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# Clinical and molecular factors for selection of nivolumab or irinotecan as third-line treatment for advanced gastric cancer

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***Abstract***

**Background:** The use of nivolumab or irinotecan as the third-line treatment for patients with advanced gastric cancer (AGC) remains controversial.

**Methods:** This study analyzed patients with AGC treated with nivolumab or irinotecan (nivolumab group or irinotecan group, respectively) from May 2016 to April 2019 following two or more previous lines of chemotherapy. Univariate survival analysis was conducted to identify the clinical and molecular factors associated with progression-free survival (PFS).

**Results:** A total of 156 patients (74 treated with nivolumab and 82 treated with irinotecan) were analyzed. The median PFS was 1.9 months in both treatment groups. The median overall survival (OS) was 7.2 and 6.2 months in the nivolumab and irinotecan groups, respectively. Eastern Cooperative Oncology Group performance status of 1 or more, liver metastasis, a large tumor size at baseline, and HER2-positive status were associated with a worse PFS in the nivolumab group compared with the irinotecan group. The nivolumab group showed a significantly longer PFS (median 3.1 *versus* 2.0 months) and OS (median 12.9 *versus* 7.8 months) than the irinotecan group in patients with 0 or 1 of these factors, whereas the irinotecan group showed a significantly longer PFS (median 1.0 *versus* 1.8 months) and a trend of longer OS (median 3.9 *versus* 6.1 months) in patients with ⩾2 of these factors.

**Conclusions:** Some clinical and molecular factors were associated with outcomes following nivolumab or irinotecan as the third- or later-line treatment in patients with AGC. These factors must be considered while selecting an optimal treatment option.

***Keywords:*** clinical and molecular factors, gastric cancer, irinotecan, nivolumab, third-line or later-line treatment

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## Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related mortality worldwide.[1] Although fluoropyrimidine and platinum-based chemotherapy combination regimens (with trastuzumab for HER2-positive cases) as the first-line therapy and taxane agents with or without ramucirumab as the second-line are the standard treatment methods for advanced gastric cancer (AGC),[2–7] the prognosis remains poor, with the median survival duration being approximately 1 year.

A phase III ATTRACTION-2 trial on anti-programmed cell death 1 (PD-1) antibody – nivolumab – demonstrated a survival benefit in patients with AGC after two or more previous lines of chemotherapy compared with placebo.[8] However, the objective response rate (ORR) was reported to be approximately 10%, and 50% of the patients exhibited early disease progression. This suggests the need to develop predictive factors that are useful for identifying patients in whom PD-1 blockade may achieve a better clinical outcome. Compared with supportive care

alone, irinotecan, a DNA topoisomerase inhibitor, has been reported to improve survival when used as the second-line or third-line treatment for AGC.[6,7] The ORR with irinotecan reportedly ranged from 3% to 18% in second-line or third-line settings.[7,9–12] In the ATTRACTION-2 trial, 75% of the overall population had been previously treated with irinotecan before study enrollment of patients.[8,13] The National Comprehensive Cancer Network Clinical Practice Guidelines and the European Society for Medical Oncology (ESMO) Clinical Practice Guidelines recommend irinotecan as the second-line or subsequent therapy treatment option for patients with AGC.[14,15] At present, the Pan-Asian-adapted ESMO Clinical Practice Guidelines and the Japanese gastric cancer treatment guidelines recommend both nivolumab and irinotecan as the third-line or later-line treatment option for patients with AGC.[16] However, it remains unclear whether to use nivolumab or irinotecan as a third- or later-line treatment. We have previously reported that several clinicopathological factors are associated with favorable or unfavorable outcomes following the use of nivolumab for AGC.[17,18] However, currently only few studies have focused on head-to-head comparison of nivolumab and irinotecan in patients with AGC in third- or later-line settings. Considering that several clinical trials on anti-PD-1 therapies for AGC are ongoing in the front-line setting, a prospective comparison of these drugs may not be investigated in the future. Therefore, the present retrospective study compared nivolumab *versus* irinotecan to clarify the clinical and molecular factors that can be used for optimal drug selection in patients with AGC.

## Methods

### Patients

This retrospective study evaluated patients with AGC treated with nivolumab or irinotecan (nivolumab or irinotecan group, respectively) following two or more previous lines of chemotherapy. The study included patients treated from May 2016 to April 2019 at the National Cancer Center Hospital East, Kashiwa, Chiba, Japan. Patients received 3 mg/kg nivolumab or 150 mg/m$^2$ irinotecan monotherapy intravenously every 2 weeks as the third- or later-line of therapy. The dose of irinotecan could be reduced at the investigator's judgment. Patients who met the following criteria were included: (a) presence of histologically proven gastric adenocarcinoma; (b) history of previous treatment with two or more regimens, including first-line fluoropyrimidine-based regimens and second-line taxane-based regimens; (c) received at least one administration of nivolumab or irinotecan; and (d) an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2. This study was performed under an institutional review board waiver in accordance with the Japanese ethical guidelines for epidemiologic research. All procedures followed in this study were in accordance with the Declaration of Helsinki of 1964 and the later versions and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. All the patients provided written informed consent prior to chemotherapy. Further, patients who underwent biomarker analysis provided written informed consent for the analysis. The study protocol of biomarker research was approved by the Institutional Review Board of National Cancer Center Japan.

### Molecular characteristics

Molecular characteristics such as the status of the human epidermal growth factor receptor 2 (HER2), programmed cell death ligand 1 (PD-L1), mismatch repair (MMR) and Epstein–Barr virus (EBV) were analyzed with formalin-fixed paraffin-embedded tissue specimens from archival tissue samples if available. Immunohistochemistry (IHC) using a monoclonal anti-HER2 antibody [PATHWAY HER2 (4B5), Ventana, Tucson, AZ, USA] and fluorescence *in situ* hybridization (FISH) using the PathVysion HER-2 probe kit (Abbott Laboratories, Abbott Park, IL, USA) were performed to assess the HER2 status. HER2 positivity was defined as an IHC score of 3+ or an IHC score of 2+ and a FISH-positive status. PD-L1 expression was assessed by IHC using an anti-PD-L1 rabbit monoclonal antibody (Clone SP142 or SP263, Ventana) and measured using the combined positive score (CPS), defined as the number of PD-L1-positive cells (tumor cells, lymphocytes and macrophages) as a proportion of the total number of tumor cells multiplied by 100. MMR status was assessed by IHC using monoclonal antibodies for anti-mutL homolog 1 (MLH1, ES05), anti-mutS homolog 2 (MSH2, FE11), anti-postmeiotic segregation increased 2 (PMS2, EP51) and anti-mutS homolog 6 (MSH6, EP49) (Agilent Technologies, Santa Clara, CA, USA), and tumors that lacked either MLH1, MSH2, PMS2 or MSH6 expression were

considered as MMR-deficient (MMR-D) tumors, whereas those that maintained the expression of MLH1, MSH2, PMS2 and MSH6 were considered MMR-proficient tumors. Chromogenic *in situ* hybridization was performed for EBV-encoded RNA (EBER) using fluorescein-labeled oligonucleotide probes (INFORM EBER Probe, Ventana) to evaluate the EBV status.[19] All the specimens in the present study were reviewed by a single author (Takeshi Kuwata).

### *Outcomes*

We assessed the ORR, disease control rate (DCR), duration of response (DOR), progression-free survival (PFS) and overall survival (OS). Tumor response was retrospectively assessed in patients with measurable lesions according to the guidelines of the Response Evaluation Criteria in Solid Tumors version 1.1. ORR was defined as the proportion of patients with the best overall complete response (CR) or partial response (PR). DCR was defined as the proportion of patients with the best overall CR, PR or stable disease. DOR was defined as the time from the date of first response (CR or PR) until the date of disease progression or death.

### *Statistical analysis*

The baseline characteristics and response rates were compared using $\chi^2$ test or Fisher's exact test. DOR, PFS and OS rates were estimated by the Kaplan–Meier method, compared between the nivolumab and irinotecan groups using Cox proportional hazards models, and presented as hazard ratios (HRs) with 95% confidence intervals (CIs). The 6-month PFS rate and the 12-month OS rate were compared between patients with nivolumab and those with irinotecan using statistical tests based on normal distribution. The predictive factors for PFS and OS were explored using subgroup analyses and interaction tests. The cut-off point for a large tumor size ($\geqslant$59.4 mm) was determined according to the sum of the diameters of the target lesion at baseline, which was associated with hyper progressive disease (HPD) during nivolumab treatment in the previous report.[18] Univariate survival analysis was conducted to identify the clinical and molecular factors associated with PFS (HR of nivolumab to irinotecan $>$ 1.25). HR $>$ 1.25 was determined according to the phase III KEYNOTE-061 trial showing a trend of worse PFS (HR 1.27) for pembrolizumab (anti-PD-1 antibody) compared with that for paclitaxel.[20] Statistical analyses were performed using the SPSS® Statistics software V26 (IBM, Armonk, NY, USA). All tests were two sided, and $p$ values of $< 0.05$ were considered statistically significant.

## Results

### *Patients' characteristics*

A total of 156 patients met all the criteria; 74 patients received nivolumab first and 82 patients received irinotecan first. Among the 74 patients in the nivolumab group, 20 patients (27%) had subsequently received irinotecan, whereas among the 82 patients in the irinotecan group, 23 (28%) received nivolumab subsequently. There was no significant difference in the patients' characteristics between the two groups (Table 1). Data on HER2, CPS, MMR and EBV were available in 156, 106, 140 and 142 patients. Among the 74 and 82 patients in the nivolumab and irinotecan groups, 10 patients in each group (14% *versus* 12%) showed HER2-positive tumors. PD-L1 expression was assessed by IHC, mainly using SP263 (74%) and partially using SP142 (26%). The proportion of patients with CPS $\geqslant$1 was not different between SP263 and SP142 (86% *versus* 79%). Overall, 47 of 54 patients (87%) and 42 of 52 patients (81%) showed CPS $\geqslant$1 in the nivolumab and irinotecan groups, respectively. MMR-D tumors were reported in four of 65 patients (6%) in the nivolumab group and two of 75 patients (3%) in the irinotecan group. An EBV-positive status was observed in three of 66 patients (5%) in the nivolumab group and six of 76 patients (8%) in the irinotecan group.

### *Response to treatment*

The Kaplan–Meier analysis estimated a median follow-up of 11.5 months (95% CI 9.1–14.0) in the nivolumab group and 12.6 months (95% CI 7.8–17.4) in the irinotecan group. Overall, 20 patients in the nivolumab group and six patients in the irinotecan group had PR, resulting in 18% and 8% ORR for each treatment, respectively ($p = 0.13$). Median DOR was not achieved with nivolumab, whereas a median DOR of 4.1 months (95% CI 2.5–5.8) was achieved with irinotecan (HR 0.22; 95% CI 0.06–0.80; $p = 0.021$). Disease control was achieved in 29 patients each in the nivolumab (43%) and the irinotecan (39%) group ($p = 1.00$) (Table 2).

**Table 1.** Patients characteristics.

| | | Nivolumab group (*n* = 74) (%) | Irinotecan group (*n* = 82) (%) | *p* value |
|---|---|---|---|---|
| Age, years | Median | 67 | 68 | 0.795 |
| Sex | Male | 51 (69) | 53 (65) | 0.613 |
| | Female | 23 (31) | 29 (35) | |
| ECOG PS | 0 | 41 (55) | 42 (51) | 0.632 |
| | ⩾1 | 33 (45) | 40 (49) | |
| Histology | Intestinal | 21 (29) | 25 (31) | 1.000 |
| | Diffuse | 51 (71) | 53 (65) | |
| Primary site | Gastroesophageal | 13 (18) | 8 (10) | 0.167 |
| | Gastric | 61 (82) | 74 (90) | |
| Number of previous chemotherapy | 2 | 60 (81) | 68 (83) | 0.836 |
| | ⩾3 | 14 (19) | 14 (17) | |
| Site of metastasis | Lymph node | 58 (78) | 56 (68) | 0.206 |
| | Peritoneum | 44 (60) | 44 (54) | 0.591 |
| | Liver | 27 (37) | 37 (45) | 0.329 |
| | Other | 16 (22) | 22 (27) | 0.463 |

ECOG PS, Eastern Cooperative Oncology Group performance status

**Table 2.** Overall response.

| | Nivolumab group *n* = 74 | Irinotecan group *n* = 82 | *p* value |
|---|---|---|---|
| Measurable lesion + | 67 | 72 | |
| CR | 0 | 0 | |
| PR | 12 | 6 | |
| SD | 17 | 23 | |
| PD | 34 | 37 | |
| NE | 4 | 7 | |
| ORR (%) | 12 (18%) | 6 (8%) | 0.13 |
| DCR (%) | 29 (43%) | 28 (39%) | 1.00 |

CR, complete response; DCR, disease control rate; NE, not evaluated; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease

The median PFS was 1.9 months both in the nivolumab (95% CI 1.4–2.3) and in the irinotecan (95% CI 1.5–2.2) group (HR 0.85; 95% CI 0.61–1.20; $p = 0.356$) (Figure 1). The 6-month PFS rate was 16% and 7% in the nivolumab and irinotecan groups, respectively ($p = 0.048$). The median OS was 7.2 (95% CI 4.7–9.2) and 6.2 months in the nivolumab and irinotecan groups (95% CI 5.4–7.0) (HR 0.74; 95% CI 0.49–1.11; $p = 0.143$) (Figure 2). The 12-month OS rate was 40% in the nivolumab and 17% in the irinotecan group ($p = 0.004$).

### *Subgroup analysis by clinical and molecular factors*

Subgroup analysis identified ECOG PS of 1 or more (HR 1.27; 95% CI 0.78–2.72), presence of liver metastasis (HR 1.61; 95% CI 0.96–2.72), a large tumor size at baseline (HR 1.90; 95% CI 0.98–3.68) and HER2-positive status (HR 3.04; 95% CI 1.06–8.67) as factors associated with a worse PFS in the nivolumab group compared with that in the irinotecan group (HR > 1.25) [Figure 3(a)]. In patients with 0 or 1 factor, the nivolumab group showed a significantly longer PFS (median 3.1 *versus* 2.0 months, HR 0.56; 95% CI 0.34–0.92, $p < 0.021$) and OS (median 12.9 *versus* 7.8 months, HR 0.45; 95% CI 0.24–0.83, $p < 0.011$) than the irinotecan group. In patients with ⩾2 factors, the irinotecan group showed a significantly longer PFS (median 1.0 *versus* 1.8 months, HR 2.11; 95% CI 1.22–3.64, $p < 0.007$) and a trend of longer OS (median 3.9 *versus* 6.1 months, HR 1.46; 95% CI 0.77–2.75, $p = 0.247$) than the nivolumab group (Figures 4 and 5). Furthermore, the HR of nivolumab to irinotecan for PFS and OS tended to be higher as the number of factors increased [Figure 3(a) and (b)]. The median PFS of patients with PD-L1 CPS ⩾1 was 1.9 and 1.8 months in the nivolumab (95% CI 1.0–2.7) and irinotecan (95% CI 1.6–2.1) groups, respectively (HR 0.77; 95% CI 0.49–1.21; $p = 0.26$). The median PFS of patients with CPS <1 was 3.1 and 1.2 months in the nivolumab (95% CI 0.0–7.5) and irinotecan (95% CI 0.8–1.6) groups, respectively (HR 0.63; 95% CI 0.22–1.82; $p = 0.40$). Table 3 shows the clinical factors and outcomes in patients with a MMR-D tumor and an EBV-positive status. Any patient with a MMR-D tumor and an EBV-positive status did not show a HER2-positive status. All the patients with MMR-D tumors and EBV-positive status (except for one EBV-positive patient

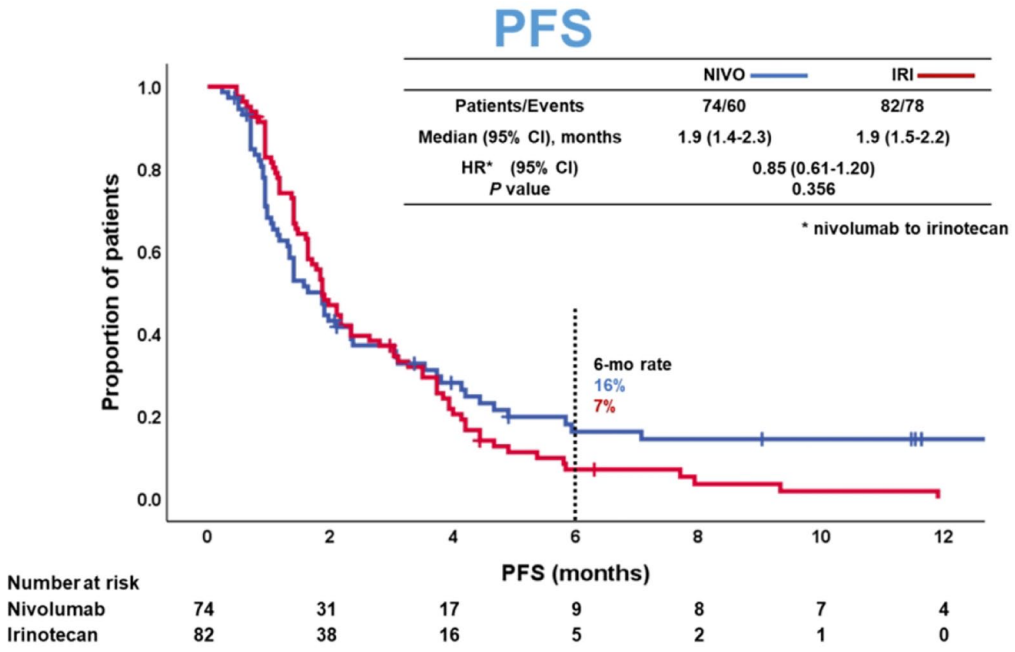


**Figure 1.** Kaplan–Meier plots of progression-free survival.
CI, confidence interval; HR, hazard ratio; IRI, irinotecan; mo, month; NIVO, nivolumab; PFS, progression-free survival

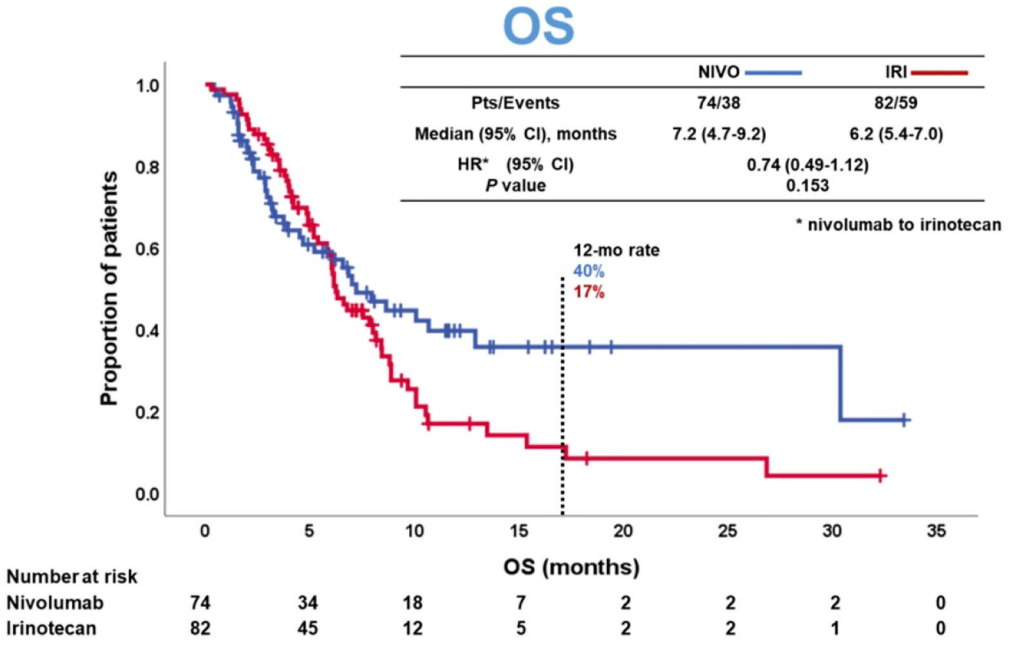


**Figure 2.** Kaplan–Meier plots of overall survival.
CI, confidence interval; HR, hazard ratio; IRI, irinotecan; mo, month; NIVO, nivolumab; OS, overall survival; Pts, patients

without a CPS status) showed CPS ⩾1. Among the four patients with MMR-D tumors, three showed a durable response in the nivolumab group, whereas two patients with MMR-D tumors did not show an objective response in the irinotecan group. Moreover, two of the three patients in the nivolumab group and one of the five patients in the irinotecan group with an EBV-positive status showed an objective response.

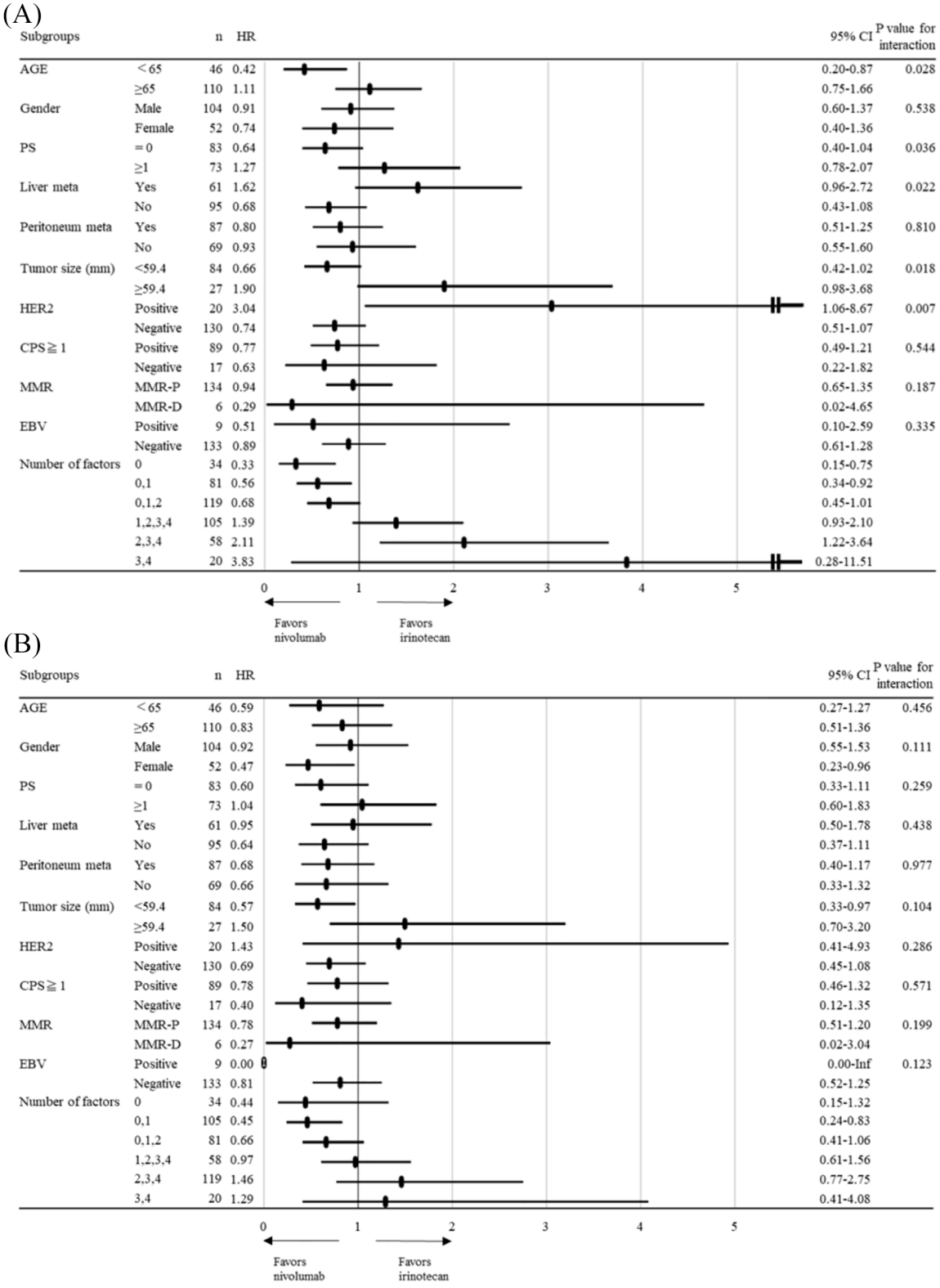


**Figure 3.** Subgroup analyses by clinical and molecular factors. (a) Forest plot of progression-free survival. (b) Forest plot of overall survival.

CI, confidence interval; CPS, combined positive score; EBV, Epstein–Barr virus; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; meta, metastasis; MMR, mismatch repair; MMR-D, MMR deficient; MMR-P, MMR proficient; PS, performance status.

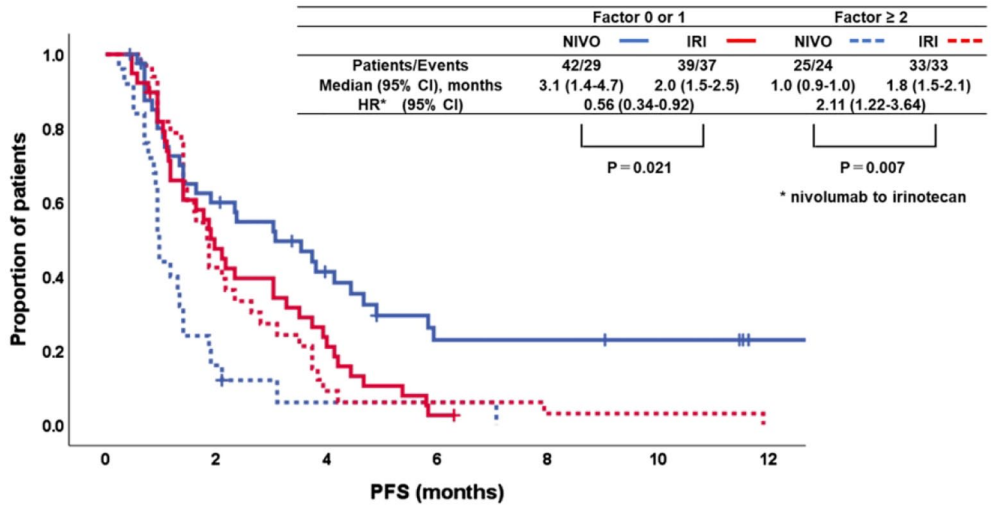


**Figure 4.** Kaplan–Meier plots of progression-free survival according to the number of factors.
CI, confidence interval; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IRI, irinotecan; meta, metastasis; NIVO, nivolumab; PFS, progression-free survival; PS, performance status.

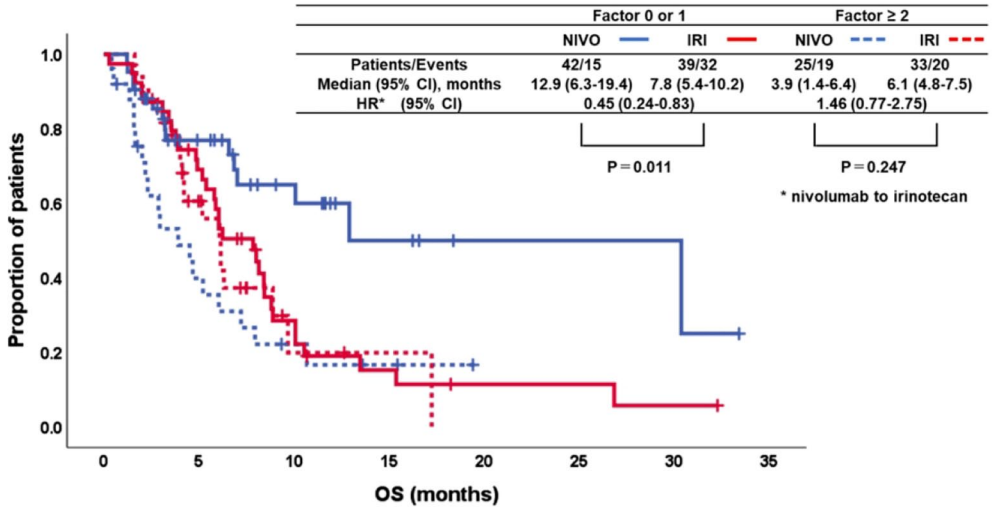


**Figure 5.** Kaplan–Meier plots of overall survival according to the number of factors.
CI, confidence interval; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IRI, irinotecan; meta, metastasis; NIVO, nivolumab; OS, overall survival; PFS, progression-free survival; PS, performance status.

## Discussion

This study retrospectively investigated the outcomes of using nivolumab or irinotecan as the third- or later-line treatment in patients with AGC. To the best of our knowledge, this is the first report to provide detailed information on the clinical and molecular features comparing these drugs in patients with AGC.

In our patient cohort, survival outcomes were not significantly different between patients who received nivolumab and those who received irinotecan. This observation is almost consistent with the results of a previous randomized study that compared avelumab as an anti-PD-L1 monoclonal antibody *versus* a standard third-line chemotherapy.[12] The Kaplan–Meier plots of PFS and OS in the overall population suggested that compared with those who received irinotecan, some patients who received nivolumab exhibited early disease progression with a poor prognosis. The curves of PFS and OS crossed at

**Table 3.** Clinical factors and outcomes in patients with MMR-D tumors and EBV-positive status.

| Treatment | MMR-D | EBV | Age (years) | Sex | PS | CPS ⩾1 | Best response | PFS (months) | OS (months) |
|---|---|---|---|---|---|---|---|---|---|
| Nivolumab | + | – | 79 | Female | 0 | + | PR | 16.6+ | 16.6+ |
| Nivolumab | + | – | 84 | Male | 1 | + | PR | 12.1+ | 12.1+ |
| Nivolumab | + | – | 77 | Male | 1 | + | PR | 4.9 | 6.2+ |
| Nivolumab | + | – | 68 | Female | 1 | + | PD | 0.8 | 1.5 |
| Irinotecan | + | – | 76 | Female | 1 | + | PD | 1.6 | 4.9 |
| Irinotecan | + | – | 62 | Male | 1 | + | SD | 0.8 | 6.2 |
| Nivolumab | – | + | 43 | Male | 0 | + | PR | 33.4+ | 33.4+ |
| Nivolumab | – | + | 69 | Male | 0 | + | SD | 3.8 | 30.4 |
| Nivolumab | – | + | 72 | Male | 0 | + | PR | 3.5 | 5.6+ |
| Irinotecan | – | + | 66 | Female | 1 | + | SD | 3.9 | 5.2 |
| Irinotecan | – | + | 67 | Male | 0 | + | SD | 3 | 13.5 |
| Irinotecan | – | + | 56 | Male | 0 | + | PR | 4.1 | 15.4 |
| Irinotecan | – | + | 61 | Male | 1 | + | PD | 1.1 | 3.4 |
| Irinotecan | – | + | 81 | Male | 1 | NA | SD | 4.7 | 8.4 |

CPS, combined positive score; EBV, Epstein–Barr virus; MMR-D, mismatch repair deficient; NA, not available; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; PS, performance status; SD, stable disease

approximately 3 and 6 months, respectively, and then the separation in favor of nivolumab was sustained, probably due to the durability of benefit in patients who achieved a response. The long-term survival benefit in patients who received nivolumab compared with those who received irinotecan was also suggested by the 6-month PFS rates of 16% *versus* 7% and the 12-month OS rates of 40% *versus* 17%. The crossing of survival curves has also been observed in a phase III KEYNOTE-061 study comparing the efficacy of paclitaxel and pembrolizumab as the second-line treatment in patients with AGC and a phase III KEYNOTE-062 study comparing the efficacy of cytotoxic agents and pembrolizumab monotherapy in patients with untreated AGC.[20,21] Such previous studies highlight the wide range of the survival benefit of anti-PD-1 treatments for AGC.

We also conducted a subgroup analysis according to clinical and molecular factors and found that an ECOG PS of 1 or more, liver metastasis, a large tumor size at baseline, and a HER2-positive status were associated with a worse PFS in the nivolumab group compared with that in the irinotecan group. As stated previously, we have previously reported that an ECOG PS of 1 or more, liver metastasis, and a large tumor size at baseline were significantly associated with HPD when nivolumab was administered in patients with AGC.[18] Another study has demonstrated that an ECOG PS of 1 or more and the presence of two or more metastatic sites were associated with a trend of higher frequencies of HPD, although there was no significant difference.[22] Furthermore, the results of the subgroup analysis of phase II and III trials of pembrolizumab have shown that a better performance status (PS) is associated with a higher response rate and longer OS.[20,23] Although the exact explanations for the correlation between PS and the clinical outcomes of PD-1 blockade have not been established, it is sometimes difficult to continue treatment in patients with a poor PS for sufficient duration to achieve a response. Liver metastasis has also been suggested to decrease the probability of a response to anti-PD-1/PD-L1 therapies

because of liver-induced immune tolerance.[24–27] Tumor burden has also shown to negatively affect tumor response and survival after anti-PD-1 blockade, particularly when T-cell re-invigoration in the peripheral blood was not sufficient.[28,29] It has been reported that HER2 alterations in gastric cancer are associated with decreased immunogenicity in terms of immune-related gene mRNA expression, immune infiltrates and neoantigen levels,[30,31] although an exploratory subgroup analysis of a phase III ATTRACTION-2 trial has demonstrated that compared with placebo, nivolumab improves OS, PFS and ORR regardless of prior trastuzumab use in patients with AGC.[32] Meanwhile, a preclinical study has reported that the combination of anti-PD-1 and anti-HER2 therapy induces T-cell activation and augments antibody-dependent cellular cytotoxicity, which might lead to promising results following the addition of trastuzumab + pembrolizumab to the first-line chemotherapy in the phase II study.[33] The impact of an HER2-positive status on the efficacy of anti-PD-1 monotherapy or combined activities of anti-PD-1 and anti-HER2 therapy warrants further investigations. Importantly, the prognostic effect exerted by the combination of these clinical and molecular factors in the present study was significantly pronounced in patients who received nivolumab compared with those who received irinotecan. This suggests that these clinicopathogical factors affect the efficacy of immunotherapy rather than cytotoxic chemotherapy. The HR of nivolumab to irinotecan for PFS and OS tended to be higher as the number of these factors increased. Therefore, further analysis is warranted to determine why the prognostic effect differs between two treatments.

Owing to the overall small number of patients with MMR-D tumors and EBV-positive status, we could not evaluate the exact impact of these molecular factors on the selection of nivolumab or irinotecan. However, three of the four patients with MMR-D tumors and two of the three patients with an EBV-positive status in the nivolumab group achieved an objective response, which is consistent with that reported previously.[34] Although these results tend to support the prior use of nivolumab to irinotecan as the third- or later-line of therapy in patients with AGC with MMR-D tumors and EBV-positive status, further evaluation is warranted in a larger cohort.

The importance of patient selection is also suggested by the first-line trial of pembrolizumab.[34] In the phase III KEYNOTE-062 trial, an ECOG PS of 0 (HR 0.87), small tumor size (HR 0.78) and MMR-D tumor (HR 0.29) were associated with a trend of better OS with pembrolizumab compared with chemotherapy in the first-line setting. These observations were almost comparable to the results of our present study.

This study had some limitations. First, this was a single-institution study with a limited sample size. Second, PD-L1 expression, MMR status and EBV status were not analyzed in all the patients. These limitations can be overcome by a larger cohort analysis. Third, the selection of nivolumab or irinotecan after the approval of nivolumab in Japan was based on the investigator's judgment, thus inducing potential selection bias.

## Conclusion

In the present study, we identified clinical and molecular factors associated with the outcomes of nivolumab or irinotecan therapy in patients with AGC. Importantly, the HR of nivolumab to irinotecan for PFS and OS tended to be higher with an increasing number of factors. Combining these factors may be useful in drug selection. For instance, nivolumab might be suitable for patients with a good PS and a small tumor size, whereas irinotecan can be more appropriate than nivolumab in patients with a poor PS and a large liver metastasis. Trifluridine/tipiracil was recently approved in the United States and Japan owing to its effectiveness as the third- or later-line treatment for AGC.[35] Thus, the optimal selection of nivolumab, irinotecan or trifluridine/tipiracil should be investigated in future studies.

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## Availability of data and materials

All the data analyzed during this study has been included within this article.

## Conflict of interest statement

TI has nothing to disclose. AK reports grants from Ono, grants from Sumitomo Dainippon, grants from Taiho, outside the submitted work. AS has nothing to disclose. SM has nothing to disclose. KS has nothing to disclose. YN reports grants from Ono, grants from Taiho, outside the submitted work; DK reports personal fees from Chugai, personal fees from Lilly, personal

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## References

1. Ferlay J, Soerjomataram I, Dikshit R, *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: E359–E386.
2. Koizumi W, Narahara H, Hara T, *et al.* S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008; 9: 215–221.
3. Cunningham D, Starling N, Rao S, *et al.* Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; 358: 36–46.
4. Wilke H, Muro K, Van Cutsem E, *et al.* Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; 15: 1224–1235.
5. Fuchs CS, Tomasek J, Yong CJ, *et al.* Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; 383: 31–39.
6. Thuss-Patience PC, Kretzschmar A, Bichev D, *et al.* Survival advantage for irinotecan versus best supportive care as second-line chemotherapy in gastric cancer–a randomised phase III study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). *Eur J Cancer* 2011; 47: 2306–2314.
7. Kang JH, Lee SI, Lim DH, *et al.* Salvage chemotherapy for pretreated gastric cancer: a randomized phase III trial comparing chemotherapy plus best supportive care with best supportive care alone. *J Clin Oncol* 2012; 30: 1513–1518.

8. Kang YK, Boku N, Satoh T, *et al*. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; 390: 2461–2471.
9. Hironaka S, Ueda S, Yasui H, *et al*. Randomized, open-label, phase III study comparing irinotecan with paclitaxel in patients with advanced gastric cancer without severe peritoneal metastasis after failure of prior combination chemotherapy using fluoropyrimidine plus platinum: WJOG 4007 trial. *J Clin Oncol* 2013; 31: 4438–4444.
10. Kawakami T, Machida N, Yasui H, *et al*. Efficacy and safety of irinotecan monotherapy as third-line treatment for advanced gastric cancer. *Cancer Chemother Pharmacol* 2016; 78: 809–814.
11. Nishimura T, Iwasa S, Nagashima K, *et al*. Irinotecan monotherapy as third–line treatment for advanced gastric cancer refractory to fluoropyrimidines, platinum, and taxanes. *Gastric Cancer* 2017; 20: 655–662.
12. Bang YJ, Ruiz EY, Van Cutsem E, *et al*. Phase III, randomised trial of avelumab versus physician's choice of chemotherapy as third-line treatment of patients with advanced gastric or gastro-oesophageal junction cancer: primary analysis of JAVELIN Gastric 300. *Ann Oncol* 2018; 29: 2052–2060.
13. Kato K, Satoh T, Muro K, *et al*. A subanalysis of Japanese patients in a randomized, double-blind, placebo-controlled, phase 3 trial of nivolumab for patients with advanced gastric or gastro-esophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2). *Gastric Cancer* 2019; 22: 344–354.
14. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Gastric cancer (version 4.2019), https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf (2019, accessed 18 March 2020).
15. Smyth EC, Verheij M, Allum W, *et al*. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; 27: v38–v49.
16. Muro K, Van Cutsem E, Narita Y, *et al*. Pan-Asian adapted ESMO clinical practice guidelines for the management of patients with metastatic gastric cancer: a JSMO-ESMO initiative endorsed by CSCO, KSMO, MOS, SSO and TOS. *Ann Oncol* 2019; 30: 19–33.
17. Mishima S, Kawazoe A, Nakamura Y, *et al*. Clinicopathological and molecular features of responders to nivolumab for patients with advanced gastric cancer. *J Immunother Cancer* 2019; 7: 24.
18. Sasaki A, Nakamura Y, Mishima S, *et al*. Predictive factors for hyperprogressive disease during nivolumab as anti-PD1 treatment in patients with advanced gastric cancer. *Gastric Cancer* 2019; 22: 793–802.
19. Kawazoe A, Shitara K, Kuboki Y, *et al*. Clinicopathological features of 22C3 PD-L1 expression with mismatch repair, Epstein-Barr virus status, and cancer genome alterations in metastatic gastric cancer. *Gastric Cancer* 2019; 22: 69–76.
20. Shitara K, Ozguroglu M, Bang YJ, *et al*. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2018; 392: 123–133.
21. Tabernero J, Cutsem EV, Bang YJ, *et al*. Pembrolizumab with or without chemotherapy versus chemotherapy for advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma: the phase III KEYNOTE-062 study. *J Clin Oncol* 2019; 37(Suppl. 18): Abstract LBA4007.
22. Aoki M, Shoji H, Nagashima K, *et al*. Hyperprogressive disease during nivolumab or irinotecan treatment in patients with advanced gastric cancer. *ESMO Open* 2019; 4: e000488.
23. Fuchs CS, Doi T, Jang RW, *et al*. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. *JAMA Oncol* 2018; 4: e180013.
24. Bearz A, Perin T, Cancian L, *et al*. Immune checkpoint inhibitors and response analysis: a tough challenge. A case report. *BMC Res Notes* 2016; 9: 349.
25. Massard C, Gordon MS, Sharma S, *et al*. Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer. *J Clin Oncol* 2016; 34: 3119–3125.
26. Funazo T, Nomizo T and Kim YH. Liver metastasis is associated with poor progression-free survival in patients with non-small cell lung cancer treated with nivolumab. *J Thorac Oncol* 2017; 12: e140–e141.
27. Tumeh PC, Hellmann MD, Hamid O, *et al*. Liver metastasis and treatment outcome with anti-PD-1 monoclonal antibody in patients with

melanoma and NSCLC. *Cancer Immunol Res* 2017; 5: 417–424.

28. Huang AC, Postow MA, Orlowski RJ, *et al*. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* 2017; 545: 60–65.
29. Joseph RW, Elassaiss-Schaap J, Kefford R, *et al*. Baseline tumor size is an independent prognostic factor for overall survival in patients with melanoma treated with pembrolizumab. *Clin Cancer Res* 2018; 24: 4960–4967.
30. Bo Wei XX, Ziyu Li, Quanli Han, *et al*. Tumor mutation burden and immunogenicity in gastric cancer with HER2 alterations. *J Clin Oncol* 2019; 37(Suppl. 15): 4024.
31. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; 513: 202–209.
32. Satoh T, Kang YK, Chao Y, *et al*. Exploratory subgroup analysis of patients with prior trastuzumab use in the ATTRACTION-2 trial: a randomized phase III clinical trial investigating the efficacy and safety of nivolumab in patients with advanced gastric/gastroesophageal junction cancer. *Gastric Cancer*. Epub ahead of print 16 May 2019. DOI: 10.1007/s10120-019-00970-8.
33. Stacchiotti S, Schoffski P, Jones R, *et al*. Safety and efficacy of tazemetostat, a first-in-class EZH2 inhibitor, in patients (pts) with epithelioid sarcoma (ES) (NCT02601950). *J Clin Oncol* 2019; 37: 11003–11003.
34. Kim ST, Cristescu R, Bass AJ, *et al*. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018; 24: 1449–1458.
35. Shitara K, Doi T, Dvorkin M, *et al*. Trifluridine/tipiracil versus placebo in patients with heavily pretreated metastatic gastric cancer (TAGS): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2018; 19: 1437–1448.