

Peripheral Lymphocyte Count as a Surrogate Marker of Immune Checkpoint Inhibitor Therapy Outcomes in Patients with Non-Small-Cell Lung Cancer

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Abstract

Background

Degree of expression of programmed death-ligand 1 (PD-L1) is related with Immune check point inhibitors (ICIs) response but is not obligate predictive marker and needs sufficient tissue. Therefore, there is unmet need for easily accessible peripheral blood (PB) biomarkers and evaluation of the prognostic value of this marker is needed. We investigated the application of serum peripheral lymphocyte count (PLC) as a predictive PB biomarker for ICI response in patients with NSCLC.

Methods

We conducted a retrospective study and reviewed the medical charts of patients with NSCLC who were treated with ICIs at Seoul National University Hospital. We evaluated the association between PLC and progression-free survival using a Cox proportional hazard model. The PLC before and after 1 month of immunotherapy was collected. The quartile groups of PLC were compared using the Kruskal-Wallis statistical test.

Results

A total of 231 patients were treated with immunotherapy for NSCLC. The median follow-up period was 4.7 months. During the follow-up period, the disease progressed in 138 patients (59.7%). The post-treatment PLC groups Q2-4 showed significantly lower disease progression than group Q1 in our adjusted model (Q4 hazard ratio: 0.41, 95% confidence interval: 0.25–0.68, $p < 0.001$). The overall survival also showed similar results. An association between adverse events and PLC was not observed in this study.

Conclusion

We revealed that an increased post-treatment PLC was associated with favorable progression-free and overall survival with NSCLC patients treated with ICIs. Therefore, PLC could be a surrogate marker for ICI responses in NSCLC.

Introduction

The introduction of immune checkpoint inhibitors (ICIs), programmed cell death receptor (PD-1) inhibitors (nivolumab or pembrolizumab), and programmed death-ligand 1 inhibitors (PD-L1) inhibitors (atezolizumab) for the standard treatment of advanced non-small-cell lung cancer (NSCLC), has improved survival rates. However, only limited patients receiving ICIs are benefitted [1, 2], and absolute and clear predictive markers are absent, except for one representative biomarker based on PD-L1 expression. PD-L1 expression measured by immunohistochemistry (IHC) has been used as a biomarker in initial clinical trials of PD-1 and PD-L1 inhibitors [3, 4]. However, it is ineffective as a single predictive biomarker for ICI treatment; various PD-L1 expression assays with different antibodies, platforms, and cutoffs have been studied, and their outcomes vary from one clinical trial to another. In addition, the

heterogeneous expression of PD-L1 in tumors is another limiting factor. Therefore, more accurate and robust markers are required for identifying patients who will benefit from ICIs.

Currently, tumor mutational burden (TMB) is another biomarker used in serial CheckMate trials [5, 6]. However, using TMB as a biomarker is also challenging because of the long turnaround time, large number of tissue samples required for accurate analysis, and significant cost involved for the interpretation of next-generation sequencing data used to measure the number of somatic mutations [7].

Tumor-infiltrating T lymphocytes (TILs) are also considered as biomarkers for tumor-immune system interactions; the higher the TIL concentration, the better the effects of ICIs [8, 9]. Shortcomings of the TIL-based prognostic biomarker include lack of quantitative IHC approaches, poor interobserver reproducibility, and time-consuming procedures for the measurement of TIL.

Therefore, multiple studies have examined peripheral blood (PB) biomarkers owing to their easy accessibility and low costs [10]. The neutrophil-to-lymphocyte ratio (NLR) is a widely studied blood-based biomarker used for various types of tumors treated with ICIs [11]. Previous studies have shown that a high NLR is associated with poor outcomes in lung cancer [11–13]. However, the association between peripheral lymphocyte count (PLC) and ICIs has not yet been established. We hypothesized that PLC may influence ICI outcomes because the mechanism of ICIs is dependent on the activities of T lymphocytes. Therefore, PLC may be a biomarker for determining ICI responses in NSCLC. In this study, we investigated the application of serum peripheral lymphocyte count (PLC) as a predictive PB biomarker for ICI response in patients with NSCLC.

Methods

Study design and participants

We conducted a retrospective study from April 1, 2016, to March 31, 2019. Enrolled patients were aged \geq 18 years, diagnosed with histologically proven NSCLC, received ICI-based (pembrolizumab, nivolumab, atezolizumab, or durvalumab) treatment, and underwent PD-L1 IHC at Seoul National University Hospital (SNUH). The study was approved by the Institutional Review Board (IRB no: H-1902-063-1010) of SNUH. Informed consent was waived due to the retrospective chart review study. The patients were treated with pembrolizumab at a dose of 200 mg intravenously once every 3 weeks, nivolumab at a dose of 3 mg/kg of body weight once every 2 weeks, or atezolizumab at a dose of 1200 mg intravenously once every 3 weeks until disease progression.

Variables

All patient records were retrospectively reviewed. Demographic variables (age at the first prescription of ICIs, sex, Eastern Cooperative Oncology Group (ECOG) status, and smoking status), tumor histology, anti-PD-L1 agents used, lines of treatment, and PD-L1 expression levels were noted [14]. The overall tumor response, including, complete, partial, stable, pseudo-progression, or progression, was defined as per the

Immune Response Evaluation Criteria in Solid Tumors criteria [15]. Data on the objective response rate (ORR)—the proportion of patients who exhibited a partial or complete response to therapy—and PLC detected in the complete blood count test, before and after (within 4 weeks) immunotherapy, were collected. Pre- and post-treatment PLC included values measured within 1 week and at 4 weeks after the initiation of immunotherapy, respectively. We also noted immune-related adverse events (irAEs); adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0, and grades 3–5 of irAEs were defined as serious AEs.

Analysis

The primary endpoint was progression-free survival (PFS) with immunotherapy, according to PLC. The secondary endpoints were overall survival (OS), ORR of ICIs, and AEs. Categorical variables were compared using the chi-square test or Fisher's exact test, and continuous variables were compared using an independent unpaired t-test or Mann–Whitney test if variables were nonparametric. PLC was presented as medians with interquartile ranges. The quartile groups of PLC were compared using the Kruskal-Wallis statistical test. A multivariate Cox proportional hazard model was used to determine the differences between PFS and OS between the quartiles of PLC. We adjusted the values for age, sex, smoking status, ECOG status, histology, epidermal growth factor receptor (EGFR) mutation status, and PD-L1 expression. The log-rank test was used to derive the trend in survival across the quartile groups of PLC. All statistical analyses were performed using Stata 13.0 (StataCorp, College Station, TX, USA), and a p-value of <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 270 patients were diagnosed with lung cancer during the study period at SNUH. Among them, 4 were diagnosed with small cell lung cancer (SCLC), 26 did not undergo PD-L1 IHC assay, and 9 did not have PLC data. Therefore, 39 patients were excluded; 231 patients were finally included, and their data were analyzed in this study. Baseline characteristics of enrolled patients are described in Table 1. Their median age was 66 years (37–86), and 181 (78.5%) patients were men. Most of the patients had good performance status, and 183 (79.6%) were ever-smokers. Twenty-four patients (9.9%) had activating EGFR mutations. Forty-nine patients (21.2%) were treated with ICIs, although they had negative PD-L1 expression. ICI was used as the first- or second-line of therapy in about half of the patients. Most patients were treated with nivolumab (75.3%), and the objective response was observed in 70 (30.3%) patients. During the median observation time, from the first ICI prescription, of 4.7 months (the longest was 30.6 months), 138 (59.7%) cases of progression were observed. The median pre- and post-treatment PLCs were 1525.9 cells/ μ L (1041.9–2070) and 1676.7 cells/ μ L (1178.6–2190), respectively.

Treatment outcomes

We found that an increased pre-treatment PLC correlated with slower lung cancer progression and longer PFS after adjusting for age, sex, smoking status, ECOG status, tumor histology, EGFR mutation, and PDL-1 expression (hazard ratio: 0.99, 95% confidence interval: 0.99–1.00 (Supplementary Table 1). Pre- and post-treatment PLCs were stratified into quartile groups (Q1–4) for multivariate analysis. PFS was significantly higher in the Q3–4 groups than in the Q1 group for pre-treatment PLC, calculated after adjusting for the factors mentioned above (Table 2). The same result was observed for post-treatment PLC, where the Q3–4 groups had a significantly lower progression rate than the reference group, Q1 (Table 3, Figure 1). Progression decreased for both pre- and post-treatment PLCs across quartile categories (p [for the trend] = 0.006 and 0.001 for pre- and post-treatment PLCs, respectively). Regardless of the pre- and post-treatment PLC values, patients aged > 65 years and ever-smokers were associated with a longer PFS. Besides, patients with EGFR mutations, poor ECOG performance status ≥ 2 , and PD-L1 expression levels of at least 1% showed higher progression rates than those non-conforming to the above criteria. We did not include the line of ICIs in the multivariable analysis because age and the line of ICIs were significantly correlated (the correlation coefficient (r) = -0.13, p = 0.04). In other words, older patients were more likely to receive ICIs as the first line of treatment.

We also assessed the effect of PLC on the OS of patients. Pre-treatment PLCs were significantly associated with favorable outcomes in the Q2–4 groups compared with in the Q1 group (Table 2). Post-treatment PLCs exhibited better OS in the Q3–4 groups than in the Q1 group (Table 3). Similar to PFS data, survival increased across both pre- and post-treatment PLC quartile groups (p [for the trend] = 0.035 and 0.005 for pre- and post-treatment PLC, respectively). Patients with poor performance status (ECOG ≥ 2), NSCLC—not otherwise specified, and a PD-L1 expression of at least 1% were associated with poor OS in the multivariate analysis model in both pre- and post-treatment PLC quartile groups, regardless of adjustment. The objective response rate (ORR) increased with the post-treatment PLC level. The ORR was 40.3% in the post-treatment PLC Q4 group, which was more than twice that of the Q1 group (Figure 2).

Sixty-one patients had irAEs; pneumonitis was the most frequently observed adverse consequence. The frequency and distribution of serious irAEs, fatal or leading to discontinuation of drugs, were similar between both pre- and post-treatment PLC quartile groups (Table 4 and Supplementary Table 2).

Discussion

Immunotherapy has been rapidly adopted as a standard treatment for NSCLC. Only a limited number of patients are benefitted from ICIs; therefore, biomarkers for identifying this population are critical. We evaluated the role of PLC in predicting the response of ICIs in NSCLC; an elevated PLC after ICI treatment was associated with prolonged PFS and OS in patients with NSCLC.

Besides, post-treatment PLC was a more statistically significant biomarker than the pre-treatment PLC, in terms of PFS, OS, and ORR. This may be attributed to the release of neoantigen into peripheral blood, post tumor cell death [16]. This was coherent with results from previous studies that reported that ICIs

used in combination with radiotherapy were more effective than ICIs alone [17]. Radiotherapy shows an additive effect with ICIs; the increased release of neoantigens after tumor lysis due to radiotherapy stimulates systemic anti-tumor immunity [18]. Therefore, if patients had a good response to ICIs, peripheral activated effector T-cell expansion may occur due to the emission of neoantigens after tumor cell lysis; this may be reflected as lymphocytosis after treatment. Therefore, lymphocytosis after immunotherapy indirectly refers to the successful treatment of NSCLC.

High-density engagement of TILs, including effector T cells and NK cells, stimulated by tumor-associated antigens (neoantigens), is important for achieving a suitable response to immunotherapy [19]. The amount of TILs is also assessed through tumor biopsy, similar to the analysis of TMB. Lee et al. demonstrated that the amount of CD8 + TILs was associated with PLC in patients with breast cancer [20]. In melanoma patients with tumor-reactive and specific lymphocytes, PD-1 positive CD8 + lymphocytes were enriched in the peripheral blood[21]. Based on these results, elevated pretreatment PLC could mean increased TIL levels in the tumor microenvironment. This may attribute to patients with increased pretreatment PLC having a favorable prognosis in our study, although only the Q4 group had a significantly increased PFS among the pre-treatment quartile groups.

Our study showed that old people responded better to immunochemotherapy. This result contradicted results from the CheckMate 227 trial [5]. This may be associated with the difference in the number of patients > 75 years in the two studies; 13 (9%) patients were > 75 years in the CheckMate study, while 37 (15%) patients were > 75 years in ours. A recent systematic review showed that elderly patients achieved better outcomes than younger patients; however, the study did not focus exclusively on NSCLC (five NSCLC and four other solid tumors were included) [22]. Changes in the gut microbiome [23] or the balance between regulatory and cytotoxic T cells [24] with age are some proposed theories in this regard, although not specific to lung cancer. Age is commonly known as a poor prognostic factor affecting survival in lung cancer. Interestingly, elderly patients aged > 75 years had a better OS than those aged < 65 years. This result could be explained in three ways. First, the OS in this study was not the rate calculated at the time of diagnosis, but the survival rate determined from the date the ICIs were first administered. Therefore, old age cannot be generalized as a good prognostic factor for lung cancer. Second, ICIs were used as the first-line treatment for elderly patients with lung cancer compared with patients aged < 65 years (25.0% vs. 17.3%, $p = 0.001$). Third, elderly patients used ICIs for the first time; therefore, the probabilities of bone marrow failure or side effects due to toxicity from previous systemic chemotherapy were minimal. Consequently, elderly patients showed longer survival. Therefore, our results confirm that older patients had a better response than younger patients.

Our study also showed that ever smokers had a significantly lower progression rate than never smokers. This finding was consistent with two previous studies, one that showed the effect of nivolumab in advanced NSCLC[25] and another being a meta-analysis[26]. This might be due to the high TMB in ever smokers compared with never smokers among patients with lung cancer[27]. In addition, patients with EGFR mutations had a more rapid progression rate those without. This result is well-known and

supported by a lot of clinical and experimental evidence[28]. Therefore, the data from these patients were excluded from analyses, although we did not exclude them from the randomized controlled study.

We did not find an association between irAEs and lymphocytosis both pre- and post-treatment. The association between PLC and irAEs remains controversial. Diehl et al. showed that patients with a PLC > 2000 cells/ μ L at baseline were associated with an increased risk of irAEs. Kamran et al. found that lymphopenia is a predictor for irAEs[29]. Therefore, we were unable to identify if lymphocytosis or lymphopenia was associated with irAEs.

This study has some limitations. First, this study was retrospective and conducted at a single institution. Second, the mechanism behind lymphocytosis influencing TIL and neoantigen levels is still ambiguous. Third, patients with EGFR mutations could not be excluded from the study. Lastly, we could not suggest a clinically useful cutoff for lymphocytosis because we used quartile analysis. Despite these limitations, this is the first study to determine whether PLC, not NLR, could be used as a biomarker and to evaluate the cutoff value of PLC that showed significance in a larger cohort compared with previous studies. In addition, the present study shortened the duration of response assessment significantly, from 6–12 weeks to 4 weeks[15]. Therefore, the effectiveness of ICI based treatment in patients with NSCLC can be determined soon after initiation of therapy.

In conclusion, PFS was significantly longer with elevated post-treatment PLCs in patients with advanced NSCLC treated with ICIs. Lymphocytosis pre- and post-treatment was not associated with irAEs. However, further studies are necessary to support these findings.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Review Committee of Seoul National University Hospital (IRB no: H-1902-063-1010). Informed consent was waived due to the retrospective chart review study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Nothing to declare

Author's contributions

Study conception YJL and EYH; Study design: YJL, YSP, TYP, and JKL; Manuscript preparation: YJL; Review and editing of manuscript: YJL, YSP, HWL, TYP, and EYH. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics of study patients.

Characteristics	Total (N=231)
Age, median (range)	66 [37-86]
Sex (%)	
Male	186 (78.5%)
ECOG (%)	
0	40 (17.2%)
1	132 (56.9%)
≥2	60 (24.8%)
Smoking (ever) (%)	183(79.6%)
EGFR activating mutation (%)	24 (9.9%)
Tumor histologic type (%)	
Adenocarcinoma	125 (54.1%)
Squamous	56 (24.2%)
Other NSCLC	50 (21.6%)
PD-L1 expression level (%)	
≥ 1%	182 (78.8%)
Line of ICIs used (%)	
1 st – 2 nd	113 (48.9%)
3 rd or more	118 (51.1%)
Type of ICIs (%)	
Nivolumab	174 (75.3%)
Pembrolizumab	54 (23.4%)
Atezolizumab	3 (1.3%)
Objective response (OR)* (%)	70 (30.3%)
Progression (%)	138 (59.7%)
PLC ⁺ , / uL	
Pre-treatment, median [IQR]	1525.9 [1041.9-2070]
Post-treatment, median [IQR]	1676.7 [1178.6-2190]

*OR: Partial response and complete response

+ PLC: peripheral lymphocyte count

Table 2. Multivariate Cox proportional hazard regression analysis for progression-free survival (PFS) and overall survival (OS) according to pre-treatment peripheral lymphocyte count

	PFS		OS	
	aHR (95% CI)	P	aHR (95% CI)	P
Age, years †				
<65	1 (reference)		1 (reference)	
65-74	0.57 (0.35,0.94)	0.03	0.71 (0.42,1.19)	0.19
≥75	0.56 (0.32,0.97)	0.04	0.7 (0.38,1.28)	0.25
Sex				
Male	1 (reference)		1 (reference)	0.58
Female	1.14 (0.68,1.91)	0.41	1.1 (0.63,1.89)	
Smoking status				0.75
Never	1 (reference)		1 (reference)	
Ever	0.59 (0.35,1.02)	0.04	0.85 (0.47,1.52)	
ECOG (ref 0)				
0	1 (reference)		1 (reference)	
1	1.54 (0.85,2.81)	0.18	1.93 (0.96,3.91)	0.12
≥ 2	2.37 (1.25,4.51)	0.01	3.61 (1.78,7.32)	0.001
Histology				
Adenocarcinoma	1 (reference)		1 (reference)	
Squamous cell carcinoma	1.14 (0.59,2.20)	0.63	1.00 (0.49,2.03)	0.86
Other NSCLC	1.41 (0.83,2.38)	0.32	2.02 (1.19,3.42)	0.01
EGFR activating mutation	3.24 (1.80,5.82)	<0.001	1.90 (0.99,3.66)	0.10
PD-L1 expression (≥1%)	0.43 (0.27,0.69)	0.001	0.47 (0.28,0.77)	0.002
Pre-treatment PLC				
Quartile [25th-75th]				
1 [561-955]	1 (reference)		1 (reference)	
2 [1187-1458]	0.68 (0.40,1.17)	0.26	0.53 (0.3,0.93)	0.004
3 [1612-1900]	0.37 (0.20,0.70)	0.004	0.25 (0.12,0.51)	<0.001
4 [2347-3126]	0.40 (0.23,0.71)	0.002	0.36 (0.2,0.66)	0.002
P for trend	0.006		0.035	

PFS: progression-free survival; OS: overall survival; aHR: adjusted hazard ratio; CI: confidence interval;
PLC: peripheral lymphocyte count;

Table 3. Multivariate Cox proportional hazard regression analysis for progression-free survival (PFS) and overall survival (OS) according to post-treatment peripheral lymphocyte count

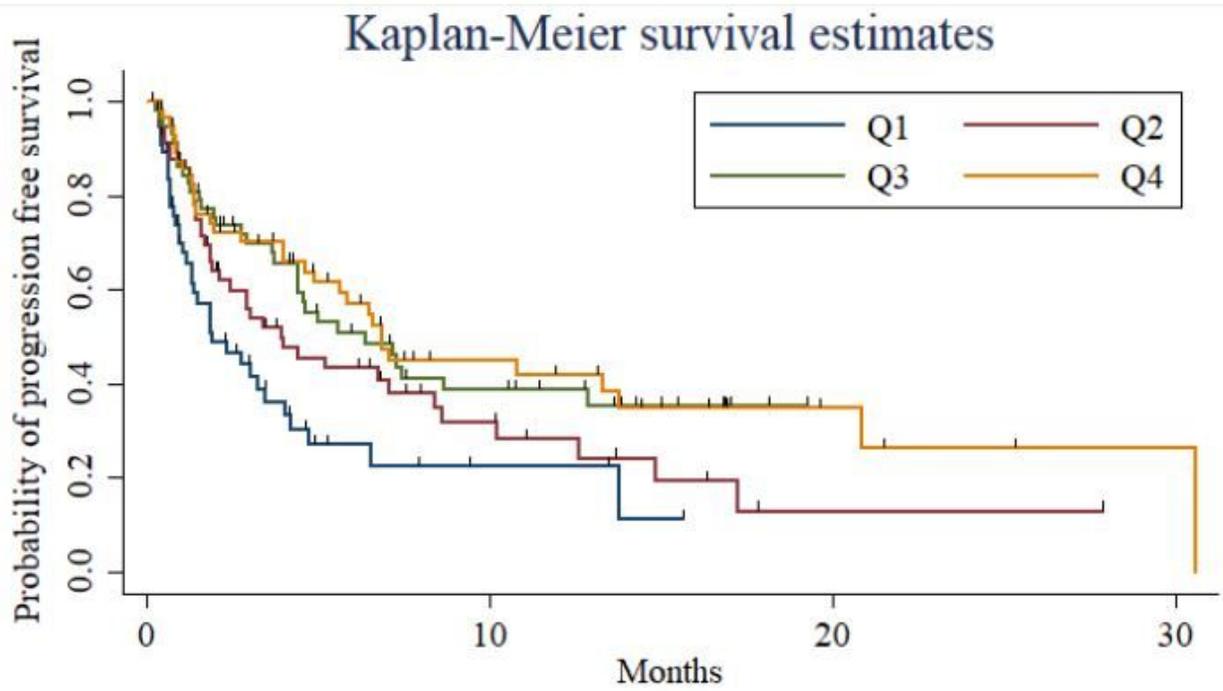
	PFS		OS	
	aHR (95% CI)	p	aHR (95% CI)	p
Age, years †				
<65	1 (reference)		1 (reference)	
65-74	0.49 (0.29,0.81)	0.006	0.67 (0.39,1.12)	0.10
≥75	0.43 (0.24,0.75)	0.004	0.51 (0.28,0.93)	0.02
Sex				
Male	1 (reference)		1 (reference)	
Female	0.93 (0.54,1.57)	0.87	0.94 (0.54,1.64)	0.84
Smoking status				
Never	1 (reference)		1 (reference)	
Ever	0.49 (0.28,0.85)	0.006	0.65 (0.36,1.19)	0.2
ECOG (ref 0)				
0	1 (reference)		1 (reference)	
1	2.04 (1.09,3.78)	0.02	2.56 (1.24,5.26)	0.02
≥ 2	3.00 (1.53,5.92)	0.001	4.37 (2.11,9.08)	<0.001
Histology				
Adenocarcinoma	1 (reference)		1 (reference)	
Squamous cell carcinoma	1.23 (0.64,2.35)	0.54	1.16 (0.57,2.37)	0.57
Other NSCLC	1.52 (0.90,2.56)	0.18	2 (1.18,3.40)	0.01
EGFR activating mutation	2.78 (1.54,5.01)	0.001	1.86 (0.97,3.61)	0.07
PD-L1 expression (≥1%)	0.48 (0.31,0.78)	0.004	0.55 (0.34,0.9)	0.01
Post-treatment PLC, Quartile [25th -75th]				
1 [598.7-1005]	1 (reference)		1 (reference)	
2 [1325-1582]	0.61 (0.35,1.06)	0.082	0.59 (0.32,1.06)	0.09
3 [1789-2018]	0.40 (0.22,0.73)	0.005	0.29 (0.15,0.57)	<0.001
4 [2419-3167]	0.28 (0.16,0.52)	<0.001	0.35 (0.19,0.65)	<0.001
P for trend	0.001		0.005	

PFS: progression-free survival; OS: overall survival; aHR: adjusted hazard ratio; CI: confidence interval; PLC: peripheral lymphocyte count;

Table 4. Treatment related adverse events according to posttreatment PLC quartile groups.

Posttreatment PLC	Quart1 (n=58)	Quart2 (n=58)	Quart3 (n=58)	Quart4 (n=57)	P
Adverse event (n=61)	11 (19.0%)	15 (24.6%)	20 (31.6%)	19(32.1%)	0.30
Pneumonitis	6 (54.6%)	3 (20.0%)	6 (33.3%)	1 (5.3%)	0.43
Skin lesion	1 (9.1%)	2 (13.3%)	4 (20.0%)	3 (15.7%)	
Hepatitis	1 (9.1%)	2 (13.3%)	2(10.0%)	2 (10.5%)	
TFT abnormality	0 (0.0%)	1 (6.7%)	3 (15.0%)	2 (10.5%)	
Arthritis	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (15.7%)	
Fever	0 (0.0%)	0 (0.0%)	1 (5.0%)	2 (10.5%)	
Anorexia	0 (0.0%)	2 (13.3%)	1 (5.0%)	1 (5.3%)	
Others	3 (27.3%)	5 (33.3%)	3 (15.0%)	5 (26.3%)	
Serious adverse event	6 (10.3%)	5 (8.6%)	7 (12.1%)	3 (5.3%)	0.67

Figures



Number at risk		0	10	20	30
Q1	58		3	0	0
Q2	57		10	1	0
Q3	58		15	0	0
Q4	57		15	4	1

Figure 1

Kaplan-Meier curves showing progression-free survival (PFS) stratified by quartiles of post-treatment PLC

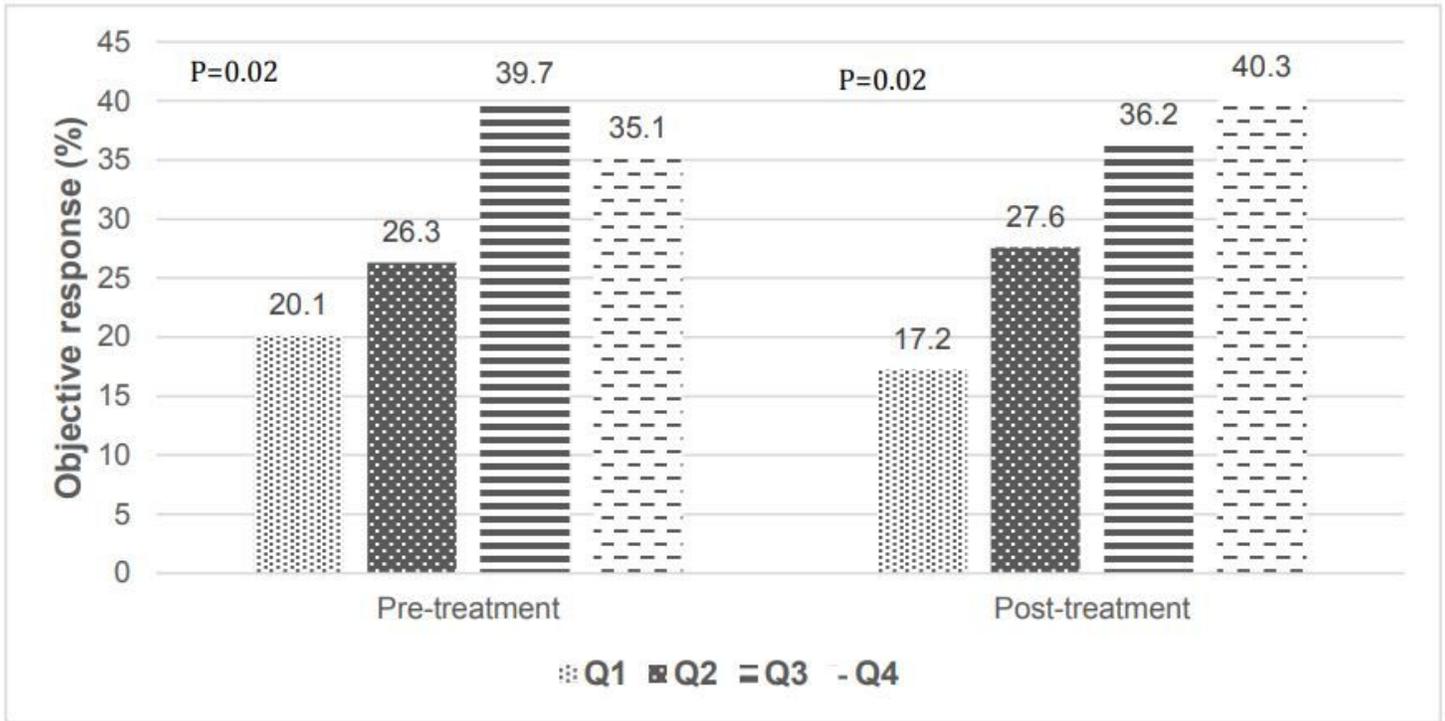


Figure 2

Tumor response rate according to pre- and post-treatment PLC

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarytable.docx](#)
- [ourrawdata.xlsx](#)