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ORIGINAL ARTICLE

Molecular determinants of clinical outcomes with pembrolizumab versus paclitaxel in a randomized, open-label, phase III trial in patients with gastroesophageal adenocarcinoma☆

K. Shitara[1*], M. Özgüroğlu[2], Y.-J. Bang[3], M. Di Bartolomeo[4], M. Mandalà[5], M.-H. Ryu[6], C. Caglevic[7], H. C. Chung[8], K. Muro[9], E. Van Cutsem[10], J. Kobie[11], R. Cristescu[11], D. Aurora-Garg[11], J. Lu[11], C.-S. Shih[11], D. Adelberg[11], Z. A. Cao[11] & C. S. Fuchs[12]

[1]National Cancer Center Hospital East, Kashiwa, Japan; [2]Istanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, Istanbul, Turkey; [3]Seoul National University College of Medicine, Seoul, South Korea; [4]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan; [5]University of Perugia, Unity of Medical Oncology, Perugia, Italy; [6]University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea; [7]Cancer Research Department, Instituto Oncológico Fundación Arturo López Perez, Santiago, Chile; [8]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, South Korea; [9]Aichi Cancer Center Hospital, Nagoya, Japan; [10]University Hospitals Gasthuisberg Leuven, KU Leuven, Leuven, Belgium; [11]Merck & Co., Inc., Kenilworth; [12]Yale Cancer Center, Smilow Cancer Hospital, New Haven, USA

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Background: In the phase III KEYNOTE-061 trial (NCT02370498), pembrolizumab did not significantly improve overall survival versus paclitaxel as second-line therapy for gastric/gastroesophageal junction (GEJ) adenocarcinoma with programmed death-ligand 1 (PD-L1) combined positive score (CPS) $\geq$1 tumors. The association of tissue tumor mutational burden (tTMB) status and clinical outcomes was determined, including the relationship with CPS and microsatellite instability-high (MSI-H) status.

Patients and methods: In patients with whole exome sequencing (WES) data [420/592 (71%); pembrolizumab, 218; paclitaxel, 202], the association of tTMB with objective response rate (ORR; logistic regression), progression-free survival (PFS; Cox proportional hazards regression), and overall survival (OS; Cox proportional hazards regression) were measured using one-sided (pembrolizumab) and two-sided [paclitaxel] *P* values. tTMB was also evaluated using FoundationOne®CDx [205/592 (35%)]. Prespecified equivalent cut-offs of 175 mut/exome for WES and 10 mut/Mb for FoundationOne®CDx were used.

Results: WES-tTMB was significantly associated with ORR, PFS, and OS in pembrolizumab-treated (all $P < 0.001$) but not paclitaxel-treated patients (all $P > 0.6$) in univariate analysis. The area under the receiver operating characteristics curve for WES-tTMB and response was 0.68 [95% confidence interval (CI) 0.56-0.81] for pembrolizumab and 0.51 (95% CI 0.39-0.63) for paclitaxel in univariate analysis. There was low correlation between WES-tTMB and CPS in both treatment groups ($r \leq 0.16$). WES-tTMB remained significantly associated with all clinical endpoints with pembrolizumab after adjusting for CPS and with PFS and OS after excluding known MSI-H tumors ($n = 26$). FoundationOne®CDx-tTMB demonstrated a positive association with ORR, PFS, and OS in pembrolizumab-treated patients (all $P \leq 0.003$) but not PFS or OS in paclitaxel-treated patients ($P > 0.1$).

Conclusion: This exploratory analysis from KEYNOTE-061 is the first to demonstrate a strong association between tTMB and efficacy with pembrolizumab but not paclitaxel in patients with gastric/GEJ adenocarcinoma in a randomized setting. Data further suggest tTMB is a significant and independent predictor beyond PD-L1 status.

Key words: pembrolizumab, chemotherapy, tumor mutational burden, gastroesophageal adenocarcinoma, gastric cancer

INTRODUCTION

Outcomes remain poor for patients with advanced gastric cancer (GC), despite advances in therapy.[1] The Cancer Genome Atlas (TCGA) identified four molecular subtypes of GC based on DNA or RNA profiling: those with microsatellite instability (MSI), Epstein—Barr virus (EBV)-positive cancer, genomically stable (GS) tumors, and tumors with chromosomal instability (CIN).[2-4] Tumors with high MSI (MSI-H) are associated with high tumor mutational burden (TMB),

*Correspondence to: Dr Kohei Shitara, Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. Tel: +81-4-7133-1111; Fax: +81-4-7134-6865

E-mail: kshitara@east.ncc.go.jp (K. Shitara).

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although some patients have GCs that are TMB-high and microsatellite stable (MSS).[5] Highly mutated tumors are more likely to harbor neoantigens, making them targets of activated immune cells.[5] Evidence indicates that TMB and neoantigen load are promising biomarkers that correlate with response to immune checkpoint inhibitors (ICI), although at least one study has suggested no clear relationship between TMB and benefit with combination immunotherapy in lung cancer.[5-12] TMB warrants further investigation in ICI-treated patients with GC.[8,9,13,14]

Based on data from cohort 1 of the phase II KEYNOTE-059 study,[15] pembrolizumab received accelerated approval in the USA for the treatment of patients with recurrent locally advanced or metastatic gastric/gastroesophageal junction (GEJ) adenocarcinoma whose tumors express programmed death-ligand 1 (PD-L1) [combined positive score (CPS) >1] with disease progression on or after ≥2 lines of therapy.[16] Pembrolizumab also received accelerated approval for the treatment of unresectable or metastatic MSI-H or mismatch-repair-deficient solid tumors that progressed following prior treatment.[16,17]

KEYNOTE-061 (ClinicalTrials.gov Identifier: NCT02370498) was a randomized, open-label phase III trial of pembrolizumab versus paclitaxel as second-line therapy in patients with advanced gastric/GEJ adenocarcinoma.[18] Initially, patients were enrolled irrespective of PD-L1 assessment, but later enrollment was restricted to patients whose tumors expressed CPS ≥1. Analysis of the dual primary endpoints in the primary analysis population (CPS ≥1) revealed that overall survival (OS) and progression-free survival (PFS) were not significantly prolonged with pembrolizumab versus paclitaxel. However, pembrolizumab was associated with more durable responses and a better safety profile than paclitaxel.[18] Additional *post hoc* analyses in patients with PD-L1 CPS ≥10 demonstrated prolonged OS, higher response rates, and durable responses with pembrolizumab ($n = 53$) versus paclitaxel ($n = 55$); however, pembrolizumab is not approved in the second-line setting for advanced gastric/GEJ adenocarcinoma.[19] In the protocol-specified subgroup with CPS <1, the hazard ratio (HR) for pembrolizumab versus paclitaxel was 1.20 (OS) and 2.05 (PFS). The crossing of the Kaplan—Meier curves for the CPS ≥1 population highlights the importance of additional biomarker analysis. Recently, a small study in GC revealed that MSI-H, EBV positivity, and high tissue TMB (tTMB) are associated with high response rates to pembrolizumab.[20] We present an exploratory analysis from KEYNOTE-061 evaluating the association of tTMB with PD-L1 and MSI-H and their relationship with clinical outcomes in patients with advanced gastric/GEJ cancer receiving second-line pembrolizumab or paclitaxel.

PATIENTS AND METHODS

Study design and participants

The phase III KEYNOTE-061 study was conducted at 148 medical centers in 30 countries; study details have been reported.[18] Eligible patients had metastatic or locally advanced unresectable adenocarcinoma of the stomach or GEJ that progressed after first-line platinum and fluoropyrimidine-based therapies. Patients were randomly assigned (1 : 1) to receive pembrolizumab 200 mg intravenously every 3 weeks (Q3W) for 35 cycles or paclitaxel 80 mg/m^2 intravenously on days 1, 8, and 15 of a 4-week cycle until disease progression, intolerable toxicity, physician decision, or patient withdrawal of consent.[18]

The study protocol and all amendments were approved by the institutional review board or ethics committee at each institution. Patients provided written informed consent before enrollment, and the study was conducted in accordance with the protocol, its amendments, and good clinical practice guidelines.

Procedures

Radiographic imaging was performed every 6 weeks (Q6W). Responses were assessed per RECIST v1.1 by masked and independent central review.

PD-L1 expression in archival or newly collected tumor samples was determined at a central laboratory using PD-L1 IHC 22C3 pharmDx (Agilent, Santa Clara, CA) by measuring the CPS, calculated as the number of PD-L1-staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. DNA mismatch repair in five mononucleotide repeat markers (*NR21*, *NR24*, *BAT25*, *BAT26*, and *MONO27*) was analyzed in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples and blood (normal control) using the MSI analysis system version 1.2 (Promega, Madison, WI). Tumors in which ≥2 markers were changed compared with normal controls were considered to have high levels of MSI. tTMB was measured using whole exome sequencing (WES) and the FoundationOne®CDx (Foundation Medicine, Cambridge, MA). EBV, CIN, and GS subgroup analyses were based on the previously described genomics classification using DNA sequencing analysis.[2]

For the WES analysis, 420/592 (71%) tumor tissue and matched normal blood were analyzed as described.[12] WES reads were mapped to the reference human genome GRCh37 using a long-read Burrows—Wheeler aligner algorithm (BWA-MEM).[21] Preprocessing [duplicate marking, insertion and deletion (indel) realignment, and base recalibration with Picard v1.114 and Genome Analysis Toolkit, v2[22]] were carried out to produce analysis-ready binary alignment/map (BAM) files of tumor and matched normal samples, which were compared to generate somatic single nucleotide variant (SNV) calls using MuTect. SNVs were eliminated if they were present in the Single Nucleotide Polymorphism Database (dbSNP, v141) but not in the Catalogue of Somatic Mutations in Cancer (COSMIC, v68)[23] or if they had mutant reads of <4 in tumor samples. WES-tTMB was defined as the sum of somatic nonsynonymous SNVs that met all criteria described for each patient.

FoundationOne®CDx is a next-generation sequencing (NGS)-based *in vitro* diagnostic device that detects substitutions, indel alterations, copy number alternations, select gene rearrangements, and genomic signatures,

including MSI and TMB.[24] Using FoundationOne®CDx v3.3.8 and v3.3.9, DNA was isolated from 205/592 (35%) FFPE tumor tissue specimens, and FoundationOne®CDx-tTMB was calculated by counting the number of synonymous and nonsynonymous mutations across a 0.8 Mb region spanning 324 genes, with computational germline status and oncogenic driver filtering, and reporting the result as mut/Mb.[25]

Outcomes

In this exploratory analysis, prespecified primary objectives included assessment of whether WES-tTMB (as a continuous $\log_{10}$-transformed variable) is associated with improved clinical efficacy with pembrolizumab or clinical efficacy with paclitaxel, estimating the relative treatment effects of pembrolizumab versus paclitaxel in WES-tTMB-high versus non-high subgroups via a prespecified cut-off of 175 mut/exome, and assessment of clinical response to pembrolizumab versus paclitaxel in each of the four TCGA subtypes: MSI, EBV, CIN, and GS. The secondary objectives were to compare the clinical utility of the tTMB cut-off of 175 mut/exome to CPS cut-offs of 10 and 1, to evaluate whether WES-tTMB and CPS (as continuous $\log_{10}$- and square-root-transformed variables, respectively) are independent predictors of response to pembrolizumab and paclitaxel, separately, in a multivariable model, and to determine whether WES-tTMB is associated with clinical efficacy with pembrolizumab and paclitaxel, separately, in the non-MSI-H subgroup [defined as no MSI detected (i.e. MSS) or unknown MSI-H status]. An evaluation of Foundation®OneCDx-tTMB was also conducted.

Statistical analysis

This exploratory analysis included all treated patients irrespective of PD-L1 status with available WES-tTMB data and/or Foundation®OneCDx-tTMB data that passed quality control; analyses followed a statistical analysis plan written before merging clinical and tTMB exploratory biomarker data, specifying where statistical testing would be used and tTMB cut-offs that would define subgroups for pembrolizumab versus paclitaxel efficacy comparisons. tTMB endpoints were generated before the merging of tTMB and clinical data; thus, tTMB data were masked to treatment group and clinical outcome.

The exploratory WES-tTMB cut-off of $\geq$175 mut/exome was also identified as the optimal cut-off for predicting response to pembrolizumab across multiple tumor types using the WES platform.[25-27] The WES-TMB score most concordant with a FoundationOne®CDx cut-off of 10 mut/Mb was determined by identifying the score that maximized average positive and negative agreement to 10 mut/Mb in a multistudy, multitumor cohort of patient samples [$n = 338$ (not including patient samples from KEYNOTE-061)] evaluated using both platforms.[25]

For testing of the association of tTMB and clinical benefit, one-sided (pembrolizumab; positive association hypothesized) and two-sided (paclitaxel; no assumed direction hypothesized) P values were calculated using logistic and Cox proportional hazards survival regression models, adjusted for Eastern Cooperative Oncology Group (ECOG) performance status. No model selection took place. A prespecified subgroup analysis using a tTMB cut-off was performed to understand the potential clinical utility, categorizing patients into two groups using the predefined tTMB cut-offs (WES, 175 mut/exome; Foundation®OneCDx, 10 mut/Mb) and estimating the efficacy of pembrolizumab versus paclitaxel in those subgroups. Within each subgroup, a Cox proportional hazard regression model was used to estimate the HR and 95% confidence interval (CI) for pembrolizumab versus paclitaxel. Missing tTMB and PD-L1 data were assumed to be missing at random. Models were run in 'complete case'.

The clinical data cut-off date for this analysis was 26 October 2017.

RESULTS

Between 4 June 2015 and 26 July 2016, 592 patients were randomly assigned to receive pembrolizumab or paclitaxel. Median follow-up in the total KEYNOTE-061 population was 7.9 months (interquartile range, 3.4-14.6 months). Of those enrolled, 420 patients (71%) had WES-tTMB data available ($n = 218$, pembrolizumab; $n = 202$, paclitaxel) and were included in this analysis (Supplementary Figure S1A, available at https://doi.org/10.1016/j.annonc.2021.05.803); biomarker overlap is shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2021.05.803. Baseline characteristics in the WES-tTMB analysis population were similar to those of the total study population and generally well balanced between treatment groups (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2021.05.803).

In pembrolizumab-treated patients, WES-tTMB (continuous $\log_{10}$-transformed variable) was significantly associated with clinical outcomes (one-sided $P < 0.001$) (Table 1; Figure 1); these data include patients with MSI-H and PD-L1-positive tumors. In contrast, WES-tTMB was not significantly associated with clinical outcomes in paclitaxel-treated patients (two-sided $P > 0.6$) (Table 1; Figure 1). After adjusting for CPS, WES-tTMB remained significantly associated with clinical outcomes following pembrolizumab treatment, indicating that WES-tTMB was an independent predictor of improved clinical benefit in response to pembrolizumab monotherapy (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2021.05.803); these data include patients with MSI-H tumors. Neither WES-tTMB nor CPS were associated with clinical response to paclitaxel in the overall or non-MSI-H population (Supplementary Tables S2 and S3, available at https://doi.org/10.1016/j.annonc.2021.05.803).

Patients classified as MSI-H ($n = 26$) exhibited the highest values of WES-tTMB; 24/26 patients had WES-tTMB $\geq$175 mut/exome. EBV, CIN, and GS status showed no differential impact on response (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2021.05.803).

Table 1. Association *P* values for WES-tTMB (log_{10} scale) and response rate, PFS, and OS for pembrolizumab versus paclitaxel

	Pembrolizumab (n = 218)			Paclitaxel (n = 202)		
	Objective response[a]	PFS[b]	OS	Objective response[a]	PFS[b]	OS
n (%)[c]	27 (12.4)	199 (91.3)	174 (79.8)	26 (12.9)	184 (91.1)	177 (87.6)
P[d,e]	0.0007	<0.0001	<0.0001	0.7	0.8	0.7
AUROC (95% CI)	0.68 (0.56-0.81)	NA	NA	0.51 (0.39-0.63)	NA	NA

AUROC, area under the receiver operating characteristics curve; BICR, blinded independent central review; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; NA, not applicable; OS, overall survival; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; TMB, tumor mutational burden; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

[a] Responder: Confirmed complete response or partial response per central review using RECIST v1.1.

[b] By BICR per RECIST v1.1.

[c] The number of responders for objective response and the number of events for PFS and OS.

[d] One-sided Wald test *P* value from logistic regression for objective response and Cox proportional hazards regression for PFS and OS adjusted for ECOG performance status (binary 0 versus 1) in pembrolizumab-treated patients.

[e] Two-sided Wald test nominal *P* value for TMB (log_{10} scale) from logistic regression for objective response and Cox proportional hazards regression for PFS and OS adjusted for ECOG performance status (binary 0 versus 1) in paclitaxel-treated patients.

In pembrolizumab-treated patients with available WES-tTMB and CPS data (n = 418), the area under the receiver operating characteristics curve (AUROC; 95% CI) for discriminating objective response was 0.68 (0.56-0.81) for WES-tTMB and 0.70 (0.61-0.80) for CPS (Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2021.05.803). After excluding patients with known MSI-H tumors, AUROC (95% CI) decreased to 0.61 (0.47-0.76) for WES-tTMB and 0.67 (0.56-0.77) for CPS (Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2021.05.803). There was little to no correlation between WES-tTMB and CPS ($r \leq 0.16$) (Figure 2).

When assessing the clinical utility of WES-tTMB, pembrolizumab offered a survival advantage to the WES-tTMB ≥175 mut/exome subgroup (Table 2; Figure 3). The HR (95% CI) of pembrolizumab versus paclitaxel for OS was 0.46 (0.27-0.81) in the WES-tTMB ≥175 mut/exome subgroup and 1.12 (0.89-1.41) in the WES-tTMB <175 mut/exome subgroup. When the clinical utility of CPS in the WES analysis population was assessed, a positive trend for OS was observed with pembrolizumab in the CPS ≥1 subgroup and the CPS ≥10 subgroup. Using a CPS cut-off of 1, the HR (95% CI) for OS for pembrolizumab versus paclitaxel was 0.82 (0.63-1.06) in the CPS ≥1 subgroup and 1.27 (0.89-

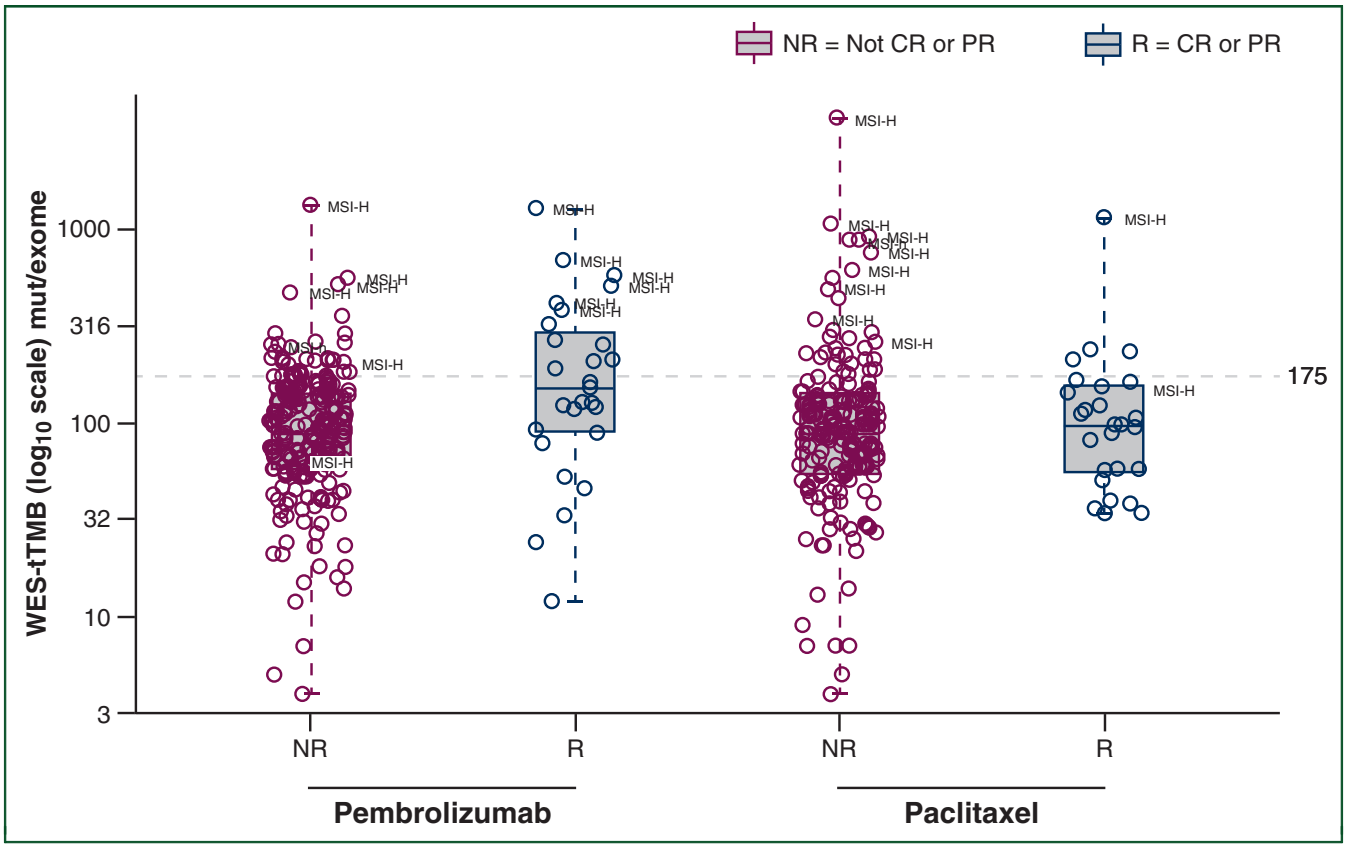

Figure 1. Boxplot of WES-tTMB (log_{10} scale) by response and treatment group, indicating patients with MSI-H status.
CR, complete response; MSI-H, microsatellite instability-high; NR, no response; PR, partial response; R, response; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

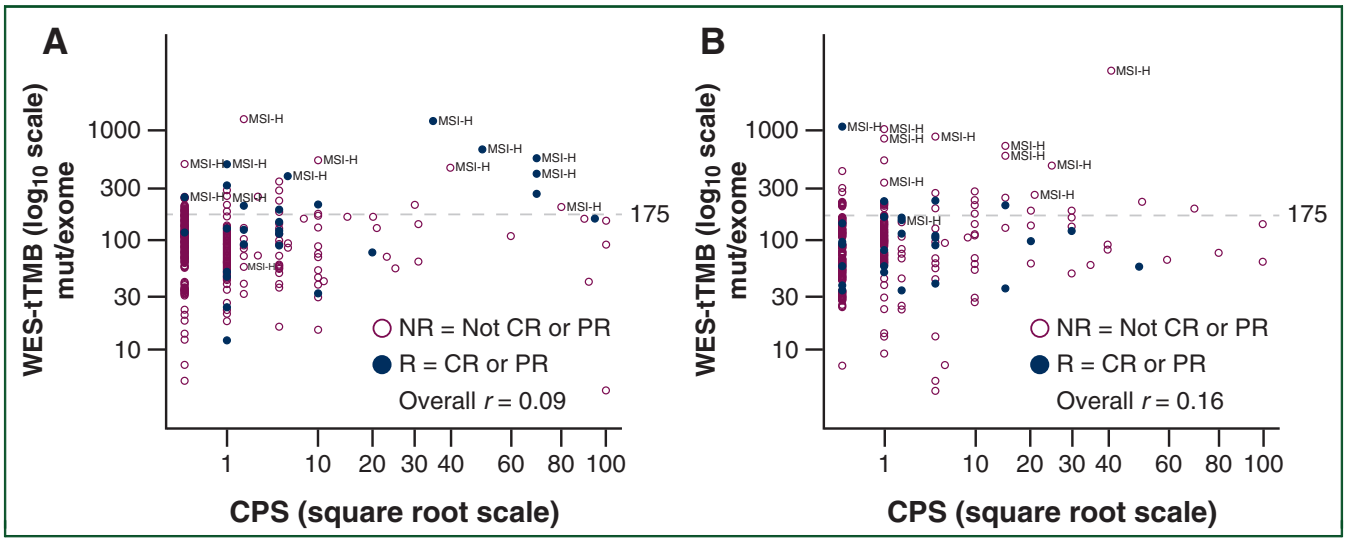

Figure 2. Scatterplot of WES-tTMB (log_{10} scale) versus CPS (square root scale) with (A) pembrolizumab and (B) paclitaxel, indicating patients with response and MSI-H status (data shown for patients with both WES-tTMB and PD-L1 CPS data available).
CPS, combined positive score; CR, complete response; MSI-H, microsatellite instability-high; NR, no response; PD-L1, programmed death-ligand 1; PR, partial response; R, response; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

1.83) in the CPS <1 subgroup. Using a CPS cut-off of 10, the HR (95% CI) for OS for pembrolizumab versus paclitaxel was 0.63 (0.38-1.06) in the CPS ≥10 subgroup and 1.03 (0.82-1.30) in the CPS <10 subgroup. OS HR estimates in the CPS subgroups in the WES analysis population were consistent with those reported in the total study population.[18] Dual biomarker subgroup analyses (tTMB and PD-L1) in the MSI-H and non-MSI-H populations are reported in Table 3; this analysis was exploratory in nature, and sample sizes were small.

WES-tTMB remained significantly associated with PFS and OS in the pembrolizumab group when patients with known MSI-H tumors were excluded (Table 4; Figure 4). In the WES-tTMB ≥175 mut/exome subgroup, the HR (95% CI) for OS for pembrolizumab versus paclitaxel increased with the exclusion of MSI-H patients from 0.46 (0.27-0.81) to 0.60 (0.31-1.16).

Using a more limited set of samples on which Foundation®OneCDx-tTMB calculated using Foundation®OneCDx (n = 205; pembrolizumab, n = 109; paclitaxel, n = 96) was available (Supplementary Figure S1B, available at https://doi.org/10.1016/j.annonc.2021.05.803), FoundationOne®CDx-tTMB demonstrated a positive association with response rate (P < 0.0006; AUROC, 0.68), PFS (P < 0.0001), and OS (P = 0.0031) in pembrolizumab-treated patients; these data include patients with MSI-H and PD-L1-positive tumors. In paclitaxel-treated patients, FoundationOne®CDx-tTMB was associated with response rate (P = 0.0469; AUROC, 0.30) but not PFS (P = 0.6) or OS (P = 0.1). When assessing the clinical utility of FoundationOne®CDx-tTMB, pembrolizumab offered a survival advantage to the FoundationOne®CDx-tTMB ≥10 mut/Mb subgroup (Table 2; Supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2021.05.803). After excluding patients with known MSI-H tumors, HRs (95% CI) by treatment group for OS by the FoundationOne®CDx cut-off were 0.38 (0.13-1.13) for FoundationOne®CDx-tTMB ≥10 mut/Mb (n = 21) versus 0.98 (0.71-1.35) for FoundationOne®CDx-tTMB <10 mut/Mb (n = 170).

DISCUSSION

This exploratory analysis from KEYNOTE-061 is the first to directly demonstrate a relationship between WES-tTMB and clinical efficacy with second-line pembrolizumab in advanced gastric/GEJ cancer and a lack of association with clinical efficacy with paclitaxel. These findings provide evidence that WES-tTMB is predictive of clinical efficacy to pembrolizumab but not to paclitaxel. Prespecified hypothesis testing confirmed a significant association between WES-tTMB and clinical outcomes after adjusting for PD-L1 CPS, suggesting that WES-tTMB is a significant and independent predictor of clinical outcome beyond PD-L1 status in patients with gastric/GEJ cancer treated with second-line pembrolizumab. There was no evidence of an association between WES-tTMB and OS with paclitaxel.

Patients with MSI-H tumors generally exhibited the highest values of WES-tTMB, the majority of which were greater than or equal to the prespecified 175 mut/exome cut-off. However, the association of WES-tTMB with clinical efficacy was not driven entirely by MSI-H status; Kaplan–Meier estimates and receiver operating characteristic (ROC) curves still showed associations between WES-tTMB levels and efficacy in the expected directions in the non-MSI-H population, although the magnitude of trends seen for AUROC and HR estimates were somewhat lessened. Exclusion of patients with MSI-H tumors reduced the clinical utility of both WES-tTMB and CPS. Prevalence of WES-tTMB ≥175 mut/exome and CPS ≥10 was similar (~18% of patients), although WES-tTMB ≥175 mut/exome appeared to achieve a higher level of ORR enrichment than CPS ≥10 in the pembrolizumab group. Of note, the OS HR was

Table 2. Univariate analysis on the association between tTMB subgroup and clinical outcomes using WES and FoundationOne®CDx, including MSI-H tumors and unadjusted for PD-L1 CPS

	WES-tTMB ≥175 mut/exome *n* = 76 (18%)		WES-tTMB <175 mut/exome *n* = 344 (82%)		FoundationOne® CDx-tTMB ≥10 mut/Mb *n* = 35 (17%)		FoundationOne® CDx-tTMB <10 mut/Mb *n* = 170 (83%)	
	Pembrolizumab *n* = 40 (10%)	Paclitaxel *n* = 36 (9%)	Pembrolizumab *n* = 178 (42%)	Paclitaxel *n* = 166 (40%)	Pembrolizumab *n* = 20 (10%)	Paclitaxel *n* = 15 (7%)	Pembrolizumab *n* = 89 (43%)	Paclitaxel *n* = 81 (40%)
ORR[a], % (95% CI)	30 (17-47)	11 (3-26)	8 (5-14)	13 (8-19)	40 (19-64)	13 (2-40)	10 (5-18)	15 (8-24)
PFS[b], months, median (95% CI)	4.1 (2.1-8.6)	4.1 (3.0-8.2)	1.5 (1.4-1.6)	4.1 (3.1-4.3)	5.7 (1.4-NR)	6.5 (4.1-NR)	1.5 (1.4-2.1)	3.4 (2.8-4.2)
HR (95% CI)	0.73 (0.44-1.22)		1.78 (1.43-2.22)		0.69 (0.31-1.51)		1.46 (1.07-1.99)	
OS, months, median (95% CI)	16.4 (10.8-NR)	8.1 (6.8-12.1)	5.7 (4.7-8.7)	8.8 (8.3-9.9)	NR (9.1-NR)	8.1 (6.5-14.4)	5.0 (3.6-7.7)	7.8 (5.8-9.4)
HR (95% CI)	0.46 (0.27-0.81)		1.12 (0.89-1.41)		0.34 (0.14-0.83)		0.98 (0.71-1.35)	

BICR, blinded independent central review; CI, confidence interval; CPS, combined positive score; HR, hazard ratio; MSI-H, microsatellite instability high; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; NR, not reached; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

[a] Responder: Confirmed complete response or partial response per central review using RECIST v1.1.

[b] By BICR per RECIST v1.1.

lower than the PFS HR in the subgroups of patients with tTMB-high and tTMB-non-high tumors, a finding similar to that of the overall patient population. Limited sample size notwithstanding, the data suggest that the efficacy of pembrolizumab was lower in patients with tTMB-non-high than tTMB-high tumors.

In a recent systematic review and meta-analysis, the pooled prevalence of EBV in >20 000 patients with GC was 8.77% (95% CI 7.73-9.92). When broken down by tumor stage, EBV prevalence was 7.39% for patients with stage I or II disease compared with 8.80% in patients with stage III or IV disease.[28] Evaluation of EBV status in the current analysis (28/592; 4.7%) yielded response rates of 13% in pembrolizumab-treated patients (2/15) and 15% in paclitaxel-treated patients (2/13) in EBV-positive tumors. Given the limited sample size, it is difficult to develop conclusions on the lack of difference in ORR between the treatment groups. In biomarker analyses from KEYNOTE-059, five patient tumors were identified as EBV-positive, but none responded to pembrolizumab monotherapy.[20] Data with PD-1 inhibitors, toripalimab and nivolumab, demonstrated EBV-positive tumors in 4/55 patients and 4/80 patients, respectively; in both analyses, only one patient had achieved a partial response.[29,30] Other reports of PD-1

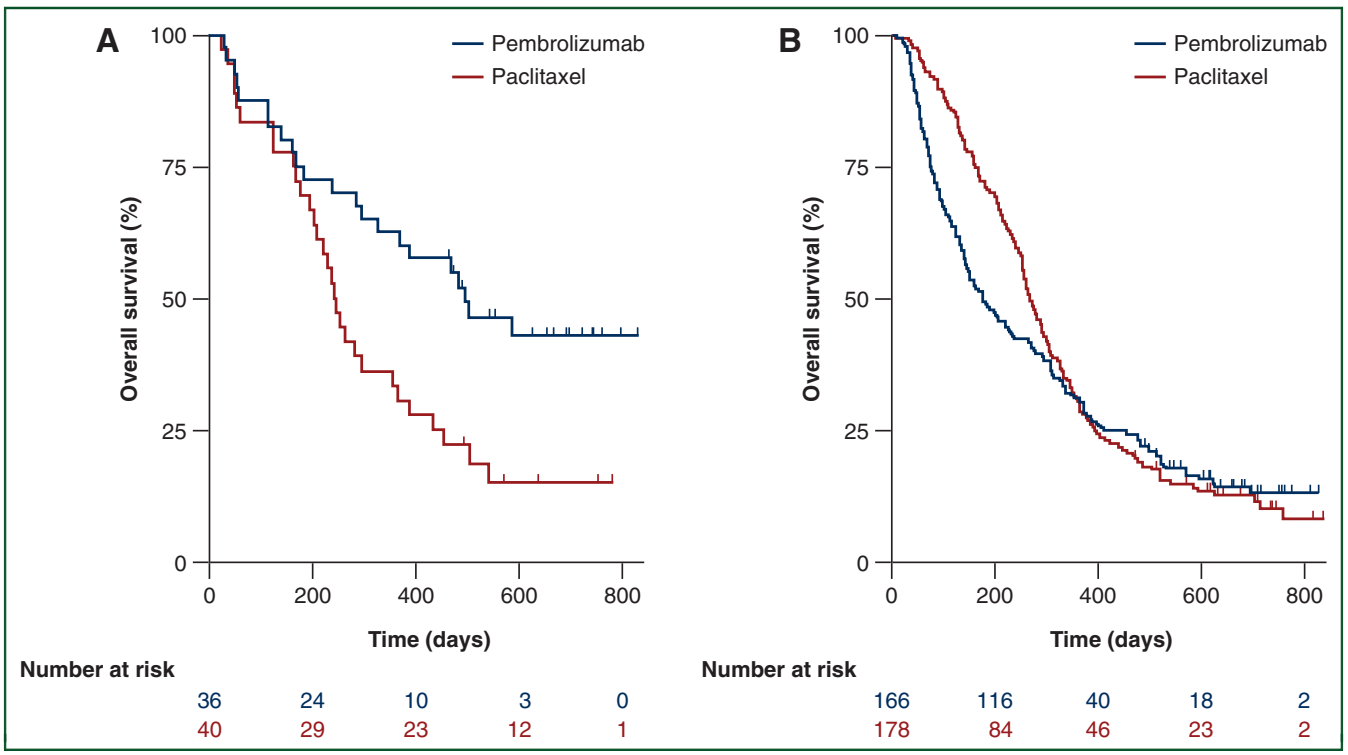

Figure 3. Kaplan–Meier survival curves with pointwise 95% CIs of pembrolizumab versus paclitaxel by WES-tTMB (A) ≥175 mut/exome and (B) <175 mut/exome using univariate analysis including MSI-tumors and unadjusted for PD-L1 CPS.

CI, confidence interval; CPS, combined positive score; mut/exome, mutations per exome; PD-L1, programmed death-ligand 1; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

Table 3. Hazard ratios (95% CI) of OS for pembrolizumab versus paclitaxel by WES-tTMB and CPS status by MSI-H status[a]

Quadrant	MSI-H included or non-MSI-H only[b]	Treatment	OS HR (95% CI)	Quadrant	MSI-H included or non-MSI-H only[b]	Treatment	OS HR (95% CI)
CPS ≥1, WES-tTMB ≥175	MSI-H included	Pembrolizumab $n = 29$ Paclitaxel $n = 27$	0.40 (0.20-0.80)	CPS ≥10, WES-tTMB ≥175	MSI-H included	Pembrolizumab $n = 10$ Paclitaxel $n = 15$	0.19 (0.05-0.69)
	Non-MSI-H only	Pembrolizumab $n = 17$ Paclitaxel $n = 18$	0.54 (0.24-1.25)		Non-MSI-H only	Pembrolizumab $n = 3$ Paclitaxel $n = 10$	NA
CPS ≥1, WES-tTMB <175	MSI-H included	Pembrolizumab $n = 116$ Paclitaxel $n = 108$	0.98 (0.74-1.30)	CPS ≥10, WES-tTMB <175	MSI-H included	Pembrolizumab $n = 29$ Paclitaxel $n = 25$	0.96 (0.53-1.72)
	Non-MSI-H only	Pembrolizumab $n = 115$ Paclitaxel $n = 107$	0.96 (0.72-1.27)		Non-MSI-H only	Pembrolizumab $n = 29$ Paclitaxel $n = 25$	0.96 (0.53-1.72)
CPS <1, WES-tTMB ≥175	MSI-H included	Pembrolizumab $n = 11$ Paclitaxel $n = 8$	0.66 (0.24-1.84)	CPS <10, WES-tTMB ≥175	MSI-H included	Pembrolizumab $n = 30$ Paclitaxel $n = 20$	0.66 (0.34-1.30)
	Non-MSI-H only	Pembrolizumab $n = 9$ Paclitaxel $n = 7$	0.75 (0.25-2.26)		Non-MSI-H only	Pembrolizumab $n = 24$ Paclitaxel $n = 14$	0.69 (0.32-1.50)
CPS <1, WES-tTMB <175	MSI-H included	Pembrolizumab $n = 61$ Paclitaxel $n = 58$	1.45 (0.98-2.13)	CPS <10, WES-tTMB <175	MSI-H included	Pembrolizumab $n = 148$ Paclitaxel $n = 141$	1.15 (0.90-1.47)
	Non-MSI-H only	Pembrolizumab $n = 61$ Paclitaxel $n = 58$	1.45 (0.98, 2.13)		Non-MSI-H only	Pembrolizumab $n = 147$ Paclitaxel $n = 140$	1.14 (0.89-1.45)

Of note, $n = 3$ for TMB ≥175/CPS ≥10 non-MSI-H pembrolizumab group. This sample size does not support OS HR estimation; thus, 'NA' is reported.
CI, confidence interval; CPS, combined positive score; HR, hazard ratio; MSI-H, microsatellite instability-high; NA, not applicable; OS, overall survival; TMB, tumor mutational burden; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.
[a] From Cox proportional hazards regression for OS, unadjusted.
[b] Non-MSI-H includes only patients with no MSI detected (i.e. microsatellite stable) or with unknown MSI-H status.

inhibitors have observed a wide range of response rates (25%-100%), albeit in a very small number of patients ($n = 4$-6) who may have received ≤2 prior lines of therapy.[20,30,31]

The comprehensive WES platform is the gold standard for sequencing when studying cancer genetics, including somatic alterations, and is the benchmark method used in ongoing TMB assessment harmonization efforts.[32] However, NGS panels such as the Foundation®OneCDx are more easily implemented in clinical practice. It is important to show that findings using WES are successfully translated to FoundationOne®CDx. Similar to WES-tTMB, FoundationOne®CDx-tTMB demonstrated a positive association with clinical outcomes with pembrolizumab but not paclitaxel. The findings from this analysis using WES and FoundationOne®CDx in patients with GC are in agreement with a previously reported monotherapy study in non-small-cell lung cancer that demonstrated that tTMB was associated with improved clinical response to pembrolizumab.[26]

This analysis is limited by small patient numbers compared with the total study population; tTMB results were not available for all patients enrolled in the KEYNOTE-061 trial, and, for analysis by FoundationOne®CDx, the sample size was further reduced. Additionally, when the protocol was developed and approved, paclitaxel was the standard of care and thus selected as the comparator for KEYNOTE-061. During the course of the study, combination therapy with anti-VEGF ramucirumab plus paclitaxel was approved as a treatment option in some countries; however, this global study and current analysis was restricted to paclitaxel.

This exploratory analysis of KEYNOTE-061 where tTMB endpoints were masked to treatment group, indicates an association between tTMB and clinical efficacy with second-line pembrolizumab in advanced gastric/GEJ cancer. The association remained after adjustment for other variables (e.g. ECOG) and PD-L1 CPS expression in the tumor

Table 4. Univariate analysis on the association between WES-tTMB subgroup and clinical outcomes in the non-MSI-H population[a]

	WES-tTMB ≥175 mut/exome, non-MSI-H population $n = 52$		WES-tTMB <175 mut/exome, non-MSI-H population $n = 342$	
	Pembrolizumab $n = 27$	Paclitaxel $n = 25$	Pembrolizumab $n = 177$	Paclitaxel $n = 165$
ORR[a], % (95% CI)	22 (9-42)	12 (3-31)	8 (5-14)	13 (8-19)
PFS[b], median (95% CI), months	3.0 (1.5-5.6)	6.8 (4.1-8.7)	1.5 (1.4-1.6)	4.1 (3.0-4.3)
HR (95% CI)	1.16 (0.64-2.11)		1.75 (1.40-2.19)	
OS, median (95% CI), months	15.5 (9.8-NA)	8.2 (6.8-15.1)	5.7 (4.7-7.7)	8.8 (8.3-9.9)
HR (95% CI)	0.60 (0.31-1.16)		1.11 (0.88-1.39)	

CI, confidence interval; HR, hazard ratio; MSI-H, microsatellite instability-high; NA, not available; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.
[a] Non-MSI-H population includes only patients with no MSI detected (i.e. microsatellite stable) or with unknown MSI-H status.
[b] By BICR per RECIST v1.1.

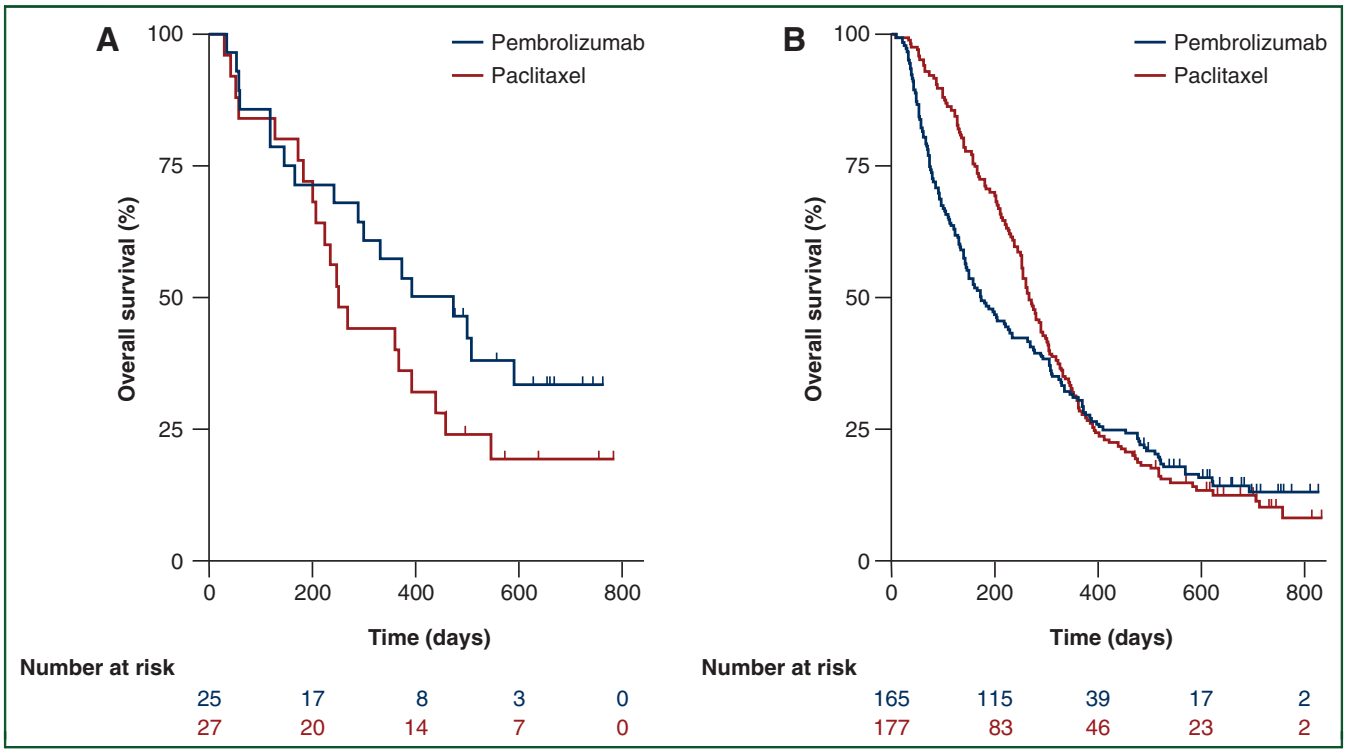

Figure 4. Kaplan–Meier survival curves with pointwise 95% CIs of pembrolizumab versus paclitaxel by WES-tTMB (A) ≥175 mut/exome and (B) <175 mut/exome in the non-MSI-H population and unadjusted for PD-L1 CPS.

CI, confidence interval; CPS, combined positive score; MSI-H, microsatellite instability-high; mut/exome, mutations per exome; PD-L1, programmed death-ligand 1; tTMB, tissue tumor mutational burden; WES, whole exome sequencing.

microenvironment, which suggest that tTMB is a significant and independent predictor beyond PD-L1 status; exploratory evaluation of combination biomarkers (tTMB/PD-L1/MSI-H or tTMB/PD-L1/non-MSI-H) was limited because of sample size and thus further analysis is needed to confirm these findings. Specifically, the benefit of tTMB ≥175 mut/exome in the non-MSI-H population by PD-L1 CPS status requires further analyses with more samples because of this limitation. Pembrolizumab has been approved by the United States Food and Drug Administration for the treatment of patients with unresectable or metastatic TMB-high (≥10 mut/Mb) solid tumors (including GC) that have progressed following prior treatment and who have no satisfactory alternative treatment options. We continue to explore TMB in other regimens and/or lines of therapy.

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DA-G is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and stockholder of Merck & Co., Inc., Kenilworth, NJ, USA and received travel, accommodations, or expenses from Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. JL is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. C-SS is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc, and received researching funding and travel, accommodations, or expenses for Exelixis. DA is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and stockholder of Merck & Co., Inc., Kenilworth, NJ, USA. ZAC is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and has stock ownership interests in Merck & Co., Inc., Kenilworth, NJ, USA, and in Bristol Myers Squibb. 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DATA SHARING

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (MSD), is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers.

The MSD data-sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

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