IMMUNE NETWORK

Original Article

Check for updates

Immunological Characteristics of Hyperprogressive Disease in Patients with Non-small Cell Lung Cancer Treated with Anti-PD-1/PD-L1 Abs

Kyung Hwan Kim [1,2,†], Joon Young Hur [3,†], Jiae Koh[4,5], Jinhyun Cho [6], Bo Mi Ku[4], June Young Koh [2], Jong-Mu Sun[7], Se-Hoon Lee[7], Jin Seok Ahn[7], Keunchil Park [7], Myung-Ju Ahn [5,7,*], Eui-Cheol Shin [2,*]

[1]Department of Radiation Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul 03722, Korea
[2]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea
[3]Division of Hematology and Oncology, Department of Internal Medicine, Hanyang University Guri Hospital, Guri 11923, Korea
[4]Research Institute for Future Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Korea
[5]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06355, Korea
[6]Division of Hematology-Oncology, Department of Internal Medicine, Inha University Hospital, Inha University School of Medicine, Incheon 22332, Korea
[7]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Korea

OPEN ACCESS

*Correspondence to
Eui-Cheol Shin
Laboratory of Immunology and Infectious Diseases, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Daejeon 34141, Korea.
E-mail: ecshin@kaist.ac.kr

Myung-Ju Ahn
Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea.
E-mail: silkahn@skku.edu

†These authors contributed equally to this work as first authors.

ORCID iDs
Kyung Hwan Kim
https://orcid.org/0000-0002-6713-1350
Joon Young Hur
https://orcid.org/0000-0003-0092-8370
Jinhyun Cho
https://orcid.org/0000-0002-0641-5210

ABSTRACT

Hyperprogressive disease (HPD) is a distinct pattern of progression characterized by acceleration of tumor growth after treatment with anti-PD-1/PD-L1 Abs. However, the immunological characteristics have not been fully elucidated in patients with HPD. We prospectively recruited patients with metastatic non-small cell lung cancer treated with anti-PD-1/PD-L1 Abs between April 2015 and April 2018, and collected peripheral blood before treatment and 7-days post-treatment. HPD was defined as ≥2-fold increase in both tumor growth kinetics and tumor growth rate between pre-treatment and post-treatment. Peripheral blood mononuclear cells were analyzed by multi-color flow cytometry to phenotype the immune cells. Of 115 patients, 19 (16.5%) developed HPD, 52 experienced durable clinical benefit (DCB; partial response or stable disease ≥6 months), and 44 experienced non-hyperprogressive progression (NHPD). Patients with HPD had significantly lower progression-free survival (p<0.001) and overall survival (p<0.001). When peripheral blood immune cells were examined, the pre-treatment frequency of CD39+ cells among CD8+ T cells was significantly higher in patients with HPD compared to those with NHPD, although it showed borderline significance to predict HPD. Other parameters regarding regulatory T cells or myeloid derived suppressor cells did not significantly differ among patient groups. Our findings suggest high pre-treatment frequency of CD39+CD8+ T cells might be a characteristic of HPD. Further investigations in a larger cohort are needed to confirm our results and better delineate the immune landscape of HPD.

Keywords: Hyperprogressive disease; PD-1-PD-L1 blockade; Lung cancer; Peripheral blood human mononuclear cells; CD39; T cell

Generated by xmlinkpress

June Young Koh (iD)
https://orcid.org/0000-0002-2043-6624
Keunchil Park (iD)
https://orcid.org/0000-0002-4846-7449
Myung-Ju Ahn (iD)
https://orcid.org/0000-0002-5376-5109
Eui-Cheol Shin (iD)
https://orcid.org/0000-0002-6308-9503

Abbreviations
CR, complete response; DCB, durable clinical benefit; HPD, hyperprogressive disease; ICI, immune checkpoint inhibitor; MDSC, myeloid derived suppressor cell; NHPD, non-hyperprogressive progressive disease; NSCLC, non-small cell lung cancer; OS, overall survival; PBMC, peripheral blood mononuclear cell; PFS, progression-free survival; PR, partial response; SD, stable disease; SLD, sum of the longest diameter; TGK, tumor growth kinetics; TGR, tumor growth rate.

INTRODUCTION

Immune checkpoint inhibitors (ICIs), including anti-PD-1/PD-L1 monoclonal Abs, have changed treatment paradigm in broad ranges of solid tumors due to the high and durable response (1). Despite such efficacy, most patients treated with ICIs suffer from progression, and even hyperprogression. Hyperprogressive disease (HPD) is characterized by a paradoxical acceleration of tumor growth after treatment with ICIs. Although questions have been raised as to whether HPD is a unique entity in response to ICIs, it is now well-established as a distinct type of progression in multiple types of cancer (2). However, the pathogenesis of this phenomenon has not been fully elucidated.

Recently, a role of tumor-associated macrophages or regulatory T cells (Tregs) has been suggested in the pathogenesis of HPD (3,4). In addition, PD-1 expression on cancer cells has been suggested to promote HPD (5). However, most of the mechanistic studies have been limited to mouse tumor models, which represent general tumor progression rather than HPD. Given the lack of HPD-specific mouse models, more human-based investigations are needed. A number of clinical factors, such as older age, higher number of metastatic lesions, locoregional disease recurrence, and specific genomic alterations, have been proposed to be associated with HPD (6-8). However, limited data exist on the immune responses associated with HPD.

In the present study, we aimed to uncover the immunological characteristics specific to HPD using peripheral blood obtained before and early after anti-PD-1/PD-L1 treatment in patients with non-small cell lung cancer (NSCLC). We mainly focused on CD8^{+} and CD4^{+} T cells, which are the main targets of anti-PD-1/PD-L1 therapy, and suppressive immune cells commonly detected in peripheral blood, such as Tregs and myeloid derived suppressor cells (MDSCs). The pre-treatment and post-treatment measures of and fold change in immune cell parameters were analyzed to evaluate the correlation with HPD.

MATERIALS AND METHODS

Study cohort

Patients with histologically confirmed metastatic NSCLC treated with anti-PD-1/PD-L1 monotherapy between April 2015 and April 2019 were included in this study (n=115). Peripheral blood was collected before treatment and 7 days after treatment initiation. Among the 115 patients, 82 had both pre-treatment and post-treatment blood available, whereas 52 patients had only pre-treatment blood available. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by density gradient centrifugation. This study was approved by the Institutional Review Board of Samsung Medical Center. All patients provided informed consent prior to inclusion in the study.

Treatment response evaluation

Tumor response was assessed by computer tomography or magnetic resonance imaging every 8–9 weeks according to the RECIST 1.1 criteria. Tumor growth kinetics (TGK) were defined as the change in the sum of the longest diameter (SLD) of target lesions per month (9). The TGK ratio (TGK_R) was defined as the ratio of post-treatment TGK (TGK_{POST}) and pre-treatment TGK (TGK_{PRE}). Tumor growth rate (TGR) was defined as the estimated increase in tumor volume per month (10). Briefly, TGR is derived from the assumption that an increase

IMMUNE
NETWORK

in tumor volume follows an exponential law in which the tumor volume is approximated from the SLD of target lesions. The TGR ratio (TGR_R) was defined as the ratio of post-treatment TGR (TGR_{POST}) and pre-treatment TGR (TGR_{PRE}). HPD was defined as patients with both TGK_R and $TGR_R \geq 2$. Durable clinical benefit (DCB) was defined as a complete response (CR), partial response (PR), or stable disease (SD) lasting longer than 6 months. Patients not classified as either HPD or DCB were classified as non-hyperprogressive progression (NHPD).

Flow cytometry

Multi-color flow cytometry was performed using the PBMCs collected pre-treatment and post-treatment (11). MDSCs were analyzed on the same day as PBMC isolation because MDSCs are prone to cell death following freezing and thawing (12). A total of 102 fresh samples were analyzed for MDSCs. Other immune cell populations were analyzed using cryopreserved PBMCs. Anti-CD8 (SK1 and RPA-T8), anti-CD3 (HIT3a), anti-CD4 (SK3), anti-CD25 (M-A251), anti-CD45RA (HI100), anti-CD28 (CD28.2), anti-CD56 (NCAM16.2), anti-CD15 (HI98), anti-HLA-DR (G46-6), anti-IFN-γ (B27), anti-TNF-α (MAb11), and anti-IL17A (N49-653) were obtained from BD Biosciences; anti-PD-1 (EH.12.2H7), anti-CD127 (A019D5), anti-CD11b (ICRF44), anti-CD39 (A1), anti-CD73 (AD2), and anti-Ki-67 (Ki-67) from BioLegend; anti-FoxP3 (PCH101), anti-CD14 (61D3), and anti-CD19 (HIB19) from eBioscience; and anti-human IgG4 Fc (HP6025) from Southern Biotech. In post-treatment PBMCs, PD-1$^+$ cells were detected by anti-human IgG4 staining due to the therapeutic binding of pembrolizumab or nivolumab (human IgG4) to cell surface PD-1. The LIVE/DEAD Fixable Red Dead Cell Stain Kit (Invitrogen) was used to gate dead cells. MDSCs were defined according to previous recommendations (13). Intracellular staining was performed using a FoxP3 transcription factor staining buffer set (eBioscience) and specific Abs. Intracellular cytokine staining was performed by stimulating PBMCs with an anti-CD3 Ab (1 μg/mL; OKT3, eBioscience) for 6 h. Brefeldin A (GolgiPlug, BD Biosciences) was added 1 h after anti-CD3 stimulation.

Statistical analysis

Continuous variables in patients with DCB, NHPD, and HPD were compared by one-way ANOVA and post-hoc analysis was performed by Tukey's multiple comparisons test. Categorical variables were compared using the χ^2 test or Fisher's exact test as indicated. Progression-free survival (PFS) was defined as the time from the start of anti-PD-1/PD-L1 therapy to either disease progression (according to RECIST v1.1) or death from any cause. Receiver Operating Characteristics (ROC) curve was generated to evaluate the predictive value for HPD. Overall survival (OS) was defined as the time from the start of anti-PD-1/PD-L1 therapy to death from any cause. Survival curves were estimated using the Kaplan–Meier method and comparisons made by the log-rank test. All statistical analyses were performed in Prism software version 7.0 (GraphPad) and IBM SPSS Statistics 25 (IBM Corp).

RESULTS

Patient characteristics

A total of 115 patients treated with anti-PD-1/PD-L1 monotherapy were analyzed. The characteristics of the cohort are shown in **Table 1.** None of the patients received prior ICI. Patients received median 1 line (range, 0–9 lines) of previous systemic treatment. Most patients received anti-PD-1 monotherapy while 10 patients (8.7%) received anti-PD-L1 monotherapy. The best response after anti-PD-1/PD-L1 treatment was CR in 1 (0.9%), PR in 34 (29.5%), SD in 37 (32.2%), and PD in 43 (37.4%) patients.

Table 1. Patient characteristics

Characteristics	Total (n=115)	DCB (n=52)	NHPD (n= 44)	HPD (n=19)	p-value
Age, median (range) (yr)	62 (35–88)	66 (41–88)	61 (35–80)	62 (47–80)	0.047*
Sex					0.212
Male	93 (80.9)	45 (86.5)	32 (72.7%)	16 (84.2%)	
Female	22 (19.1)	7 (13.5)	12 (27.3%)	3 (15.8%)	
ECOG performance status					0.142
0–1	102 (88.7)	49 (94.2)	38 (86.4%)	15 (78.9%)	
2	13 (11.3)	3 (5.8)	6 (13.6%)	4 (21.1%)	
Histology					0.197
Adenocarcinoma	68 (59.1)	28 (53.8)	31 (70.5%)	9 (47.4%)	
Squamous cell carcinoma	39 (33.9)	21 (40.4)	11 (25.0%)	7 (36.8%)	
Others	8 (7.0)	3 (5.8)	2 (4.5%)	3 (15.8%)	
Tumor burden (cm)	4.5 (0.5–15.7)	4.8 (0.5–12.2)	4.5 (1.0–15.7)	3.6 (1.5–12.9)	0.656
PD-L1 (%)					0.565
<1	17 (14.8)	5 (9.6)	9 (20.5)	3 (15.8)	
≥1 and <50	20 (17.4)	9 (17.3)	8 (18.2)	3 (15.8)	
≥50	63 (54.8)	33 (63.5)	21 (47.7)	9 (47.4)	
Not performed	15 (13.0)	5 (9.6)	6 (13.6)	4 (21.1)	
Number of previous treatment	1 (0–9)	1 (0–5)	2 (0–9)	2 (0–7)	0.049*
Anti-PD-1 agent					0.072
Atezolizumab/Avelumab	10 (8.7)	7 (13.5)	2 (4.6%)	1 (5.2%)	
Nivolumab	42 (36.5)	12 (23.1)	21 (47.7%)	9 (47.4%)	
Pembrolizumab	63 (54.8)	33 (63.5)	21 (47.7%)	9 (47.4%)	

DCB, durable clinical benefit; NHPD, non-hyperprogressive progression; HPD, hyperprogression; ECOG, Eastern Cooperative Oncology Group.
*p>0.05 between patients with NHPD and HPD.

Defining patients with HPD

To assess HPD, tumor growth dynamics before and after treatment with anti-PD-1/PD-L1 were evaluated by estimating TGR and TGK in 43 patients who had PD as their best response (**Fig. 1A and B**). TGR_R and TGK_R were assessed to account for an acceleration in tumor growth following anti-PD-1/PD-L1 treatment. Despite the difference in definitions, TGR_R and TGK_R were tightly correlated (r=0.95; p<0.001; **Fig. 1C**). However, 4 patients only satisfied either TGR_R or TGK_R ≥2.0. HPD was defined as fulfilling both TGR_R and TGK_R ≥2.0 (**Fig. 1C**). Among the 115 patients, 52 (45.2%) achieved DCB, 19 experienced HPD (16.5%), and 44 (38.3%) exhibited no durable benefit but did not fulfill the HPD criteria (NHPD). Patients with DCB had significantly better performance status and fewer previous lines of treatment compared to patients with NHPD or HPD, however we found no significant differences in
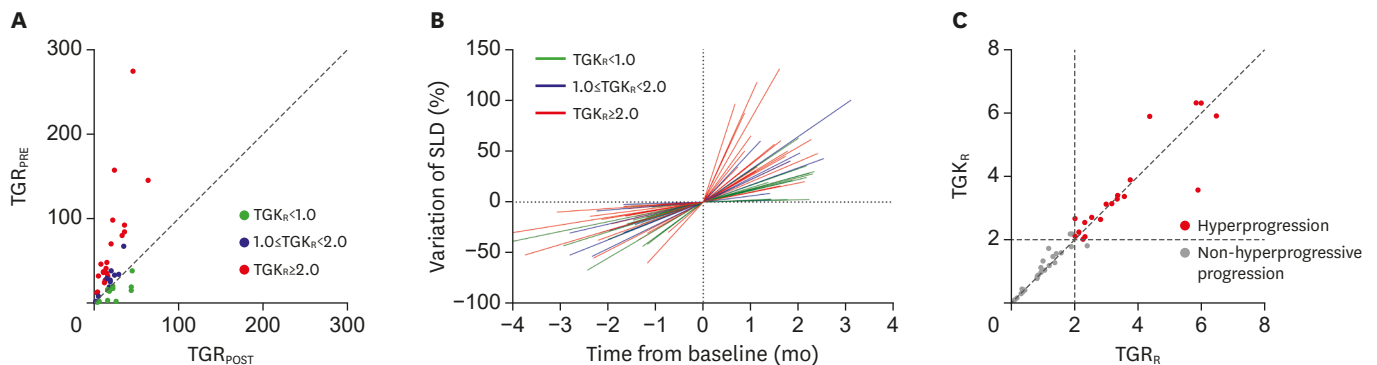

Figure 1. TGR and TGK in patients with progressive disease as the best response according to RECIST 1.1 criteria (n=43). (A) TGR before anti-PD-1/PD-L1 treatment (TGR_{PRE}) and during treatment (TGR_{POST}). The dashed line indicates $TGR_{PRE}=TGR_{POST}$. Red dots indicate TGR ratio ($TGR_R=TGR_{POST}/TGR_{PRE}$)≥2.0. Blue indicates $1.0≤TGR_R<2.0$, and green indicates $TGR_R<1.0$. (B) Percentage change in SLD before and during anti-PD-1/PD-L1 treatment. (C) Correlation between TGR_R and TGK_R. Patients fulfilling both $TGR_R≥2.0$ and $TGK_R≥2.0$ were defined as hyperprogressive disease.
TGR, tumor growth rate; TGK, tumor growth kinetics; SLD, sum of the longest diameter.
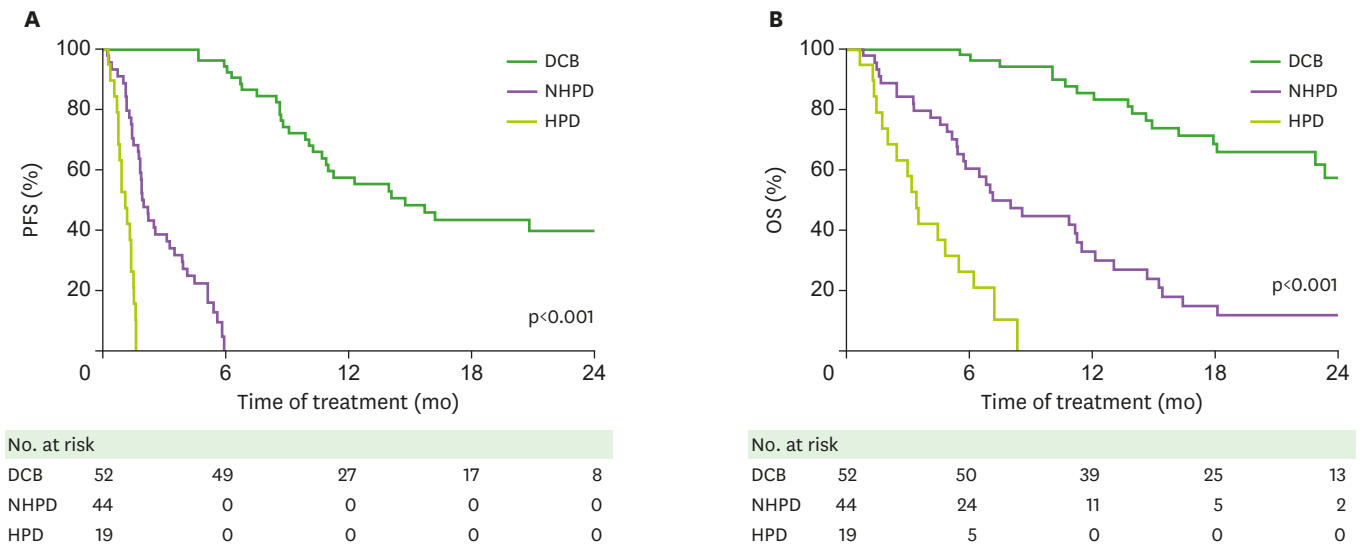
Figure 2. PFS and OS among the patients in this study. (A) PFS and (B) OS in patients with DCB (n=52), NHPD (n=44), and HPD (n=19). PFS, progression-free survival; OS, overall survival; DCB, durable clinical benefit; NHPD, non-hyperprogressive progressive disease.

baseline patient characteristics between patients with NHPD and HPD (**Table 1**). Regarding the anti-PD-1/PD-L1 agents administered, pembrolizumab was more commonly administered in patients with DCB than those with NHPD and HPD, but the distribution of the anti-PD-1/PD-L1 agents were similar between patients with NHPD and HPD (**Table 1**).

At a median follow-up of 18.2 months (range, 4.2–54.3 months), median PFS of patients with DCB, NHPD, and HPD was 14.8 months (95% confidence interval [CI], 10.0–19.5 months), 1.9 months (95% CI, 1.5–2.3 months), and 1.1 months (95% CI, 0.7–1.5 months), respectively (p<0.001; **Fig. 2A**). The median OS of patients with NHPD and HPD was 7.2 months (95% CI, 4.6–9.7 months) and 3.4 months (95% CI, 2.7–4.2 months), respectively (p<0.001; **Fig. 2B**). For patients with DCB, OS was not reached.

Correlation between HPD and peripheral blood immune cells following anti-PD-1/PD-L1 therapy

Next, we examined the peripheral blood immune cells pre-treatment and 1-week post-treatment using flow cytometry. The gating strategies are provided in **Supplementary Fig. 1**. The frequencies and phenotypes of CD8$^+$ T cells, CD4$^+$ T cells, and MDSCs were analyzed. T-cell markers related to exhaustion (PD-1, CD39, and CD73), differentiation status (CD28 and CD45RA), proliferation (Ki-67), and cytokine secretion (IFN-γ, TNF-α, and IL-17A) were included. The frequency of MDSCs, including the subpopulations PMN-MDSCs (Lin-CD15$^+$CD14$^-$CD11b$^+$HLA-DR$^{-/lo}$) and M-MDSCs (Lin- CD14$^+$CD15$^-$HLA-DR$^{-/lo}$), were evaluated. Tregs were also evaluated and analyzed according to the subsets naïve (Fraction I) Tregs, effector (Fraction II; FII) Tregs, and non-suppressive cells (Fraction III) (14). The pre-treatment and post-treatment measures of, and fold change in each parameter were correlated with treatment response to identify HPD-specific markers. The values of each examined parameter are summarized in **Supplementary Table 1**. Among the parameters evaluated, the pre-treatment frequency of CD39$^+$ cells among CD8$^+$ T cells and fold change in the frequency of Ki-67$^+$ cells among PD-1$^+$CD8$^+$ T cells post-treatment exhibited significant differences among patients with DCB, NHPD, and HPD (**Supplementary Table 1**). The frequency of MDSCs, which was reported to predict treatment outcome after anti-PD-1 therapy (12), did not significantly differ among
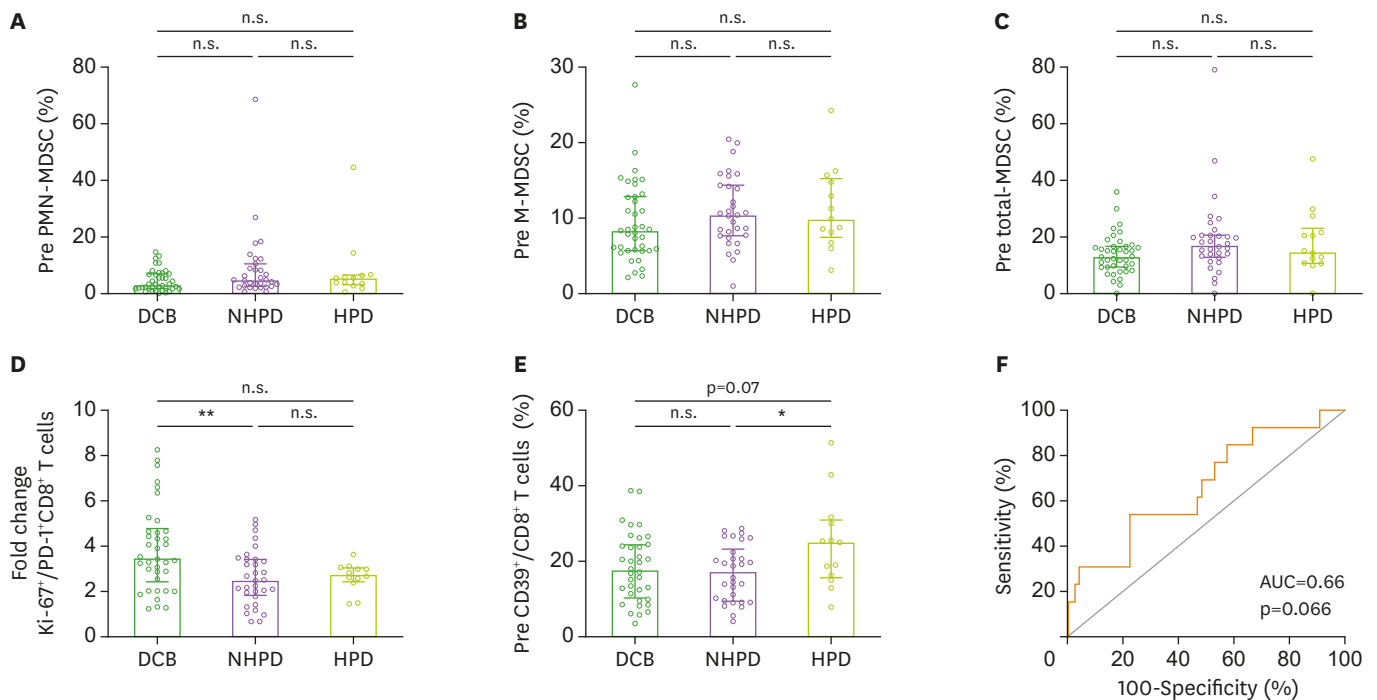
Figure 3. Immune cell parameters that significantly differ among patients with DCB, NHPD, and HPD. (A) Frequency of pre-treatment PMN-MDSCs (Lin⁻CD14⁻CD15⁺CD11b⁺HLA⁻DR$^{-/lo}$) among live single cells. (B) Frequency of pre-treatment M-MDSCs (Lin⁻CD14⁺HLA⁻DR$^{-/lo}$) among live single cells. (C) Frequency of pre-treatment MDSCs (frequency of M-MDSCs and PMN-MDSCs) among live single cells. (D) Fold change in the frequency of Ki-67⁺ cells among PD-1⁺CD8⁺ T cells after anti-PD-1/PD-L1 therapy. (E) Frequency of pre-treatment CD39⁺ cells among CD8⁺ T cells. (F) The receiver operating characteristics curve for prediction of HPD with the frequency of pre-treatment CD39⁺ cells among CD8⁺ T cells.

n.s., not significant; DCB, durable clinical benefit; NHPD, non-hyperprogressive progressive disease; HPD, Hyperprogressive disease; MDSC, myeloid derived suppressor cell; AUC, area under the receiver operating characteristics curve.

*p<0.05, **p<0.01.

patients with DCB, NHPD, and HPD (**Figure 3A-C**). The fold change in the frequency of Ki-67⁺ cells among PD-1⁺CD8⁺ T cells post-treatment did not show significant differences between patients with NHPD and HPD, although patients with DCB exhibited significantly higher values (**Fig. 3D**). The pre-treatment frequency of CD39⁺ cells among CD8⁺ T cells was significantly higher in patients with HPD compared to those with NHPD (**Fig. 3E**). There was a trend of higher pre-treatment frequency of CD39⁺ cells among CD8⁺ T cells in patients with HPD compared to those with DCB although it was not statistically significant. The pre-treatment frequency of CD39⁺ cells among CD8⁺ T cells showed borderline significance to predict HPD (area under the curve 0.66, p=0.066; **Fig. 3F**). Interestingly, PD-1⁺TIGIT⁺ cells, which have been shown to be more susceptible to activation-induced cell death following PD-1 blockade ([15]), were enriched in CD39⁺CD8⁺ T cells compared to CD39⁻CD8⁺ T cells (**Supplementary Fig. 2**).

Regulatory T cells in patients with HPD

A role of FII Tregs in the pathogenesis of HPD was recently reported ([4]). When we evaluated the change in frequency and proliferation of peripheral blood Tregs after anti-PD-1/PD-L1 monotherapy, we observed an increase in proliferating Ki-67⁺ FII Tregs, but it was not statistically significant between NHPD and HPD patients (**Fig. 4A**). No significant differences were observed in the fold change in Ki-67⁺ FII Tregs following anti-PD-1/PD-L1 treatment among patients with DCB, NHPD, and HPD (**Fig. 4B**). We also evaluated the proliferative response in PD-1⁺ FII Tregs, but no significant difference among patients with DCB, NHPD, and HPD was noted (**Fig. 4C**). Moreover, we found no significant differences in the fold change in the frequency of FII Tregs or PD-1⁺ FII Tregs (**Fig. 4D**).
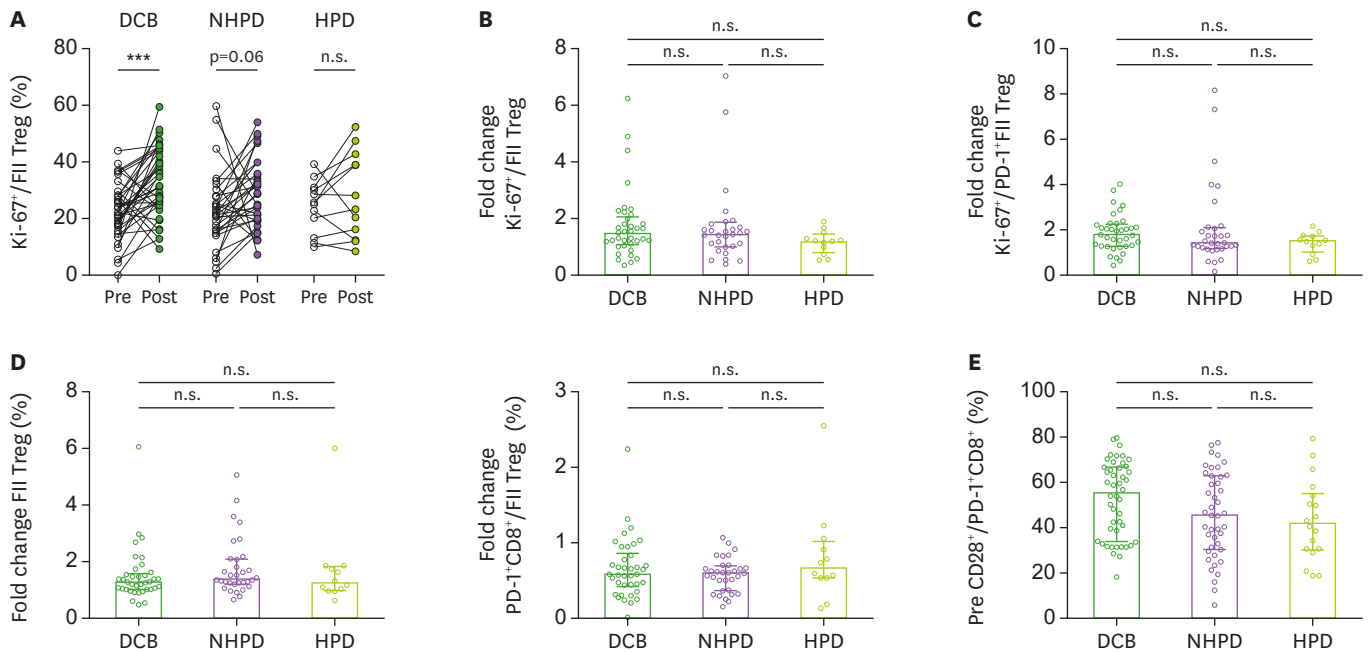
Figure 4. Regulatory and CD28+PD-1+CD8+ T cells in patients with DCB, NHPD, and HPD. (A) Frequency of Ki-67+ cells among FII Tregs (FoxP3hiCD45RA−) pre- and post-treatment. (B) Fold change in the frequency of Ki-67+ cells among FII Tregs following anti-PD-1/PD-L1 treatment. (C) Fold change in the frequency of Ki-67+ cells among PD-1+ FII Tregs following anti-PD-1/PD-L1 treatment. (D) Fold change in the frequency of FII Treg and PD-1+ FII Tregs among CD4+ T cells following anti-PD-1/PD-L1 treatment. (E) Frequency of CD28+ cells among PD-1+CD8+ T cells before treatment.
DCB, durable clinical benefit; NHPD, non-hyperprogressive progressive disease; HPD, Hyperprogressive disease; n.s., not significant.
***p<0.001.

CD28+PD-1+CD8+ T cells in patients with HPD

Our group recently reported that tumor-infiltrating CD28+PD-1+CD8+ T cells, which express TCF1 and retain stemness, are the population responding to PD-1 blockade ([16]). Based on these results, we measured the frequency of CD28+ cells among peripheral blood PD-1+CD8+ T cells and found a decreasing trend from DCB patients to HPD patients regarding the baseline frequency of CD28+ cells among PD-1+CD8+ T cells, but the difference was not significant (**Fig. 4E**).

DISCUSSION

In the present study we aimed to evaluate the immunological characteristics specific for HPD that are distinct from non-hyperprogressive progression using peripheral blood obtained before and after treatment. Compared to previous studies that defined HPD with either TGK_R or TGR_R only ([9,10,17]), we more stringently defined HPD as fulfilling both TGK_R and $TGR_R \geq 2$. For immune cell analysis, we mainly focused on CD8+ and CD4+ T cells, which are the main effectors and target cells of anti-PD-1/PD-L1 therapy, and other suppressive cell populations that have been shown to correlate with a poorer response to PD-1 blockade, such as MDSCs and Tregs ([4,18,19]). We observed a significant difference in PFS and OS between NHPD and HPD patients, implying a distinct clinical course of HPD, and found that the pre-treatment frequency of CD39+ cells among CD8+ T cells was significantly higher in patients with HPD compared to patients with NHPD.

CD39 is an ectonucleotidase expressed on the cell surface and CD39 expression has been reported to identify terminally exhausted CD8+ T cells ([20,21]). Severely or terminally

exhausted CD8^{+} T cells have been demonstrated as a subpopulation that does not respond to PD-1 blockade (22). Although not specific to HPD, our group recently demonstrated that the frequency of CD39^{+} cells among CD8^{+} T cells in peripheral blood predicts the efficacy of anti-PD-1 therapy in patients with NSCLC (12). It can be hypothesized that an increase in terminally exhausted CD39^{+}CD8^{+} T cells may lead to a lack of response to PD-1 blockade, resulting in HPD. However, HPD, where the growth rate of tumor cells increase after treatment, is a distinct process from lack of treatment response (2). Although the underlying mechanisms of HPD is not fully understood, some hypotheses regarding the association of CD39^{+}CD8^{+} T cells and HPD may be suggested. A recent report showed that higher frequency of severely exhausted PD-1^{+}TIGIT^{+}CD8^{+} T cells in peripheral blood at baseline can predict HPD in NSCLC patients, and demonstrated that these cells were more vulnerable to activation-induced cell death following T-cell receptor stimulation with PD-1 blockade (15). We found that the frequency of PD-1^{+}TIGIT^{+} cells were significantly higher in CD39^{+}CD8^{+} T cells compared to CD39^{−}CD8^{+} T cells. Therefore, CD39^{+}CD8^{+} T cells may also be vulnerable to activation-induced cell death following PD-1 blockade and may result in a loss of tumor-specific T cells resulting in a paradoxical tumor progression. Another study demonstrated the immunosuppressive role of CD39^{+}CD8^{+} T cells via the ectonucleotidase activity of CD39 (21). Therefore, anti-PD-1-induced proliferation of CD39^{+}CD8^{+} T cells may lead to further suppression of anti-tumor immune responses through increased ATP hydrolysis and adenosine. However, we measured the frequency of CD39^{+}CD8^{+} T cells in the peripheral blood which may not accurately reflect the composition of CD39^{+} cells among CD8^{+} T cells in the tumor microenvironment. Further studies utilizing pre-treatment tissue biopsies may further elucidate the importance of this terminally exhausted CD8^{+} T cell subpopulation in the development of HPD.

The magnitude of CD8^{+} T-cell reinvigoration measured by the increase in proliferating PD-1^{+}CD8^{+} T cells has been demonstrated to predict the response to anti-PD-1 therapy in multiple types of cancers, including melanoma and NSCLC (23,24). In this study, we found a significantly higher fold change in the frequency of Ki-67^{+} cells among PD-1^{+}CD8^{+} T cells after anti-PD-1/PD-L1 treatment in patients with DCB compared to patients with NHPD or HPD, which is consistent with previous results. However, the fold change was not significantly different between patients with HPD and NHPD. We also detected a significantly lower baseline frequency of MDSCs in patients with DCB, which is in line with a recent study by Ko *et al.* (12). However, the frequency of MDSCs at baseline were similar between NHPD and HPD patients. These data imply that the response of PD-1^{+}CD8^{+} T cells and baseline frequency of MDSCs, which predict treatment response after anti-PD-1, do not specifically predict HPD. Moreover, these findings indicate that PD-1^{+}CD8^{+} T cells and MDSCs may not be involved in the process of HPD.

We also evaluated the frequency of CD28^{+} cells among PD-1^{+}CD8^{+} T cells. Recently, our group identified that tumor-infiltrating CD28^{+}PD-1^{+}CD8^{+} T cells highly express TCF1 and respond to PD-1 blockade, whereas CD28^{−}PD-1^{+}CD8^{+} T cells are terminally exhausted and poorly respond to PD-1 blockade (16). TCF1, a Wnt family transcription factor, has been demonstrated to be a key marker of anti-PD-1 responsive T cells (25,26). In regards to the previous studies, we hypothesized that the lack of anti-PD-1-responding tumor-infiltrating CD8^{+} T cells may be one of the causes of HPD. A trend of decreased frequency of CD28^{+} cells among PD-1^{+}CD8^{+} T cells was shown in HPD. These indicate that an increase in terminally exhausted CD8^{+} T cells may be associated with HPD, and further investigations evaluating the role of CD28^{−} or CD39^{+}CD8^{+} T cells in the development of HPD are required.

We also examined the change in the frequency and proliferative response of Tregs, which were recently claimed to play a major role in HPD (4). Tregs can be subdivided into naïve (Fraction I) Tregs, effector (Fraction II) Tregs, and non-suppressive cells (Fraction III) (14). FII Tregs mainly exert the well-known suppressive functions of Tregs. In our study, we found an increase in Ki-67 expression among FII Tregs regardless of the response to treatment. Moreover, the ratio of post-treatment and pre-treatment frequency of Ki-67^{+} cells among FII Tregs was similar among DCB, NHPD, and HPD patients. The results were also similar when Ki-67 expression was evaluated among PD-1^{+} FII Tregs. In contrast, Kamada *et al.* (4) found that the frequency of Ki-67^{+} cells among tumor-infiltrating FII Tregs was only increased in HPD patients. They utilized paired tissue samples from gastric cancer patients obtained before and 4-6 weeks after anti-PD-1 therapy. In addition, they demonstrated the role of PD-1 in Tregs and found that loss of PD-1 in Tregs can lead to tumor growth in a mouse model. The proliferative response or frequency of Tregs analyzed using peripheral blood in our study may not properly reflect the status or dynamic change in the tumor tissue microenvironment after treatment. Further investigations with paired tissue samples may be needed to elucidate the association between Tregs and HPD in humans.

Previous study reported that several clinicopathological factors such as Eastern Cooperative Oncology Group performance status, previous lines of treatment, and type of anti-PD-1/PD-L1 agent were significantly different among DCB, NHPD, and HPD patients. However, no significant differences were observed between NHPD and HPD regarding these clinical factors in our study.

The present study has several limitations. First, the number of patients was small, which limits the statistical power of this study. In addition, paired peripheral blood samples were only available in 61% of patients, and the rest had only baseline peripheral blood samples. Second, immune cell analysis was limited to MDSCs and T cells. Applying a more unbiased method, such as CyTOF or single-cell RNA sequencing, in a larger population may shed light on the novel immune signatures of HPD. Furthermore, paired tissue samples before and after ICI treatment, rather than peripheral blood, are needed to better characterize HPD-specific changes in the immune microenvironment of tumors.

In conclusion, we propose that a high pre-treatment frequency of CD39^{+} cells among CD8^{+} T cells might be a characteristic of HPD. Further investigations with a larger cohort are needed to validate our results and to better delineate the immune landscape of patients experiencing HPD in order to improve the prediction and management of this devastating pattern of disease progression.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Peripheral blood immune cell parameters

Click here to view

Supplementary Figure 1

Gating strategy of CD8⁺ T cells (A), CD4⁺ T cells (B), cytokine producing T cells (C), and MDSCs (D).

Click here to view

Supplementary Figure 2

Frequency of PD-1⁺TIGIT⁺ cells among CD39⁺ and CD39⁻CD8⁺ T cells (A) and FACS plot of a representative patient (B). Statistical analysis performed by paired t-test.

Click here to view

REFERENCES

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018;359:1350-1355.
   PUBMED | CROSSREF

2. Champiat S, Ferrara R, Massard C, Besse B, Marabelle A, Soria JC, Ferté C. Hyperprogressive disease: recognizing a novel pattern to improve patient management. *Nat Rev Clin Oncol* 2018;15:748-762.
   PUBMED | CROSSREF

3. Lo Russo G, Moro M, Sommariva M, Cancila V, Boeri M, Centonze G, Ferro S, Ganzinelli M, Gasparini P, Huber V, et al. Antibody-Fc/FcR interaction on macrophages as a mechanism for hyperprogressive disease in non-small cell lung cancer subsequent to PD-1/PD-L1 blockade. *Clin Cancer Res* 2019;25:989-999.
   PUBMED | CROSSREF

4. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, Sato E, Fukuoka S, Tada Y, Tanaka A, et al. PD-1⁺ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U S A* 2019;116:9999-10008.
   PUBMED | CROSSREF

5. Du S, McCall N, Park K, Guan Q, Fontina P, Ertel A, Zhan T, Dicker AP, Lu B. Blockade of tumor-expressed PD-1 promotes lung cancer growth. *OncoImmunology* 2018;7:e1408747.
   PUBMED | CROSSREF

6. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after immunotherapy: analysis of genomic alterations associated with accelerated growth rate. *Clin Cancer Res* 2017;23:4242-4250.
   PUBMED | CROSSREF

7. Kim Y, Kim CH, Lee HY, Lee SH, Kim HS, Lee S, Cha H, Hong S, Kim K, Seo SW, et al. Comprehensive clinical and genetic characterization of hyperprogression based on volumetry in advanced non-small cell lung cancer treated with immune checkpoint inhibitor. *J Thorac Oncol* 2019;14:1608-1618.
   PUBMED | CROSSREF

8. Wang X, Wang F, Zhong M, Yarden Y, Fu L. The biomarkers of hyperprogressive disease in PD-1/PD-L1 blockage therapy. *Mol Cancer* 2020;19:81.
   PUBMED | CROSSREF

9. Saâda-Bouzid E, Defaucheux C, Karabajakian A, Coloma VP, Servois V, Paoletti X, Even C, Fayette J, Guigay J, Loirat D, et al. Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Ann Oncol* 2017;28:1605-1611.
   PUBMED | CROSSREF

10. Champiat S, Dercle L, Ammari S, Massard C, Hollebecque A, Postel-Vinay S, Chaput N, Eggermont A, Marabelle A, Soria JC, et al. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clin Cancer Res* 2017;23:1920-1928.
    PUBMED | CROSSREF

11. Kim KH, Hur JY, Cho J, Ku BM, Koh J, Koh JY, Sun JM, Lee SH, Ahn JS, Park K, et al. Immune-related adverse events are clustered into distinct subtypes by T-cell profiling before and early after anti-PD-1 treatment. *OncoImmunology* 2020;9:1722023.
    PUBMED | CROSSREF

12. Koh J, Kim Y, Lee KY, Hur JY, Kim MS, Kim B, Cho HJ, Lee YC, Bae YH, Ku BM, et al. MDSC subtypes and CD39 expression on CD8⁺ T cells predict the efficacy of anti-PD-1 immunotherapy in patients with advanced NSCLC. *Eur J Immunol* 2020;50:1810-1819.
    PUBMED | CROSSREF

IMMUNE NETWORK

13. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* 2016;7:12150.
PUBMED | CROSSREF

14. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity* 2009;30:899-911.
PUBMED | CROSSREF

15. Kim CG, Kim KH, Pyo KH, Xin CF, Hong MH, Ahn BC, Kim Y, Choi SJ, Yoon HI, Lee JG, et al. Hyperprogressive disease during PD-1/PD-L1 blockade in patients with non-small-cell lung cancer. *Ann Oncol* 2019;30:1104-1113.
PUBMED | CROSSREF

16. Kim KH, Kim HK, Kim HD, Kim CG, Lee H, Han JW, Choi SJ, Jeong S, Jeon M, Kim H, et al. PD-1 blockade-unresponsive human tumor-infiltrating CD8+ T cells are marked by loss of CD28 expression and rescued by IL-15. *Cell Mol Immunol* 2020. doi: 10.1038/s41423-020-0427-6.
PUBMED | CROSSREF

17. Ferrara R, Mezquita L, Texier M, Lahmar J, Audigier-Valette C, Tessonnier L, Mazieres J, Zalcman G, Brosseau S, Le Moulec S, et al. Hyperprogressive disease in patients with advanced non-small cell lung cancer treated with PD-1/PD-L1 inhibitors or with single-agent chemotherapy. *JAMA Oncol* 2018;4:1543-1552.
PUBMED | CROSSREF

18. Kim HR, Park SM, Seo SU, Jung I, Yoon HI, Gabrilovich DI, Cho BC, Seong SY, Ha SJ, Youn JI. The ratio of peripheral regulatory T cells to Lox-1+ polymorphonuclear myeloid-derived suppressor cells predicts the early response to anti-PD-1 therapy in patients with non-small cell lung cancer. *Am J Respir Crit Care Med* 2019;199:243-246.
PUBMED | CROSSREF

19. Kim KH, Kim CG, Shin EC. Peripheral blood immune cell-based biomarkers in anti-PD-1/PD-L1 therapy. *Immune Netw* 2020;20:e8.
PUBMED | CROSSREF

20. Gupta PK, Godec J, Wolski D, Adland E, Yates K, Pauken KE, Cosgrove C, Ledderose C, Junger WG, Robson SC, et al. CD39 expression identifies terminally exhausted CD8+ T cells. *PLoS Pathog* 2015;11:e1005177.
PUBMED | CROSSREF

21. Canale FP, Ramello MC, Núñez N, Araujo Furlan CL, Bossio SN, Gorosito Serrán M, Tosello Boari J, Del Castillo A, Ledesma M, Sedlik C, et al. CD39 expression defines cell exhaustion in tumor-infiltrating CD8+ T cells. *Cancer Res* 2018;78:115-128.
PUBMED | CROSSREF

22. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* 2015;36:265-276.
PUBMED | CROSSREF

23. Kim KH, Cho J, Ku BM, Koh J, Sun JM, Lee SH, Ahn JS, Cheon J, Min YJ, Park SH, et al. The first-week proliferative response of peripheral blood PD-1+CD8+ T cells predicts the response to anti-PD-1 therapy in solid tumors. *Clin Cancer Res* 2019;25:2144-2154.
PUBMED | CROSSREF

24. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, Xu W, Harmon S, Giles JR, Wenz B, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* 2017;545:60-65.
PUBMED | CROSSREF

25. Jansen CS, Prokhnevska N, Master VA, Sanda MG, Carlisle JW, Bilen MA, Cardenas M, Wilkinson S, Lake R, Sowalsky AG, et al. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. *Nature* 2019;576:465-470.
PUBMED | CROSSREF

26. Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, Yates KB, Lako A, Felt K, Naik GS, et al. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* 2019;20:326-336.
PUBMED | CROSSREF