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Association between gene expression signatures and clinical outcomes of pembrolizumab versus paclitaxel in advanced gastric cancer: exploratory analysis from the randomized, controlled, phase III KEYNOTE-061 trial

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ABSTRACT

Background In the randomized, controlled, phase III KEYNOTE-061 trial, second-line pembrolizumab did not significantly prolong overall survival (OS) versus paclitaxel in patients with PD-L1-positive (combined positive score ≥1) advanced gastric/gastroesophageal junction (G/GEJ) cancer but did elicit a longer duration of response and offered a favorable safety profile. This prespecified exploratory analysis was conducted to evaluate associations between tumor gene expression signatures and clinical outcomes in the phase III KEYNOTE-061 trial.

Methods Using RNA sequencing data obtained from formalin-fixed, paraffin-embedded baseline tumor tissue samples, we evaluated the 18-gene T-cell-inflamed gene expression profile ($Tcell_{inf}$GEP) and 10 non-$Tcell_{inf}$GEP signatures (angiogenesis, glycolysis, granulocytic myeloid-derived suppressor cell (gMDSC), hypoxia, monocytic MDSC (mMDSC), MYC, proliferation, RAS, stroma/epithelial-to-mesenchymal transition/transforming growth factor-β, WNT). The association between each signature on a continuous scale and outcomes was analyzed using logistic (objective response rate (ORR)) and Cox proportional hazards regression (progression-free survival (PFS) and OS). One-sided (pembrolizumab) and two-sided (paclitaxel) p values were calculated for $Tcell_{inf}$GEP (prespecified α=0.05) and the 10 non-$Tcell_{inf}$GEP signatures (multiplicity-adjusted; prespecified α=0.10).

Results RNA sequencing data were available for 137 patients in each treatment group. $Tcell_{inf}$GEP was positively associated with ORR (p=0.041) and PFS (p=0.026) for pembrolizumab but not paclitaxel (p>0.05). The $Tcell_{inf}$GEP-adjusted mMDSC signature was negatively associated with ORR (p=0.077), PFS (p=0.057), and OS (p=0.033) for pembrolizumab, while the $Tcell_{inf}$GEP-adjusted glycolysis (p=0.018), MYC (p=0.057), and proliferation (p=0.002) signatures were negatively associated with OS for paclitaxel.

Conclusions This exploratory analysis of tumor $Tcell_{inf}$GEP showed associations with ORR and PFS for pembrolizumab but not for paclitaxel. $Tcell_{inf}$GEP-adjusted mMDSC signature was negatively associated with ORR, PFS, and OS for pembrolizumab but not paclitaxel. These data suggest myeloid-driven suppression may play a role in resistance to PD-1 inhibition in G/GEJ cancer and support a strategy of considering immunotherapy combinations which target this myeloid axis.

Trial registration number NCT02370498.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Despite multiple systemic therapeutic options, prognosis for patients with advanced gastric or gastroesophageal junction (G/GEJ) cancer remain poor globally. One potential explanation for the poor prognosis may be the constantly evolving molecular expression patterns and interactions between tumor cells and immune cells in the tumor microenvironment. As a result, there is a need to identify molecular determinants that may predict response to systemic therapy in patients with advanced G/GEJ cancer.

WHAT THIS STUDY ADDS

⇒ This exploratory study from the randomized, controlled, phase III KEYNOTE-061 trial of pembrolizumab versus paclitaxel as second-line therapy in patients with advanced G/GEJ cancer found that tumor 18-gene T-cell-inflamed gene expression profile ($Tcell_{inf}$GEP) as a continuous variable showed a positive association with objective response rate (ORR) and progression-free survival (PFS) in patients treated with pembrolizuamb, and that the $Tcell_{inf}$GEP-adjusted monocytic myeloid-derived suppressor cell (mMDSC) signature when analyzed as a continuous variable showed a negative association with ORR, PFS, and overall survival.

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HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Together, these data indicate a potential role for Tcell$_{inf}$GEP and mMDSC signatures in predicting anti-PD-1 therapy outcomes in patients with advanced G/GEJ cancer. Furthermore, the negative associations between mMDSC and outcomes supports a strategy of considering immunotherapy combinations intended to target the myeloid axis in advanced G/GEJ cancer.

INTRODUCTION

Clinical outcomes remain poor for patients with advanced gastric or gastroesophageal junction (G/GEJ) cancer globally despite multiple systemic therapeutic options.[1–3] An estimated 769,000 deaths due to gastric cancer occurred in 2020, and the 5-year relative survival rate for advanced or metastatic disease in the USA is 6%.[4 5] Such poor prognosis for patients with G/GEJ cancer may result from the constantly evolving molecular expression patterns and interactions between tumor cells and immune cells in the tumor microenvironment (TME).[6–8] As a result, there is a need to identify biomarkers that may predict response to systemic therapy in patients with advanced G/GEJ cancer.

Pembrolizumab is a programmed cell death protein 1 (PD-1) inhibitor that is recommended for patients with microsatellite instability-high (MSI-H)/deficient mismatch repair tumors and tumor mutational burden (TMB) high (TMB-H; ≥10 mutations/megabase) tumors when no other satisfactory alternative therapeutic options exist.[3 9] It was recently suggested that programmed death ligand 1 (PD-L1) expression using Combined Positive Score (CPS), TMB, and MSI-H are predictors of response to pembrolizumab in patients with previously treated advanced G/GEJ cancer.[1 10 11] However, given the complexity of the TME, a greater understanding of the TME beyond TMB is needed for a more robust and reliable prediction of patient response to pembrolizumab. Exploratory studies showed that certain other molecular determinants in the TME such as an interferon gamma (IFN-γ)-related 18-gene T-cell-inflamed gene expression profile (Tcell$_{inf}$GEP) signature and other non-Tcell$_{inf}$GEP consensus signatures associated with key cell types, certain biological processes, or oncogenic pathways are associated with pan-tumor response to pembrolizumab.[12–16] In one such pan-tumor study involving seven tumor types, it was found that Tcell$_{inf}$GEP along with 10 other TME-associated non-Tcell$_{inf}$GEP consensus RNA expression signatures for angiogenesis, glycolysis, granulocytic myeloid-derived suppressor cells (gMDSC), hypoxia, monocytic MDSCs (mMDSC), MYC, proliferation, RAS, stromal/epithelial-to-mesenchymal transition (EMT)/transforming growth factor-β (TGFβ), and WNT had a high concordant coexpression pattern across tumor types; some of these consensus gene expression signatures were significantly associated with response to pembrolizumab.[12] At present, the association of these gene expression signatures with clinical response to pembrolizumab has not been specifically explored in the G/GEJ cancer setting.

The randomized, open-label, phase 3 KEYNOTE-061 trial was designed to evaluate the efficacy and safety of pembrolizumab versus paclitaxel as second-line therapy in patients with advanced G/GEJ cancer that progressed on platinum and fluoropyrimidine-based chemotherapy.[17 18] Overall survival (OS) and progression-free survival (PFS) in the primary population (PD-L1 CPS≥1) were not significantly prolonged with pembrolizumab versus paclitaxel; however, pembrolizumab showed more durable responses and had a manageable adverse event profile compared with paclitaxel.[18] We evaluated the association between tumor gene expression signatures and clinical outcomes of pembrolizumab versus paclitaxel in previously treated patients with advanced G/GEJ cancer from the KEYNOTE-061 trial.

MATERIALS AND METHODS

Trial design and patients

Details of the KEYNOTE-061 (ClinicalTrials.gov, NCT02370498) trial design and eligibility criteria have been published.[18] Briefly, key eligibility criteria included age ≥18 years, histologically or cytologically confirmed unresectable advanced or metastatic gastric or GEJ adenocarcinoma that progressed after first-line therapy with a platinum agent and fluoropyrimidine or after first-line trastuzumab for human epidermal growth factor receptor 2-positive tumors, measurable disease per Response Evaluation Criteria in Solid Tumors, V.1.1 (RECIST V1.1), by investigator assessment, and Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients with squamous cell or undifferentiated gastric cancer histology, previous therapy with an anti-PD-1/L1 agent, and active autoimmune disease that necessitated systemic treatment were excluded.

Patients were randomly assigned (1:1) to receive pembrolizumab 200 mg intravenously every 3 weeks or paclitaxel 80 mg/m² intravenously on days 1, 8, and 15 of 4-week cycles for 35 cycles (approximately 2 years; pembrolizumab only) or until disease progression, unacceptable toxicity, physician decision, or patient withdrawal of consent. The trial protocol and all amendments were approved by the institutional review board or ethics committee at each participating institution. The name of each ethics committee/institutional review board at each participating center including approval numbers are shown in online supplemental table 1. The trial was conducted in accordance with the protocol, its amendments, the ethical principles originating from the Declaration of Helsinki, and Good Clinical Practice guidelines. Written informed consent was provided by all patients before enrollment.

Outcomes

In this exploratory post hoc analysis, prespecified objectives included: (1) assessment of whether Tcell$_{inf}$GEP (as a continuous variable) is associated with clinical efficacy with pembrolizumab or paclitaxel, (2) assessment of

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whether the 10 non-Tcell$_{inf}$GEP signatures (angiogenesis, glycolysis, gMDSC, hypoxia, mMDSC, MYC, proliferation, RAS, stroma/EMT/TGFβ, and WNT) adjusted for the Tcell$_{inf}$GEP (as continuous variables) are associated with clinical efficacy with pembrolizumab or paclitaxel, (3) estimation of the relevant treatment effects of pembrolizumab versus paclitaxel in Tcell$_{inf}$GEP subgroups based on a prespecified cut-off of the first tertile, and (4) estimation of the relevant treatment effects of pembrolizumab versus paclitaxel in non-Tcell$_{inf}$GEP signatures that showed an association with OS when analyzed as continuous variables via a prespecified cut-off of the median.

Procedures

RNA sequencing was performed on formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples provided at screening using the HiSeq 4000 platform (Illumina, California, USA). Of note, the gene expression signatures performed similarly in gene expression data obtained from freshly frozen and FFPE samples.[12] The RNA-sequencing raw reads were processed using a customized RNA-sequencing data analysis pipeline in OmicSoft ArraySuite version 9 (Qiagen, Hilden, Germany) as previously described.[12] First, the raw reads were quality-filtered and then aligned to the reference genome Human.B37.3 using the Omicsoft sequence aligner.[19] Thereafter, gene expression levels, defined as raw read counts and fragments per kilobase of exon per million mapped fragments, were quantified using the RNA sequencing by expectation maximization algorithm with the Ensembl.R75 gene model.[20]

Tcell$_{inf}$GEP score was calculated as the weighted sum of normalized expression values for the 18 genes determined as predictors of response in the pan-tumor setting on the NanoString platform.[12 13] The 10 non-Tcell$_{inf}$GEP signature scores were calculated as the average of the genes (on the logarithmic scale) in each signature gene set as previously described.[12 13]

TMB was determined via whole exome sequencing (mut/exome) of tumor samples and matched DNA. TMB was calculated as the number of somatic non-synonymous single nucleotide variants and indels that met prespecified criteria as previously described.[16 21]

Radiologic tumor imaging was performed every 6 weeks and survival follow-up assessed every 12 weeks. Objective response rate (ORR) was defined as the proportion of patients in the analysis population who had a confirmed complete response or partial response per RECIST V.1.1 by blinded independent central review. PFS was defined as the time from randomization to the first documented disease progression per RECIST V.1.1 by investigator assessment or death due to any cause, whichever occurred first. OS was defined as the time from randomization to death due to any cause.

Statistical analysis

This prespecified exploratory analysis included all treated patients in KEYNOTE-061 who had available RNA sequencing data.

The association between continuous scores for the Tcell$_{inf}$GEP and 10 non-Tcell$_{inf}$GEP signatures and clinical outcomes was evaluated using logistic regression (ORR) and Cox proportional hazards regression (PFS and OS), with adjustments for ECOG performance status; the 10 non-Tcell$_{inf}$GEP scores were also adjusted for Tcell$_{inf}$GEP. Adjustment for the Tcell$_{inf}$GEP was performed to understand the additional explanatory value that any non-Tcell$_{inf}$GEP signatures had for clinical outcome, an approach equivalent to evaluating the association between clinical outcome and the residuals of consensus signatures after detrending for their relationship with the Tcell$_{inf}$GEP. For the Tcell$_{inf}$GEP, one-sided (pembrolizumab; positive association hypothesized) and two-sided (paclitaxel; no direction hypothesized) p values were calculated; these hypotheses were informed by prior evidence in other tumor types that the Tcell$_{inf}$GEP may be positively associated with response to pembrolizumab[22 23] and because the association between Tcell$_{inf}$GEP and paclitaxel has not been substantiated. P values were calculated (prespecified significance level, α=0.05). For the 10 non-Tcell$_{inf}$GEP signatures, one-sided (pembrolizumab; negative association hypothesized except for proliferation) and two-sided (paclitaxel; no direction hypothesized) multiplicity-adjusted p values were calculated (prespecified significance level, α=0.10).

Descriptive subgroup analyses to estimate efficacy of pembrolizumab versus paclitaxel and understand potential clinical utility were performed using prespecified cutoffs for the Tcell$_{inf}$GEP (≥ first tertile (Tcell$_{inf}$GEP^{nonlow}) and <first tertile (Tcell$_{inf}$GEP^{low}) as previously defined and validated[13] and non-Tcell$_{inf}$GEP signatures (≥ median and <median, where median is signature specific, Tcell$_{inf}$GEP-detrended). Within each subgroup, the exact binomial method was used to estimate difference in ORR whereas the Cox proportional hazards regression model, with adjustment for ECOG performance status (Tcell$_{inf}$GEP and non-Tcell$_{inf}$GEP signatures) and Tcell$_{inf}$GEP (non-Tcell$_{inf}$GEP signatures only), was used to estimate the OS and PFS HRs) and corresponding 95% CIs for pembrolizumab versus paclitaxel. OS was also evaluated by dual cutoffs of the Tcell$_{inf}$GEP signature and TMB (<175 mut/exome [TMB^{nonhigh}] and ≥175 mut/exome [TMB^{high}]; this cut-off of 175 mut/exome had been determined to be optimal for predicting response to pembrolizumab across several tumor types and is concordant with 10 mut/Mb via FoundationOneCDx.[24–26]

RESULTS

Patients

Between June 4, 2015, and July 26, 2016, 592 patients were randomly assigned to receive either pembrolizumab or paclitaxel. The median follow-up duration, defined as

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Table 1 Baseline characteristics and clinical outcomes in patients with evaluable RNA sequencing data

	Pembrolizumab n=137	Paclitaxel n=137
Age, median (IQR), years	63 (16.0)	60 (16.0)
Male	94 (68.6)	95 (69.3)
ECOG performance status		
0/1	61 (44.5)/76 (55.5)	63 (46.0)/74 (54.0)
Histology*		
Adenocarcinoma	106 (77.4)	107 (78.1)
Tubular adenocarcinoma	8 (5.8)	13 (9.5)
Poorly cohesive carcinoma	6 (4.4)	6 (4.4)
Signet-ring cell carcinoma, diffuse type	5 (3.6)	6 (4.4)
Histological subtype†		
Diffuse	46 (33.6)	33 (24.1)
Intestinal	21 (15.3)	31 (22.6)
Mixed	3 (2.2)	7 (5.1)
Primary location		
Stomach	95 (69.3)	92 (67.2)
Gastroesophageal junction	42 (30.7)	45 (32.8)
Previous gastrectomy		
Total	26 (19.0)	36 (26.3)
Subtotal	19 (13.9)	21 (15.3)
Partial	13 (9.5)	11 (8.0)
None	79 (57.7)	69 (50.4)
PD-L1 CPS		
≥1	90 (65.7)	105 (76.6)
<1	47 (34.3)	32 (23.4)
MSI status‡		
MSI-H	8 (5.8)	9 (6.6)
Non-MSI-H	124 (90.5)	122 (89.1)
TTP on first therapy		
<6 months	87 (63.5)	82 (59.9)
≥6 months	50 (36.5)	55 (40.1)
HER2 positive	28 (20.4)	26 (19.0)
Current disease stage		
Metastatic	30 (21.9)	46 (33.6)
Locally advanced	107 (78.1)	91 (66.4)
Peritoneal metastasis	30 (21.9)	46 (33.6)
Presence of ascites	18 (13.1)	15 (10.9)
Clinical outcomes		
Responders§	16 (11.7)	20 (14.6)
PFS,¶ median (95% CI), months	1.5 (1.4 to 1.9)	4.0 (3.0 to 4.2)
OS, median (95% CI), months	6.1 (4.6 to 10.1)	8.4 (7.8 to 8.9)

Values are n (%) unless stated otherwise.
*There were 12 patients (8.8%) and 5 patients (3.6%) with other histological types in the pembrolizumab and paclitaxel groups, respectively.
†There were 67 patients (48.9%) and 66 patients (48.2%) with unknown histological subtypes in the pembrolizumab and paclitaxel groups, respectively.
‡There were five patients and six patients with unknown MSI tumor status in the pembrolizumab and paclitaxel groups, respectively.
§Based on confirmed complete response or partial response per RECIST V.1.1 by BICR.
¶Per RECIST V.1.1 by investigator assessment.
BICR, blinded independent central review; CPS, combined positive score; ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2; MSI-H, microsatellite instability-high; OS, overall survival; PD-L1, programmed death ligand 1; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; TTP, time to progression.

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Table 2 Association p values of gene expression signatures with clinical outcomes

	Pembrolizumab n=137			Paclitaxel n=137		
	ORR	PFS	OS	ORR	PFS	OS
$Tcell_{inf}GEP$*	**0.041**	**0.026**	0.178	0.822	0.207	0.644
Adjusted for $Tcell_{inf}GEP$†						
Angiogenesis	**0.077**‡	0.298	0.623	0.220	0.462	0.263
Glycolysis	0.934	0.861	0.956	0.223	**0.097**‡	**0.018**‡
gMDSC	0.626	0.573	0.623	0.694	0.352	0.263
Hypoxia	0.934	0.861	0.956	0.223	0.220	0.440
mMDSC	**0.077**‡	**0.057**‡	**0.033**‡	0.694	0.932	0.263
MYC	0.934	0.861	0.956	0.223	0.327	**0.057**‡
Proliferation	0.934	0.861	0.956	0.223	**0.071**‡	**0.002**‡
RAS	0.934	0.861	0.956	0.223	0.327	0.440
Stroma/EMT/TGFβ	0.306	0.366	0.522	0.694	0.462	0.263
WNT	0.934	0.861	0.956	**0.097**‡	0.327	0.440

All models include additional covariates of ECOG performance status.
*Bolded p values (one sided for pembrolizumab with hypothesized positive association and two sided for paclitaxel with no hypothesized association) indicate nominal statistical significance (α=0.05); model includes additional covariates of ECOG performance status.
†Bolded p values (one sided for pembrolizumab with hypothesized negative association except for proliferation with hypothesized positive association and two sided for paclitaxel with no hypothesized association) indicate multiplicity-adjusted statistical significance (α=0.10); model includes additional covariates of ECOG performance status and $Tcell_{inf}GEP$.
‡Negative association observed.
ECOG, Eastern Cooperative Oncology Group; EMT, epithelial-to-mesenchymal transition; gMDSC, granulocytic myeloid-derived suppressor cells; mMDSC, monocytic MDSC; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; $Tcell_{inf}GEP$, T-cell-inflamed gene expression profile.

time between randomization and the October 26, 2017, data cut-off, in the total KEYNOTE-061 population was 7.9 months (IQR 3.4–14.6 months).[18] Of those treated, 274 patients (pembrolizumab, n=137; paclitaxel, n=137) had evaluable RNA sequencing data and were included in this analysis. Baseline characteristics in the RNA sequencing analysis population were generally well balanced between treatment groups (table 1) and similar to those of the total KEYNOTE-061 trial population.[18] Clinical outcomes for patients with evaluable RNA sequencing data are presented in table 1.

Association between gene expression signatures and clinical outcomes

$Tcell_{inf}GEP$ as a continuous variable was positively associated with ORR and PFS for pembrolizumab (one-sided p=0.041 and 0.026, respectively); no significant associations were observed between $Tcell_{inf}GEP$ and clinical outcomes for paclitaxel (table 2). The distribution of $Tcell_{inf}GEP$ scores trended higher in responders (n=16) than nonresponders (n=121) for pembrolizumab, but no difference was observed between responders (n=20) and nonresponders (n=117) for paclitaxel (figure 1A). The area under the receiver operating characteristics curve for discriminating $Tcell_{inf}GEP$ as a predictor of objective response was 0.64 (95% CI 0.50 to 0.78) for pembrolizumab and 0.50 (95% CI 0.37 to 0.62) for paclitaxel (figure 1B).

After adjusting for the $Tcell_{inf}GEP$, the angiogenesis and mMDSC signatures were negatively associated with outcomes with pembrolizumab (one-sided multiplicity-adjusted p values for angiogenesis: ORR 0.077; mMDSC: ORR 0.077; PFS 0.057; OS 0.033); none of the remaining signatures were statistically significantly associated with outcomes for pembrolizumab after multiplicity adjustment (table 1). After adjusting for the $Tcell_{inf}GEP$, the glycolysis, MYC, proliferation, and WNT signatures were associated with negative outcomes with paclitaxel (two-sided multiplicity-adjusted p values for glycolysis: PFS 0.097; OS, 0.018; MYC: OS 0.057; proliferation, PFS 0.071; OS 0.002; WNT: ORR 0.097; table 2).

Efficacy estimates by $Tcell_{inf}GEP$

We assessed the clinical utility of the $Tcell_{inf}GEP$ using a prespecified cut-off of the first tertile ($Tcell_{inf}GEP^{nonlow}$, n=183; $Tcell_{inf}GEP^{low}$, n=91). ORRs were similar between pembrolizumab and paclitaxel in the $Tcell_{inf}GEP^{nonlow}$ subgroup (15.9% (95% CI 9.0% to 25.2%) vs 15.8% (95% CI 9.1% to 24.7%), respectively) and numerically lower with pembrolizumab versus paclitaxel in the $Tcell_{inf}GEP^{low}$ subgroup (4.1% (95% CI 0.5% to 14.0%) vs 11.9% (95% CI 4.0% to 25.6%), respectively; figure 2A). The HR of pembrolizumab versus paclitaxel for PFS was lower in the $Tcell_{inf}GEP^{nonlow}$ subgroup compared with the $Tcell_{inf}GEP^{low}$ subgroup (1.28 (95% CI 0.94 to 1.74) vs 1.70 (95% CI 1.11 to 2.60); figure 2B) and the HR of pembrolizumab versus paclitaxel for OS was similar between the $Tcell_{inf}GEP^{nonlow}$ and $Tcell_{inf}GEP^{low}$ subgroups (0.88 (95% CI 0.64 to 1.22) vs 0.83 (95% CI 0.53 to

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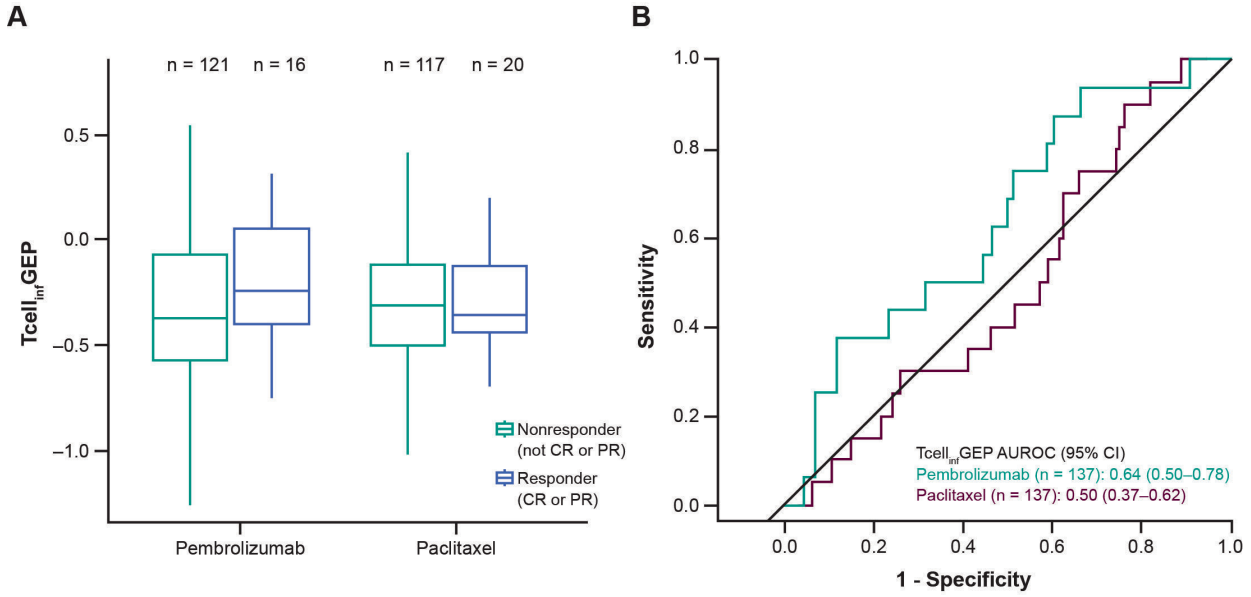


Figure 1 Association between Tcell$_{inf}$GEP and response. (A) Response for Tcell$_{inf}$GEP in the pembrolizumab and paclitaxel groups. (B) Receiver operating characteristics curve for sensitivity and specificity. AUROC, area under the receiver operating characteristics curve; CR, complete response; PR, partial response; Tcell$_{inf}$GEP, T-cell-inflamed gene expression profile.

1.30); figure 2C). When OS was evaluated by dual Tcell$_{inf}$GEP and TMB cutoffs, survival probability was generally higher in patients with Tcell$_{inf}$GEP^{nonlow} and TMB^{high} tumors compared with other subgroups based on the dual Tcell$_{inf}$GEP and TMB cutoffs with pembrolizumab; OS probability was similar across subgroups based on dual Tcell$_{inf}$GEP and TMB cutoffs with paclitaxel (online supplemental figure 1).

Efficacy estimates by selected non-Tcell$_{inf}$GEP signatures

For illustrative purposes, we also evaluated the four non-Tcell$_{inf}$GEP signatures (mMDSC, glycolysis, proliferation, and MYC), which showed an association with OS for pembrolizumab or paclitaxel when analyzed as a continuous variable, using a prespecified cut-off of the signature specific, Tcell$_{inf}$GEP-adjusted median. When Tcell$_{inf}$GEP-adjusted mMDSC was evaluated using a prespecified cut-off of the median (mMDSC≥median, n=137; mMDSC<median, n=137), ORR was numerically lower for pembrolizumab versus paclitaxel (7.1% (95% CI 2.4% to 15.9%) vs 16.4% (95% CI 8.5% to 27.5%)), respectively) in the mMDSC≥median subgroup and higher with pembrolizumab versus paclitaxel in the mMDSC<median subgroup (16.4% (95% CI 8.5% to 27.5) vs 12.9% (95% CI 6.1% to 23.0%), respectively; figure 3A). The PFS and OS HRs for pembrolizumab versus paclitaxel were lower in the mMDSC<median subgroup compared with the mMDSC≥median subgroup (PFS: 1.14 (95% CI 0.80 to 1.64) vs 1.83 (95% CI 1.28 to 2.61); OS: 0.61 (95% CI 0.42 to 0.88) vs 1.26 (95% CI 0.87 to 1.81), figure 3B,C). When the evaluation of the Tcell$_{inf}$GEP-adjusted mMDSC signatures was restricted to patients with non-MSI-H tumors (mMDSC≥median, n=129; mMDSC<median, n=128), the OS HR for pembrolizumab versus paclitaxel was lower in the mMDSC<median subgroup versus the mMDSC≥median subgroup (0.66 (95% CI 0.45 to 0.96) vs 1.44 (95% CI 0.99 to 2.10), respectively; online supplemental figure 2), consistent with the MSI-H inclusive results.

For the Tcell$_{inf}$GEP-adjusted glycolysis signature, the OS HR for pembrolizumab versus paclitaxel was lower in the glycolysis≥median subgroups compared with the <median subgroup (0.58 (95% CI 0.40 to 0.85) vs 1.24 (95% CI 0.86 to 1.81), respectively; online supplemental figure 3A).

For the Tcell$_{inf}$GEP-adjusted proliferation and MYC signatures, OS HRs for pembrolizumab versus paclitaxel were lower in the proliferation and MYC≥median subgroups compared with the respective <median subgroups (proliferation: 0.63 (95% CI 0.43 to 0.91) vs 1.12 (95% CI 0.77 to 1.63), respectively, and MYC: 0.63 (95% CI 0.43 to 0.92) vs 1.17 (95% CI 0.81 to 1.70), respectively; online supplemental figure 3B and 3C).

DISCUSSION

In this exploratory analysis of patients with previously treated advanced G/GEJ cancer from the KEYNOTE-061 trial, the tumor Tcell$_{inf}$GEP as a continuous variable showed some associations with clinical outcomes for pembrolizumab (ORR and PFS) and no associations with clinical outcomes for paclitaxel. For pembrolizumab, negative associations were observed between the Tcell$_{inf}$GEP-adjusted mMDSC signature as a continuous variable and all three clinical outcomes (ORR, PFS, and OS), and between the Tcell$_{inf}$GEP-adjusted angiogenesis signature as a continuous variable and ORR. For

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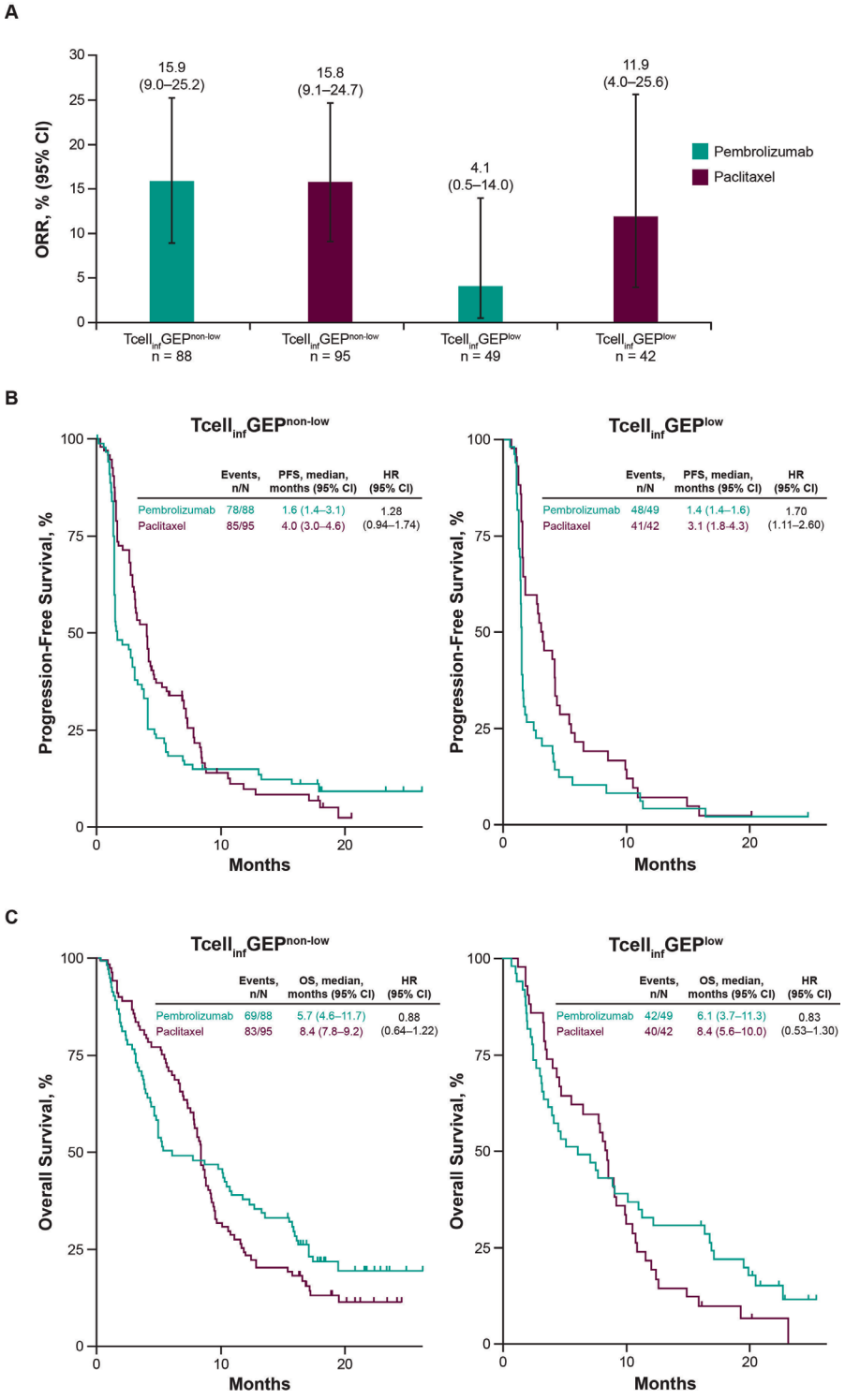


Figure 2 Efficacy of pembrolizumab versus paclitaxel by Tcell$_{inf}$GEP cutoff. (A) Objective response rate. (B) Progression-free survival. (C) Overall survival. ORR, objective response rate; OS, overall survival; PFS, progression-free survival; Tcell$_{inf}$GEP, T-cell-inflamed gene expression profile.

paclitaxel, the Tcell$_{inf}$GEP-adjusted glycolysis, MYC, and proliferation signatures as continuous variables were negatively associated with OS. Evaluation of the efficacy estimates of selected non-Tcell$_{inf}$GEP signatures, adjusted for Tcell$_{inf}$GEP, by prespecified cutoffs of the median showed improved OS HRs of pembrolizumab versus paclitaxel for the mMDSC<median, proliferation≥median, and MYC≥median subgroups compared with their respective alternative subgroups.

The significant positive association between Tcell$_{inf}$GEP and ORR with pembrolizumab is consistent with findings in the pan-tumor setting[12 15 16] and suggests that Tcell$_{inf}$GEP may predict response to second-line pembrolizumab in patients with advanced G/GEJ cancer. Such a

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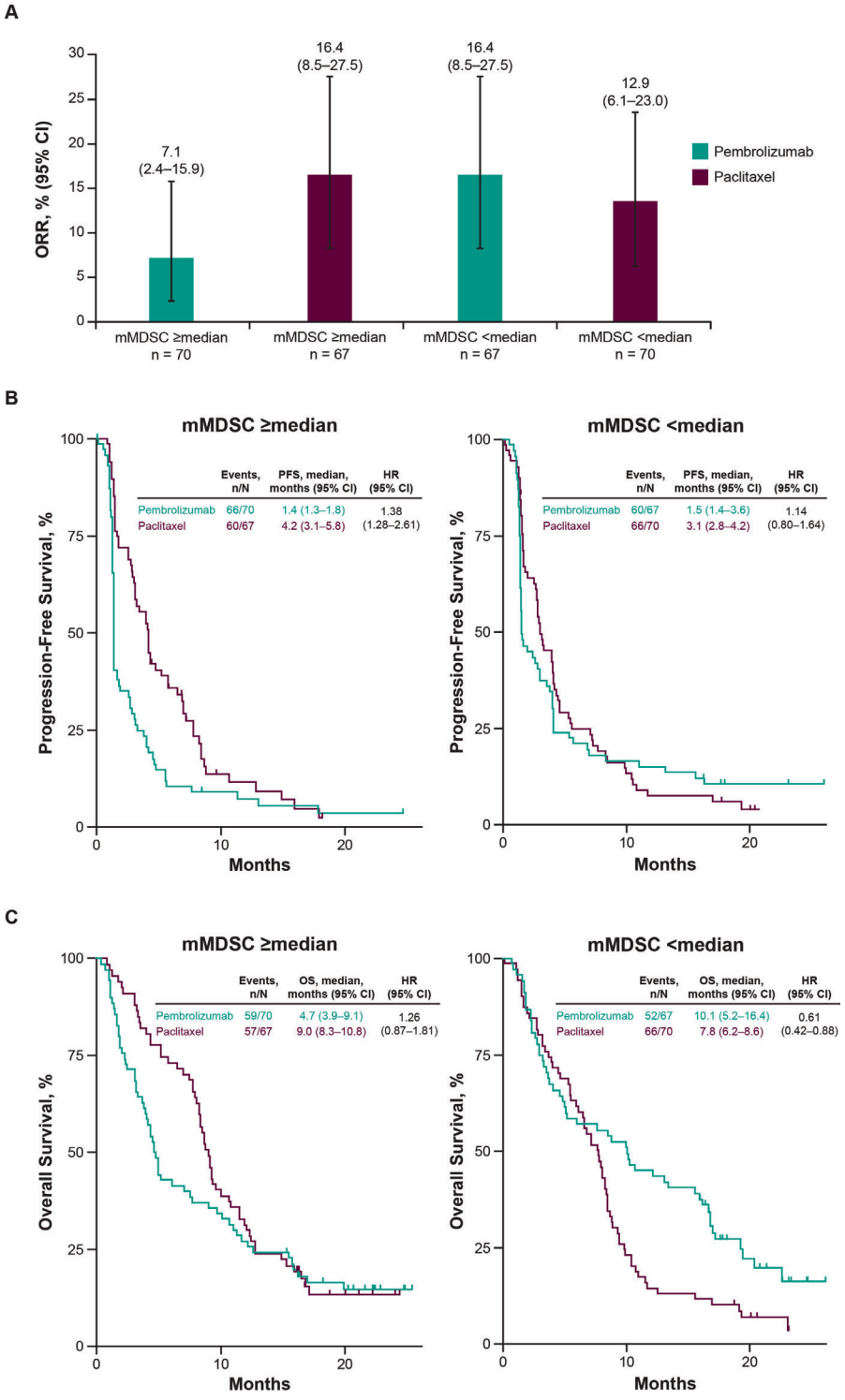


Figure 3 Efficacy of pembrolizumab versus paclitaxel by mMDSC cut-off after adjusting for $Tcell_{inf}GEP$. (A) Objective response rate. (B) Progression-free survival. (C) Overall survival. mMDSC, monocytic myeloid-derived suppressor cells; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; $Tcell_{inf}GEP$, T-cell-inflamed gene expression profile.

positive association between the $Tcell_{inf}GEP$ and response to pembrolizumab could be expected given that the $Tcell_{inf}GEP$ includes RNA expressions for PD-L1 (*CD274*[13]), whose expression has been widely shown to correlate with response to anti-PD-1/L1 therapy in several tumors.[27] A moderate but statistically significant correlation has also been demonstrated between the $Tcell_{inf}GEP$ and PD-L1 CPS (via immunohistochemistry) in a pan-tumor dataset (Spearman ρ=0.40; p<0.001)[15]; however, the $Tcell_{inf}GEP$ and PD-L1 CPS are regarded as nonequivalent biomarkers.[12] Conversely, the lack of significant associations between $Tcell_{inf}GEP$ as a continuous variable and clinical outcomes for paclitaxel is consistent with a previous report[22] and suggests that the $Tcell_{inf}GEP$ may

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not be a predictor of a patient's response to systemic chemotherapy.

Similar to results obtained in the pan-tumor setting,[16] our evaluation of the clinical utility of the Tcell$_{inf}$GEP in predicting response to pembrolizumab showed relatively higher response rates in the Tcell$_{inf}$GEP^{nonlow} subgroup versus the Tcell$_{inf}$GEP^{low} subgroup, suggesting that the Tcell$_{inf}$GEP signature may be better suited in predicting response to second-line pembrolizumab in patients with advanced G/GEJ cancer with a Tcell$_{inf}$GEP score of at least or greater than the first tertile versus less than the first tertile, although formal testing is needed for verification. In contrast, the minimal difference in ORR between the Tcell$_{inf}$GEP cut-off subgroups for paclitaxel suggests a low sensitivity of the Tcell$_{inf}$GEP cut-off to predict response to paclitaxel consistent with testing results. Although pembrolizumab did not show PFS benefits versus paclitaxel in both Tcell$_{inf}$GEP subgroups, the HR for PFS was lower in the Tcell$_{inf}$GEP^{nonlow} subgroup compared with the Tcell$_{inf}$GEP^{low} subgroup suggesting that Tcell$_{inf}$GEP$^{non\text{-}low}$ may have clinical utility in this setting. We speculate that such clinical utility of the Tcell$_{inf}$GEP may be best when used in conjunction with other biomarkers such as TMB, which has been suggested to be a significant predictor of response to pembrolizumab in this patient population.[1] In this regard, our subgroup analysis based on dual Tcell$_{inf}$GEP and TMB status showed highest OS probability in the subgroup of patients with Tcell$_{inf}$GEP^{nonlow} and TMB^{high} tumors.

Of all the non-Tcell$_{inf}$GEP signatures evaluated in this study, only the Tcell$_{inf}$GEP-adjusted mMDSC signature showed significant negative associations with all three clinical outcomes (ORR, PFS, and OS) of pembrolizumab treatment. This observation suggests that the mMDSC signature may be a strong negative predictor of clinical outcomes of pembrolizumab. Consistent with our findings, a negative association between Tcell$_{inf}$GEP-adjusted mMDSC signature and response to pembrolizumab has been observed in the pan-tumor setting.[12] MDSCs are known to be associated with antigen-specific tolerance and suppression of T-cell response in the TME[7 28]; thus, the observed negative associations between the mMDSC signature and clinical outcomes suggests that myeloid-driven suppression may play a role in resistance to PD-1/L1 inhibition. Illustration of the potential clinical utility of the mMDSC signature for pembrolizumab treatment showed higher ORR and an OS benefit versus paclitaxel in the Tcell$_{inf}$GEP-adjusted mMDSC<median subgroup than the mMDSC≥median subgroup, with the OS benefit observed in the mMDSC<median subgroup robust to the exclusion of patients with MSI-H tumors. Conversely, trends in ORR and median OS between the Tcell$_{inf}$GEP-adjusted mMDSC subgroups for paclitaxel treatment suggest poor discriminatory potential of the mMDSC signature for paclitaxel in this setting consistent with testing results. Altogether, observations for the mMDSC signature for pembrolizumab suggest that immunotherapy targeting the myeloid axis may be an effective anti-tumor strategy to overcome anti-PD-1/L1 resistance across many tumor types, including advanced G/GEJ cancer. Such a strategy is currently being explored in a phase 1 trial (ClinicalTrials.gov, NCT03918278) of a novel humanized immunoglobulin G4 monoclonal antibody MK-0482 targeting the immunoglobulin-like transcript 3 receptor on MDSCs or tolerogenic dendritic cells.[29] Preliminary results from this ongoing phase 1 trial have shown modest efficacy with MK-0482 in combination with pembrolizumab in patients with heavily pretreated advanced solid tumors.[29]

Furthermore, the negative association observed between the Tcell$_{inf}$GEP-adjusted angiogenesis signature as a continuous variable and ORR for pembrolizumab also supports the rationale for considering immunotherapy combinations that target the angiogenesis axis. At present, the LEAP program is being conducted to evaluate the safety and efficacy of the multikinase inhibitor lenvatinib in combination with pembrolizumab across several advanced solid tumors.[30] In the population with advanced gastric cancer, the randomized phase 3 LEAP-015 trial (ClinicalTrials.gov, NCT04662710) is being conducted to evaluate the safety and efficacy of lenvatinib in combination with pembrolizumab and chemotherapy (CAPOX or mFOLFOX) as first-line therapy in patients with advanced/metastatic gastroesophageal adenocarcinoma.[31] Preliminary results from the safety run-in phase of LEAP-015 have demonstrated anti-tumor activity.

Limitations of the prespecified exploratory analysis from the KEYNOTE-061 trial reported here include the small sample sizes of the subgroups and lack of statistical power which hinders making definitive conclusions. Additionally, the low proportion of patients who responded to pembrolizumab (11.7%) or paclitaxel (14.7%) in this RNA sequencing population may be another limitation. Last, paclitaxel was used as the comparator arm in this analysis because it was the standard of care at the time the KEYNOTE-061 trial was designed and approved. Since then, combination therapy with the vascular endothelial growth factor receptor 2 inhibitor, ramucirumab, plus paclitaxel is now the approved standard-of-care second-line therapy for patients with advanced G/GEJ in many countries.[32]

In conclusion, this exploratory analysis from the phase 3 KEYNOTE-061 trial showed that tumor Tcell$_{inf}$GEP as a continuous variable was associated with clinical outcomes with second-line pembrolizumab in advanced G/GEJ cancer. The Tcell$_{inf}$GEP-adjusted mMDSC signature as a continuous variable was negatively associated with clinical outcomes with pembrolizumab. This analysis suggests that myeloid-driven suppression may play a role in resistance to anti-PD-1 therapy and supports a strategy of

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considering immunotherapy combinations intended to target the myeloid axis.

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Competing interests KS: reports receiving research funding paid to his institution from Astellas Pharma, Ono Pharmaceutical, Daiichi Sankyo, Taiho Pharmaceutical, Chugai Pharma, MSD, Amgen, Eisai, and Medi Science; consulting fees for advisory role from Eli Lilly and Company, Bristol Myers Squibb, Takeda Pharmaceuticals, Pfizer, Ono Pharmaceutical, Novartis, AbbVie, Daiichi Sankyo, Taiho Pharmaceutical, GlaxoSmithKline, Amgen, Boehringer Ingelheim, MSD, Astellas, Guardant Health Japan, and Janssen; and honoraria for lectures from Bristol Myers Squibb, Takeda Pharmaceuticals, and Janssen. MDB: reports participation on a data safety monitoring board/advisory board for MDS. MM: reports no disclosures. M-HR: reports receiving consulting fees from Ono Pharmaceutical, Bristol Myers Squibb, MSD, Eli Lilly, Taiho, Novartis, AstraZeneca, and Daiichi Sankyo. CC: reports receiving payment or honoraria from MSD and Roche, and other financial or nonfinancial interests paid to his institution for participating as a principal investigator or subinvestigator for MSD, Medivation, AstraZeneca, Roche, Astellas Pharma, Bristol Myers Squibb, GlaxoSmithKline, Athenex, Daiichi Sankyo, and Sanofi. TO: reports no disclosures. HCC: reports receiving study material and medical writing support for the present work from Merck; grants or contracts paid to his institution by Eli Lilly, GSK, MSD, Merck Serono, Bristol Myers Squibb/Ono Pharmaceutical, Taiho, Amgen, BeiGene, Incyte, and Zymeworks; consulting fees from Taiho, Celltrion Healthcare, MSD, Eli Lilly, Bristol Myers Squibb/Ono Pharmaceutical, Merck-Serono, Beigene/Amgen, and Zymeworks; and payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, or educational events from Merck Serono and Eli Lilly; and participation on a data safety monitoring board or advisory board for MSD. KM: reports receiving research funding paid to his institution from Solasia Pharma, Merck Serono, Daiichi Sankyo, Parexel International, Pfizer, MSD, Amgen, Ono Pharmaceutical, Astellas, Sanofi, Taiho, and Eisai; consulting fees from AstraZeneca, Ono Pharmaceutical, and Amgen; honoraria for lectures from Ono Pharmaceutical, Taiho, Bristol Myers Squibb, and Eli Lilly; and participation on an advisory board for Ono Pharmaceutical, Amgen, AstraZeneca, Eli Lilly, and Takeda. EG: reports no disclosures. RSM: reports no disclosures. WM: reports no disclosures. ZW: reports receiving support for the present work from Merck; grants paid to his institution from Novartis, Bristol Myers Squibb, Plexxikon, and Arcus; consulting fees from AstraZeneca, Arcus, Amgen, Bristol Myers Squibb, Novartis, Eli Lilly, Bayer, Daiichi, Merck, and Astellas; support for attending meetings and/or travel from Amgen; and participation on a data safety monitoring board or advisory board for Mirati and Pfizer. C-SS: employee of Merck Sharp & Dohme, a subsidiary of Merck & Co, Rahway, New Jersey, USA. JK: employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, New Jersey, USA, and has stock in Merck & Co., Inc., Rahway, NJ, USA. MN: employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, New Jersey, USA, and has stock in Merck & Co., Inc., Rahway, NJ, USA. RC: employee of Merck Sharp & Dohme, a subsidiary of Merck & Co., Rahway, New Jersey, USA, and has stock in Merck & Co., Rahway, New Jersey, USA; and reports a pending patent (WO 2020/167619) related to the application of Angiogenesis and mMDSC gene expression-based biomarker of tumor response to PD-1 antagonists. ZAC: employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, New Jersey, USA, and has stock in Merck & Co., Inc., Rahway, NJ, USA. AL: employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, New Jersey, USA, and has stock in Merck & Co., Inc., Rahway, NJ, USA. MÖ: reports no disclosures.

Patient consent for publication Not applicable.

Ethics approval The trial protocol and all amendments were approved by the institutional review board or ethics committee at each participating institution. The name of each ethics committee/institutional review board at each participating center including approval numbers are shown in online supplemental table 1. The trial was conducted in accordance with the protocol, its amendments, the ethical principles originating from the Declaration of Helsinki, and Good Clinical Practice guidelines. Written informed consent was provided by all patients before enrolment.

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Data availability statement Data are available on reasonable request. Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, 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 USA and the European Union 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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