Short Communication

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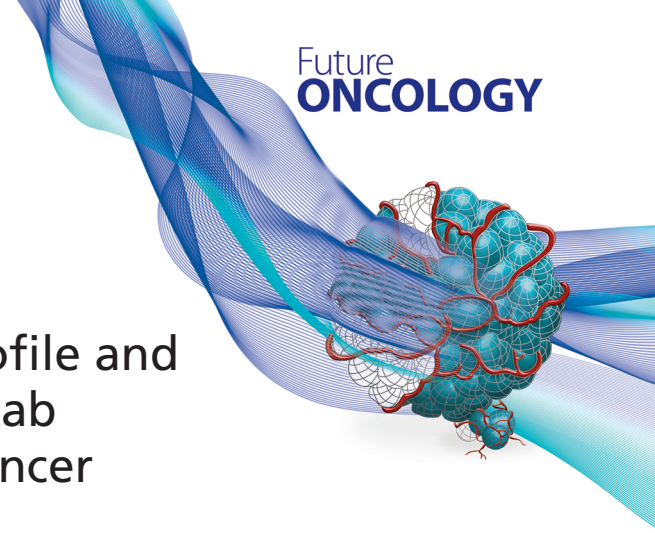

T cell-inflamed gene expression profile and PD-L1 expression and pembrolizumab efficacy in advanced esophageal cancer

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Aim: Investigate the relationship between response to pembrolizumab and expression of the 18-gene T cell-inflamed gene expression profile ($Tcell_{inf}$GEP) or PD-L1 combined positive score (CPS) in esophageal cancer. **Materials & methods:** This analysis included heavily pretreated patients with advanced/metastatic esophageal/gastroesophageal junction adenocarcinoma or squamous cell carcinoma who received pembrolizumab in the single-arm, phase II study KEYNOTE-180. PD-L1 CPS was evaluated with PD-L1 IHC 22C3 pharmDx. **Results:** In patients with squamous cell carcinoma, trends toward enrichment for responders were observed for patients with PD-L1 CPS ≥10 tumors. In patients with adenocarcinoma, a trend was observed for $Tcell_{inf}$GEP but not for PD-L1. **Conclusion:** $Tcell_{inf}$GEP and PD-L1 CPS may enrich for responders to pembrolizumab in patients with esophageal cancer.

Clinical Trial Registration: NCT02559687 (ClinicalTrials.gov)

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Keywords: esophageal cancer • gene expression profile • pembrolizumab • programmed death ligand 1 • tumor microenvironment

Biomarkers currently used or under investigation that predict immunotherapy response include expression of PD-L1 [1], tumor mutational burden [2,3] and gene expression profiles (GEPs) [2–4]. The T cell-inflamed GEP ($Tcell_{inf}$GEP) [2–4] was developed using data from clinical studies of pembrolizumab [5]. The GEP has been examined in esophageal tumor samples, including those of 18 patients who were included in the population used to develop the $Tcell_{inf}$GEP [4]. In pembrolizumab-treated patients with advanced esophageal cancer in KEYNOTE-

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028, the gene signature score (as a continuous variable) showed trends toward statistical significance in response (one-sided p = 0.107) and progression-free survival (PFS; one-sided p = 0.053) [6].

KEYNOTE-180 (NCT02559687) was a single-arm, phase II study of pembrolizumab in heavily pretreated patients with advanced/metastatic esophageal/gastroesophageal junction cancer, irrespective of PD-L1 status (n = 121); objective response rate (ORR) was 9.9%, median duration of response was not reached, and median overall survival (OS) was 5.8 months [7].

We explored the relationship between clinical outcomes of pembrolizumab and the $Tcell_{inf}$GEP score and PD-L1 combined positive score (CPS) status by histology (squamous cell carcinoma [SCC] and adenocarcinoma) in patients with esophageal cancer from KEYNOTE-180.

Materials & methods

The design of KEYNOTE-180 has been described [7]; details are included in the supplement. In the current analysis, outcomes were assessed based on $Tcell_{inf}$GEP score and PD-L1 CPS status; analysis by histologic subgroup was exploratory.

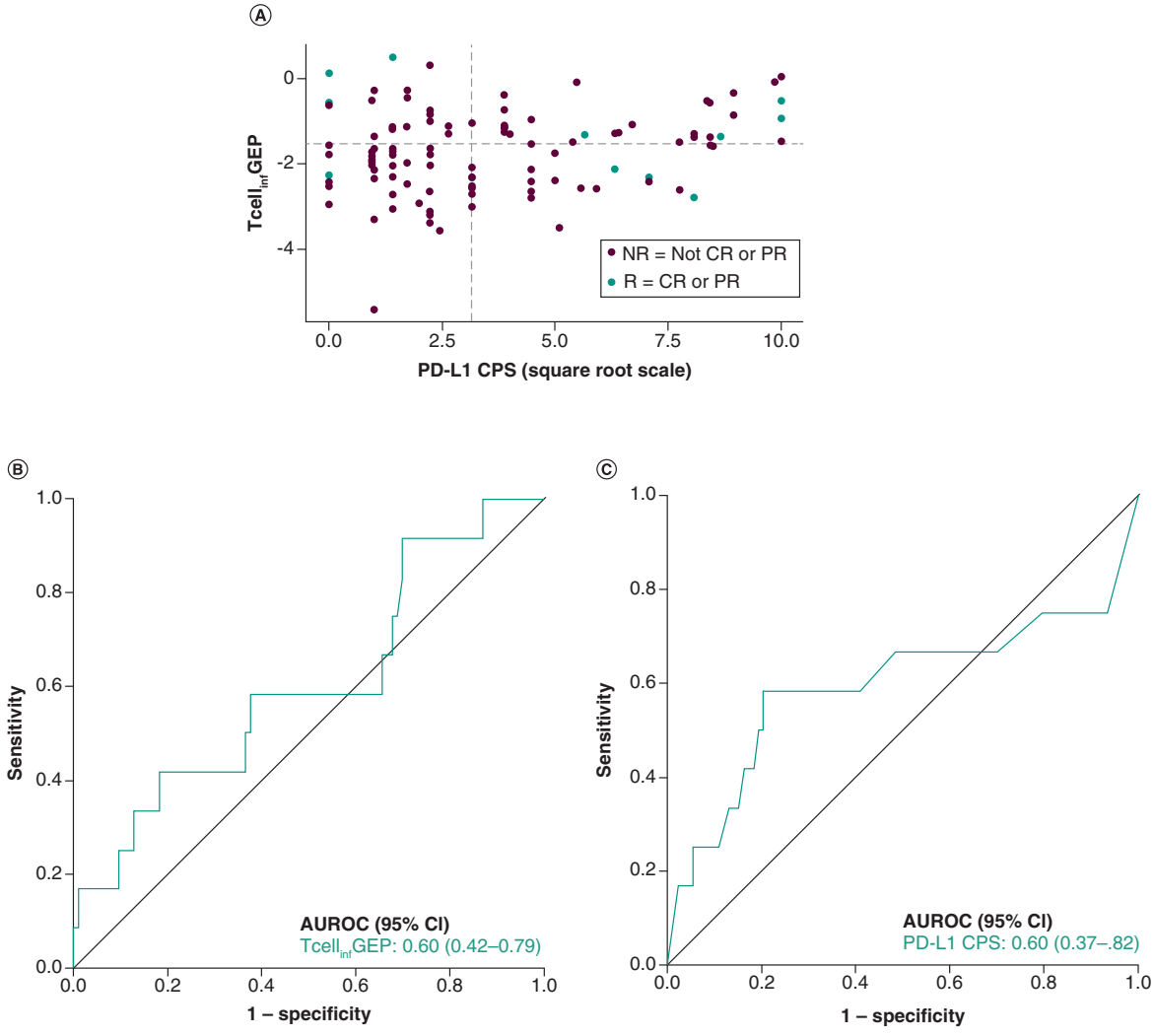

Figure 1. Correlation of T cell-inflamed gene expression profile and PD-L1 combined positive score and associations with response to pembrolizumab. (A) The Spearman correlation was 0.17. AUROC for **(B)** $Tcell_{inf}$GEP score and **(C)** PD-L1 CPS status.
AUROC: Area under the receiver operating curve; CPS: Combined positive score; CR: Complete response; NR: Nonresponder; PR: Partial response; R: Responder; $Tcell_{inf}$GEP: T cell-inflamed gene expression profile.

Table 1. T cell-inflamed gene expression profile score by PD-L1 combined positive score status.

	$Tcell_{inf}GEP^{non\text{-}low}$	$Tcell_{inf}GEP^{low}$
PD-L1 CPS ≥10	31 (25.6%)	26 (21.5%)
PD-L1 CPS <10	20 (16.5%)	41 (33.9%)

$Tcell_{inf}$GEP scores were missing for three patients.
CPS: Combined positive score; $Tcell_{inf}$GEP: T cell-inflamed gene expression profile.

Table 2. Response summary based on central radiology review per RECIST v1.1 by T cell-inflamed gene expression profile score or PD-L1 combined positive score status and histology.

	SCC N = 63				AC N = 55			
	$Tcell_{inf}GEP^{non\text{-}low}$ n = 26		$Tcell_{inf}GEP^{low}$ n = 37		$Tcell_{inf}GEP^{non\text{-}low}$ n = 25		$Tcell_{inf}GEP^{low}$ n = 30	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Confirmed objective response†	4 (15.4)	4.4–34.9	5 (13.5)	4.5–28.8	3 (12.0)	2.5–31.2	0	–
Complete response	0	–	2 (5.4)	0.7–18.2	0	–	0	–
Partial response	4 (15.4)	4.4–34.9	3 (8.1)	1.7–21.9	3 (12.0)	2.5–31.2	0	–
Stable disease	7 (26.9)	11.6–47.8	9 (24.3)	11.8–41.2	4 (16.0)	4.5–36.1	3 (10.0)	2.1–26.5
Disease control‡	11 (42.3)	23.4–63.1	14 (37.8)	22.5–55.2	7 (28.0)	12.1–49.4	3 (10.0)	2.1–26.5
Progressive disease	14 (53.8)	33.4–73.4	20 (54.1)	36.9–70.5	15 (60.0)	38.7–78.9	21 (70.0)	50.6–85.3
No assessment§	1 (3.8)	0.1–19.6	3 (8.1)	1.7–21.9	3 (12.0)	2.5–31.2	6 (20.0)	7.7–38.6
	SCC N = 63				AC N = 58			
	CPS ≥10 n = 35		CPS <10 n = 25		CPS ≥10 n = 23		CPS <10 n = 35	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Confirmed objective response†	7 (20.0)	8.4–36.9	2 (7.1)	0.9–23.5	1 (4.3)	0.1–21.9	2 (5.7)	0.7–19.2
Complete response	1 (2.9)	0.1–14.9	1 (3.6)	0.1–18.3	0	–	0	–
Partial response	6 (17.1)	6.6–33.6	1 (3.6)	0.1–18.3	1 (4.3)	0.1–21.9	2 (5.7)	0.7–19.2
Stable disease	7 (20.0)	8.4–36.9	9 (32.1)	15.9–52.4	6 (26.1)	10.2–48.4	3 (8.6)	1.8–23.1
Disease control‡	14 (40.0)	23.9–57.9	11 (39.3)	21.5–59.4	7 (30.4)	13.2–52.9	5 (14.3)	4.8–30.8
Progressive disease	21 (60.0)	42.1–76.1	13 (46.4)	27.5–66.1	12 (52.2)	30.6–73.2	25 (71.4)	53.7–85.4
No assessment§	0	–	4 (14.3)	4.0–32.7	4 (14.7)	5.0–38.8	5 (14.3)	4.8–30.3

†Objective response defined as complete response plus partial response.
‡Disease control defined as complete response plus partial response plus stable disease.
§Patients who had a baseline assessment but no postbaseline assessment on the data cutoff date because of missing data, discontinuation from the study or death before the first postbaseline imaging.
AC: Adenocarcinoma; CPS: Combined positive score; RECIST v1.1: Response Evaluation Criteria in Solid Tumors: version 1.1; SCC: Squamous cell carcinoma; $Tcell_{inf}$GEP: T cell-inflamed gene expression profile.

Tumor expression levels of 18 genes were determined using the NanoString nCounter Analysis System from tumor samples, and the individual expression levels of the genes were combined as a weighted average to obtain a single $Tcell_{inf}$GEP score. A prespecified, validated cutoff was used to divide tumors into 'low' and 'non-low' categories [4]. PD-L1 expression was characterized using PD-L1 IHC 22C3 pharmDx (Agilent) and measured using CPS.

We report efficacy data in all patients who received ≥1 dose of pembrolizumab and had evaluable $Tcell_{inf}$GEP or PD-L1. Only confirmed objective responses, defined as complete response plus partial response, were reported.

Results

Between 12 January 2016 and 21 March 2017, 121 patients were enrolled (data cutoff: 30 July 2018); 51 patients (42.1%) had $Tcell_{inf}GEP^{non\text{-}low}$ tumors (Supplementary Figure 1) and 58 (47.9%) had PD-L1 CPS ≥10 tumors (Supplementary Figure 2). Baseline characteristics were generally well balanced between $Tcell_{inf}$GEP (Supplementary Table 1) and PD-L1 CPS (Supplementary Table 2) subgroups.

PD-L1 CPS and the $Tcell_{inf}$GEP scores showed a modest positive correlation (Figure 1A). The prevalence of $Tcell_{inf}$GEP and PD-L1 CPS by region and histology are reported in Supplementary Table 3; 46 tumors (38.0%) were discordant for PD-L1 CPS and $Tcell_{inf}$GEP score (Table 1).

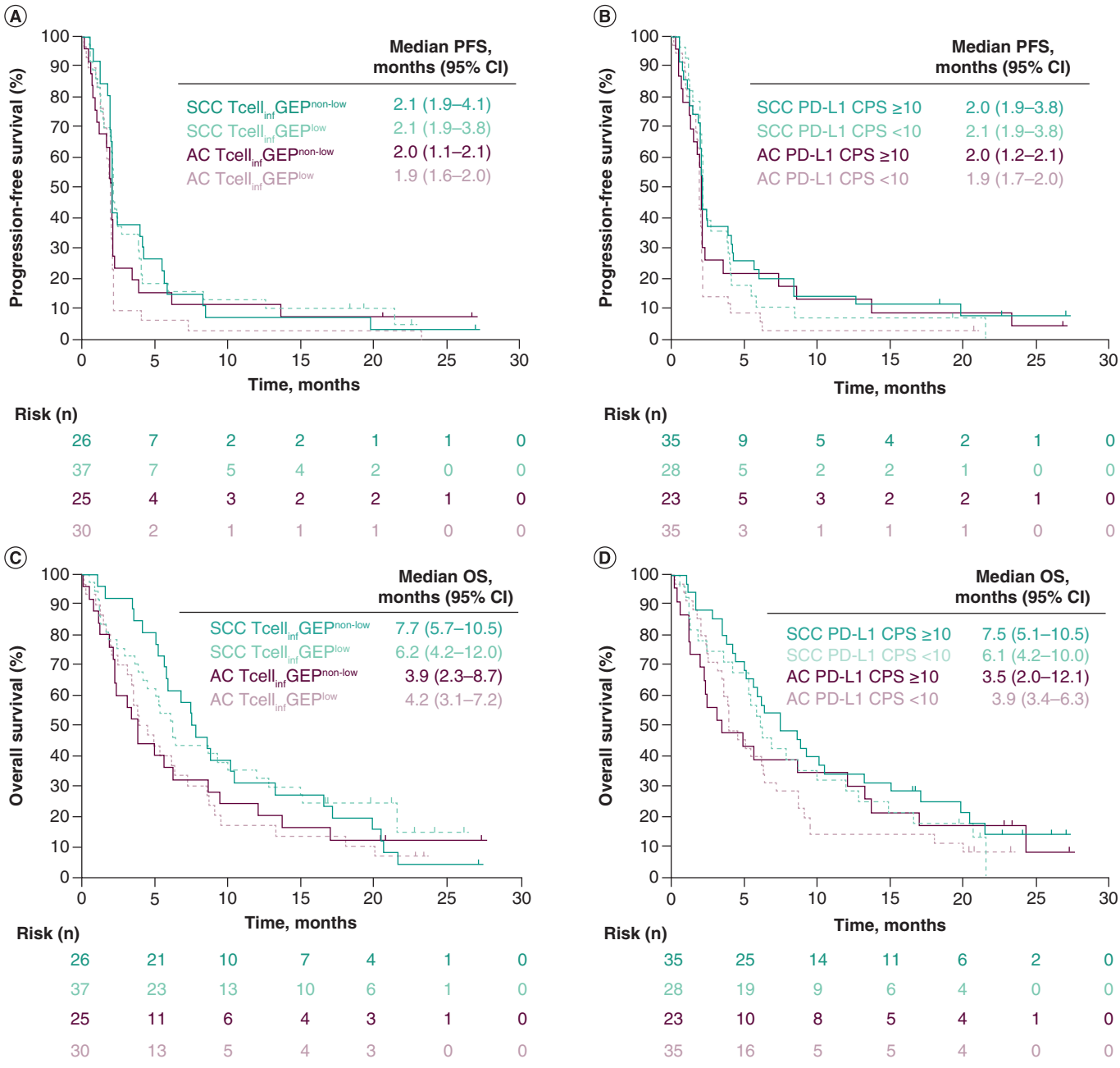

Figure 2. Kaplan–Meier estimates of survival outcomes and the T cell-inflamed gene expression profile score and PD-L1 combined positive score status by histology. Kaplan–Meier estimates of PFS by histology, **(A)** Tcell$_{inf}$GEP score, and **(B)** PD-L1 CPS status and Kaplan–Meier estimates of OS by histology, **(C)** Tcell$_{inf}$GEP score and **(D)** PD-L1 CPS status.
AC: Adenocarcinoma; CPS: Combined positive score; OS: Overall survival; PFS: Progression-free survival; SCC: Squamous cell carcinoma; Tcell$_{inf}$GEP: T cell-inflamed gene expression profile.

A summary of responses is reported in Table 2. AUROC estimates were modest overall for Tcell$_{inf}$GEP and PD-L1 when pooling histologies, but trends suggest an association with increased response to pembrolizumab (Figure 1). The clinical utility of the PD-L1 CPS cutoff is shown in Supplementary Table 4.

In patients with SCC, median PFS was 2.1 and 2.1 months by Tcell$_{inf}$GEP$^{non-low}$ and Tcell$_{inf}$GEP^{low} score and 2.0 and 2.1 months by PD-L1 CPS ≥10 and CPS <10 status (Figure 2A–B). Median OS was 7.7 and 6.2 months by Tcell$_{inf}$GEP$^{non-low}$ and Tcell$_{inf}$GEP^{low} score and 7.5 and 6.1 months by PD-L1 CPS ≥10 and CPS <10 status

(Figure 2C–D). PFS and OS medians were similar across $Tcell_{inf}GEP$ and PD-L1 CPS subgroups in patients with adenocarcinoma.

Discussion

In the primary analysis of KEYNOTE-180, pembrolizumab provided durable antitumor activity and an acceptable safety profile in some patients with heavily pretreated advanced/metastatic esophageal cancer [7]. Patients with SCC demonstrated higher ORR than patients with adenocarcinoma (14.3 vs 5.2%) [7]. Response was also evaluated by PD-L1; ORR was 13.8 versus 6.3% in patients with CPS ≥10 versus <10 tumors [7]. These data suggest the possibility that biomarkers and disease characteristics can be used to enrich for higher efficacy, which was further investigated in the current analysis.

Higher levels of PD-L1 expression have generally been shown to correlate with PD-1/PD-L1 inhibitor response across tumor types, with some variability, possibly because of differing definitions of PD-L1 positivity and assays [8]. PD-L1 CPS has been incorporated into regulatory approvals for multiple indications of pembrolizumab, including CPS ≥10 for esophageal cancer [5]. The phase III KEYNOTE-181 trial of pembrolizumab established PD-L1 CPS ≥10 as a cutoff for efficacy in SCC [9]. Other biomarkers may also be useful in predicting response to pembrolizumab in esophageal cancer.

In this analysis, we present clinical outcomes based on $Tcell_{inf}GEP$ score and PD-L1 CPS status by histology. Although both $Tcell_{inf}GEP$ and PD-L1 are viewed as indicative of IFN-γ-driven inflammation, there is considerable discordancy between these two biomarkers, with lower correlation than has been observed in other tumor types [3,10], indicating different aspects of the tumor microenvironment are captured by each marker. Consistent with the findings in the primary analysis, response rates were numerically higher among patients with SCC than among those with adenocarcinoma regardless of $Tcell_{inf}GEP$ score or PD-L1 CPS status. In the analysis of ORR, $Tcell_{inf}GEP^{non\text{-}low}$ appeared to enrich for response among patients with adenocarcinoma. PD-L1 CPS ≥10 enriched for response among patients with SCC but not for patients with adenocarcinoma. Estimates of median PFS were similar across all biomarker subgroups, whereas median OS was marginally longer among patients with SCC whose tumors were classified as $Tcell_{inf}GEP^{non\text{-}low}$ versus $Tcell_{inf}GEP^{low}$ and whose tumors expressed PD-L1 CPS ≥10 versus CPS <10. Patients with adenocarcinoma had similar median OS across the $Tcell_{inf}GEP$ and PD-L1 CPS subgroups.

Limitations of the current study include the single-arm nonrandomized study design, the modest sample sizes and the small number of responders, leading to wide CIs; all results should be interpreted with caution. Larger randomized studies will facilitate better interpretation of the relationship between these inflammatory biomarkers and time-to-event end points.

Conclusion

Our findings suggest that these measures of inflammation – PD-L1 and $Tcell_{inf}GEP$ – may enrich for positive clinical outcomes from treatment with pembrolizumab. In SCC, a trend toward enrichment was observed for patients with PD-L1 CPS ≥10 tumors. In adenocarcinoma, a trend was observed for $Tcell_{inf}GEP$ but not for PD-L1 CPS. Additional studies are needed to facilitate understanding of the molecular correlates in adenocarcinoma. The cytokine IFN-γ has an important role in immune regulation that can be exploited by cancer cells [4]; work in a pan-tumor setting led to the development of an 18-gene $Tcell_{inf}GEP$ as a biomarker for pembrolizumab efficacy [2,4]. Findings in KEYNOTE-180 are consistent with analyses using esophageal tumor samples from KEYNOTE-028 showing an improved propensity for response to pembrolizumab with higher levels of IFN-γ-related gene expression [6].

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2021-1134

Author contributions

M Ayers, P Enzinger and MJ Marton contributed to conception, design or planning of the study. H-T Arkenau, F El-Hajbi, P Enzinger, M Gupta, A Hollebecque, T Kojima, AC Lockhart, F Lordick, P Pfeiffer, J Raimbouurg, MA Shah and M Tajika contributed to acquisition of data. H-T Arkenau, D Hochhauser, F Lordick, MA Shah, J Lunceford and S Suryawanshi contributed to data analysis. H-T Arkenau, P Bhagia, ZA Cao, K Kato, S-B Kim, AC Lockhart, MJ Marton, MA Shah, D Hochhauser, A Hollebecque,

J Lunceford, P Pfeiffer and S Suryawanshi contributed to the interpretation of the data. ZA Cao, J Lunceford and MA Shah drafted the manuscript. All authors participated in critically reviewing or revising the manuscript for important intellectual content and approved the final manuscript for submission.

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Ethical conduct of research

The protocol was approved by all participating institutions. The study was conducted in accordance with the Declaration of Helsinki and International Good Clinical Practice Guidelines. All patients provided written informed consent.

Data sharing statement

Merck Sharp & Dohme Corp., 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

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Summary points

- We explored the relationship between clinical outcomes of pembrolizumab and the 18-gene T cell-inflamed gene expression profile ($Tcell_{inf}GEP$) score and PD-L1 status by histology in patients with esophageal cancer.
- Heavily pretreated patients with advanced/metastatic esophageal/gastroesophageal junction adenocarcinoma or squamous cell carcinoma received pembrolizumab in the single-arm, phase II study KEYNOTE-180.
- In patients with esophageal squamous cell carcinoma, trends toward enrichment for responders were observed for patients with PD-L1 combined positive score $\geq$10 tumors.
- In patients with esophageal adenocarcinoma, a trend was observed for $Tcell_{inf}GEP$ but not for PD-L1 combined positive score.
- Our findings suggest that these measures of inflammation – $Tcell_{inf}GEP$ and PD-L1 – may enrich for positive clinical outcomes in esophageal cancer from treatment with pembrolizumab.
- Additional studies are needed to facilitate understanding of the molecular correlates in esophageal adenocarcinoma.

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