improvement in clinical efficacy (40% objective response rate [ORR], 5.26 month median progression-free survival [PFS], and 15.2 month median OS) than other subgroups (table 1). In addition to immune-related genes in the microenvironment, DEG analysis also revealed that tumor-related genes were highly expressed in non-responders, such as intrinsic genes related to angiogenesis (VEGFA [P=0.07], KDR [P=0.07]), the mTOR pathway (MTOR [P=0.015]), and DNA damage repair (REV3L [P=0.007]). MTOR and REV3L were associated with shorter PFS (P=0.02; P=0.003) and OS (P=0.03; P =0.008), respectively.

Conclusions By using GEP, T-cell and MHC I GS were identified as potentially predictive biomarkers of response to tislelizumab monotherapy in PD-L1+ UC in this retrospective analysis. By combining these two GS scores, patients with optimal efficacy responses could be identified. Conversely, high expression of tumor intrinsic genes related to angiogenesis and the mTOR pathway may indicate resistance and suggest potential future drug combinations for these patients. Both findings warrant further validation in a phase 3 study (NCT03967977).

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79 TUMOR-IMMUNE SIGNATURES ASSOCIATED WITH RESPONSE OR RESISTANCE TO TISLELIZUMAB (ANTI-PD-1) IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA (ESCC)

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Background Tislelizumab, an anti-PD-1 monoclonal antibody, showed promising clinical outcomes for patients with ESCC. Here, the tumor and immune microenvironment is investigated using gene expression profiles (GEP) and gene signatures associated with clinical efficacy in patients with ESCC receiving tislelizumab either as monotherapy (NCT02407990, NCT04068519) or in combination with chemotherapy (5-fluorouracil plus cisplatin; NCT03469557).

Methods Baseline tumor samples were subjected to GEP using a 1392-gene HTG EdgeSeq panel. Signature scores were calculated using the Gene Set Variation Analysis package with publicly available gene signatures. Differential gene signature (DEG) analysis was performed between responders and non-responders using the Wilcoxon rank-sum test. Associations between gene signatures and survival were evaluated using the Cox proportional hazards model.

Results In GEP-evaluable patients receiving monotherapy (n=43), DEG analysis showed toll-like receptor (TLR) signature scores, driven by TLR8, TLR6, TIRAP, and TLR4, were positively correlated with response and survival, while Treg scores, driven by FOXP3, EBI3, TNFRSF18, and BATF, showed a negative correlation. After combining TLR-high and Treg-low scores (as defined by median cutoff), the prediction of clinical efficacy was further improved (table 1). In addition to Treg scores, non-responders (NR) to tislelizumab monotherapy could be further clustered into four subgroups (NR1, NR2, NR3, and NR4), each exhibiting distinct resistance signatures. Despite a high level of immune infiltration, NR1 expressed a higher exhaustion signature (CD96, CTLA4, TIGIT, HAVCR2, etc.) versus responders (P=0.01). Both NR2 and NR3 demonstrated a trend of enhanced cell-cycle signatures versus responders (P=0.07 and P=0.08, respectively), accompanied by a lower NK signature (KIR2DS4, KIR.panL, CD56) in NR2 and a lack of immune infiltration in NR3. In the NR4 subgroup, a trend toward higher TH17 (P<0.01) and IL-17F signatures ($Log_2FC=0.56$, P=0.10) versus responders was observed. GEP-evaluable patients (n=12) receiving tislelizumab in combination with chemotherapy had an objective response rate of 58% (n=7), with a different gene signature pattern than those observed in patients receiving monotherapy. Responders to combination therapy showed higher DNA repair expression versus NR (P=0.07), while angiogenesis signatures were significantly higher in NR vs responders (P=0.01). Consistent with this, NR exhibited higher expression of VEGFC at a single gene level ($Log_2FC=2.46$, P <0.01).

Abstract 79 Table 1 Tumor-immune signatures associated with clinical efficacy of tislelizumab in patients with ESCC

Tislelizumab monotherapy	GEP-evaluable	Single TLR signature		Single Treg signature		Combined signature	
Subgroup	n=43	TLR-high (n=21)	TLR-low* (n=22)	Treg-high (n=21)	Treg-low* (n=22)	TLR-high and Treg-low (n=10)	Others* (n=33)
ORR, n (%)	6 (14.0)	5 (23.8)	1 (4.5)	1 (4.8)	5 (22.7)	4 (40.0)	2 (6.1)
DCR, n (%)	15 (34.9)	11 (52.4)	4 (18.2)	4 (19.0)	11 (50.0)	8 (80.0)	7 (21.2)
Median PFS, mo (95% CI)	2.09 (2.00-4.17)	2.50 (2.04-8.02)	2.00 (1.64-2.63)	2.04 (1.87-2.63)	2.50 (2.00-8.02)	6.31 (2.50-NR)	2.00 (1.87-2.27)
Hazard ratio (95% CI)	NA	0.51 (0.27-0.99)		1.74 (0.89-3.4)		0.40 (0.18-0.89)	
Median OS, mo (95% CI)	4.76 (3.65-8.44)	7.92 (4.14-NR)	3.98 (2.00-8.08)	6.31 (2.63-10.25)	4.76 (2.50-12.95)	8.51 (4.14-NR)	4.44 (2.63-8.44)
Hazard ratio (95% CI)	NA	0.52 (0.26-1.04)		1.14 (0.58-2.28)		0.56 (0.24-1.29)	

*Subgroups were used as reference for hazard ratio analysis.
Abbreviation: CI, confidence interval; DCR, disease control rate; GEP, gene expression profiling; NA, not applicable; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TLR, toll-like receptor.

Conclusions While higher TLR signaling was associated with clinical benefit of tislelizumab monotherapy, elevated Treg, exhaustion, cell cycle, and TH17 signatures may indicate resistance. Signatures predictive for combination therapy may vary. Both immune- and tumor-related features may be considered for validation in phase 3 studies (NCT03430843, NCT03783442).

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80 EVALUATION OF THE TRUSIGHT ONCOLOGY 500 ASSAY FOR ROUTINE CLINICAL TESTING OF TUMOR MUTATIONAL BURDEN (TMB) AND CLINICAL UTILITY FOR PREDICTING RESPONSE TO PEMBROLIZUMAB

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Background Various biomarkers have been investigated for their ability to identify patients more likely to respond to

immunotherapy. Recently, the PD-1 inhibitor pembrolizumab was approved by the FDA for treating patients with unresectable or metastatic solid tumors with high TMB (TMB-H) who have no satisfactory alternative treatment options following progression on prior treatment. The FDA contemporaneously approved the FoundationOne®CDx (F1CDx; Foundation Medicine) as the companion diagnostic for TMB assessment for pembrolizumab. However, multiple comprehensive genomic profiling panels that can measure TMB are currently available or in development. We evaluated the performance of TruSight™ Oncology 500 (TSO500; Illumina) for assessing TMB and its clinical utility using F1CDx and whole exome sequencing (WES) as reference methods.

Methods Pretreatment archival tumor samples from patients enrolled in 8 clinical trials of pembrolizumab monotherapy were evaluated for TMB by TSO500, F1CDx QSR pipeline v3.2.0, and WES. Correlation was assessed using Spearman's rank correlation coefficient (ρ). The F1CDx and WES TMB cutpoints were 10 mut/Mb and 175 mut/exome, respectively. The TSO500 cutpoint was selected using the Youden index criterion. Concordance was assessed by calculating area under the receiver-operating curve (AUROC), positive percentage agreement (PPA), and negative percentage agreement (NPA). Statistical significance of the association of TMB measured by TSO500 with ORR was assessed using logistic regression adjusted for ECOG performance status and cancer type. Clinical utility of the selected TSO500 TMB cutpoint for discriminating responders and nonresponders was assessed by calculating sensitivity, specificity, positive predictive value, negative predictive value, ORR enrichment, and prevalence.

Results TMB scores were valid for 294/294 patients assessed by TSO500, 269/270 assessed by F1CDx, and 293/294 assessed by WES. TMB assessed by TSO500 had good correlation with TMB assessed by F1CDx (ρ=0.76) and WES (ρ=0.74). Using Youden index criterion, 10 mut/Mb was the TSO500 cutpoint that corresponded with both the F1CDx and WES cutpoints. TSO500 reliably predicted TMB-H and non–TMB-H status as determined by the F1CDx (AUROC=0.99, PPA=97.4%, NPA=93.0%) and WES (AUROC=0.95, PPA=76.2%, NPA=96.1%) cutpoints. TMB measured by TSO500 was significantly associated with ORR (one-sided P<0.0001). Clinical utility metrics were generally similar for TSO500 and F1CDx (table 1) and TSO500 and WES (table 2).

Conclusions TMB assessed by TSO500 is highly correlated and concordant with TMB assessed by F1CDx and WES. Similar to the validated and approved F1CDx TMB cutpoint of 10 mut/Mb, the TSO500 TMB cutpoint of 10 mut/Mb is predictive of response to pembrolizumab monotherapy.

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Trial Registration NA

Ethics Approval The protocols and all amendments for the studies included in this analysis were approved by the appropriate ethics committee at each participating institution.

Consent NA

Abstract 80 Table 1 Clinical Utility Metrics for the TSO500 TMB Cutpoint Compared with the F1CDx TMB Cutpoint (n=269)

	TSO500 at 10 mut/Mb	F1CDx at 10 mut/Mb
Sensitivity	0.425	0.350
Specificity	0.838	0.890
Positive predictive value[a]	0.315	0.359
Negative predictive value[b]	0.888	0.883
ORR enrichment[c]	2.820	3.058
Prevalence of TMB-H	20.0%	14.5%

[a]Response rate for patients with TMB-H = positive predictive value * 100.
[b]Response rate for patients with non-TMB-H = (1 – negative predictive value) * 100.
[c]Enrichment = positive predictive value / (1 – negative predictive value).

Abstract 80 Table 2 Clinical Utility Metrics for the TSO500 TMB Cutpoint Compared with the WES TMB Cutpoint (n=293)

	TSO500 at 10 mut/Mb	WES at 175 mut/exome
Sensitivity	0.419	0.465
Specificity	0.844	0.828
Positive predictive value[a]	0.316	0.317
Negative predictive value[b]	0.894	0.900
ORR enrichment[c]	2.981	3.175
Prevalence of TMB-H	19.5%	21.5%

[a]Response rate for patients with TMB-H = positive predictive value * 100.
[b]Response rate for patients with non-TMB-H = (1 – negative predictive value) * 100.
[c]Enrichment = positive predictive value / (1 – negative predictive value).

REFERENCE

NA

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81 INCLUSION OF PD-L1-EXPRESSING TUMOR CELLS IN THE COMBINED POSITIVE SCORE ALGORITHM YIELDS SUPERIOR IDENTIFICATION OF POSITIVE SPECIMENS AROUND DIAGNOSTIC CUT-OFFS ACROSS MULTIPLE INDICATIONS

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Background PD-L1 IHC 22C3 pharmDx uses Tumor Proportion Score (TPS) and Combined Positive Score (CPS) scoring algorithms for the immunohistochemical (IHC) evaluation of PD-L1 protein expression in human cancer tissues; both algorithms include PD-L1 staining tumor cells (TC) in scoring and CPS also includes scoring of PD-L1 staining mononuclear inflammatory cells to aid in the identification of patients for treatment with pembrolizumab (KEYTRUDA®) using indication-specific diagnostic cut-offs. This study evaluated contribution of TC in determining specimen diagnostic status based on the CPS scoring algorithm by looking into four tumor indications approved for use with KEYTRUDA®: gastric or gastroesophageal junction (GEJ) adenocarcinoma (GC/GEJ), urothelial carcinoma (UC), head and neck squamous cell carcinoma (HNSCC), and esophageal squamous cell carcinoma (ESCC). Detection of specimens expressing PD-L1 is significantly dependent on the PD-L1 staining TC component.

Methods A retrospective analysis was done looking at Dako's internal tumor bank of the mentioned indications that were all stained with PD-L1 IHC 22C3 pharmDx and scored using the TPS, CPS and Quantitative Immune Cell Density (QID) methods described in figure 1. Statistical analysis encompassed looking at the scores generated that met the following criteria: CPS>0, TPS>0 and CPS≠TPS and then evaluating the percentage of those samples that changed from positive to negative diagnostic status upon removal of the TC component from the scoring.

Results A noticeable downward trend was observed in all four indications in the total number of positives with the removal of the TC component. Table 1 aptly captures this by showing the number of specimens for each indication that had changed from positive to negative around each indication's diagnostic cut-off(s). The three indications that showed the highest