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Clinical and molecular predictors of response to immune checkpoint inhibitors in patients with advanced esophagogastric cancer

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Abstract

Purpose: Immune checkpoint inhibitors (ICIs) are effective in only a minority of patients with esophagogastric cancer (EGC). Here, we aimed to identify predictors of durable clinical benefit to ICI in EGC.

Experimental Design: Patients with advanced EGC treated with ICIs at Memorial Sloan Kettering Cancer Center were identified. Clinicopathologic variables were assessed. In patients profiled by MSK-IMPACT targeted sequencing, outcomes were correlated with tumor genomic features.

Results: One-hundred sixty-one patients were treated with ICIs (110 with anti-PD-1/PD-L1 antibodies and 51 with anti-CTLA-4 and PD-1/PD-L1 antibodies). The median progression-free survival (PFS) and overall survival (OS) were 1.7 and 4.9 months, respectively. Greater number of disease sites (≥3), liver metastases, treatment with ≥3 prior therapies and ECOG performance status ≥2 were associated with poorer PFS and OS. Patients treated with combination ICI and those with PD-L1 positive tumors had improved outcomes. There was no difference in outcomes between patients treated with antibiotics during or in the 2 months preceding ICI treatment versus those who were not. Occurrence of irAEs was associated with improved OS. In genomically profiled tumors (n=89), survival was associated with increasing tumor mutation burden (TMB). However, in multivariable analyses and when microsatellite unstable (MSI) patients were excluded, a significant association was no longer observed.

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Conclusions: In patients with advanced EGC, heavily pre-treated patients, those with high-volume disease and/or poor PS were less likely to benefit from ICI. irAEs were associated with improved OS. TMB correlated with improved survival, but this association was not observed when MSI-high patients were excluded.

Keywords

advanced esophagogastric carcinoma; immune checkpoint inhibitors; tumor mutational burden; immune-related adverse events; antibiotic use

Introduction

Esophagogastric cancer (EGC) is the third most common cancer worldwide with 1.6 million patients diagnosed annually.[1] Metastatic disease has historically been associated with poor outcomes, although recent advances in treatment have led to incremental improvements.

Most notably, pembrolizumab, an anti-programmed cell death protein (PD)-1 antibody, was recently approved in the United States for the treatment of patients with chemorefractory gastric or gastroesophageal junction (GEJ) adenocarcinoma whose tumors express programmed death ligand 1 (PD-L1).[2] This approval was based on a single-arm phase II study, the results of which are similar to those for nivolumab, another anti-PD-1 antibody, which was studied in the phase III ATTRACTION-2 study[3], which enrolled East Asian patients. Nivolumab is approved in Japan regardless of PD-L1 status.

Although some EGC patients achieve dramatic and durable responses to ICI therapy, only a minority of patients benefit. To improve the efficacy of these therapies, it will be critical to understand the molecular and clinical factors that dictate the variable response of ICIs. This would allow selection of patients likely to respond to single-agent ICI therapy and lead to the development of combination strategies that overcome response-limiting mechanisms.

Non-synonymous somatic mutations give rise to neoantigens which may in turn mediate T-cell responses and immune-mediated tumor cell death. Recent studies suggest that tumor mutational burden (TMB), defined as the total number of mutations per coding area of a tumor genome as measured by next generation sequencing (NGS), is a predictive biomarker of response to anti-PD-1/PD-ligand 1 (PD-L1) therapies in melanoma, non-small cell lung carcinoma (NSCLC), and urothelial carcinoma. [4–8]. In a recent study from Memorial Sloan Kettering Cancer Center (MSKCC) which evaluated tumor mutational load as a predictor of survival following ICI treatment across multiple cancer types, a trend toward improved overall survival (OS) was observed in patients with EGC who had a TMB cut-off greater than 8.8.[9] Another analysis from our group suggested that TMB may be of value in EGC as a quantitative marker to identify patients who obtain durable benefit from ICI.[10] Patients with microsatellite unstable (MSI) tumors were included in both of these analyses.

It has also been hypothesized that development of immune-related adverse events (irAEs) may be associated with response in patients with melanoma and NSCLC, as such events may correlate with effective de-inhibition of immune responses, although studies to date have yielded conflicting results.[11–14] In addition, a recent study suggested that antibiotic use

may compromise the efficacy of ICIs in certain cancers by altering the composition of the gut microbiome.[15]

In this study, we sought to identify clinical and molecular predictors of outcome in patients with metastatic EGC treated with ICIs.

Methods

We identified patients with metastatic EGC who had received anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), anti-PD-1 or anti-PD-L1 therapy at MSKCC between September 2013 and May 2018, alone or in combination, within the context of a therapeutic clinical trial, as compassionate-use or as standard therapy. A waiver of authorization to review these data was approved by the Institutional Review Board. For all patients, pathology was reviewed and confirmed at MSKCC. PD-L1 status was assessed by centrally performed immunohistochemistry (IHC) in patients enrolled on clinical studies or using the E1L3N antibody from Cell Signaling at MSKCC followed by calculation of the tumor proportion score (TPS) or combined positive score (CPS). Tumor mismatch repair (MMR) was evaluated using immunohistochemical staining for MLH1, MSH2, MSH and PMS1 proteins. Tumors with loss of expression of any one of the four proteins were identified as MMR deficient (MMRd) by IHC. Epstein-Barr virus (EBV) was tested by Epstein-Barr encoding region (EBER) in situ hybridization.

Patients who received at least one dose of ICI therapy were included. Clinicopathologic characteristics were collected for all patients. For antibiotic usage, pharmacy records were reviewed to identify any antibiotic use within 60 days of commencing ICI therapy or during treatment with ICIs. Home medication lists were also reviewed to capture any oral antibiotics prescribed outside of our institution. The antibiotic class, indication for treatment, route of administration and duration were collected. For irAEs, the type, grade, time to occurrence and use of steroids was recorded.

The MSKCC Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) NGS assay was performed as previously described.[16] Briefly, genomic DNA was extracted from tumors and patient-matched blood samples to generate barcoded libraries. After capture of exons and selected introns of the genes included in the sequencing panel using custom biotinylated DNA probes and streptavidin-conjugated beads, pooled libraries were sequenced on the Illumina HiSeq 2500 system. The panel included either 341, 410, or 468 genes. The resulting sequences were run through an optimized informatics pipeline to identify somatic mutations, copy number alterations, and select structural rearrangements.[16,17]

To calculate TMB, the total number of somatic nonsilent protein-coding mutations in the sequenced genes was determined and normalized to the exonic coverage of the respective MSK-IMPACT panel in megabases. TMB was analyzed both as a continuous variable and by quartiles. Low purity cases were excluded (n=12). For gene alteration analysis, low purity and hypermutated cases were excluded. Utilizing OncoKB and Cancer Hotspots, variants of unknown significance were excluded. If a sample had either a mutation, copy number

alteration or fusion, the gene was considered altered and assigned a binary value for analysis.

MSI status was assessed using the MSIsensor algorithm, which calculates the percentage of microsatellite loci covered by the MSK-IMPACT assay that are unstable in the tumor as compared to the patients matched normal. [18] In-house PD-L1 IHC, MSK-IMPACT and MSI testing via MSIsensor are all clinically validated assays performed in Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratories.

Responses were based on RECIST 1.1 for patients enrolled on clinical trials or by manual clinical radiographic assessment in patients who received treatment on a compassionate-use basis or FDA-approved-use basis.

Statistical analysis

Disease and treatment characteristics were summarized using the frequency and percentage for categorical variables and median and inter-quartile range (IQR) for continuous variables. Best objective response rate (ORR) was defined as overall best response of complete responders (CR) + partial responders (PR). Disease control rate (DCR) was defined as PR+ CR+ stable disease (SD). Duration of response was calculated among patients who had CR +PR from the date of best response until date of disease progression and estimated using the Kaplan-Meier method.

Progression-free survival (PFS) was calculated from the start of ICI treatment to the date of progression or death, whichever occurred first. OS was calculated from the start of ICI treatment to date of death. Patients who did not experience the event of interest were censored at the date of last follow-up (December 18, 2018). In patients who received multiple lines of ICI, the first treatment was used for analysis. PFS and OS were estimated using Kaplan-Meier methods and compared between subgroups using the log-rank test.

A Cox proportional hazards model was used to examine the association with antibiotic exposure or occurrence of irAES, and certain clinical variables such as gender, age (<65 vs. ≥65), number of disease sites (1–2 vs. ≥3), ECOG performance status (PS) (0–1 vs. 2–3), primary site, number of prior therapies (<3 vs. ≥3), ICI type (single-agent vs. combination) and PD-L1 status (positive or negative), with survival outcomes. A multivariable model was then constructed to evaluate survival after adjusting for other potential confounders that were significantly associated with outcomes in univariate analyses. Antibiotic exposure and occurrence of irAES were entered as time-dependent covariates in the regression models to account for these events occurring at different time points after the start of ICI treatment. Patients who received antibiotics within 60 days prior to ICI treatment were included as exposed at day 1. A separate analysis was undertaken to examine the impact of receiving antibiotics in the 30 days prior to commencing treatment with ICIs. Patients who received antibiotics outside of this 30-day period were considered as not having an antibiotic exposure for the purposes of this analysis.

Analysis of the effect of TMB on survival outcomes was undertaken in the subgroup of patients who underwent tumor genomic profiling. TMB was transformed using natural

logarithm transformation and entered as a continuous variable in the regression models. Cox regression analysis was used to examine the association between TMB (log-transformed) with PFS and OS. As a separate analysis, TMB was also categorized into 4 groups by quartiles and then dichotomized into upper quartile vs everything else to examine the association with survival outcomes.

For gene alteration analysis, survival analyses were performed on genes which were altered in ≥10% of cases. PFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test.

All statistical analyses were performed with SAS version 9.3 (SAS Institute, INC., Cary, NC, USA) or R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Between September 2013 and July 2018, 161 patients with EGC were treated with ICIs. Baseline patient and disease characteristics are outlined in Table 1.

Most patients were male (77%) and had received at least 2 prior therapies (70%), mostly fluoropyrimidine, platinum and taxane compounds. Most patients (76%) had good performance status (PS) (ECOG 0 or 1). Just over two-thirds of patients received single-agent anti-PD-1 or anti-PD-L1 therapy; 32% received combination therapy with anti-PD-1/PD-L1 and anti-CTLA-4. Patients who received combination therapy were significantly younger (57 vs. 64 years, p=0018), had better ECOG PS (90% ECOG 0/1 vs. 70% ECOG 0/1, p=0.005) and had received fewer prior lines of therapy (p=0.006). Fourteen (9%) received ≥2 lines of immunotherapy. Fifteen patients (9.3%) were MMRd by IHC and/or MSI by NGS (MMRd = 8 [7 of whom had MSK-IMPACT testing and were MSI by MSI Sensor]; MSI = 7 [by MSI Sensor = 5, by commercial NGS testing = 2]). Sixty-nine patients' tumors (43%) were tested for PD-L1 expression by IHC, of which 45 (65%) were PD-L1-positive. Of the 15 MMRd/MSI patients, 6 tumors were tested for PD-L1, and all were positive. Seven patients underwent testing for EBV and one was positive.

The objective response rate (ORR) for ICI therapy was 11.8% (95% CI: 7.2%−17.8%) (n=19), including 12 partial responses (PR) and 7 complete responses (CR). The DCR was 21.1% (95% CI: 15.1%−28.2%). Among the 19 patients who achieved CR or PR, the median duration of response was 44.5 months with a lower confidence interval (CI) of 20.6. At a median follow-up of 11.5 months (range 1.2–59 months) among survivors, 147 patients experienced disease progression or died. The median PFS was 1.7 months (95% CI, 1.4, 2.2), with 6- and 12-month PFS of 15% (95% CI, 10%, 22%) and 9% (95% CI, 5%, 15%) respectively, as shown in Figure 1a. Median OS was 4.9 months (95% CI, 3.9, 7.9) with 12- and 24-month OS of 30% (95% CI, 24%, 39%) and 14% (95% CI 8%, 23%) respectively, as shown in Figure 1b.

Determinants of response

Clinical factors—The impact of several clinical factors on survival outcomes were evaluated in univariate analysis, as shown in Table 2. When patients were stratified by

ECOG PS, there was a significant association with inferior median PFS of 1.2 vs 1.8 months (HR 1.8 [95% CI, 1.2–2.6], p=0.002) and median OS of 2.0 vs 6.4 months (HR 2.2 [95% CI, 1.5–3.3] p < 0.001) in patients with poor performance status (ECOG ≥2) versus patients with good performance status (ECOG ≤1).

Patients with a greater number of metastatic sites (≥3) also had worse outcomes than patients with fewer (≤2) metastatic sites; median PFS 1.4 vs. 2.1 months (HR 1.6 [95% CI, 1.1–2.3], p=0.006) and median OS 3.6 vs 8.4 (HR 2.1 [95% CI, 1.5–3.0], p<0.001). Similarly, patients who had received ≥3 prior therapies for metastatic disease had poorer outcomes than patients who had received ≤2 previous treatments (median PFS 1.4 vs 1.8 months, HR 1.7 [95% CI, 1.2–2.4], p=0.003; median OS 3.4 vs 7.2 months, HR 2.0 [95% CI, 1.4–3.0], p<0.001). We also observed inferior survival in patients who had liver metastases at commencement of ICI therapy versus those who did not; median PFS (1.4 vs. 2.1 months, HR 1.5 [95% CI, 1.1–2.1], p=0.016) and OS (3.1 vs 8.3 months, HR 2.11 [95% CI, 1.5–3.0], p<0.001].

When patients were stratified by receipt of single-agent anti-PD-1/PD-L1 versus combination checkpoint inhibition with anti-CTLA-4 plus anti-PD-1/PD-L1, there was no significant difference in median PFS (1.6 vs 1.9 months, HR 0.8 [95% CI, 0.6–1.9], p=0.208). However, median OS was significantly improved in those patients treated with combination therapy (8.8 vs 4.3 months, HR 0.6 [95% CI, 0.4–0.9], p=0.008) compared to those who received single-agent therapy. In multivariable OS and PFS analyses, only performance status, number of disease sites and number of prior therapies remained significantly associated with outcomes as shown in Table 3.

PD-L1 status—Forty-three percent (n=69) of the cohort had PD-L1 testing. There were no differences in baseline characteristics between PD-L1 tested patients and those who did not undergo PD-L1 testing. In PD-L1 tested patients, PD-L1 negative patients had inferior median PFS (1.3 vs. 2.7 months, HR 2.5 [95% CI, 1.4–4.2], p=0.001) and median OS (2.9 vs. 6.7 months, HR 2.3 [95% CI 1.3–4.0], p=0.004) versus PD-L1 positive patients.

Antibiotics use—Antibiotics were prescribed in 62 of 162 (38%) patients during or within the 60 days preceding treatment with ICIs. The most commonly used antibiotics were beta-lactams, quinolones, macrolides, vancomycin, and sulfonamides. They were mainly prescribed to treat respiratory tract infections, cellulitis, dental infections, or empirically in patients with fever. Antibiotics were administered orally in 56% (n=35), whereas 32% (n=20) received intravenous antibiotics and 11% (n=7) received both. In univariate analysis treating antibiotics exposure as a time dependent covariate, we found no difference in PFS (HR 1.1; 95% CI, 0.78–1.55; p=0.581) or OS (HR 1.26; 95% CI, 0.87–1.81; p=0.219) between those patients treated with antibiotics versus those who were not.

Outcomes of patients who received antibiotics in the 30 days prior to commencing ICIs (n=14) were separately evaluated. While there was no significant difference in median PFS observed in patients who received antibiotics in this time period versus those who did not (1.8 vs 0.6 months, HR 1.5 [95% CI, 0.8–2.6], p=0.204), we did observe that receipt of antibiotics in the 30-day interval prior to ICIs was significantly associated with OS; median 0.9 vs. 5.8 months in patients who received antibiotics versus those who did not (HR 2.4

[95% CI, 1.3–4.2], p=0.003), in a univariate analysis. Of note these patients had a numerically poorer ECOG PS than that of the total study population (36% vs. 24% had a PS of ≥2). However, other baseline variables including age, number of prior therapies and sites of metastatic disease and PD-L1 status were similar between both groups. Half of the patients (n=7) treated with antibiotics in the 30 days prior to initiation of ICI received them while hospitalized.

irAEs—Of the 43 patients who developed irAEs, 20 had grade 3/4 toxicities, the most common of which were hepatitis (n=7) and colitis (n=7). Skin toxicity and hypothyroidism were the most common of all irAEs, occurring in 20 (47%) and 8 (19%) patients respectively. Steroids were administered to 22 (51%) patients. The median time to the development of an irAE was 1.38 months (range, 0.32–13.11). While there was no significant association between occurrence of irAEs and PFS (HR 0.68; 95% CI, 0.4–1.1; p=0.082), OS was improved in patients who developed irAEs (HR 0.43; 95% CI, 0.3–0.7; p<0.001), irrespective of irAE grade. A significant association remained after adjusting for potential confounders in multivariable analysis (HR 0.58; 95% CI, 0.34–0.97; p=0.038).

MMRd/MSI patients

Among 15 patients who were MMRd/MSI, fourteen (93%) received single agent anti-PD-1 therapy and one received combination anti-CTLA-4 plus anti-PD-1 therapy. Seven of these patients (47%) had an objective response, including 3 CRs and 4 PRs with a median response duration of 11.6 months. Two patients had stable disease resulting in a DCR of 60%. The 12-month OS rate was 33%. There was no difference in baseline variables between MMRd/MSI patients who were responders versus non-responders, although there was a trend toward younger median age in responders (64 vs. 70 years, p=0.072). In twelve patients who had their tumors sequenced, the median TMB was 38.6 in responders versus 45.3 in non-responders. Three of the six responders had β2-microglobulin truncating mutations (all complete responses) and 3 had *MLH1* truncating mutations or deletions, neither of which were observed in the non-responders.

Tumor mutation burden

The tumors of 101 (62%) patients were subjected to targeted capture next generation sequencing using the MSK-IMPACT platform. We observed no difference in baseline characteristics between patients who underwent genomic profiling with MSK-IMPACT vs. those who did not. After excluding those with low-purity samples, we sought to correlate tumor molecular features identified on MSK-IMPACT with treatment outcomes in the remaining 89 patients.

The median TMB was 5.6 (interquartile range: 3.3–8.8). In PD-L1 positive patients, the median TMB was 6.1 (range 1.1–62) while in PD-L1 negative patients the median TMB was 3.7 (range 1.7–9.8); p=0.038. For each 1 unit increase of TMB, there was an association with improved PFS (HR 0.97; 95% CI, 0.95–0.99, p=0.003) and OS (HR 0.72; 95% CI, 0.55–0.94, p=0.016). However, we did not observe a significant interaction between TMB and outcomes with respect to PD-L1 status or type of immune checkpoint inhibitor therapy (single agent vs. combination therapy) received. In multivariable analysis, a significant

relationship between TMB as a continuous variable and PFS (HR 0.79; 95% CI, 0.62–1.01, p=0.058) or OS (HR 0.96; 95% CI, 0.71–1.30, p=0.802) was not maintained, as shown in Table 3b. Furthermore, when MSI patients (n=12) were excluded from the TMB analysis, we observed no significant difference in PFS (HR 0.89; 95% CI, 0.62–1.26, p=0.502) or OS (HR 0.78; 95% CI, 0.53–1.15, p=0.211) per 1 unit increase in TMB.

When TMB was categorized by quartiles, we again detected a significant association with improvement in PFS (p=0.025) and OS (p=0.022) as shown in Figure 2. When dichotomized by the upper quartile Q3 (>8.78) vs. everything else we also observed a significant improvement in PFS (p=0.007) and OS (p=0.08). However, when MSI patients were excluded from the analysis, we did not detect a significant difference in PFS (p=0.13) or OS (p=0.103) at the upper level of Q3 (>6.69), similar to the results obtained when TMB was analyzed as continuous variable.

Of note, several patients with low TMB obtained durable benefit (greater than 6 months) from ICI therapy. One of these patients was a 70-year-old man with metastatic gastric cancer to lymph nodes and peritoneum who had received 3 prior lines of therapy. His tumor was MMR proficient and MSS by MSI Sensor and PD-L1 positive, CPS 60. He had CD274 (PD-L1) amplification on MSK-IMPACT and was EBV negative. His TMB was 1.1. He was treated with single-agent anti-PD-1 therapy and had SD for almost 9 months. Also, a 48-year-old male patient with metastatic gastroesophageal junction adenocarcinoma (diffuse type) to lymph nodes received 2 prior lines of therapy. His tumor was MMR proficient and MSS by MSI Sensor and EBV positive. PD-L1 status by IHC is unknown; MSK-IMPACT did not reveal a CD274 (PD-L1) amplification. His TMB was 6.8. He was treated with combination anti-CTLA-4 plus anti-PD-1 antibodies on a clinical trial for 4 cycles, followed by continuation of anti-PD-1 therapy with a durable complete response which is ongoing. The patient remains on treatment now more than 4 years.

The swimmers plot shown in Figure 3 summarizes outcomes as stratified by the above molecular and clinical variables.

Molecular alterations

Univariate survival analysis was undertaken in genes in the MSK-IMPACT assay that were altered in ≥10% of patients. Low purity and MSI-high samples were excluded. Analyses were undertaken in all patients, patients who received single-agent therapy only and in patients treated with combination therapy only. We did not observe any significant difference in survival outcomes by gene alteration analyzed, as shown in supplementary figures (Appendix 1).

Post-progression therapy

After excluding patients who received first-line ICI (followed by subsequent chemotherapy) and those who are continuing ICIs with ongoing benefit, 29% of patients (n=42) received chemotherapy immediately following progression on ICIs. An additional 8% (n=11) received other immunotherapies in this setting. The median number of lines of post-ICI chemotherapy was 1 (range 1–5). The median PFS and OS in this group, from the start of post-ICI chemotherapy, were 3.0 and 6.5 months, respectively.

Discussion

This review of patients with advanced EGC treated with ICIs identified several key factors associated with more favorable OS in univariate analysis: fewer sites of metastatic disease and absence of liver metastases, fewer prior therapies, better performance status, tumor PD-L1 positivity, receipt of combination ICI therapy and development of irAEs. In multivariable analysis, the association of treatment with combination therapy on outcomes was not seen as the choice to administer combination ICI on-study or on a compassionate-use basis was likely driven by specific patient factors, including a more robust performance status.

While increasing TMB, measured either as a continuous variable or by quartiles, was associated with improved PFS and OS in univariate analysis, this observation was not maintained after adjusting for potential confounders in multivariable analyses. Furthermore, when MSI patients were excluded, the effect was lost, suggesting that the positive effect of TMB in univariate analysis may have been largely driven by these patients. It is well recognized that The Cancer Genome Atlas (TCGA) MSI subtype has elevated mutation burden and as a result is more likely to respond to single-agent ICIs. A recent study also reported an ORR of 100% in EBV-positive patients and 86% in MSI patients compared to only 12% and 5% in the genomically stable (GS) and chromosomal instability (CIN) subtypes.[19] The ability to demonstrate a positive association of TMB and outcomes in patients with microsatellite stable tumors may however be limited by the sample size. Given these results, the merits of TMB as a clinically useful biomarker to guide treatment with ICIs in patients with EGC requires further prospective investigation.

Antibiotic use has been suggested to limit the effectiveness of ICIs, potentially by eradicating microorganisms in the gut which interact with the immune system. [15] We did not initially observe a significant association between antibiotic exposure and outcomes in patients with metastatic EGC in patients who received antibiotics in the 60 days prior to or during treatment with ICIs. This finding contrasts with that of Routy et al., who reported reduced efficacy of ICIs in patients with lung, renal, and urothelial carcinoma in patients who had been treated with antibiotics 2 months preceding and during the first month of ICI therapy.[15] This difference could be explained by the use by Routy and colleagues of the Kaplan-Meier method to evaluate this association, which may not be valid in this context. Instead, we used Cox regression analysis with a time-dependent covariate, which accounts for antibiotic exposure occurring at variable time points during treatment with ICIs.

However, when we performed a separate analysis in patients prescribed antibiotics only in the 30 days prior to treatment with ICIs, there was a significant negative impact on OS observed in univariate analysis. This preliminary finding is consistent with two other recent reports which found a dramatic reduction in survival in patients with multiple cancer types (mainly NSCLC, melanoma and renal cell carcinoma) who received antibiotics in the 30 days before starting an ICI.[15,20] However, all of these retrospective analyses should be interpreted with considerable caution. Our particular analysis included only 14 patients exposed to antibiotics 30 days prior to ICI and half of this group received antibiotics while hospitalized, which is likely a surrogate marker for a sicker population. Indeed, more patients had ECOG PS 2–3 in this cohort when compared to the total study population and

our univariate analysis does not adjust for potential confounding factors. However, given the apparent striking difference in survival between these groups, these findings merit prospective evaluation as they may identify a subgroup of patients who should not be offered ICIs. Ultimately, any future analysis of the impact of antibiotics on ICI outcomes must include the prospective collection of stool samples and analysis of microbiomes pre- and post-antibiotic use since deleterious changes in the microbiome are the putative mechanism by which antibiotics exert an effect on response to ICIs.

To our knowledge, this is the first study in EGC to show an association between irAEs and improved survival. This association makes mechanistic sense; the development of an inadvertent immune response against autoantigens may also result in an anti-tumor immune response. Though results in melanoma have been inconsistent,[11–13] two recent studies in NSCLC have linked early development of irAEs with improved outcomes.[14,21]

We also observed comparatively promising outcomes for chemotherapy administered after progression on ICIs (frequently as fourth- and fifth-line therapy), with a median PFS and OS of 3.0 and 6.5 months respectively. By comparison, randomized studies of second-line chemotherapy report median OS of only about 5 months.[22,23] Recent retrospective studies in NSCLC have demonstrated higher than expected ORRs to chemotherapy after administration of ICIs.[24–26] Park et al. reported that 53% of patients responded to salvage chemotherapy after PD-1/PD-L1 inhibitors, compared with a 34.9% ORR to the last chemotherapy regimen administered prior to ICI therapy.[23] Taken together, these data suggest that initial augmentation of the immune system by ICIs, though insufficient for an anti-tumor response, may enhance subsequent cell death caused by cytotoxic chemotherapy. A strategy of first-line chemotherapy followed by ICI as a “maintenance” strategy is currently being evaluated in a completely-accrued phase III study (JAVELIN 100, clinicaltrials.gov NCT02625610).

In addition to its retrospective nature, this study had several other limitations. The cohort included patients treated with both single-agent PD-1/PD-L1 blockade and combination therapy. Nevertheless, the ORR, PFS, and OS of the entire cohort closely matches that of ICI-treated patients in the KEYNOTE-059 and ATTRACTION-2 studies, suggesting generalizability of our conclusions. Also, because PD-L1 testing was not standard-of-care at the time many of these patients were treated, only 43% of our cohort underwent PD-L1 testing.

In conclusion, this study provides new insights into potential molecular and clinical determinants of response to ICIs in EGC. We hypothesize that heavily pre-treated patients with a high disease burden and poor performance status may be unlikely to respond to ICIs. In line with other data, PD-L1 was associated with improved outcomes. Occurrence of irAEs is also positively associated with survival. Though TMB was associated with outcome in univariate analyses, this association did not persist after adjusting for other risk factors and when MSI tumors were excluded. Ongoing correlative efforts to identify predictive biomarkers and mechanisms of resistance to immunotherapies are crucial to build on our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

A minority of patients with advanced esophagogastric cancer benefit from treatment with immune checkpoint inhibitors. Beyond microsatellite instability (MSI), the only other biomarker that has yet been validated to guide use of these therapies is PD-L1, which is imperfect. In addition to confirming that patients with PD-L1 positive tumors had superior outcomes, factors most associated with poor outcomes included high-volume disease, a high number of prior therapies and poor performance status. We also found that immune-related adverse events were associated with improved survival and recent or concurrent antibiotic exposure did not correlate with outcomes. While tumor mutation burden was associated with improved survival, this association was not maintained after excluding MSI-high patients. We did not find any genetic alterations on next generation sequencing that was associated with outcomes. These findings improve our understanding of the clinical and molecular factors that may predict benefit from immune checkpoint inhibitors in this disease.

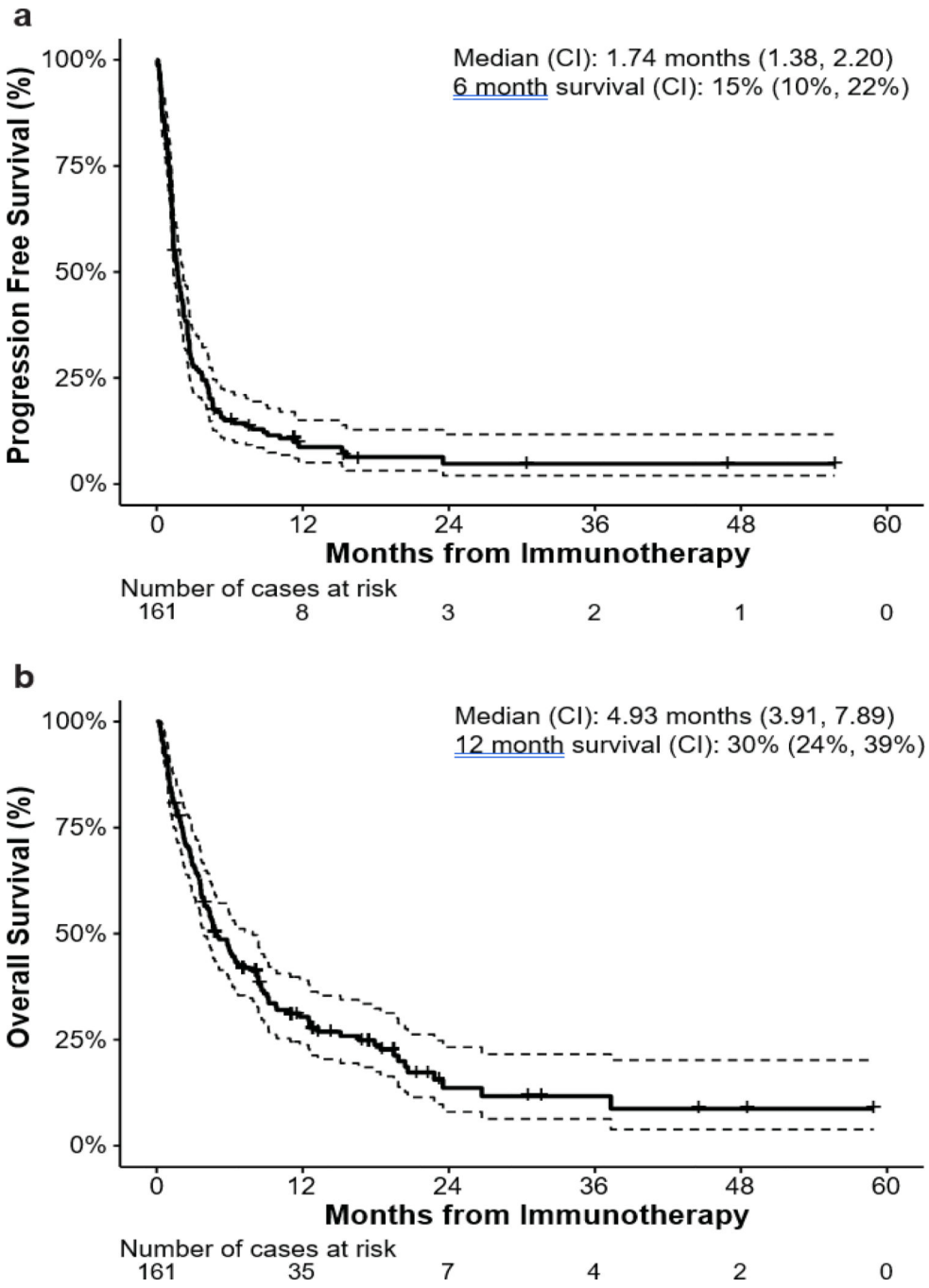

Figure 1a and 1b: Progression-free and overall survival in all patients treated with immune checkpoint inhibitors

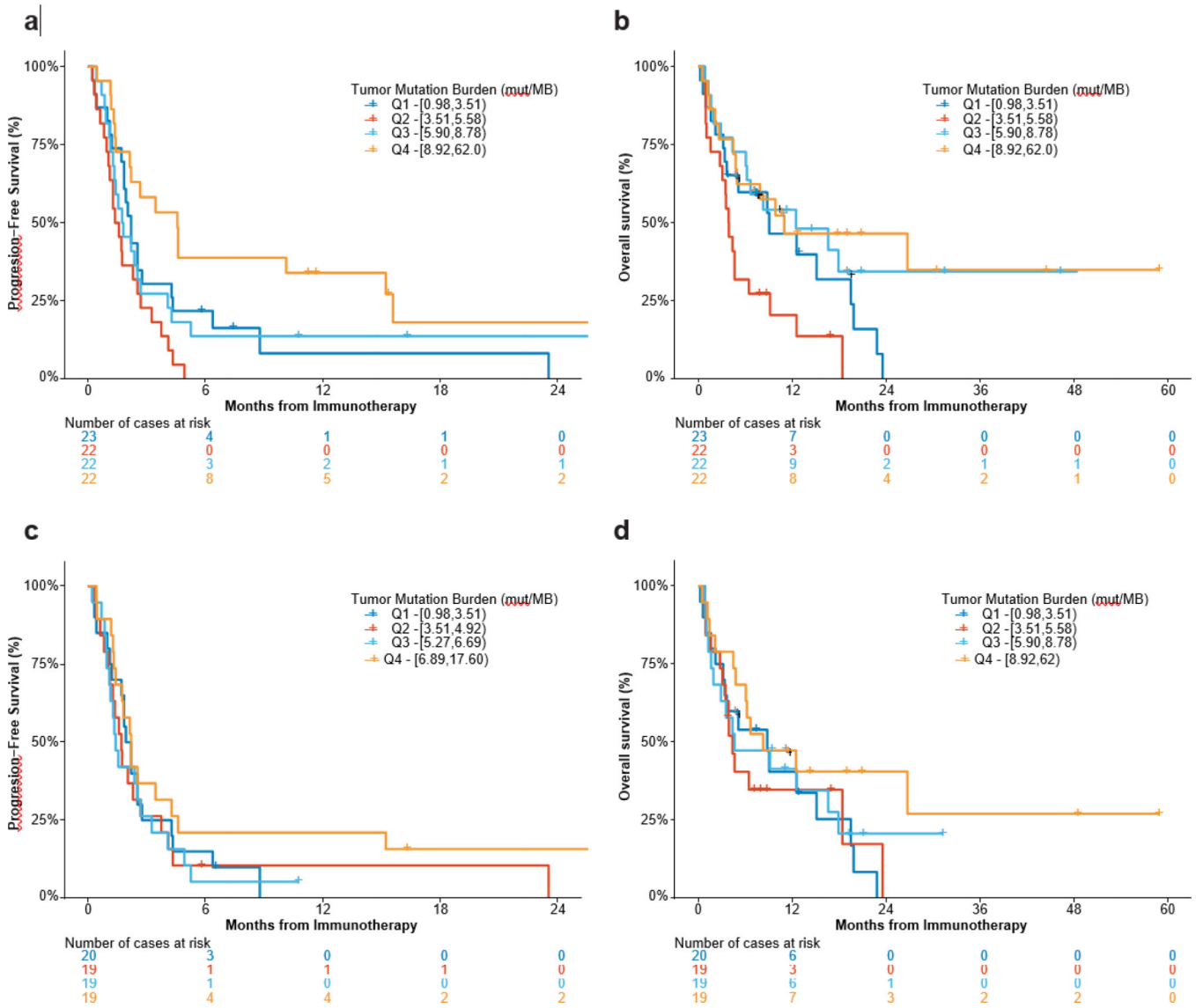

Fig 2a-d:

Progression-free (a) and overall survival (b) stratified by tumor mutation burden quartiles in all patients and progression-free (c) and overall survival (d) stratified by tumor mutation burden quartiles in microsatellite stable patients.

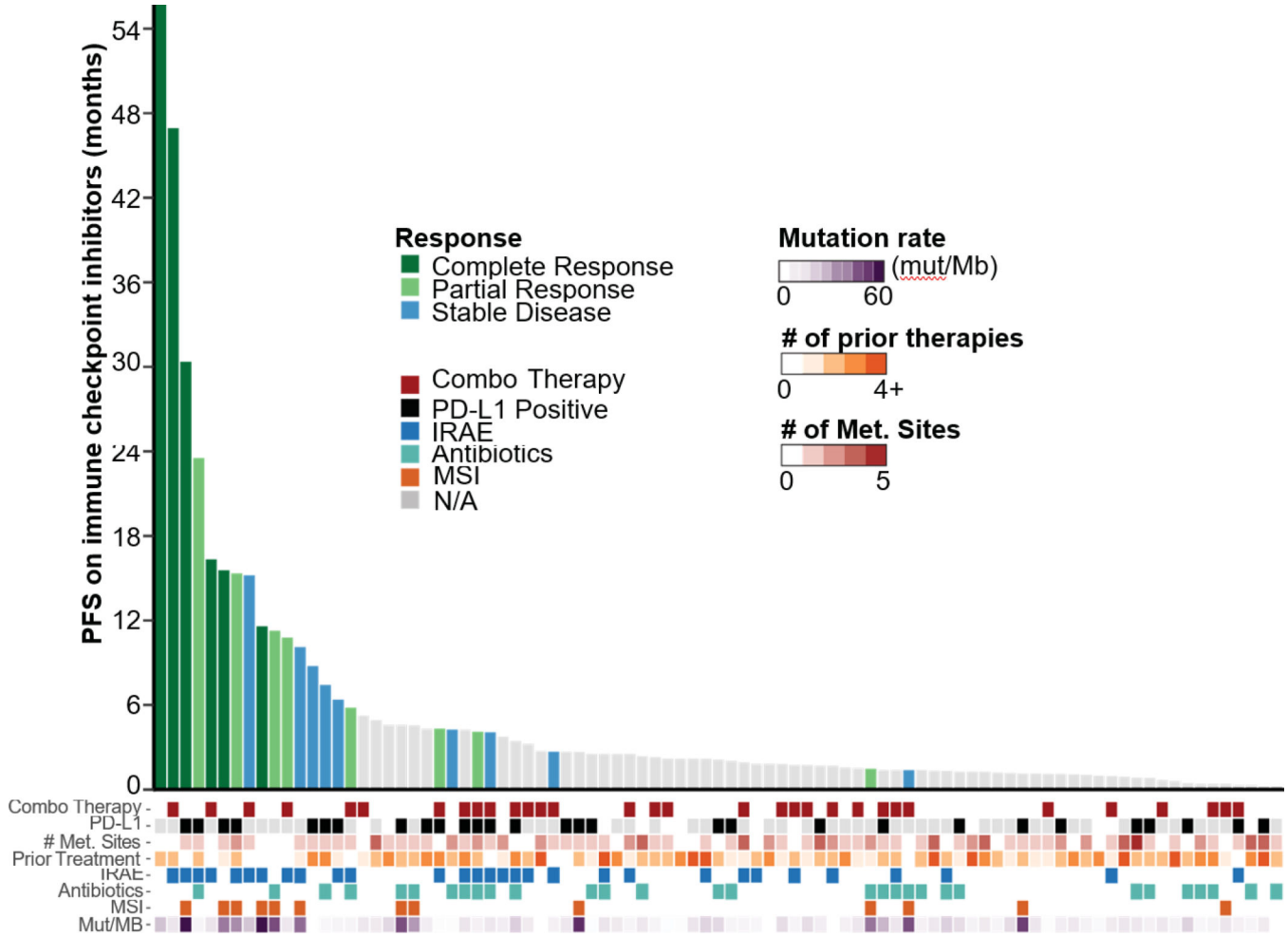

Fig 3: Swimmers plot demonstrating progression-free survival on ICI treatment as stratified by key molecular and clinical variables in patients who underwent genomic profiling by MSK-IMPACT (n=89).

PFS, progression-free survival; Combo Therapy, combination therapy; IRAE, immune-related adverse event; MSI, microsatellite unstable; N/A, not-applicable (PD-L1 untested patients); #, number; Met., metastatic.

Table 1.

Patient and disease characteristics.

	n (% of total 161 patients)
Age	
Median (range)	62 (23–88)
Patients <65 years	94 (58%)
Sex	
Male	124 (77%)
Female	37 (23%)
ECOG performance status	
0–1	123 (76.4%)
≥2	38 (23.6%)
Primary site	
Esophagus/gastroesophageal junction	85 (53%)
Gastric	76 (47%)
Grade	
Moderately differentiated	51 (31.7%)
Poorly differentiated	106 (65.8%)
Unknown	4 (2.5%)
HER-2 status	
Positive	18 (11.2%)
Negative	139 (86.3%)
Unknown	4 (2.5%)
MMR/MSI status	
MMR-deficient/MSI	15 (9.3%)
MMR intact/MSS	106 (65.8%)
Unknown	40 (24.8%)
PD-L1 status	
PD-L1 positive	45 (28%)
PD-L1 negative	24 (14.9%)
Unknown	92 (57.1%)
Number of prior regimens	
0–2	112 (69.6%)
≥3 (range 3–6)	49 (30.4%)
Previous therapies	
Fluoropyrimidine	137 (85%)
Platinum	135 (84%)
Taxane	96 (60%)
Ramucirumab	65 (40%)
Irinotecan	51 (32%)
Trastuzumab	21 (13%)
Gemcitabine	9 (6%)

	n (% of total 161 patients)
Anthracycline	5 (3%)
Other	14 (9%)
Number of sites of disease at start of ICI	
1–2	105 (65.2%)
≥3	56 (34.8%)
Type of ICI	
Single-agent anti-PD-1/PD-L1	110 (68.3%)
Combination anti-CTLA-4 plus anti-PD-1/PD-L1	51 (31.7%)

MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; PD-L1, programmed death-ligand-1; ICI, immune checkpoint inhibitors; anti-PD-1, anti-programmed cell death protein-1; anti-CTLA-4, anti-cytotoxic T-lymphocyte-associated protein-4.

Table 2

Univariate analysis of effect of clinical variables on survival outcomes

		Progression-free survival			Overall survival		
Variable	**No. of patients**	**Median, months (95% CI)**	**HR (95% CI)**	***p*-value**	**Median, months (95% CI)**	**HR (95% CI)**	***p*-value**
Age				0.409			0.891
<65 years	94	1.4 (1.3–2.2)	1.00 *		5.1 (3.6–8.6)	1.00 *	
≥65 years	67	2.0 (1.6–2.7)	0.87 (0.6–1.2)		4.6 (3.7–9.1)	1.03 (0.7–1.5)	
Gender				0.614			0.948
Male	124	1.68 (1.4–2.2)	1.00 *		5.1 (3.9–8.4)	1.00 *	
Female	37	2.01 (1.3–2.7)	1.1 (0.8–1.6)		4.9 (2.9–20.4)	0.99 (0.6–1.5)	
ECOG				**0.002**			**<0.001**
0–1	123	1.8 (1.6–2.5)	1.00 *		6.4 (4.8–9.1)	1.00	
≥2	38	1.2 (0.8–2.2)	1.81 (1.2–2.6)		2.0 (1.0–3.7)	2.23	
Primary site				0.73			0.9
Esophageal	25	1.4 (1.2–4.1)	1.00 *		5.9 (3.91-NR)	1.00 *	
GE junction	60	1.8 (1.5–2.7)	0.98 (0.6–1.6)		4.5 (3.1–8.3)	1.1 (0.7–1.9)	
Gastric	76	1.7 (1.4–2.5)	1.1 (0.7–1.8)		4.9 (3.6–9.2)	1.1 (0.7–1.9)	
No. of metastatic sites				**0.006**			**<0.001**
1–2 ≥3	105	2.1 (1.6–2.7)	1.00 *		8.4 (5.8–11.8)	1.00 *	
≥3	56	1.4 (1.2–1.9)	1.6 (1.1–2.3)		3.6 (2.9–4.6)	2.1 (1.5–3.0)	
Liver metastases				**0.016**			**<0.001**
No	102	2.1 (1.7–2.7)	1.00 *		8.2 (5.1–12.5)	1.00 *	
Yes	59	1.4 (1.2–1.8)	1.5 (1.1–2.1)		3.1 (2.2–4.6)	2.11 (1.5–3.0)	
No. of prior therapies				**0.003**			**<0.001**
1–2	112	1.8 (1.6–2.6)	1.00 *		7.2 (4.9–9.9)	1.00 *	
≥3	49	1.4 (1.1–2.2)	1.7 (1.2–2.4)		3.4 (2.2–4.8)	2.0 (1.4–3.0)	
Type of ICI				0.208			**0.008**
Single-agent anti-PD-1/PD-L1	110	1.6 (1.3–2.2)	1.00 *		4.3 (3.6–6.0)	1.00 *	
Anti-CTLA-4 + anti-PD-1/PD-L1	51	1.9 (1.6–3.3)	0.8 (0.6–1.9)		8.8 (5.8–20.6)	0.6 (0.4–0.9)	
PD-L1 status $				**0.001**			**0.004**
PD-L1 positive	45	2.7 (2.0–4.3)	1.00 *		6.7 (3.9–19.9)	1.00 *	

		Progression-free survival			Overall survival		
Variable	**No. of patients**	**Median, months (95% CI)**	**HR (95% CI)**	***p*-value**	**Median, months (95% CI)**	**HR (95% CI)**	***p*-value**
PD-L1 negative	24	1.3 (1.0–2.2)	2.47 (1.4–4.2)		2.9 (2.0–6.2)	2.3 (1.3–4.0)	

HR, hazard ratio; CI, confidence interval;

*Denotes reference HR; GE junction, gastroesophageal junction, ICI, immune checkpoint inhibitors; anti-PD-1, anti-programmed cell death protein-1; PD-L1, PD-ligand-1; anti-CTLA-4, anti-cytotoxic T-lymphocyte-associated protein-4; NR, not reached;

$patients with unknown PD-L1 status (n=92) were excluded from analysis; TMB, tumor mutation burden;

¥Among patients who underwent genomic profiling (n=89); NA, not applicable; significant values highlighted in bold

Table 3a.

Multivariable model for PFS and OS of significant clinical variables in univariate analysis

	PFS		OS	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
ECOG PS				
≥2 vs. 0–1	1.67 (1.1–2.5)	**0.004**	2.08 (1.37–3.16)	**0.001**
No. of metastatic sites				
≥3 vs. <3	1.7 (1.2–2.3)	**0.004**	2.36 (1.62–3.45)	**<0.001**
No. of prior therapies				
≥3 vs. <3	1.57 (1.1–2.3)	**0.014**	1.73 (1.16–2.6)	**0.008**
Type of ICI therapy				
Combination vs. single-agent	NA	NA	0.7 (0.5–1.07)	0.096

Table 3b.

Multivariable model for PFS and OS in patients who underwent genomic profiling (n=89)

	PFS		OS	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
TMB (log-scale)	0.79	0.058	0.96 (0.7–1.3)	0.802
ECOG PS ≥2 vs. 0–1	1.74 (0.1–3.2)	0.075	2.35 (1.2–4.7)	**0.015**
No. of metastatic sites ≥3 vs. <3	1.66 (0.1–2.9)	0.078	2.71 (1.4–5.2)	**0.002**
No. of prior therapies ≥3 vs. <3	NA	NA	1.67 (0.9–3.0)	0.087

PS, performance status; No., number; ICI, immune checkpoint inhibitor; NA, not applicable (not significant in univariate analysis); TMB, tumor mutation burden. Significant values highlighted in bold.