Clinical implication of serologic indexes and compositional changes of immune cells on hyperprogressive disease in non-small cell lung cancer patients receiving immunotherapy

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Abstract

Background

Immune checkpoint blockers (ICBs) are characterized by a durable clinical response and better tolerability in patients with a variety of advanced solid tumors. However, we not infrequently encounter patients with hyperprogressive disease (HPD) exhibiting paradoxically accelerated tumor growth with poor outcomes. This study aimed to determine differences in factors among patients with non-small cell lung cancer (NSCLC) displaying different tumor responses to immunotherapy.

Methods

This study evaluated 231 NSCLC patients receiving ICBs between January 2014 and May 2018. HPD was defined as a > 2-fold tumor growth kinetics ratio during ICB therapy and time-to-treatment failure of ≤ 2 months. We analyzed clinical data, imaging studies, periodic serologic indexes, and immune cell compositions in tumors and stromata using multiplex immunohistochemistry.

Results

Twenty-six patients (11.3%) met the HPD criteria. HPD was more frequent in patients with oncogenic driver mutations, ≥ 3 metastatic sites, ≥ 4 prior systemic treatments, and low PD-L1 expression (< 10%). Serological indexes at the first response evaluation were significantly associated with HPD. The number of CD4 + effector T cells and CD8 + cytotoxic T cells, and CD8+/PD-1 + tumor-infiltrating lymphocytes tended to be smaller, especially in stromata of HPD group. While in the stromal region, there were more M2-type macrophages expressing CD14, CD68 and CD163, and remarkably fewer intratumoral CD56 + NK cells in HPD.

Conclusions

These results indicate that oncogenic driver mutations, multiple metastatic sites, serological markers, fewer CD8+/PD-1 + cells, and more M2 macrophages in the tumor microenvironment predict HPD in advanced/metastatic NSCLC patients undergoing immunotherapy.

Background

Immune checkpoint blockers (ICBs), which blocks CTLA-4, PD-1 or PD-L1, exert anti-tumor activities through re-invigorating exhausted T-lymphocytes [1–3]. Clinical excitement regarding these ICBs has resulted from their different advantages, including the unprecedented number of durable clinical responses and better tolerability among patients with a variety of advanced cancer types [4–6]. However, in some cases, patients not infrequently exhibit a paradoxically accelerated tumor growth with poor

outcome; such cases are designated as hyperprogressive disease (HPD). Even though the definition of and predisposing factors to HPD somewhat differ depending on the source, one consistent finding is that the growth kinetics at first tumor assessment more than double compared to that at the beginning of immunotherapy [7, 8].

The tumor is surrounded by a complex and heterogeneous tumor microenvironment (TME), comprised of several types of immune cells, fibroblasts, and a tumor-specific extracellular matrix [3, 9, 10]. There is abundant evidence that tumor cells and the TME constantly interact to modulate tumor growth [4, 9]. Regarding tumor-associated factors, certain genetic aberrations such as *MDM2/4* amplification and *KRAS-SKT11* mutation, have been reported to be associated with HPD. In addition, down-regulated T cell signatures and a high density of M2-type macrophages and myeloid derived suppressor cells, which exist in TME, have shown negative impacts on immunotherapy [10–13].

Earlier studies demonstrated that tumor-infiltrating lymphocytes (TILs) were strongly associated with local PD-L1 expression in the tumor biopsies of melanoma patients [14]. To suppress local effector T-cell function, tumor cells upregulate PD-L1 expression in response to interferon-γ released by TILs as an adaptive immune-resistance mechanism [2, 7, 15, 16]. In addition, increased levels of CD3 and CD8 + TILs were associated with better outcome in a large series of patients with non-small cell lung cancer (NSCLC) [15]. Moreover, PD-L1 can also be expressed constitutively on cancer cells through poorly characterized oncogenic signaling pathways [17]. Indeed, PD-L1 expression is observed in various cancers including lung, melanoma, breast, kidney cancer, and Hodgkin lymphoma, and it is widely accepted as key biomarker for predicting clinical response to anti–PD-1/PD-L1 therapy [5, 18, 19].

On the basis of PD-L1 status and presence or absence of TILs, malignant disease can be classified into four groups: type I (PD-L1 positive with TILs driving adaptive immune resistance), type II (PD-L1 negative with no TIL indicating immune ignorance), type III (PD-L1 positive with no TIL indicating intrinsic induction), and type IV (PD-L1 negative with TIL indicating the role of other suppressor(s) in promoting immune tolerance) [2, 20]. In addition to natural killer (NK) cells and dendritic cells, cancer associated fibroblasts, which regulate the dynamic process of M2 transformation, can affect the response to cancer immunotherapy [10]. Furthermore, inflammatory reaction indices in the peripheral blood do not directly reflect the local immune responses occurring at tumor; nonetheless, the systemic indices closely connected with the tumor response to immunotherapy [21, 22].

Accordingly, we hypothesized that there may be meaningful distinctions in clinical features, serologic markers, and compositional changes of immune cells among patient groups displaying different tumor response to immunotherapy. We therefore conducted a retrospective analysis of clinical data, periodically monitored serological indices, and quantitatively analyzed immune cell compositions of the intratumoral and stromal regions.

Methods

Study population and design

Data were retrospectively collected from all consecutive eligible patients with advanced NSCLC who were treated with ICBs between January 2014 and May 2018, at five St. Mary's Hospitals in Seoul, Suwon, Uijeongbu, Bucheon, and Yeouido, Korea. This study was approved by the Institutional Review Board of Catholic Medical Center [KC18SESI0440]. ICBs including nivolumab, pembrolizumab, avelumab, atezolizumab, or durvalumab, and were prescribed under coverage by health insurance or an early access program. We excluded patients who were lost to follow-up while showing a favorable response to ICBs or who did not have information available regarding the previous treatment.

Clinical data included age at diagnosis, sex, primary tumor location, TNM stage at diagnosis, number of prior systemic treatments, best tumor response during immunotherapy, baseline and post-immunotherapy imaging, patterns of recurrence, and location of distant metastases. Patients were divided into four groups: HPD, non-HPD progressive disease (non-HPD PD), stable disease (SD), and partial/complete response (PR/CR) displaying different tumor response to immunotherapy. We recorded time-series laboratory data including serum C-reactive protein, erythrocyte sedimentation rate, albumin, lactate dehydrogenase (LDH), and white blood cell count immediately before starting treatment, at the beginning of immunotherapy, and at the first tumor response assessment, i.e., 6-8 weeks after initiation of immunotherapy. The neutrophil-to-lymphocyte ratio (NLR) was defined as the absolute neutrophil count divided by absolute lymphocyte count, and the platelet-to-lymphocyte ratio (PLR) was defined as thrombocyte count divided by the lymphocyte counts. The C-reactive protein-to-albumin ratio (CAR) was calculated by dividing the C-reactive proteins level by the albumin level.

Tumor growth kinetics

Radiological changes were evaluated based on the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST ver. 1.1) [23] and irRECIST [24]. We defined HPD as having (1) a tumor growth kinetics ratio (TGKr) exceeding the tumor growth rate by two-fold between the reference period (before immunotherapy) and the experimental periods during anti–PD-1/PD-L1 therapy and (2) a time-to-treatment failure (TTF) less than 2 months [7, 13, 25, 26]. We reviewed all pre- and post-immunotherapy images and determined the two points for determining tumor growth kinetics (i.e., before starting immunotherapy [TGK_{PRE}] and after immunotherapy [TGK_{POST}]) [12, 13, 21]. T_{PRE}, T_0, and T_{POST} denote the time of the reference period's baseline, experimental period's baseline, and the experimental period's first-post imaging, respectively. S_{PRE}, S_0, and S_{POST} denote the sum of the largest diameter of target lesions at the reference period's baseline, experimental period's baseline, and first follow-up image of the experimental periods, respectively. TGK_{PRE} was defined as the difference in the sum of the largest diameters of the target lesions per unit of time between the reference period and experimental baseline imaging: $(S_0S_{PRE})/(T_0T_{PRE})$. Similarly, TGK_{POST} was defined as $(S_0S_{POST})/(T_0T_{POST})$. TGKr was defined as the ratio of TGK_{POST} to TGK_{PRE}. TGKr >1 indicated tumor growth acceleration, whereas $0 < TGKr \leq 1$ and $TGKr \leq 0$ indicated tumor deceleration and tumor shrinkage, respectively [13, 25-27].

Assessment of PD-L1 expression level using immunohistochemistry

We used archival tumor tissues obtained by core needle biopsy or excisional biopsy at the initial diagnosis. PD-L1 expression is widely used as a key predictive biomarker for PD-1/PD-L1 blockade and has been approved as a companion diagnostic test for pembrolizumab (Kytruda®; Merck, Kenilworth, NJ, USA). PD-L1 expression was assessed using immunohistochemistry (IHC) in formalin-fixed paraffin-embedded tumor tissue using the PD-L1 IHC 22C3 pharmDx assay (Dako, Santa Clara, CA, USA) at a hospital pathology laboratory. These data were determined by means of a Combined Positive Score, which includes the number of PD-L1 positive cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.

Analysis of immune cell composition using multiplex IHC

To examine the TME, we used a quantitative multispectral imaging method using the Opal Multiplex IHC kit (Perkin-Elmer, Waltham, MA, USA) and Vectra automated quantitative pathology imaging system (Perkin-Elmer). Multiplex IHC staining for immune cells and antagonists of the PD-1/PD-L1 pathway was performed using a Leica Bond Rx™ Automated Stainer (Leica Biosystems, Newcastle, UK). We analyzed scanned images using inForm image analysis software (Perkin-Elmer) and TIBCO Spotfire software (TIBCO, Palo Alto, CA, USA).

We analyzed differences in the immune composition of the TME using multiplex IHC. T cell markers, including CD4, CD8, FOXP3, CD45RO, and CD3 were placed on panel 1, and co-inhibitory signal markers including TIM3, LAG3, PD-1, and PD-L1 were placed on panel 2. We also examined the degree of penetration of CD14, CD68, CD163, and CD206 as macrophage markers on panel 3 as well as CD11c as a myeloid-derived cell marker, CD16, CD56, CD86, and CD103 as NK cell and dendritic cell markers on panel 4.

Statistical analysis

The correlation of HPD with the clinicopathologic factors was analyzed using Pearson's chi-squared test and Student’s t-test. The quantified multiplex IHC data were analyzed using the Mann Whitney U test and compared using the Kruskal Wallis test. Overall survival and progression-free survival were calculated from the start of ICB administration until the date of death and disease progression, respectively. For survival analysis, living patients or those with no disease progression were censored from the last follow-up date. Multivariate analyses were identified by cox regression and overall survival and progression-free survival were performed using logistic regression and the log-rank test. All statistical analyses were performed using SPSS software (version 21; IBM Corp., Armonk, NY, USA) and R-studio package (version 1.8; https://rstudio.com/ Boston, MA, USA). A two-sided *P* value of <0.05 was considered statistically significant. Spider plots, scatterplots, and Kaplan-Meier survival curve were generated using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Baseline clinical characteristics

Altogether 231 patients were included in the tumor growth kinetics analysis, with a mean age of 64.2 years, male sex composition of 74.9% and ex- or current smoker composition of 70.2%. Among the smokers, heavy smokers (≥ 20 packs per year) comprised 88.9%. The majority of patients (80.1%) were treated with at least second-line ICBs. Thirty-seven patients were heavily treated (≥ 4th line treatment). PR/CR and SD were achieved in 50 (21.6%) and 79 (34.2%) patients, respectively. Twenty patients (8.7%) did not have a response evaluation due to rapid progression with early death, and were subsequently classified as the NE (non-evaluable) group (Supplementary Fig. 1). Of 82 patients exhibiting PD, 26 (31.7%) met the criteria for HPD (Supplementary Fig. 2). The baseline characteristics of all patients are listed in Supplementary Table 1, Supplementary Figs. 1 and 2.

Correlation between clinical and pathological parameters and tumor response pattern

When compared with good response group (defined as SD, and PR/CR), HPD was markedly frequent in patients carrying oncogenic driver mutations (30.8%, $P = 0.018$). Furthermore, there were significant differences in age ($P = 0.002$), presence of ≥ 3 metastatic sites before the beginning of immunotherapy ($P = 0.005$), and number of previous treatments ($P = 0.139$) between good responders (SD + PR/CR) and poor responders (HPD) (Table 1). The PD-L1 expression level in 176 patients was different among the four tumor response groups ($P = 0.166$). PD-L1 expression in the HPD group tended to be lower than that in the PR/CR group ($P = 0.060$) and HPD occurred more frequently in patients displaying low PD-L1 expression (< 10%) ($P = 0.026$) (Supplementary Fig. 3). Additionally, there was an obviously shortened median overall survival time in poor responders than in good responders (5.5 months and 6.1 months vs 16.2 months and 18.3 months, respectively, $P = 0.000$) (Supplementary Fig. 4).

Table 1

Clinical characteristics according to tumor response pattern (n = 155)[†]

Total patients	HPD (n = 26)	SD/PR/CR (n = 129)	*P* value
Age (y)	58.96 ± 10.2	65.19 ± 9.1	0.002
Gender (Female:Male)	3:23	35:94	0.092
Smoking history (pack/y)	29.3 ± 19.6	24.41 ± 21.6	0.284
< 20	5	51	0.049
≥ 20	21	78	
Histologic subtype			0.061
Adenocarcinoma	14	93	
Squamous cell carcinoma	11	34	
Others	1	2	
Oncogenic driver mutations			
Yes:No	8:18	16:113	0.018
EGFR mutation	5	7	
ALK, ROS1, other gene mutations	3	9	
Number of metastatic site			0.005
< 3	17	113	
≥ 3	9	16	
Treatment lines before immunotherapy			0.139
< 3	21	117	
≥ 3	5	12	

Values are presented as mean ± standard deviation, ratio, or number.

[†]Excluding non-evaluable group and Non-HPD PD group.

ALK Anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor, *HPD* hyperprogressive disease, *Non-HPD PD* non-HPD progressive disease, *OS* overall survival, *PR/CR* partial/complete response, *SD* stable disease.

Changes in inflammation-related serologic markers in the four tumor response groups

Figure 1 contains the associations of tumor response pattern with NLR, PLR, CAR, and LDH at serial time points. At the time of initial response evaluation, all inflammation-related serologic markers were

significantly associated with HPD. Even at the beginning of initiation of treatment and immunotherapy, CAR still had significant correlations with the tumor response pattern.

Predictive Factors For Hpd In Univariate And Multivariate Analyses

In the univariate analysis, age, number of metastatic sites, presence of oncogenic driver mutations, and post-immunotherapy serological markers (NLR, PLR, CAR, and LDH) were significantly associated with HPD. In multivariate logistic regression, only the number of metastatic sites, and post-immunotherapy NLR at first response evaluation independently predicted HPD (Table 2).

Table 2

Predictive factors associated with HPD as determined by univariate and multivariate analysis (n = 76)[†]

Variable	HR (95% CI)	*P* value
Univariate analysis		
Age < 55 /≥55	3.252 (1.044–10.130)	0.036
Sex, male/female	2.421 (0.617–9.499)	0.195
Smoking history, < 20 /≥20	2.800 (0.9097–8.647)	0.068
No of metastatic sites, < 3 /≥3	3.252 (1.044–10.130)	0.036
Oncogenic driver mutant, none/yes	4.000 (1.153–13.876)	0.023
PD-L1 (22C3), < 10 /≥10	3.986 (1.124–14.138)	0.026
No. of treatments before IO, < 3/≥3	2.143 (0.559–8.212)	0.259
Baseline_CAR, < 0.5 /≥0.5	2.844 (1.069–7.568)	0.034
Post_NLR, < 5 /≥5	17.00 (4.982–58.009)	0.000
Post_PLR < 150 /≥150	4.603 (1.577–13.438)	0.004
Post_CAR, < 0.5 /≥0.5	4.554 (1.656–12.524)	0.002
Post_LDH < 400 /≥400	3.371 (1.006–11.290)	0.042
Multivariate analysis		
No. of metastatic sites, < 3 /≥3	6.712 (1.277–35.273)	0.025
Post_NLR, < 5 /≥5	15.788 (4.190-59.485)	0.000
Post_LDH, < 400 /≥400	4.011 (0.805–19.976)	0.090

[†]Including HPD group and PR/CR group

CAR C-reactive protein-albumin ratio, *LDH* Lactate dehydrogenase, *NLR* neutrophil-to-lymphocyte ratio, *PLR* platelet-to-lymphocyte ratio.

Analysis of immune cell composition in the TME by multiplex IHC

To understand the cross-talk between the tumor and its accompanying heterogeneous TME, we analyzed the expression of several types of immune cells using multiplex IHC as explorative setting, only some cases with many available tissues (Fig. 2). In the PR/CR group, the number of cells expressing T cell and TIL markers tended to be higher in the entire area, but especially in the stroma. In contrast, in the HPD group, cells expressing macrophage markers were markedly higher in both the tumor and stroma. In particular, there was a noticeable increase of M2 marker-positive cells in the stroma (Fig. 3). In the HPD group, there were fewer CD4 + effector T cells and CD8 + cytotoxic T cells ($P < 0.010$ and $P < 0.382$,

respectively), whereas, there were significantly more regulatory T (Treg) cells co-expressing CD4 + and FOXP3 + in both the tumor and stroma ($P < 0.003$ and $P < 0.015$, respectively). CD8+/PD-1 + cells, a parameter for TIL activity, were significantly lower in HPD group than in the PR/CR group, and the degree of immune cell penetration into the tumor region did not increase (Fig. 4a). In addition, there were significantly more macrophages expressing CD14, CD68, and CD163 in the TME of the HPD group, implying a tendency for M2 polarization in HPD (Fig. 4b). In the HPD group, CD11c, one of the myeloid markers expressed by cancer-associated fibroblasts, tended to increase intratumorally, but CD103 (a marker of dendritic cells) and CD56 (a maker of NK cells) decreased (Figs. 3 and 4c). Interestingly, there was a reverse composition of CD56 + NK cells in the tumor and stromal regions between the PR/CR and HPD groups. Even though there was not a significant difference in stromal CD56 + NK cells between the two groups, there were significantly more intratumoral CD56 + NK cells in PR/CR group (Fig. 4c).

Discussion

Although immunotherapy can induce favorable and durable tumor responses in some patients, in clinical practice, many patients experienced a poor prognosis with tumor flare-ups [5–7, 28]. Generally, HPD is defined as a TGKr exceeding 2 during anti–PD-1/PD-L1 therapy and a TTF of ≤ 2 months. HPD incidence is diverse, ranging between 4% and 29% [7, 13, 25–27]. This study analyzed associations of clinical and serological parameters with the tumor response pattern and evaluated the immune composition of the tumor and its microenvironment in patients with advanced/metastatic NSCLC receiving immunotherapy.

The current study, applied the stringent definition of HPD (TGKr ≥ 2 and TTF ≤ 2 months) and only NSCLC patients were included. The incidence of HPD (11.3%) was no higher than those found in the literatures [7, 27, 29]. Moreover, several clinical and serological factors were significantly associated with HPD. Among them, NSCLC patients carrying activating epidermal growth factor receptor (*EGFR*) mutations exhibited a markedly lower response to immunotherapy compared to those with wild type-*EGFR* [30]. There are several possible explanations for this result. First, tumor mutational load in *EGFR*-mutated NSCLCs is lower than that of wild type-EGFR NSCLCs. Second, the TME of NSCLCs harboring *EGFR* mutations is not inflamed, resulting in a reduced interferon γ signature [2, 30]. Consistent with the report of Ferrara et al. [27], the current data showed that HPD was significantly correlated with multiple metastatic sites (≥ 3) and heavy treatment (≥ 4 prior treatments).

PD-L1 expression is not a prerequisite for anti–PD-1/PD-L1 inhibitors due to its variability and dynamicity [4]. Beyond PD-L1 testing for immunotherapy, tumor mutation burden, cytotoxic CD8 + T cells/TILs, an “immunoscore”, T cell receptor clonality, immune gene signature/RNA repertoire, and major histocompatibility complex class polymorphisms are being investigated [18, 19]. Nevertheless, PD-L1 expression levels, which are readily available, have substantial predictive capacity, although it can sometimes be limited. In the present study, tumoral PD-L1 expression tended to be lower in the HPD group in the PR/CR group; additionally, HPD was significantly more frequent in patients with low PD-L1 expression (< 10%). Even though lower PD-L1 expression was significantly associated with HPD, these events sometimes occurred even in the patients with high PD-L1 expression. Furthermore, NLR and PLR,

hematological indicators reflecting the changes of blood cell pattern, are a secondary local response to immunotherapy [21, 22, 31]. Hence, with the exception of CAR, serologic markers before immunotherapy have no clinical implication. In contrast, CAR is an inflammatory indicator reflecting a patient's general condition and cancer progression. Several reports have noted that serologic markers (NLR ≥ 5, PLR ≥ 150, and CAR ≥ 0.5) significantly correlated with HPD [22, 31]. In support of this, the presenter results indicated that CAR (≥ 0.5) values immediately before initiating treatment, as well as before and during immunotherapy, are predictive of poor response (HPD and PD). Inoue et al. reported that a high CAR value was associated with early death after immunotherapy [28]. A recent Korean real world study demonstrated that NLR, PLR, and CAR at six weeks after administration of immunotherapy were powerful predictive markers for HPD. Regardless, there is some debate as to whether inflammation-based serologic markers obtained from systemic circulating blood denote the degree of immune response at the local tumor. Whatever the reason, many research results strongly support the evidence that local immune response manifests adequate prognostic value [21]. These serologic markers, as well as imaging studies, may provide additional information concerning the tumor response to immunotherapy in patients with advanced NSCLC.

The present study, revealed that cells expressing T cell and TIL markers were abundant in the stromal region of good responders; however, there were significantly more T regulatory cells co-expressing CD4 + and FOXP3 + in the entire tumoral and stromal area in poor responders. Previous studies have emphasized that pre-existing anti-tumor immunity and T cell exhaustion are associated with HPD [15, 16]. Moreover, Lo Russo et al. recently presented evidence that, upon immunotherapy-related Fc receptor engagement, tumor-associated macrophage reprogramming, plays a crucial role in HPD [29]. The present results show that M2-type macrophages and cancer-associated fibroblasts from the HPD group had a wide distribution in the stromal region. This study provides evidence of a novel association between HPD and M2-type macrophages throughout heterogeneous cellular components within the TME. Recently, reports have indicated that macrophages are influenced by the TME, causing them to adopt and facilitate epithelial–mesenchymal transition features, and transformed from M1 to M2 polarization, eventually resulting in rapid disease progression [2, 9, 10]. Of note, M1 and M2 signatures have important functional differences: M1 evinces an enhanced microbicidal and tumor resistant effect, while M2 plays a role in anti-parasite defense and immunoregulation [2, 32]. However, in human disease, their functional activities overlap and are far more dynamic. In the present study, there were more M2-type macrophages in HPD. Notwithstanding, since M2 macrophages are more dynamic than other immunologic indices, they are a poor prognostic biomarker for differentiating HPD.

NK cells are important cytotoxic, innate immune cells involved in the elimination of cancer cells [33]. There are two main NK cell subsets based on CD56 and CD16 expression: the $CD56^{bright}CD16-$ NK subset produces abundant cytokines, including interferon-γ and tumor necrosis factor α, whereas the $CD56^{dim}CD16+$ subset has high cytolytic activity and releases granules containing perforin and granzymes [34]. Several studies have discovered enhanced PD-1 expression on activated NK cells such as $CD56+^{bright}$NK cells [33, 35]. We took note of the composition of $CD56+^{bright}CD16-$ NK and

$CD56^{dim}CD16+$ NK subsets in tumors and stroma. $CD56+^{bright}$ NK cells were remarkably increased in intratumoral region in the PR/CR group. Intratumoral PD-1 + NK cells are related to PD-L1 expressed on cancer cells and prevent the expansion and function of effector T cells and their exhaustion, eventually leading to immune evasion by the tumor. Based on these results, we assume that increased intratumoral $CD56+^{bright}$ NK cells may play an important role in the immunotherapy-initiated revitalization of T-cells in the PR/CR group.

The present study has several limitations. First, even though this study was carried out only for patients with lung cancer, the composition and function of immune cells in the tumor and the TME may change in response to multiple cytotoxic chemotherapy compared to that at the initial diagnosis. Second, the amount of archival tissue available for this retrospective analysis was insufficient to conduct a whole genome study such as next generation sequencing. Accordingly, we applied multiplex IHC to identify various immune cell markers. Third, owing to insufficient numbers in some of the subgroups based on tumor response pattern, it is difficult to draw widely applicable conclusions from the results.

HPD is a phenomenon caused by immunotherapy rather than by differences treatment efficacy in biologically heterogeneous patients with NSCLC [16, 29]. Although the results show that serologic markers and changes in the composition of immune cells predict HPD occurring in patients with NSCLC receiving immunotherapy, these results may be stochastic. To better clarify this, we are conducting a prospective study to better explore the mechanisms underlying the development of HPD.

In conclusion, HPD is a unique biologic process distinct from other PD. The current results strongly suggest that serologic biomarkers including NLR, PLR, and CAR, are predictive of HPD. Furthermore, the composition of T-cell subsets, macrophages, and NK cells in tumors and the surrounding stromata may be useful to predict the tumor response to immunotherapy; in addition, these biomarkers may also aid in improving understanding regarding the dynamic and complex changes of immune cells within the TME.

Abbreviations

ICBs: Immune checkpoint blockers; HPD:hyperprogressive disease; NSCLC:non-small cell lung cancer; TME:tumor microenvironment; TILs:tumor-infiltrating lymphocytes; NK:natural killer; PD:progressive disease; SD:stable disease; PR/CR:partial/complete response; LDH:lactate dehydrogenase; NLR:neutrophil-to-lymphocyte ratio; PLR:platelet-to-lymphocyte ratio; CAR:C-reactive protein-to-albumin ratio; RECIST:Response Evaluation Criteria in Solid Tumors; TGKr:tumor growth kinetics ratio; TTF:time-to-treatment failure; IHC:immunohistochemistry; NE:non-evaluable; Treg cell:regulatory T cell; EGFR:epidermal growth factor receptor.

Declarations

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study disclosed information using an opt-out method and excluded patients who did not consent to the study. This study was reviewed and approved by the Institutional Review Board of Catholic Medical Center [KC18SESI0440].

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

Conceptualization and design: SR Kim, SH Chun and JH Kang, Collection and assembly of data: SR Kim, SH Chun, JR Kim, CK Jung, BM Gil, YH Ko, IS Woo, BY Shim and SH Hong, Data analysis and interpretation: SR Kim, SY Kim, JY Seo and JH Kang, Manuscript writing: All authors, Final approval of manuscript: All authors

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Figures

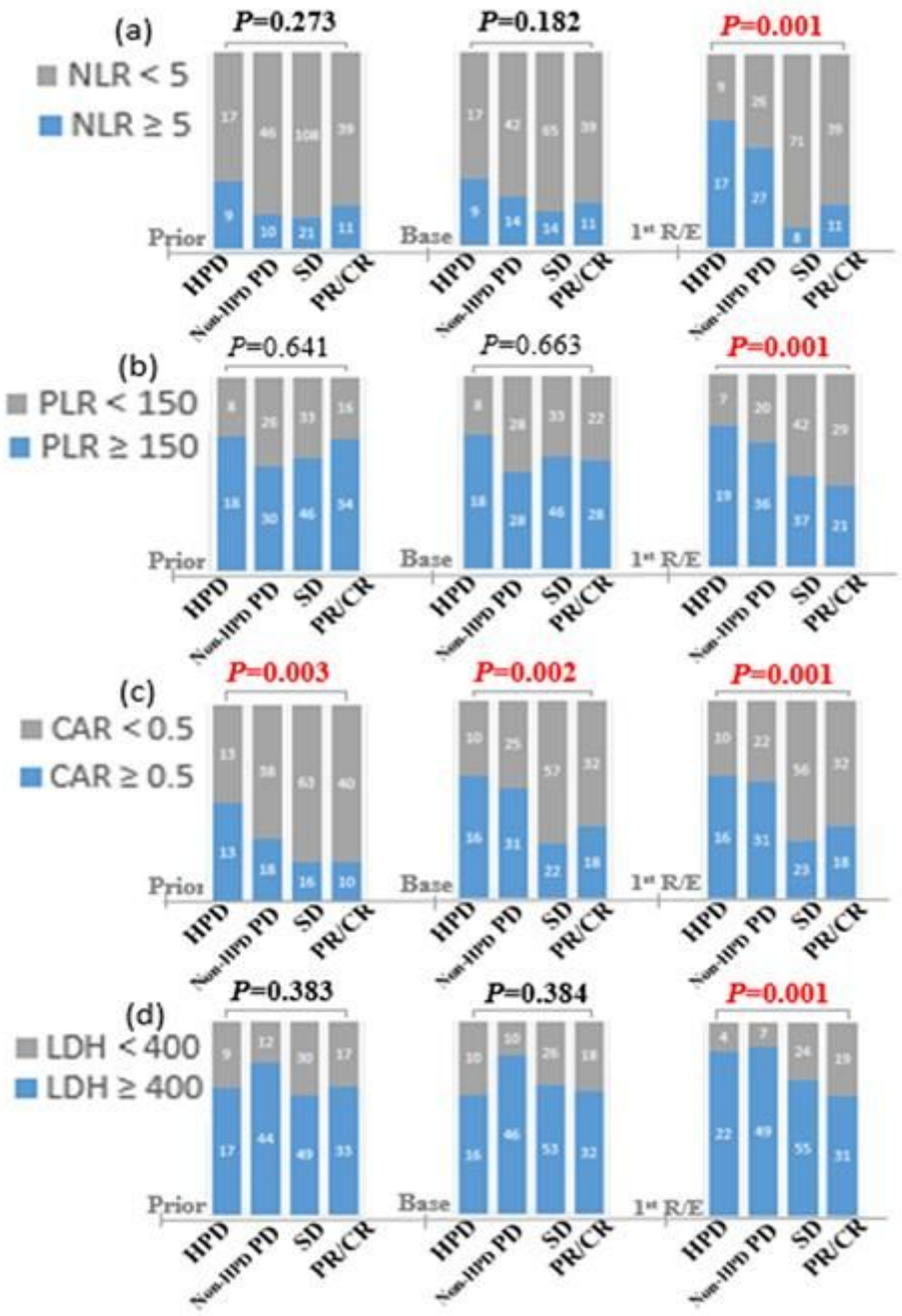

Figure 1

Change in the Serologic and inflammatory Markers in Four Tumor Response Groups (n = 211)† †Excluding non-evaluable group. prior At the beginning of prior treatment, base At the beginning of immunotherapy, 1st R/E At 1st tumor response evaluation, CAR C-reactive protein-albumin ratio, LDH Lactate dehydrogenase, NLR neutrophil-to-lymphocyte ratio, PLR platelet-to-lymphocyte ratio, HPD hyperprogressive disease, Non-HPD PD non-HPD progressive disease, SD stable disease, PR/CR partial response/complete response.

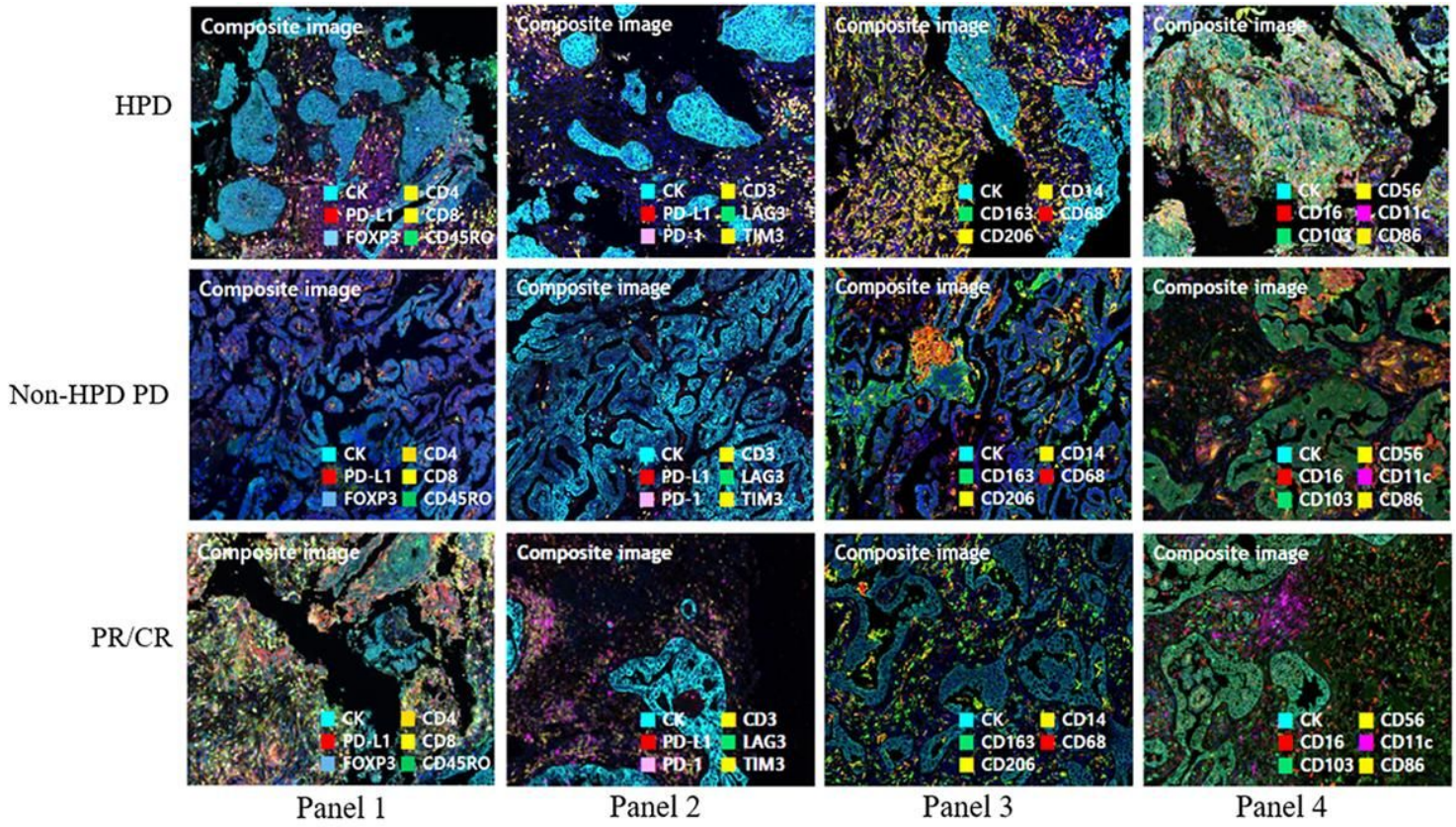

Figure 2

Multiplex immunohistochemistry for immune cell markers in HPD, non-HPD PD, and PR/CR group (n=24)† †As explorative setting, only some cases with many available tissues in HPD, non-HPD PD and PR/CR group HPD hyperprogressive disease, NK natural killer, Non-HPD PD non-HPD progressive disease.

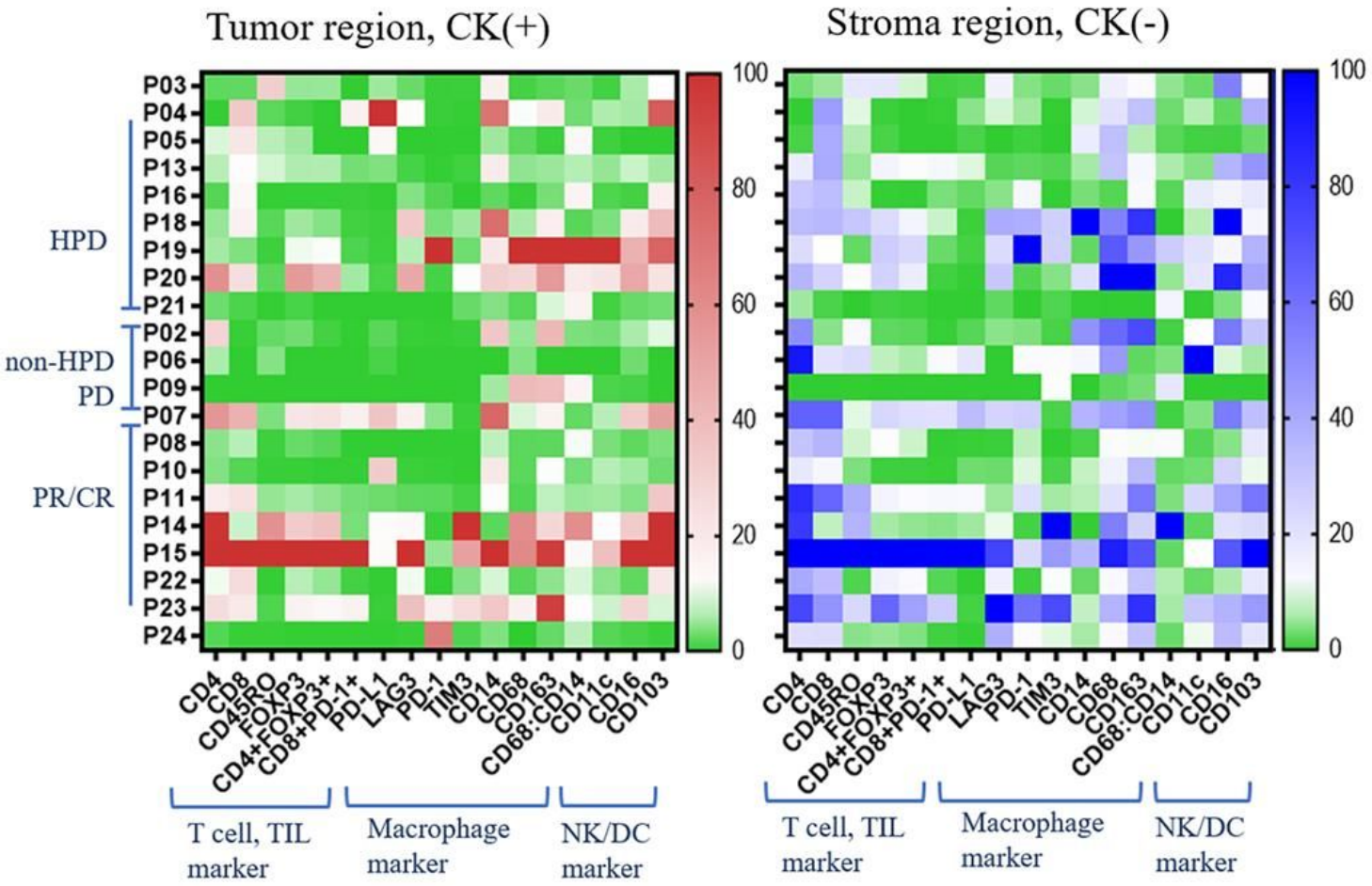

Figure 3

Multiplex immunohistochemistry panels display immune compositional change of tumor microenvironment (n=24)† †As explorative setting, only some cases with many available tissues in HPD, non-HPD PD and PR / CR group HPD hyperprogressive disease, Non-HPD PD non-HPD progressive disease, PR/CR partial response/complete response, TIL tumor infiltrating lymphocyte, NK natural killer, DC dendritic cell.

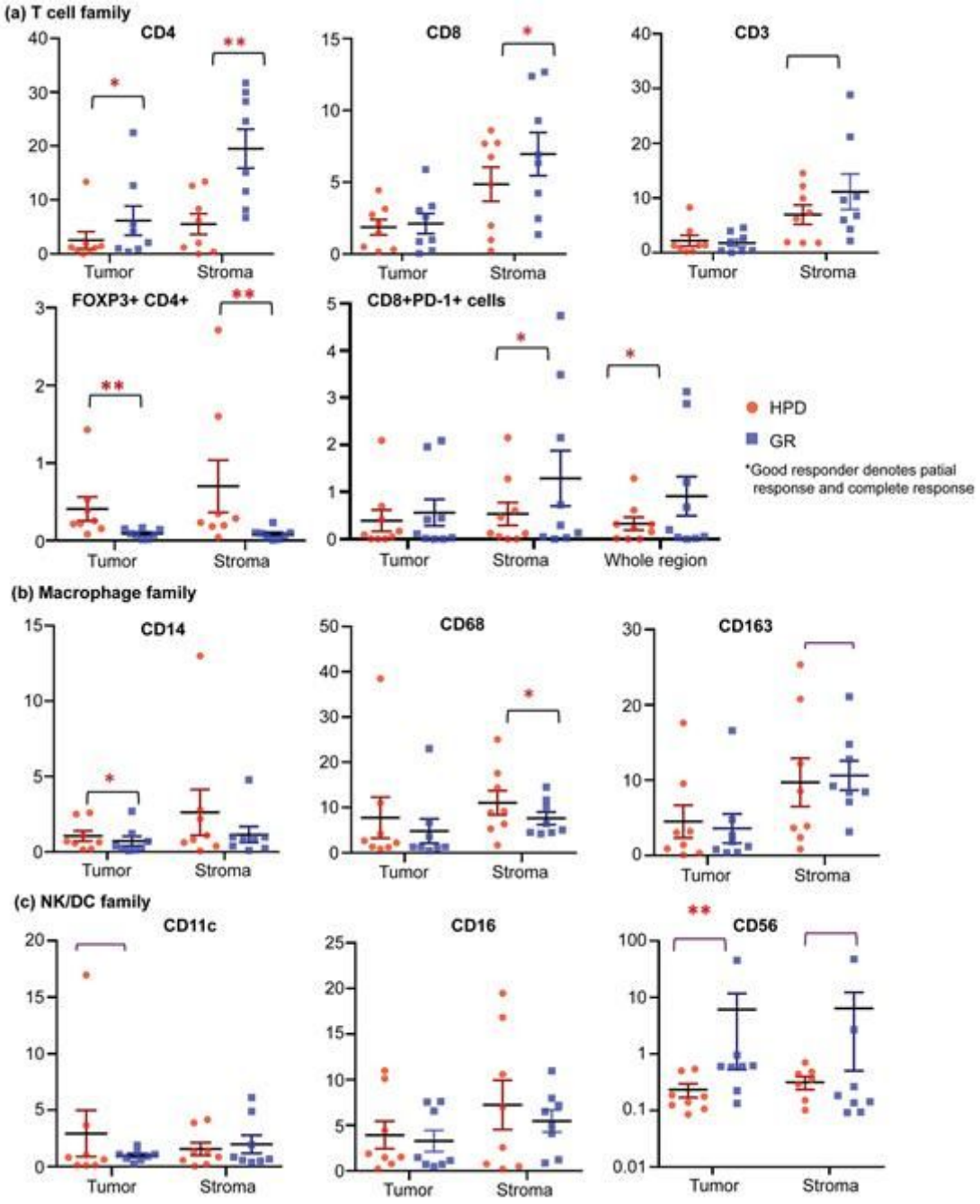

Figure 4

Analysis of Immune Cell Composition in the TME by Multiplex IHC. (a) Multiplex IHC data shows the lymphocytes, such as CD4 and CD8 in the HPD group, are smaller than in the good responder, and T reg cells marked with FOXP3 increase in the HPD group. The HPD group was supposed to be an immune desert state that was not inflamed. (b) The key observational data was a tendency to M2 polarization in patients with HPD when the macrophage M2 markers of CD68, CD163 and CD206 were observed. (c) According to this multiplex IHC data, the myeloid markers, such as CD11c, expressed by CAFs, tend to increase in the intra-tumor lesion of the HPD group, and dendritic cell markers (CD103) and NK cell marker (CD56) tend to decrease in the HPD group. IHC immunohistochemistry, HPD hyperprogressive

disease, NK natural killer, DC dendritic cell. Statistical power : ** implies p value <0.05, * implies p value <0.5, † implies p value ≥0.5 but shows a clinical correlation.

Supplementary Files

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