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).

Method 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 nonresponders 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, nonresponders (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).

Conclusion 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).

Table 1.

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.

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Trial Registration: NCT02407990, NCT04068519, NCT03469557