastric 100) and one in second-line (KEYNOTE-061). A total of 2545 patients with evaluable MSI status were included and 123 (4.8%) had MSI-high cancers.

The HR for OS benefit with anti-PD-1-based regimens compared with chemotherapy alone was 0.34 [95% confidence interval (CI): 0.21-0.54] for MSI-high cancers versus 0.85 (95% CI: 0.71-1.00) for MSS (Figure 1A). The treatment effect was significantly different in the two subgroups (*P* for interaction 0.003). Regarding PFS endpoint ($n = 4$ experimental arms), the HR in MSI-high cancers was 0.57 (95% CI: 0.33-0.97; $P = 0.04$; Figure 1B). With respect to ORR ($n = 3$ experimental arms), the odds ratio in MSI-high cancers was 1.76 (95% CI: 1.10-2.83; $P = 0.02$; Figure 1C). All trials to date have not reported PFS and ORR data in the MSS subgroup, thus impeding testing for interaction with regard to these endpoints. Visual inspection of the funnel plot (Begg test) provides no significant publication bias ($P = 0.28$); similarly, regression tests ($P = 0.09$) did not indicate any significant bias according to the size of the study.

After excluding the second-line trial and the maintenance trial, the results for the subgroup difference in terms of OS remained similar ($P < 0.01$) and are reported in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2020.100036. Also, we calculated the cumulative HRs for OS in the MSI-high subgroup in two analyses restricted to anti-PD-1 monotherapy versus chemotherapy (HR = 0.2; 95% CI: 0.17-0.63) or anti-PD-1-based chemoimmunotherapy versus chemotherapy (HR = 0.35; 95% CI: 0.18-0.69), respectively (interaction $P = 0.87$; Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2020.100036).

DISCUSSION

In the majority of the global healthcare systems, immunotherapy is not approved for the treatment of patients with advanced GC, even for previously treated subjects with MSI-high disease.[1] As already shown for MSI-high mCRC,[8] the first-line use of anti-PD-1-based therapy in patients with MSI-high advanced GC may be of paramount importance, since rapid health decline from disease progression during or immediately after initial chemotherapy may preclude, for some patients, exposure to highly effective immunotherapy in subsequent treatment lines. The CheckMate-649 first-line study recently showed clinically meaningful and statistically significant OS benefit of nivolumab plus chemotherapy

Table 1. Trials comparing anti-PD-1 agents alone or in combination with chemotherapy to chemotherapy alone in patients with gastric and gastroesophageal cancer including outcomes by MSI status

Author, year	Type of study/line	Treatment arms	Patient subgroups	Number of patients (Exp/Ctr)	Overall response rate (Exp/Ctr), %	Median OS (Exp/Ctr), months	Median PFS (Exp/Ctr), months
Shitara et al., 2020[11]	Phase III Randomized/1L	P versus CT[a]	All patients MSI-high MSS	256/250 14/19 242/231	14.8/37.2 57.1/36.8 –	10.6/11.1 –/8.5 9.5/11.2	2.0/6.4 11.2/6.6 –
Shitara et al., 2020[11]	Phase III Randomized/1L	P + CT versus CT[a]	All patients MSI-high MSS	257/250 17/19 –/231	48.6/37.2 64.7/36.8 –	12.5/11.1 NR/8.5 –/11.2	6.9/6.4 –/6.6 –
Moehler et al., 2020[13]	Phase III Randomized/1L	NIVO + CT versus CT[b]	All patients[d] MSI-high MSS	473/482 19/15 421/425	60/45 – –	14.4/11.1 –/8.8 14.1/11.1	7.7/6.0 – –
Moehler et al., 2020[14]	Phase III Randomized/1L Maintenance	A versus CT[c]	All patients MSI-high MSS	249/250 8/5 209/210	NA – –	10.4/10.9 NR/8.0 10.6/10.9	3.2/4.4 –
Shitara et al., 2018[9]	Phase III Randomized/2L	P versus PTX	All patients[e] MSI-high MSS	196/199 15/12 –	16/14 46.7/16.7 –	9.1/8.3 –/8.1 –	1.5/4.1 17.8/3.5 –

1L, first line; 2L, second line; A, avelumab; CPS, combined positive score; CT, chemotherapy; Ctr, control group; Exp, experimental group; MSI, microsatellite instability; MSS, microsatellite stable; NIVO, nivolumab; OS, overall survival; P, pembrolizumab; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PTX, paclitaxel.

[a] CT included cisplatin in combination with either 5-fluorouracil or capecitabine.

[b] CT included oxaliplatin in combination with either 5-fluorouracil (FOLFOX) or capecitabine (XELOX).

[c] CT included oxaliplatin in combination with either 5-fluorouracil (FOLFOX) or capecitabine (XELOX). Patients unfit for combination therapy could receive capecitabine, 5-fluorouracil with leucovorin or oxaliplatin.

[d] Refers to patients with PD-L1 CPS $\geq$ 5% tumors included in the analysis of primary endpoint.

[e] Refers to patients with PD-L1 CPS $\geq$ 1% tumors included in the analysis of primary endpoint.

compared with chemotherapy alone in patients with cancers bearing a programmed death-ligand 1 (PD-L1) combined positive score (CPS) $\geq$5, thus providing robust evidence for the first-line use of anti-PD-1 agents for molecularly selected subgroups inclusive of Western Countries.[13] Although most MSI-high GCs show higher tumor PD-L1 expression versus MSS ones[15,16] and may fall in the subgroup with CPS $\geq$ 5, some may not express PD-L1 at this level and could miss the opportunity of receiving first-line nivolumab.[17] However, patients with MSI-high GC and relatively lower PD-L1 expression could derive less benefit from immunotherapy and future translational studies should focus on the mechanisms of primary resistance to PD-1 blockade in MSI-high cancers.

Based on the above considerations, the need for specific data on the efficacy and activity of immunotherapy in patients with MSI-high advanced GC is clinically important. In fact, although the results of *post hoc* analyses of RCTs in this molecular subgroup are extremely promising, the number of analyzed patients is quite low, with large CIs of the HRs and odds ratio (for survival and response endpoints, respectively) crossing the 1 value. In this meta-analysis of the available RCTs with retrospective data on MSI status, anti-PD-1 agents with or without chemotherapy significantly and consistently improved OS, PFS and ORR versus chemotherapy alone in the subgroup of patients with MSI-high advanced GC. Such an effect was confirmed when restricting the analysis to the first-line setting and the interaction between treatment effect on OS and MSI status remained significant. The latter observation suggests that, even if some patients with MSS advanced GC may benefit from immunotherapy (mainly those with CPS $\geq$ 5/10, high tumor mutational burden and/or Epstein—Barr virus-positive status), the efficacy and activity of immunotherapy in the MSI-high subgroup is higher compared with the overall MSS counterpart. Finally, robust data on the role of anti-PD-1 agents as a chemotherapy-free monotherapy option were still lacking in patients with MSI-high cancers. When specifically focusing on anti-PD-1 monotherapy or anti-PD-1-based chemo-immunotherapy, the HRs of both strategies versus chemotherapy alone were similar, suggesting that immunotherapy alone may be an option particularly if the clinical risk of rapid progression is low.

Our analysis has clear limitations, including the lack of individual patient data, the heterogeneity of studies with regard to treatment line, the use of anti-PD-1 agents as monotherapy or in combination with chemotherapy and the heterogeneity of trial populations in terms of CPS status. Moreover, the test for interaction between MSI status and treatment was possible only for OS, due to the lack of PFS and ORR data reported in the MSS subgroup.

In conclusion, we formally provide statistically significant evidence of improved survival and response in patients with MSI-high advanced GC who received anti-PD-1-based therapy in the frame of RCTs, with significantly greater OS benefit compared with the subgroup with MSS tumors. Based on the available data in MSI-high GC and phase III data in MSI-high mCRC, immunotherapy should be routinely available for this molecular subgroup of patients. Finally, patients with MSI-high GC should be regarded as a specific and highly immunosensitive population worthy of dedicated clinical trials.

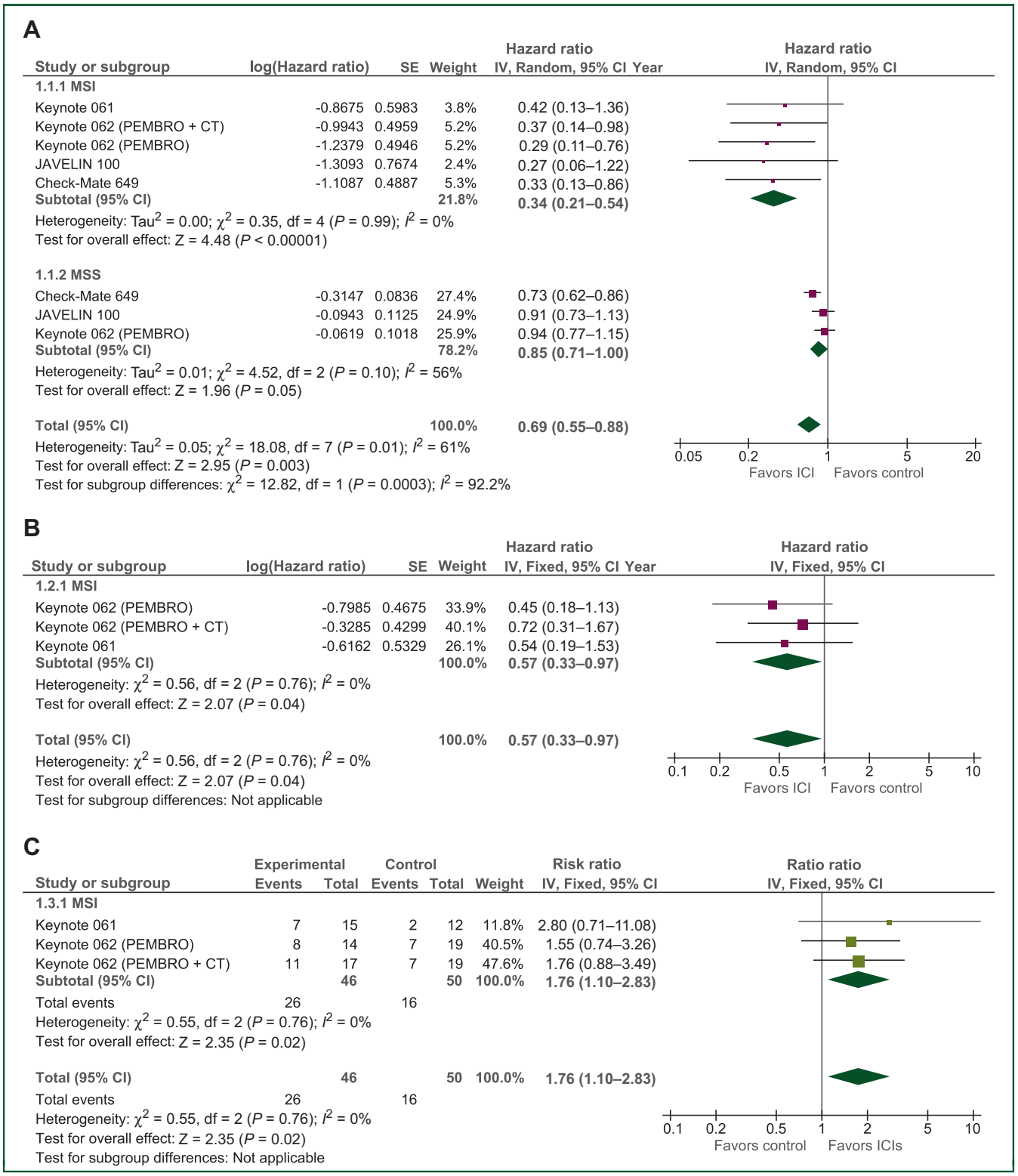

Figure 1. Forest plots showing (A) hazard ratio for overall survival, (B) progression-free survival and (C) overall response rate for anti-programmed cell death protein 1 (PD-1)-based treatment in MSI-high metastatic gastric cancer patients.
CI, confidence interval; CT, chemotherapy; df, degrees of freedom; ICI, immune checkpoint inhibitor.

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