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SITC 2021
Annual Meeting
Nov. 10–14, 2021

Tumor Microenvironment based on PD-L1 and CD8 T-cell infiltration correlated with the survival of MSS mCRC patients treated vactosertib in combination with pembrolizumab

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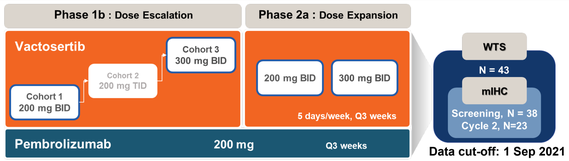
BACKGROUND

- Immune checkpoint inhibitor is an established treatment option for microsatellite instability-high (MSI-H) colorectal cancer[1,2], however, only 3.5-6.5% of metastatic colorectal cancer (mCRC) patients show MSI-H[3,4,5]
- MP-VAC-204, a phase 1b/2a study evaluating the combination of vactosertib (TGF-β receptor type 1 inhibitor) with pembrolizumab in previously treated microsatellite stable (MSS) mCRC (Clinical trial: NCT03724851) demonstrated promising anti-tumor efficacy and manageable safety at ASCO 2021[6]
  - ✓ Overall Response Rate (ORR) were 16.0% (RECIST) / 20.0% (iRECIST) and median Overall Survival (mOS) was 15.8 months without cardiac valvulopathy
- Meanwhile, expression of PD-L1 and tumor-infiltrating CD8 T cells were reported to have a decisive effect on the immunotherapy response[7]
- This study has been conducted to understand the basis of the clinical responses of anti-PD-1 and TGF-β inhibitor combination therapy in MSS mCRC, conducting a comprehensive analysis of survival outcome, whole transcriptome sequencing (WTS) data, and multiplex immunohistochemistry (mIHC) data

METHODS



Clinical Analysis

- Response assessed by RECIST and iRECIST
- Responder was defined as best overall response (BOR) complete remission (CR), partial response (PR), and iPR
- Non-responder was defined as BOR progression disease (PD)

Whole Transcriptome Sequencing (WTS)

- Screening and Cycle 2 post-treatment biopsies were used
- WTS data was generated by Illumina platform
- *CD274* and *CD8A* expression cut-off were calculated as median value in the merged data set of TCGA Pan-cancer and MP-VAC-204 study
- Tumor microenvironment immune status with a combination of *CD274* and *CD8A* gene expression (high or low) was classified into four subtypes

Subtype	*CD8A* high	*CD8A* low
CD274 high	Subtype 1	Subtype 3
CD274 low	Subtype 4	Subtype 2

Multiplex Immunohistochemistry (mIHC)

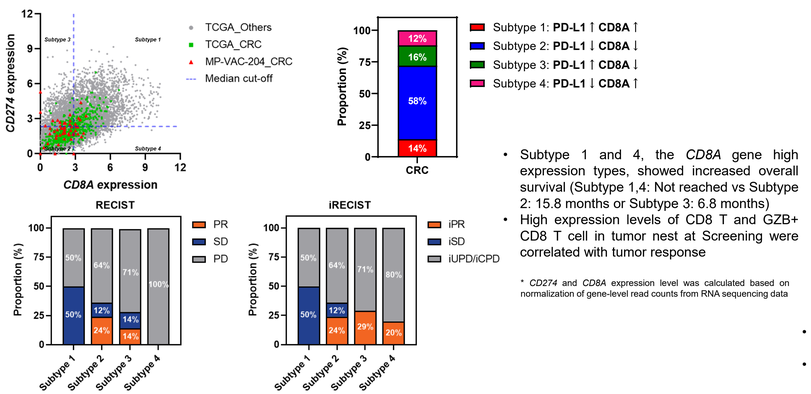
- Screening and Cycle 2 post-treatment biopsies were used
- As part of the multiplex panel, expression of PD-L1 (clone 22C3), CD8 (clone SP16), and granzyme B (GZB, clone D6E9W) were measured in tumor nest and stroma

RESULTS

Table 1. Baseline Characteristics

	Subtype 1 (N=6)	Subtype 2 (N=25)	Subtype 3 (N=7)	Subtype 4 (N=5)	Overall (N=43)
Age, years, median (range)	62 (39-72)	60 (39-83)	56 (40-67)	63 (46-70)	60 (39-83)
Sex, n (%)					
Male	5 (83)	12 (48)	3 (43)	5 (100)	25 (58)
Female	1 (17)	13 (52)	4 (57)	0 (0)	18 (42)
Race, n (%)					
Asian	6 (100)	25 (100)	7 (100)	5 (100)	43 (100)
ECOG Performance Status, n (%)					
0	3 (50)	9 (36)	1 (14)	1 (20)	14 (33)
1	3 (50)	16 (64)	6 (86)	4 (80)	29 (67)
***KRAS* mutation, n (%)**	2 (33)	13 (52)	2 (29)	0 (0)	17 (40)
***BRAF* mutation, n (%)**	0 (0)	1 (4)	0 (0)	0 (0)	1 (2)
Microsatellite Instability, n (%)					
MSS	6 (100)	25 (100)	7 (100)	5 (100)	43 (100)
TMB (var No./MBs), n (%)					
< 10	4 (67)	18 (72)	4 (57)	3 (60)	29 (67)
≥ 10	1 (17)	7 (28)	3 (43)	2 (40)	13 (30)
Unknown	1 (17)	0 (0)	0 (0)	0 (0)	1 (2)
No. of prior anticancer therapies, n (%)					
1-2	4 (67)	9 (36)	1 (14)	1 (20)	15 (35)
3	1 (17)	6 (24)	2 (29)	2 (40)	11 (26)
≥ 4	1 (17)	10 (40)	4 (57)	2 (40)	17 (40)

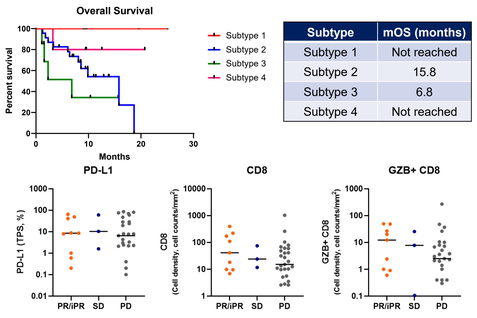
Figure 1. Tumor Microenvironment Immune Subtypes



- Subtype 1 and 4, the *CD8A* gene high expression types, showed increased overall survival (Subtype 1,4: Not reached vs Subtype 2: 15.8 months or Subtype 3: 6.8 months)
- High expression levels of CD8 T and GZB+ CD8 T cell in tumor nest at Screening were correlated with tumor response

* *CD274* and *CD8A* expression level was calculated based on normalization of gene-level read counts from RNA sequencing data

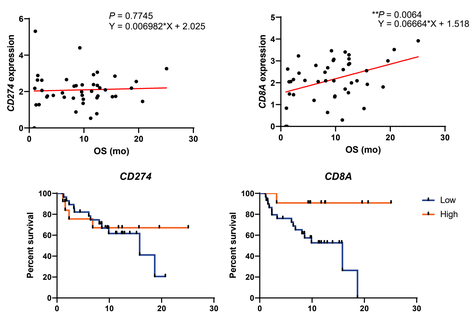
Figure 2. Survival Analysis by Tumor Microenvironment Immune Subtypes



Subtype	mOS (months)
Subtype 1	Not reached
Subtype 2	15.8
Subtype 3	6.8
Subtype 4	Not reached

- 58% of MP-VAC-204 CRC patients were categorized was Subtype 2, the "immune-cold" subtype
- 24% of patients with "immune-cold" tumor microenvironment showed PR (RECIST 1.1 and iRECIST)

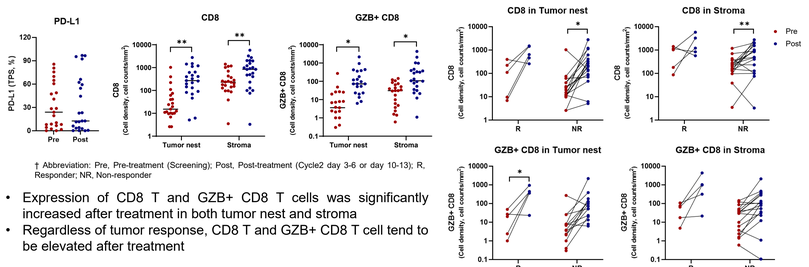
Figure 3. Correlation and Survival Analysis by expression of *CD274* and *CD8A*



Log-rank test	*CD274*		*CD8A*	
	Low	High	Low	High
mOS (months)	15.8	Not reached	15.8	Not reached
P value	0.5968 (ns)		0.0091 (**)	
Hazard Ratio	1.35		8.65	
95% CI	0.47 – 3.90		3.13 – 23.93	

- Overall survival and *CD8A* gene expression showed positive correlation (***P*=0.0064)
- Survival analysis by expression of *CD274* (PD-L1) or *CD8A* suggested that high expression of *CD8A* was associated with prolonged mOS than *CD274* (15.8 months vs Not reached, ***P*=0.0091, hazard ratio 8.65 [95% CI 3.13-23.93])

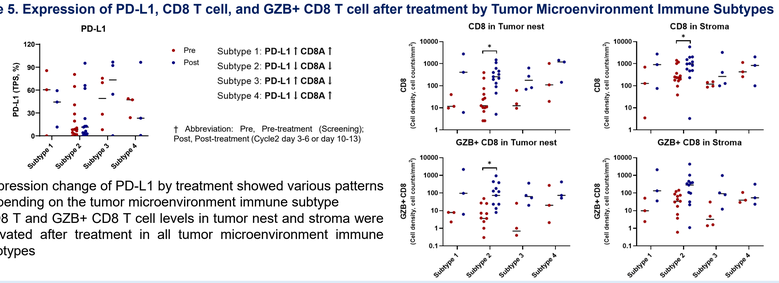
Figure 4. Changes in the expression of PD-L1, CD8 T cell, and GZB+ CD8 T cell after treatment



† Abbreviation: Pre, Pre-treatment (Screening); Post, Post-treatment (Cycle2 day 3-6 or day 10-13); R, Responder; NR, Non-responder

- Expression of CD8 T and GZB+ CD8 T cells was significantly increased after treatment in both tumor nest and stroma
- Regardless of tumor response, CD8 T and GZB+ CD8 T cell tend to be elevated after treatment

Figure 5. Expression of PD-L1, CD8 T cell, and GZB+ CD8 T cell after treatment by Tumor Microenvironment Immune Subtypes



† Abbreviation: Pre, Pre-treatment (Screening); Post, Post-treatment (Cycle2 day 3-6 or day 10-13)

- Expression change of PD-L1 by treatment showed various patterns depending on the tumor microenvironment immune subtype
- CD8 T and GZB+ CD8 T cell levels in tumor nest and stroma were elevated after treatment in all tumor microenvironment immune subtypes

SUMMARY and CONCLUSIONS

- Vactosertib and pembrolizumab combination therapy was well tolerated and demonstrated promising anti-tumor efficacy in this phase 1b/2a study with previously treated MSS mCRC patients
- With respect to overall survival, better clinical outcome with this dual inhibition regimen of TGF-β and PD-1 was associated with CD8 T cell infiltration status in tumor microenvironment
- The increase in CD8 T cell infiltration driven by vactosertib and pembrolizumab treatment was observed regardless of tumor response based on RECIST 1.1
- It is possible that this increase in TIL is one of the main contributing factors of better survival in these previously treated MSS mCRC patients
- Further investigation is warranted

REFERENCE

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ACKNOWLEDGEMENTS

- We would like to sincerely thank all patients and their families and investigators who participated in this study
- The authors thank the Merck team for supporting this study

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