Co-enrichment of CD8 T-cells and macrophages is associated with clinical benefit of tislelizumab in solid tumors

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Background

- Therapies targeting the programmed cell death protein 1 (PD-1) receptor and programmed death-ligand 1 (PD-L1) have demonstrated efficacy in a range of tumor types by modulating the immune system to control tumor growth. However, not all patients benefit from PD-(L)1 blockade, highlighting the need to identify biomarkers for implementation in precision medicine protocols[1,2]
- Functionally activated immune cells (ICs) in the tumor microenvironment (TME) are critical to antitumor efficacy:
  - CD8-positive (CD8+) T-cells infiltrating the TME are considered predictive of the clinical efficacy of immunotherapy, however, the TME is complex and understanding other cell types in the TME and their interplay with CD8+ T-cells is crucial[3,4]
  - Macrophages (Mφ) play an important role in the TME, although because of their potential to exert anti- or pro-tumor functions their prognostic role is controversial[5–7]
- Tislelizumab is an anti-PD-1 antibody specifically designed with mutations in the Fc region to minimize Fc-gamma receptor binding on Mφ[8]
- Here, we report association between ICs and the clinical efficacy of tislelizumab, by examining tumor tissues from various tumor types in three pooled Phase 1/2 studies

Methods

Clinical cohorts

- Patients with advanced solid tumors from three studies (A317-001 [NCT02407990], A317-102 [NCT04068519], and A317-204 [NCT04004221]) who had received tislelizumab monotherapy and had tissue samples available for biomarker evaluation were eligible for this retrospective analysis
- Study designs of A317-001, A317-102, and A317-204 have been previously described[8–10]
- Overall survival (OS) in the biomarker evaluable population (BEP) was pooled and analyzed to explore the association with biomarker subgroups

Biomarkers

- Available baseline tumor tissues were tested with either multiplex-immunohistochemistry (mIHC) (Opal automation Multiplex IHC kit, panels CD8, CD68, PD-L1, panCK, CD64, DAPI) or gene expression profile (GEP) (HTG EdgeSeq Precision Immuno-Oncology Panel; gene signature scores were calculated using the gene set variation analysis [GSVA] method)
- High/low cell density and high/low signature scores were defined per median score

Statistical analysis

- Median OS was estimated by the Kaplan-Meier method and log-rank test was used to compare survival curves between patients with different biomarker levels
- Boxplots were analyzed using the Fishers exact test with a multiplicity adjustment
- All statistical analysis results are *post-hoc* exploratory and thereby p values are descriptive

Results

Baseline characteristics and clinical outcomes

- 629 patients had their GEP status evaluated; and 67 patients had their mIHC status evaluated
- The baseline characteristics and median OS were comparable. However, compared with the mIHC BEP, the GEP BEP included a broader spectrum of different cancer types, shown in **Table 1**

Table 1. Baseline characteristics and overall survival

Characteristic	Overall* (n=864)	GEP BEP (n=629)	mIHC BEP (n=67)
Age, median years (range)	60 (18–82)	60 (19–81)	59 (26–78)
Sex, n (%)			
Female	537 (62.2)	396 (63.0)	45 (67.2)
Male	327 (37.8)	233 (37.0)	22 (32.8)
ECOG PS, n (%)			
0	302 (35.0)	228 (36.2)	20 (29.9)
1	562 (65.0)	401 (63.8)	47 (70.1)
Cancer type, n (%)			
Non-small cell lung cancer	105 (12.2)	57 (9.1)	25 (37.3)
Gastric cancer	78 (9.0)	58 (9.2)	13 (19.4)
Esophageal cancer	79 (9.1)	66 (10.5)	4 (6.0)
Urothelial cancer	152 (17.6)	127 (20.2)	25 (37.3)
Hepatocellular carcinoma	68 (7.9)	50 (7.9)	0 (0)
Other	382 (44.2)	271 (43.1)	0 (0)
Prior anticancer drug therapy, n (%)			
0–1	407 (47.1)	297 (47.2)	29 (43.3)
2	204 (23.6)	147 (23.4)	20 (29.9)
≥ 3	190 (22.0)	137 (21.8)	18 (26.9)
Unknown	63 (7.3)	48 (7.6)	0 (0)
Clinical outcomes			
Median OS, months (95% CI)	11.1 (9.5, 11.7)	11.1 (9.6, 11.9)	11.2 (6.2, 16.1)

**All patients enrolled in A317-001 (NCT02407990), A317-102 (NCT04068519), and A317-204 (NCT04004221).*
BEP, biomarker evaluable population; ECOG PS, Eastern Cooperative Oncology Group performance status; ESCC, esophageal squamous cell carcinoma; GEP, gene expression profiling; mIHC, multiplex-immunohistochemistry

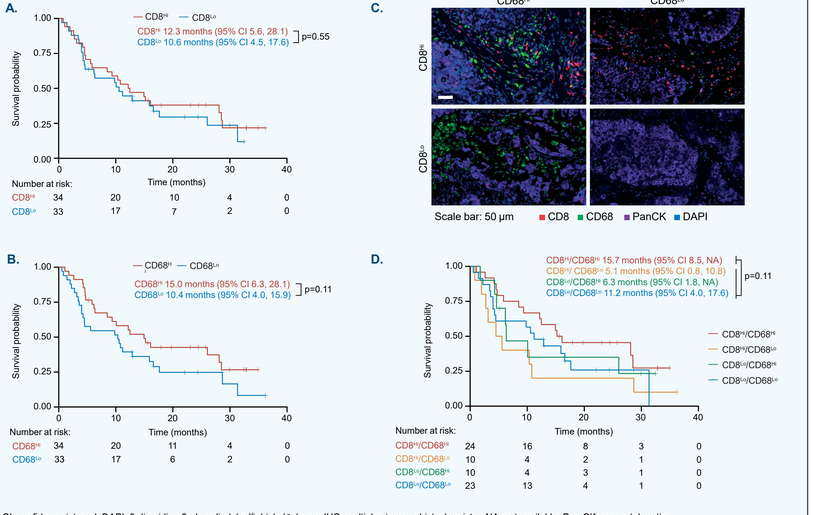
Conclusions

- **Co-enrichment of CD8 T-cells and Mφ was associated with survival benefit** in patients with various tumor types treated with tislelizumab
- This combination was also associated with an **immune-activated tumor microenvironment**
- The combination of **high CD8 and Mφ levels may aid the identification of the subset of patients who are most likely to benefit from treatment with tislelizumab**

High density of CD8+ T-cell and Mφ is positively associated with survival benefit in patients receiving tislelizumab treatment

- A numerical improvement of median OS was observed in patients with high CD8+ T-cell density ($CD8^{Hi}$) compared with patients with low CD8+ T-cell density ($CD8^{Lo}$) (mOS: 12.3 vs 10.6 months, p=0.55; **Figure 1A**)
- More prominently, patients with high Mφ density ($CD68^{Hi}$) showed a longer median OS compared with patients with low Mφ density ($CD68^{Lo}$) (15.0 months vs 10.4 months, p=0.11; **Figure 1B**)
- Considering the different functions and the potential crosstalk of CD8 T-cells and Mφ in the TME, we explored the clinical benefit in patients with a distinct density of CD8 T-cells and Mφ. The mIHC BEP was categorized into four subgroups by the density of CD8+ T-cells and Mφ using a median cutoff (**Figure 1C**)
- Patients with the $CD8^{Hi}/CD68^{Hi}$ showed the longest median OS (15.7 months) compared with other subgroups (5.1, 6.3 and 11.2 months for $CD8^{Hi}/CD68^{Lo}$, $CD8^{Lo}/CD68^{Hi}$, $CD8^{Lo}/CD68^{Lo}$, respectively; $CD8^{Hi}/CD68^{Hi}$ vs others, p=0.11) (**Figure 1D**)

Figure 1. mIHC-defined immune cell association with survival benefit of tislelizumab treatment

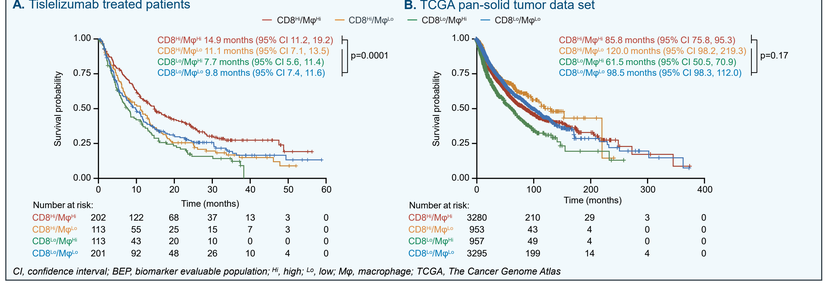


CI, confidence interval; DAPI, 6-diamidino-2-phenylindole; Hi, high; Lo, low; mIHC, multiplex immunohistochemistry; NA, not available; Pan-CK, pan-cytokeratin

Longer OS is observed in patients with $CD8^{Hi}$/ $M\varphi^{Hi}$ defined by gene signature scores

- Consistent with the data observed in the mIHC cohort, median OS was longer (14.9 months) in patients with $CD8^{Hi}/M\varphi^{Hi}$ gene signatures compared with the other three subgroups (11.1, 7.7 and 9.8 months for $CD8^{Hi}/M\varphi^{Lo}$, $CD8^{Lo}/M\varphi^{Hi}$, $CD8^{Lo}/M\varphi^{Lo}$, respectively; $CD8^{Hi}/M\varphi^{Hi}$ vs others, p=0.0001) (**Figure 2A**)
- Gene expression and clinical data of 8485 solid tumors from The Cancer Genome Atlas (TCGA) were also analyzed. In contrast to the tislelizumab treated patients, the TCGA pan-solid tumor dataset patients with $CD8^{Hi}/CD68^{Hi}$ did not exhibit prolonged survival compared with the other subgroups ($CD8^{Hi}/M\varphi^{Hi}$) vs others, p=0.17; **Figure 2B**), which indicated that the survival benefit observed may potentially be related to tislelizumab rather than a prognostic factor

Figure 2. Overall survival in gene expression BEP according to CD8+ T-cell and Mφ signature stratified subgroups

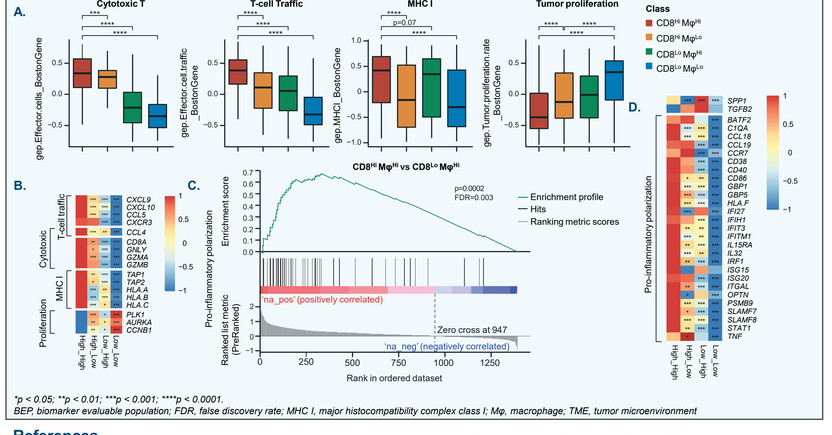


CI, confidence interval; BEP, biomarker evaluable population; Hi, high; Lo, low; Mφ, macrophage; TCGA, The Cancer Genome Atlas

Co-enrichment of CD8+ T-cell and Mφ is associated with an immune-activated TME

- Patients in the subgroup with $CD8^{Hi}/M\varphi^{Hi}$ signatures had the highest expression of immune related signatures and genes, such as those relating to cytotoxic cells (*CD8A, GNLY, GZMA, GZMB*), T-cell traffic (*CXCL9, CXCL10, CCL4, CCL5*), and major histocompatibility complex class I (MHC I) (*TAP1, TAP2, HLA.A, HLA.B, HLA.C*), while the tumor proliferation signature (*PLK1, AURKA, CCNB1*) was the lowest (**Figure 3A, B**)
- To further examine the different pro- or anti-tumor macrophage phenotypes with or without CD8 T-cell co-enrichment, we performed gene set enrichment analysis (GSEA) between $CD8^{Hi}/M\varphi^{Hi}$ and $CD8^{Lo}/M\varphi^{Hi}$. A significantly higher level of pro-inflammatory polarization signals[11,12] (*STAT1, SLAMF7/8, ISG15, IRF1, IL32, CCL18*) and lower expression of pro-angiogenic genes[13] (*SPP1, TGFB2*) was observed in patients with $CD8^{Hi}/M\varphi^{Hi}$ (p=0.0002) compared with patients with $CD8^{Lo}/M\varphi^{Hi}$ (**Figure 3C, D**), which may also be associated with the longest overall survival in the $CD8^{Hi}/M\varphi^{Hi}$ subgroup

Figure 3. Distinct TME in four signature-defined subgroups in the gene expression BEP



**p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.*
BEP, biomarker evaluable population; FDR, false discovery rate; MHC I, major histocompatibility complex class I; Mφ, macrophage; TME, tumor microenvironment

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