Tumor-immune signatures associated with response or resistance to tislelizumab in patients with previously treated advanced hepatocellular carcinoma

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Introduction

- Tislelizumab is an anti-programmed death protein-1 (PD-1) antibody that has high affinity and binding specificity for PD-1[1–3]
- Tislelizumab demonstrated clinical activity and was generally well tolerated in patients with previously treated advanced hepatocellular carcinoma (HCC) in the open-label, multicenter, Phase 2 RATIONALE-208 study (NCT03419897)[4]
  - After a median follow-up of 12.4 months (data cut-off: February 2020):[4]
    - Objective response rate (ORR) was 13.3% (95% CI: 9.3, 18.1)
    - Median progression-free survival (PFS) was 2.7 months (95% CI: 1.4, 2.8)
    - Median overall survival (OS) was 13.2 months (95% CI: 10.8, 15.0)
- Response to immune checkpoint inhibitors in HCC may be influenced by both tumor-intrinsic factors and extrinsic factors relating to the tumor microenvironment[5]
- We report an exploratory analysis of the association of gene expression profiles (GEPs) with response or resistance to tislelizumab among patients enrolled in the RATIONALE-208 study, through which we:
  - Identify gene signatures (GS) associated with clinical responses or resistance to tislelizumab
  - Define non-responder (NR) subgroups based on tumor and immune GS

Methods

RATIONALE 208 study design

- Study design has been previously described; scan QR code to read full study methods:

Analysis of GEP

- GEP analysis was performed using the HTG EdgeSeq Precision Immuno Oncology panel
- Baseline tumor sampling was optional, and 138 tumor samples were assessed (fresh tumor, n=6; archival tumor, n=132)
- Signature scores were calculated using the Gene Set Variation Analysis package with publicly available GS

Analysis of association between GEP and clinical outcomes

- GS or genes differentially expressed between responders and NRs were determined using the Wilcoxon rank-sum test and modified t-test using limma
- Association of GS with ORR was determined using Fisher's exact test
- Distributions of OS and PFS for GS subgroups were estimated by Kaplan-Meier method
- Hierarchical clustering of NRs was achieved using 1-Pearson's correlation metric and the average linkage method
- All statistical analysis results are *post-hoc* exploratory and thereby p values are descriptive

Results

Baseline patient characteristics and clinical outcomes

- As of February 2020, 249 patients were enrolled and received ≥ 1 dose of tislelizumab
  - 138 patients had evaluable GEP data
  - Demographics and baseline characteristics were similar in the GEP analysis population and overall population (**Table 1**)

Association between GS and response or resistance to tislelizumab

- Among ~450 tumor-immune signatures, the following were enriched in responders (n=19) or NRs (n=113, **Figure 1**):
  - Major histocompatibility complex (MHC) class I, cytotoxic T cell (CTL), CD8 T cell, and CD4 T cell signatures were enriched in responders
  - Cancer-associated fibroblasts (CAF), hypoxia, and angiogenesis signatures were enriched in NRs

Conclusions

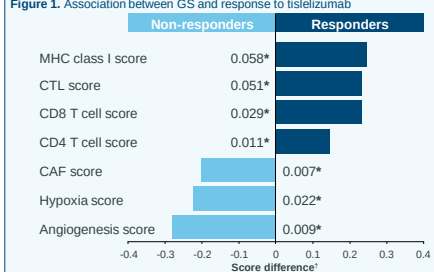
- **This exploratory analysis identified distinct GS associated with tumor response and resistance to tislelizumab monotherapy in patients with previously treated advanced HCC**
  - **High T cell and MHC class I GS, as well as the novel CD8B_PDCD1_9 GS may be associated with better response and longer PFS or OS**
  - **CAF, angiogenesis and hypoxia GS were highly expressed in NRs and may be associated with lack of response**
  - **Elevated DNA repair, cell cycle, Treg, and T cell co-inhibition signatures were also observed in distinct NR subgroups**
- **These findings increase understanding of the tumor microenvironment in HCC**
- **Due to the limitations of a single-arm study, the response and resistance mechanisms discussed in this analysis will be further explored and validated in an ongoing randomized Phase 3 study of tislelizumab vs sorafenib as first-line therapy in patients with advanced HCC (NCT03412773)**

Table 1. Baseline characteristics and clinical outcomes

Characteristic	GEP population (n=138)	Overall population (N=249)[4]
Male, n (%)	115 (83.3)	217 (87.1)
Age, n (%)		
< 65 years	89 (64.5)	149 (59.8)
≥ 65 years	49 (35.5)	100 (40.2)
Region, n (%)		
Mainland China and Taiwan	75 (54.3)	122 (49.0)
Europe	63 (45.7)	127 (51.0)
ECOG PS, n (%)		
0	60 (43.5)	129 (51.8)
1	78 (56.5)	120 (48.2)
Prior lines of therapy, n (%)		
1	84 (60.9)	138 (55.4)
≥ 2	54 (39.1)	111 (44.6)
HCC etiology, n (%)		
Hepatitis B	76 (55.1)	128 (51.4)
Hepatitis C	17 (12.3)	31 (12.4)
Non-viral	45 (32.6)	90 (36.1)
Clinical outcome		
ORR*, n (%)	19 (13.8)	33 (13.3)
Median PFS*, months (95% CI)	2.7 (1.4, 2.8)	2.7 (1.5, 2.8)
Median OS, months (95% CI)	13.8 (10.8, 18.9)	13.2 (10.8, 15.0)

*IRC-assessed; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance score; GEP, gene expression profiling; HCC, hepatocellular carcinoma; IRC, independent review committee; ORR, objective response rate; OS, overall survival; PFS, progression-free survival

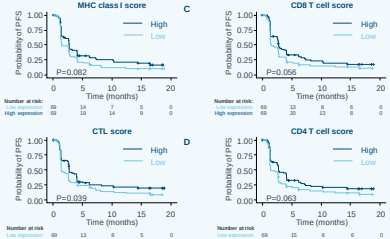
Figure 1. Association between GS and response to tislelizumab



*P value determined by Wilcoxon test; †Median of the difference; GS analysis for response or resistance was applied in 132 patients with evaluable GEP and post-baseline tumor response data
CAF, cancer-associated fibroblasts; CTL, cytotoxic T cell; GEP, gene expression profiling; GS, gene signature; MHC, major histocompatibility

- A trend towards longer PFS was seen in patients with high MHC class I or T cell signature scores (**Figure 2**)

Figure 2. Association between MHC class I or T cell signature scores and PFS



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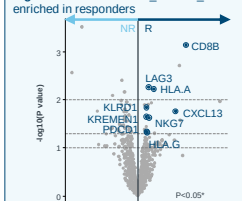
Gene signature	Median PFS, months (95% CI)	
	Low score	High score
MHC class I	1.46 (1.38, 2.76)	2.76 (2.10, 4.11)
CTL	1.45 (1.38, 2.73)	2.76 (2.50, 4.14)
CD8 T cell	1.46 (1.38, 2.76)	2.76 (2.63, 4.14)
CD4 T cell	1.41 (1.38, 2.73)	2.79 (2.63, 4.08)

P values were determined by a log-rank test
CI, confidence interval; CTL, cytotoxic T cell; MHC, major histocompatibility; PFS, progression-free survival

Identification of novel GS associated with response to tislelizumab: CD8B_PDCD1_9

- By analyzing the 1392 genes in the panel, 9 genes were identified as being highly expressed in responders, relating to functions in T/natural killer (NK) cell infiltration and activity (*CD8B, CXCL13, KLRD1, NKG7*), antigen presentation (*HLA-A, HLA-G*), checkpoint inhibition (*LAG3, PDCD1*), and tumor suppression (*KREMEN1*)
- A novel GS (*CD8B_PDCD1_9*) was generated, comprising the 9 genes (**Figure 3**)

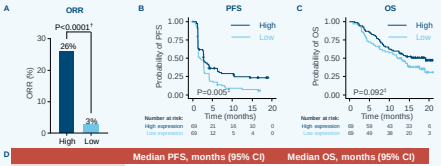
Figure 3. Genes in CD8B_PDCD1_9 were enriched in responders



*P value was determined by t-test using limma
NR, non-responder; R, responder

- Significantly higher ORR and longer PFS, and a trend toward longer OS, were observed in patients with a high vs low CD8B_PDCD1_9 score (**Figure 4**)

Figure 4. Correlation between CD8B_PDCD1_9 GS expression status* and clinical outcomes



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	Median PFS, months (95% CI)	Median OS, months (95% CI)
CD8B_PDCD1_9 Score		
Low	1.84 (1.38, 2.76)	12.00 (7.66, 14.89)
High	2.76 (1.48, 4.14)	19.10 (9.90, NE)

*High or low expression status was defined by the median score of the 9 genes comprising the CD8B_PDCD1_9 GS; †P value determined by Fisher's exact test; ‡P value determined by log-rank test; CI, confidence interval; GS, gene signature; NE, not evaluable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival

Identification of three distinct NR subgroups and associated survival

Table 2. Tumor and immune GS used for NR subgroup clustering

Anti-tumor immune activity	Immune cell-related	Signaling pathway	Tumor features
IFNγ	CD8 or CD4 T cell	TGFβ	EMT
Cytotoxicity	MDSC	Wnt	DNA repair
Inflammatory	NK cell	TNF	Angiogenesis
MHC class I	B cell	NF-κB	Hypoxia
Immune checkpoint	Treg	NOD-like pathway	CAF
TIS	Macrophage		Cell cycle
	Dendritic cell		Apoptosis
	T cell co-inhibition		

CAF, cancer-associated fibroblasts; EMT, epithelial-mesenchymal transition; GS, gene signature; IFNγ, interferon gamma; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility; NF-κB, nuclear factor kappa B; NK, natural killer; NOD, nucleotide oligomerization domain; NR, non-responder; TGF, tumor growth factor; TIS, tumor inflammation signature

- NRs were characterized according to tumor- and immune-related GS (**Table 2**), and three NR subgroups were identified (NR1, NR2, and NR3, **Table 3**)
  - NR1 was enriched with cell cycle and DNA repair GS and had a short median PFS
  - Despite having a high tumor inflammation signature, NR2 was highly enriched with Treg and T cell co-inhibition signatures, and had a numerically longer PFS compared with NR1 or NR3
  - NR3 was enriched with angiogenesis and hypoxia signatures and had the shortest median OS

Table 3. Summary of NR subgroup characteristics and clinical outcomes

Subgroup	N	Highly enriched GS	Median PFS, months (95% CI)	Median OS, months (95% CI)
NR1	36	▫ DNA repair ▫ Cell cycle	1.4 (1.4, 2.7)	14.0 (9.7, NE)
NR2	10	▫ TIS ▫ Treg signature ▫ T cell co-inhibition	5.8 (2.6, 14.4)	14.3 (3.1, NE)
NR3	67	▫ Angiogenesis ▫ Hypoxia	1.4 (1.4, 2.7)	8.6 (6.8, 12.4)

Median PFS and OS for responders were not reached as of the data cut-off (Feb 27, 2020)
CI, confidence interval; GS, gene signature; NE, not evaluable; NR, non-responder; OS, overall survival; PFS, progression-free survival; TIS, tumor inflammation signature

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