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

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BACKGROUND

- Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers associated with high mortality and has a low 5-year overall survival (OS) rate when diagnosed at an advanced stage[1-2]
- Recently, treatment with programmed cell death protein-1 (PD-1) inhibitors in ESCC have shown promising clinical benefit, but the mechanisms of response or resistance are not very clear yet and further exploration is needed[3-6]
- Tislelizumab, an anti–PD-1 monoclonal antibody, has been approved by the China National Medical Products Administration (NMPA) as a treatment for patients with classical Hodgkin lymphoma and for patients with locally advanced or metastatic urothelial carcinoma with PD-L1–high expression
- Tislelizumab also demonstrated clinical activity in patients with ESCC as a single agent (NCT02407990 and NCT04068519) and in combination with chemotherapy (NCT03469557)[7-9]
- Here we show the retrospective analysis of immune and tumor-transcriptomic features and its association with tislelizumab efficacy in ESCC

METHODS

Study Design

- Pooled analysis from three clinical trials
  - BGB-A317-001 (NCT02407990): First-in-human, multicenter, phase1a/1b dose-escalation/indication-expansion study
    - Samples were analyzed from patients with advanced or metastatic ESCC
  - BGB-A317-102 (NCT04068519): Chinese, multicenter, phase1/2 study
    - Samples were analyzed from patients with previously treated/untreated advanced or metastatic ESCC
  - BGB-A317-205 (NCT03469557): Phase 2 first-line study of tislelizumab plus fluorouracil and cisplatin in Chinese patients
    - Samples were analyzed from patients with locally advanced or metastatic ESCC

Gene Expression Profiling (GEP)

- Baseline tumor samples (formalin-fixed, paraffin-embedded blocks or cut slides) were applied to GEP by HTG EdgeSeq Precision Immuno-Oncology Panel (containing 1392 genes)
- Signature scores were calculated using the Gene Set Variation Analysis (GSVA) package with publicly available gene signatures
- Differentially expressed gene or gene signature analysis was performed between responders (Rs) and non-responders (NRs)
- Non-responder subgroups were hierarchically clustered by one minus Pearson's correlation with average linkage by columns

Statistical Analysis

- Gene signature statistical analysis was tested by two-sided Wilcoxon rank-sum test, while modified t-test with limma was used for differentially expressed gene analysis
- Associations with survival were analyzed by log-rank test and Cox proportional hazards model

RESULTS

Patient Characteristics

- Of 68 enrolled patients, 55 had samples evaluable for GEP analysis, with no significant difference in baseline disease characteristics and clinical outcomes between overall and GEP evaluable patients (Table 1)

Association of TLR and Treg Signatures With Clinical Outcomes of Tislelizumab Monotherapy

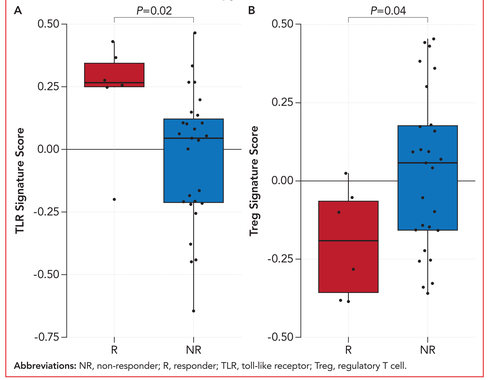
- Of 43 GEP-evaluable patients receiving tislelizumab monotherapy:
  - A 27-gene toll-like receptor (TLR) core network signature[10] (driven by *TLR8*, *TLR6*, *TIRAP*, *TLR4*) was significantly higher in baseline tumors from Rs versus NRs (P=0.02) (Figure 1A)
  - A 26-gene regulatory T cell (Treg) signature[11-12] (driven by *FOXP3*, *EBI3*, *TNFRSF18*, *BATF*) was higher in baseline tumors from NRs versus Rs (P=0.04) (Figure 1B)

Table 1: Baseline Characteristics and Clinical Outcome

Characteristic		Monotherapy Overall (n=53)	Monotherapy GEP (n=43)	Combination Therapy Overall (n=15)	Combination Therapy GEP (n=12)
Age (mean [SD])		59.92 (8.91)	59.33 (9.14)	59.87 (6.41)	59.83 (6.48)
Age <65, n (%)		37 (69.8)	30 (69.8)	10 (66.7)	8 (66.7)
Age ≥65, n (%)		16 (30.2)	13 (30.2)	5 (33.3)	4 (33.3)
Male, n (%)		41 (77.4)	33 (76.7)	14 (93.3)	11 (91.7)
Histologic grade at baseline, n (%)	Poorly differentiated	12 (32.4)	10 (33.3)	5 (35.7)	5 (41.7)
	Moderately differentiated	24 (64.9)	20 (66.7)	6 (42.9)	4 (33.3)
	Well differentiated	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)
	Other	0 (0.0)	0 (0.0)	3 (21.4)	3 (25.0)
Tumor stage at baseline, n (%)	Stage III	2 (3.9)	2 (2.4)	3 (21.4)	4 (27.3)
	Stage IV	49 (96.1)	41 (97.6)	10 (71.4)	8 (72.7)
	Other	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)
Number of lines of prior systemic anticancer therapy (mean [SD])		2.19 (1.37)	2.09 (1.21)	NA	NA
ECOG PS, n (%)	0	10 (18.9)	7 (16.3)	4 (26.7)	4 (33.3)
	1	43 (81.1)	36 (83.7)	11 (73.3)	8 (66.7)
Median follow-up (OS), months (95% CI)		14.52 (10.22, 18.66)	14.52 (12.22, 18.66)	12.98 (12.52, 14.03)	12.98 (12.52, 14.02)
Median PFS, months (95% CI)		2.10 (1.97, 2.63)	2.09 (1.97, 2.50)	10.35 (5.55, NE)	10.61 (5.55, NE)
Median OS, months (95% CI)		4.76 (3.55, 7.92)	4.76 (3.55, 8.08)	10.61 (5.55, NE)	NE
ORR, % (95% CI)		13.21 (5.48, 25.34)	13.95 (5.30, 27.93)	46.67 (21.27, 73.41)	58.33 (27.67, 84.83)

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; GEP, gene expression profiling; NA, not available; NE, not estimable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SD, standard deviation.

Figure 1: TLR and Treg Signatures Were Associated With Response to Tislelizumab Monotherapy



Abbreviations: NR, non-responder; R, responder; TLR, toll-like receptor; Treg, regulatory T cell.

Association of TLR and Treg Signatures With Clinical Outcomes of Tislelizumab Monotherapy

- Clinical outcomes in subgroups defined by TLR, Treg, or combined signatures are summarized in Table 2
- With a median TLR signature cutoff of 0.0474, higher objective response rate (ORR) and disease control rate (DCR), significant improvement of progression-free survival (PFS), and a trend of longer OS were observed in patients with TLR-high versus TLR-low signatures
- Compared to the Treg-low subgroup, the Treg-high subgroup showed lower ORR, DCR, and a shorter PFS trend, while no difference was observed in OS with a median cutoff of -0.036
- Patients with combined TLR-high and Treg-low signatures were associated with further improved clinical activities, including ORR, DCR, median PFS, and median OS

Table 2: Summary of Clinical Outcomes in Subgroups Defined by the Expression of TLR and Treg Signatures

Tislelizumab Monotherapy Subgroup	TLR Signature: TLR-high (n=21)	TLR Signature: TLR-low* (n=22)	Treg Signature: Treg-high (n=21)	Treg Signature: Treg-low* (n=22)	Combined Signatures: TLR-high and Treg-low (n=10)	Combined Signatures: TLR-low or Treg-high* (n=33)
ORR, n (%)	5 (23.8)	1 (4.5)	1 (4.8)	5 (22.7)	4 (40.0)	2 (6.1)
DCR, n (%)	11 (52.4)	4 (18.2)	4 (19.0)	11 (50.0)	8 (80.0)	7 (21.2)
Median PFS, months (95% CI)	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-NE)	2.00 (1.87-2.27)
Hazard ratio (95% CI)	0.51 (0.27-0.99)		1.74 (0.89-3.4)		0.40 (0.18-0.89)	
Median OS, months (95% CI)	7.92 (4.14-NE)	3.98 (2.00-8.08)	6.31 (2.63-10.25)	4.76 (2.50-12.95)	8.51 (4.14-NE)	4.44 (2.63-8.44)
Hazard ratio (95% CI)	0.52 (0.26-1.04)		1.14 (0.58-2.28)		0.55 (0.24-1.29)	

*The subgroups were used as reference for hazard ratio analysis.
Abbreviations: CI, confidence interval; DCR, disease control rate; NE, not estimable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TLR, toll-like receptor; Treg, regulatory T cell.

Four Subgroups of Monotherapy NRs Featured Diverse GEP Signatures

- Monotherapy NRs were clustered into four distinct subgroups according to immune and tumor gene signatures listed in Table 3

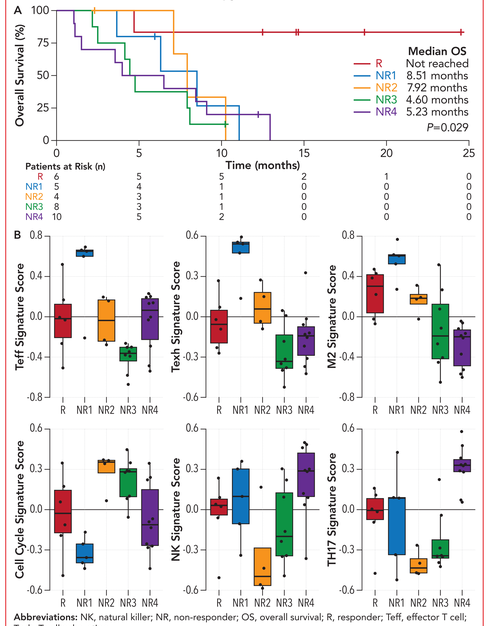
Table 3: Immune and Tumor Gene Signatures Utilized for NR Subgroup Clustering

Tumor Features			Immune Features			
Intrinsic Phenotype	TME Phenotype	Sensitivity to Immunity	Immune Activation	Cytotoxic T cell	Anti-tumor	Pro-tumor
Cell cycle	EMT	Type1 IFN	Cancer antigens	CD8T	NK	TH2
Apoptosis	Angiogenesis	Type2 IFN	Ag present	Cytotoxicity	B cell	TH17
DNA repair	Hypoxia		Infiltration & trafficking	Co-inhibitory	M1 macrophage	Treg
	CAF		TLR	Co-stimulatory	TH1	M2 macrophage
			DC	Exhaustion	γδT	MDSC
			CD45			Neutrophil

Abbreviations: Ag, antigen; CAF, cancer associated fibroblast; DC, dendritic cell; EMT, epithelial-mesenchymal transition; IFN, interferon; MDSC, myeloid-derived suppressor cells; NK, natural killer; NR, non-responder; γδT, gamma delta T cell; TLR, toll-like receptor; TME, tumor immune microenvironment; Treg, regulatory T cell.

- Overall survival and differentially expressed gene signatures among distinct NR subgroups and Rs are shown in Figure 2A and Figure 2B, respectively
  - There was no significant difference in OS between the four NR subgroups
  - Despite a high level of immune infiltration, NR1 (n=5) expressed a higher exhaustion signature (driven by *CD96*, *CTLA4*, *TIGIT*, *HAVCR2*, etc.) versus Rs; M2 macrophage signature was also highly expressed in NR1 versus Rs
  - Both NR2 (n=4) and NR3 (n=8) showed a trend of enhanced cell-cycle signature, accompanied by lower natural killer (NK) cell signature (driven by *KIR2DS4*, *KIR.panL*, *CD56*) in NR2 and lack of immune effector cell infiltration in NR3
  - In the NR4 (n=10) subgroup, a trend toward a higher TH17 signature was observed, which was driven by *IL-17F* (Log_2FC=0.56, P=0.10)

Figure 2: Tumor-Immune Profiles of Rs and NR Subgroups of Tislelizumab Monotherapy



Abbreviations: NK, natural killer; NR, non-responder; OS, overall survival; R, responder; Teff, effector T cell; Texh, T-cell exhaustion.

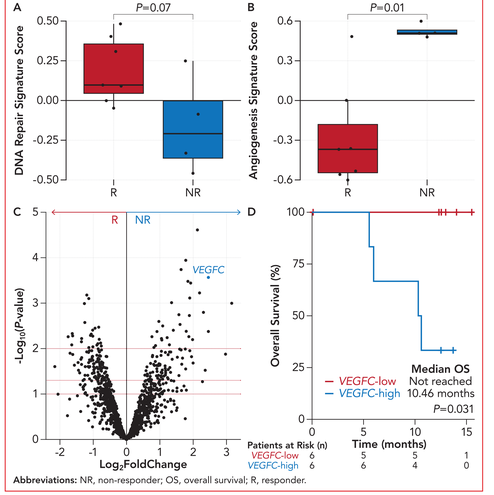
Gene Expression Associated With Clinical Outcomes of Tislelizumab Combination Therapy

- Responders to combination therapy showed a trend of higher DNA repair gene expression signatures compared to NRs (Figure 3A)
- Non-responders had numerically higher angiogenesis signatures versus Rs (P=0.01; Figure 3B); differentially expressed gene analysis revealed *VEGFC* was highly expressed in NRs (Log_2FC=2.46, P<0.01; Figure 3C), suggesting angiogenesis may potentially be associated with resistance to tislelizumab/chemotherapy
- With a median *VEGFC* cutoff of 5.124, a shorter OS was observed in patients with *VEGFC*-high versus *VEGFC*-low gene expression (P=0.031; Figure 3D)

CONCLUSIONS

- Through association analysis of tumor-immune transcription profiles with clinical efficacy, TLR and Treg signatures have been identified as potential biomarkers in patients with ESCC receiving tislelizumab monotherapy
  - A higher TLR signature was associated with favorable clinical outcomes, including higher ORR, longer PFS, and a trend of longer OS
  - An elevated Treg signature was observed in NRs and was associated with unfavorable PFS
  - Clinical efficacy outcomes were further improved in patients whose tumors had combined TLR-high and Treg-low signatures
- In addition to the Treg signature, multiple other gene signatures (eg, extremely high T-cell exhaustion, M2 macrophage, cell cycle, TH17, decreased immune infiltration or NK) may further contribute to resistance in distinct NR subgroups receiving tislelizumab monotherapy
- Gene expression profiling analysis also suggested efficacy-associated tumor-immune profiles in ESCC patients treated with tislelizumab plus chemotherapy
  - A higher DNA repair signature was associated with favorable clinical benefit in combination therapy
  - Responders tended to express lower levels of the angiogenesis signature and *VEGFC* gene expression

Figure 3: Tumor-Immune Profiles of Rs and NRs of Tislelizumab Combination Therapy



Abbreviations: NR, non-responder; OS, overall survival; R, responder.

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