Original Article

SP142 evaluation contributes to the prediction of immune checkpoint inhibitor efficacy in non-small cell lung cancer with high PD-L1 expression assessed by 22C3

Kenji Nakahama[1]^, Masahiko Osawa[2], Motohiro Izumi[3], Naoki Yoshimoto[4], Akira Sugimoto[5], Hiroaki Nagamine[5], Koichi Ogawa[5], Yoshiya Matsumoto[5], Kenji Sawa[5], Yoko Tani[6], Hiroyasu Kaneda[6], Shigeki Mitsuoka[6], Tetsuya Watanabe[5], Kazuhisa Asai[5], Tomoya Kawaguchi[5,6]

[1]Department of Respiratory Medicine, Graduate School of Medicine, Osaka City University, Osaka, Japan; [2]Department of Diagnostic Pathology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan; [3]Department of Pulmonary Medicine, Bell land General Hospital, Sakai, Japan; [4]Department of Pulmonary Medicine, Ishikiriseiki Hospital, Higashiosaka, Japan; [5]Department of Respiratory Medicine, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan; [6]Department of Clinical Oncology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan

Contributions: (I) Conception and design: K Nakahama, H Kaneda; (II) Administrative support: K Nakahama; (III) Provision of study materials or patients: K Nakahama, M Izumi, N Yoshimoto, H Nagamine; (IV) Collection and assembly of data and sample: K Nakahama, M Izumi, N Yoshimoto, H Nagamine; (V) Data analysis and interpretation: K Nakahama; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Kenji Nakahama. Department of Respiratory Medicine, Osaka City University Graduate School of Medicine, Asahimachi 1-4-3, Abeno-ku Osaka City, Osaka 545-8585, Japan. Email: nakahama_kenji@yahoo.co.jp.

Background: It remains unclear whether assessing programmed death-ligand 1 (PD-L1) expression by SP142 plus 22C3 adds value for predicting the response to immunotherapy in non-small cell lung cancer (NSCLC).

Methods: This retrospective multicenter study included patients with advanced NSCLC treated with immune-checkpoint inhibitors. We constructed tissue microarrays (TMAs) and performed immunohistochemical staining with 22C3 and SP142 assays. We denoted the PD-L1 tumor proportion score (TPS) obtained from clinical medical records based on 22C3 staining as "22C3 (C)" and that obtained with 22C3 staining using our TMA as "22C3 (TMA)". SP142 staining was evaluated in both tumor cells and immune cells. We assessed the concordance between each PD-L1 assessment method and analyzed the objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) based on the PD-L1 expression level determined using the 22C3 and SP142 assays.

Results: In total, 288 patients were included. Among those with 22C3 (TMA) ≥50%, 60% of patients showed SP142 TC3 or IC3; among patients with 22C3 (C) <1%, 9% and 18% exhibited 22C3 (TMA) ≥1% and SP142 TC1/2/3 or IC1/2/3, respectively. Among patients with 22C3 (C) ≥50% treated with immune-checkpoint inhibitor monotherapy, the SP142 TC1/2/3 or IC1/2/3 group showed significantly better ORR, PFS and OS than the SP142 TC0 and IC0 group (54% *vs.* 29%, P=0.040, median =11.0 *vs.* 3.2 months, P=0.002, median =27.9 *vs.* 12.6 months, P=0.030, respectively). Multivariate analysis revealed that SP142 TC0 and IC0 was an independent unfavorable prognostic factor for PFS and OS in patients with 22C3 (C) ≥50% treated with immune-checkpoint inhibitor monotherapy. For those with 22C3 (C) ≥50% and SP142 TC0 and IC0, immune-checkpoint inhibitor concurrent with chemotherapy tended to result in a longer PFS and OS than immune-checkpoint inhibitor monotherapy (median =13.7 *vs.* 2.3 months, P=0.054, median = not estimable *vs.* 12.0 months, P=0.064, respectively).

Conclusions: SP142 evaluation contributes to the prediction of immune-checkpoint inhibitor efficacy in

^ ORCID: 0000-0001-7423-6153.

NSCLC with high PD-L1 expression assessed by 22C3.

Keywords: Non-small cell lung cancer (NSCLC); SP142; 22C3; programmed death-ligand 1 (PD-L1); tissue microarray (TMA)

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Introduction

Lung cancer has one of the highest mortality rates among cancers worldwide, and very few patients have survived long-term after treatment with cytotoxic anticancer agents alone (1,2). However, long-term survival has been achieved in some patients with non-small cell lung cancer (NSCLC) with the advent of immunotherapy (3,4). Various biomarkers have been explored for predicting response to immunotherapy, with programmed death-ligand 1 (PD-L1) expression being the most used in clinical practice (5-8). Indeed, PD-L1 can predict response to anti-PD-1 and -PD-L1 antibodies, though not completely.

Different antibodies to evaluate the degree of PD-L1 expression have been used for each anti-PD-1/PD-L1 drug in studies examining the effect of immune checkpoint inhibitors (ICIs) (7-10). Based on the results of the CheckMate017 and CheckMate057 studies, the anti-PD-1 antibody nivolumab was first approved by the U.S. Food and Drug Administration (FDA) for patients with NSCLC who were previously treated with platinum-based chemotherapy, and the PD-L1 immunohistochemistry (IHC) 28-8 assay was used to evaluate PD-L1 expression (11,12). Then, atezolizumab, an anti-PD-L1 antibody, was approved in a similar population based on results of the OAK study in which the PD-L1 IHC SP142 assay was used to detect PD-L1 expression (9). Next, the anti-PD-1 antibody pembrolizumab was first approved for first-line NSCLC treatment in patients with a PD-L1 tumor proportion score (TPS), i.e., the proportion of PD-L1 positive tumor cells, of ≥50% based on the PD-L1 IHC 22C3 assay, as reported by the KEYNOTE024 study. In this study, a significantly longer progression-free survival (PFS) and overall survival (OS) in first-line setting for NSCLC was obtained with pembrolizumab monotherapy compared to conventional chemotherapy in patients with PD-L1 TPS ≥50% (7). The KEYNOTE042 trial also reported a significantly better outcome for patients with PD-L1 TPS ≥1% assessed by the 22C3 assay compared to chemotherapy in the first-line setting for NSCLC, and the indication of pembrolizumab monotherapy was expanded to patients with PD-L1 TPS ≥1% assessed by 22C3 staining in first-line therapy (8). In the IMpower110 study, atezolizumab led to significantly longer OS than chemotherapy in patients with PD-L1 expression on tumor or immune cells, as assessed by the SP142 assay, and atezolizumab monotherapy was approved for the first-line NSCLC treatment when PD-L1 expression was detected by the SP142 assay (13). Nevertheless, it is not known whether pembrolizumab or atezolizumab is superior as first-line therapy because no randomized control trial has directly compared the two. Several studies have shown the efficacy of ICI combination therapy with cytotoxic chemotherapy or anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibody at any level of PD-L1 expression; such therapy can be administered to NSCLC patients in the first line without PD-L1 selection, yet there is need to prove PD-L1 expression for ICI monotherapy in the first-line setting (14-18).

Thus, in the first-line setting for NSCLC, proof of PD-L1 expression obtained using the 22C3 assay is required for the administration of pembrolizumab monotherapy and proof of PD-L1 expression obtained using the SP142 assay is required for the administration of atezolizumab monotherapy; however, measuring PD-L1 using two assays increases the costs and requires sufficient tumor tissue and time if expression levels are measured sequentially. Moreover, few reports have examined the association between the combined results of the two PD-L1 immunostaining assays and the clinical outcomes of ICI therapy (19). To date, the concordance of PD-L1 expression assessed by 22C3 and SP142 assays has been investigated in several studies, with the positivity rate of the SP142 assay reportedly be lower than that of the 22C3 assay (20,21). For NSCLC, the 22C3 assay is used to assess PD-L1 expression on tumor cells only, whereas SP142 evaluates both tumor cells and immune cells, and it has been reported that PD-L1 expression on immune cells as well as tumor

cells is associated with ICI therapeutic response in some carcinomas other than lung cancer (22-24).

Furthermore, it remains unclear whether therapeutic response to ICIs differs when only one of the PD-L1 expression assessments based on 22C3 or SP142 is positive compared to when both are positive, and an important clinical question is whether there is additional clinical significance in measurements based on two PD-L1 antibodies compared to only one in NSCLC treatment. Therefore, the purpose of this study was to clarify the clinical significance of adding the SP142 assay to the 22C3 assay in evaluating NSCLC patients treated with ICIs. We present the following article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-496/rc).

Methods

Study design

This was a retrospective multicenter study. We included patients with locally advanced or metastatic NSCLC who were previously treated with ICIs between December 2015 and July 2020 at Osaka Metropolitan University, Ishikiriseiki Hospital, Bell Land General Hospital. Patients who previously received anti-CTLA-4 therapy, who previously received durvalumab as adjuvant therapy after chemoradiation, and who had insufficient residual tissue for tissue microarray (TMA) and immunostaining were excluded. We evaluated PD-L1 expression by performing a TMA with 22C3 as well as SP142 staining to assess the discrepancy in PD-L1 TPS between measurements using TMA and in clinical practice. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The protocol was approved by the institutional review boards or ethics committees of all participating institutions (approval numbers: Osaka Metropolitan University Hospital 2020-177, Ishikiriseiki Hospital 20-26, and Bell Land General Hospital 2020-020). Because the application of the opt-out method in this research is permitted under Japan's most preferential law governing clinical research, informed consent was obtained in the form of an opt-out option on the website.

Data collection

We obtained the patients' medical records, including sex, age and Eastern Cooperative Oncology Group Performance Status (ECOG PS) at the time of administration of ICIs, smoking status, histological type, TNM stage according to the eighth edition, TPS of PD-L1, which was assessed using 22C3 staining in clinical practice, molecular profile of epidermal growth factor receptor (EGFR), response to ICI treatment and date of progression (or last follow-up), and date of death or last follow-up. The patients were followed-up for disease status until March 31, 2022.

Microarray construction

We prepared the TMA using formalin-fixed paraffin-embedded tumor blocks from biopsy and surgically resected specimens, which were obtained in routine clinical practice at each participating institution. We carefully selected the most representative tumor areas with reference to matched hematoxylin and eosin-stained slides and made a mark directly on the donor block. Then, we removed a tissue sample with a 2.0-mm diameter from the marked region in each donor block with a manual tissue microarrayer and embedded it directly into the premade recipient block. The premade recipient block and manual microarrayer were obtained from Funakoshi Co., Ltd. In total, we constructed 10 TMA blocks.

IHC analysis

Four-micrometer sections from each TMA block were stained immunohistochemically using the FDA-approved VENTANA PD-L1 (SP142) Assay and DAKO IHC 22C3 pharmDx kits with the designated methods for each antibody via outsourcing through N-Lab Ltd.

PD-L1 expression was immunohistochemically stained with 22C3 and SP142 antibodies and assessed by an experienced pathologist using previously published scoring criteria (9,25) (*Figure 1*). For 22C3, the percentage of tumor cells with membrane PD-L1 staining was determined as TPS; cases with fewer than 100 tumor cells were excluded. Here, we denote PD-L1 TPS obtained from medical records evaluated by 22C3 in clinical practice as "22C3 (C)" and PD-L1 TPS stained with 22C3 using our TMA as "22C3 (TMA)". For SP142, PD-L1 expression as a percentage of total tumor cells and tumor-infiltrating immune cells expressing PD-L1 as a percentage of tumor area were scored. TC3, TC2 and TC1 indicate ≥50%, ≥5% and ≥1% tumor cells expressing PD-L1, respectively. IC3, IC2 and IC1 indicate tumor-infiltrating immune cells expressing PD-L1 found in ≥10%, ≥5% and ≥1% of the

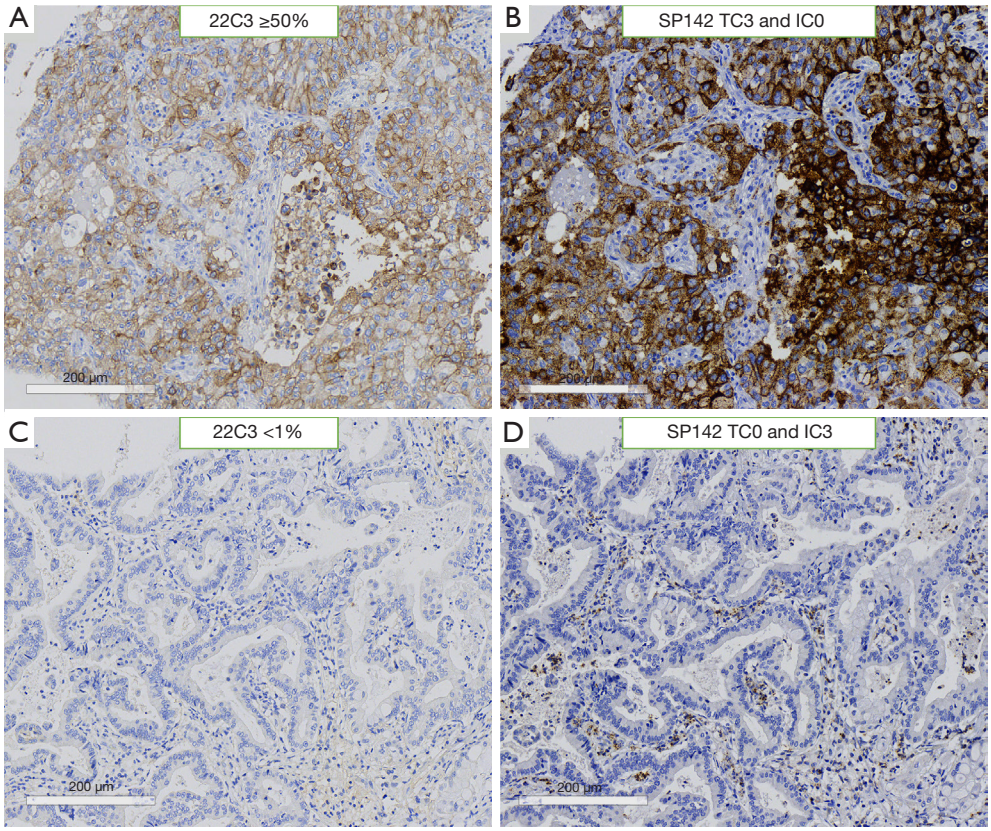

Figure 1 Representative images of immunohistochemical staining of non-small cell lung cancer in tissue microarray samples. (A,B) Tissue microarray samples from the same patient; the tissue in (A) was stained with the 22C3 antibody and determined as PD-L1 TPS ≥50%, and the tissue in (B) was stained with the SP142 antibody and determined as TC3 and IC0, respectively. (C,D) Tissue microarray samples from another patient; the tissue in (C) was stained with the 22C3 antibody and assessed as PD-L1 TPS <1%, and the tissue in (D) was stained with the SP142 antibody and assessed as TC0 and IC3. TC3 and IC0: PD-L1 expression on at least 50% of tumor cells and less than 1% of tumor-infiltrating immune cells; TC0 and IC3: PD-L1 expression on less than 1% of tumor cells and at least 10% of tumor-infiltrating immune cells. PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

tumor area, respectively.

Statistical analyses

Tumor responses were assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (26). We assessed the concordance between each PD-L1 assessment method and analyzed the objective response rate (ORR), PFS and OS based on the PD-L1 expression level stained with the 22C3 antibody and SP142 antibody. PFS was estimated from the date of the first ICI administration until disease progression or death from any cause. OS was calculated from the date of the first ICI administration until death from any cause. Fisher's exact tests were used to compare categorical variables. To estimate survival curves, the Kaplan-Meier method was applied; the log-rank test was employed to compare differences between groups. The hazard ratios (HR) and 95% confidence intervals (CIs) were calculated and univariate and multivariate analyses including the following variables were performed: PD-L1 expression status assessed based on SP142 staining, sex, smoking history, ECOG PS, histological type and ICI treatment line. Statistical significance was considered at P values less than 0.05. All statistical analyses were conducted using EZR in R commander version 1.55 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Table 1 Patients' characteristics

Characteristic	N=288
Age (years), median [range]	71 [33–87]
Sex	
Male	228 [79]
Female	60 [21]
Smoking status	
Current or former smoker	245 [85]
Never smoker	40 [14]
Unknown	3 [1]
ECOG PS	
0–1	239 [83]
≥2	49 [17]
TNM stage	
Stage III	55 [19]
Stage IV or recurrent	233 [81]
Sample	
Resection	83 [29]
Biopsy	205 [71]
Histological type	
Squamous	103 [36]
Non-squamous	185 [64]
EGFR mutation status	
Wild type	176 [61]
Mutant	17 [6]
Unknown	95 [33]
Treatment	
ICI monotherapy	205 [71]
ICI concurrent with chemotherapy	83 [29]
ICI administration line	
1	160 [56]
≥2	128 [44]
Type of ICI	
Pembrolizumab	187 [65]
Nivolumab	69 [24]
Atezolizumab	32 [11]
PD-L1 22C3 (C)	
≥50%	108 [38]
1–49%	88 [31]
<1%	45 [16]
Unknown	47 [16]
PD-L1 22C3 (TMA)	
≥50%	50 [17]
1–49%	75 [26]
<1%	163 [57]
PD-L1 SP142	
TC3 or IC3	43 [15]
TC1/2 or IC1/2	67 [23]
TC0 and IC0	178 [62]

Data presented as No. [%]. ECOG PS, Eastern Cooperative Oncology Group Performance status; TNM, tumor, node, metastasis; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor; PD-L1, programmed death-ligand 1; 22C3 (C), PD-L1 tumor proportion score (TPS) reviewed from medical records evaluated by 22C3 in clinical practice; 22C3 (TMA), PD-L1 TPS stained with evaluated by 22C3 using tissue microarray; TC3 or IC3, PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC1/2 or IC1/2, PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells and less than 50% of tumor cells or less than 10% of tumor-infiltrating immune cells; TC0 and IC0, PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells; TPS, tumor proportion score; TMA, tissue microarray.

Results

Patient characteristics

In total, 493 patients with advanced NSCLC received ICI during the study period. Among them, 288 patients who were able to construct microarrays and evaluate immunostaining results from their samples were reviewed (*Table 1*). The median age was 71 years, and 79% of the patients were male. Microarray blocks were prepared from resection samples from 29% of the patients and in biopsy samples from 71%. The histological type of 64% of patients was non-squamous cell carcinoma. Seventy-one percent of

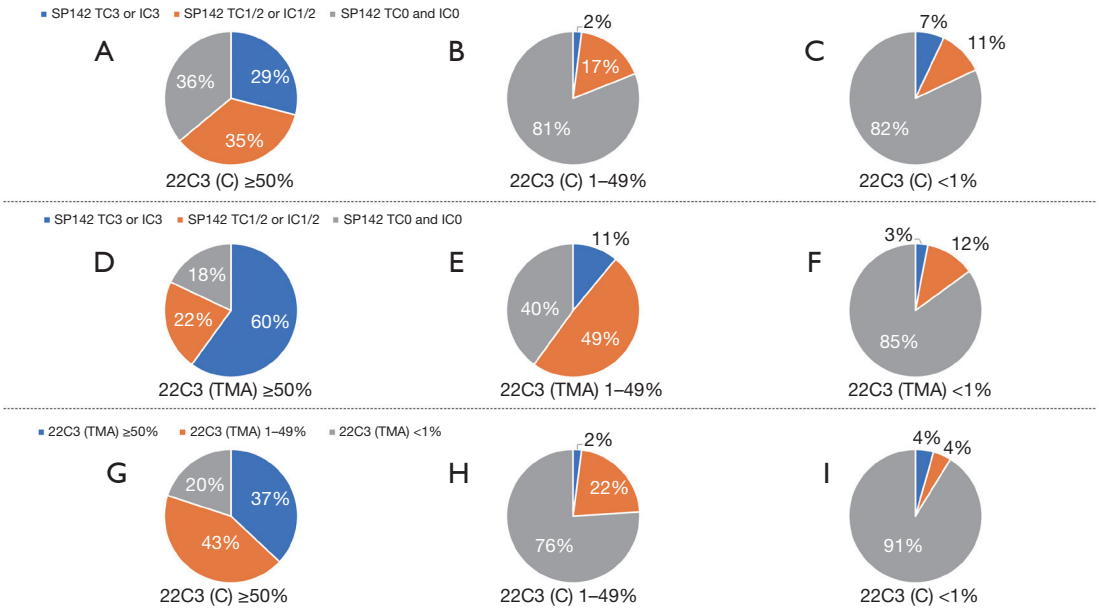

Figure 2 Concordance between each PD-L1 assessment method. The number of patients with each SP142 result among patients with 22C3 (C) ≥50% (A), 1–49% (B), and <1% (C). The number of patients with each SP142 result among patients with 22C3 (TMA) ≥50% (D), 1–49% (E), and <1% (F). The number of patients with each 22C3 (TMA) result among patients with 22C3 (C) ≥50% (G), 1–49% (H), and <1% (I). TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC 1/2 or IC1/2: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells and less than 50% of tumor cells or less than 10% of tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells; 22C3 (C): PD-L1 TPS reviewed from medical records evaluated using the 22C3 assay in clinical practice; 22C3 (TMA): PD-L1 TPS evaluated by staining with the 22C3 antibody in a tissue microarray. TMA, tissue microarray; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

the patients received ICI monotherapy, and 29% received ICI concurrent with chemotherapy. The value of 22C3 (C) was ≥50% in 38% of the patients whereas only 17% and 15% of the patients exhibited 22C3 (TMA) ≥50% and SP142 TC3 or IC3, respectively. There was no significant difference in the positive rate of PD-L1 expression in TMA between resection and biopsy samples by either the 22C3 or SP142 assay (P=0.50, P=0.62, respectively) (Table S1). Details of the ICI treatment lines in each group, divided by the 22C3 and SP142 results, are provided in Table S2.

Concordance between PD-L1 assessment methods

For 22C3 (C) ≥50%, 31 (29%), 38 (35%) and 39 (36%) of patients showed SP142 TC3 or IC3, TC1/2 or IC1/2, and TC0 and IC0, respectively (*Figure 2A*). For 22C3 (C) 1–49% and <1%, 17 (19%) and 8 (18%) of patients had positive expression of PD-L1 in SP142 (*Figure 2B,2C*). Among patients with 22C3 (TMA) ≥50%, 30 (60%), 11 (22%), and 9 (18%) showed SP142 TC3 or IC3, SP142 TC1/2 or IC1/2, and TC0 and IC0, respectively (*Figure 2D*). Positive expression of PD-L1 assessed by the SP142 assay was detected in 45 (60%) and 24 (15%) of patients with 22C3 (TMA) 1–49% and <1%, respectively (*Figure 2E,2F*). Among those with 22C3 (C) ≥50%, 22C3 (TMA) ≥50% was observed for 40 patients (37%) (*Figure 2G*). On the other hand, among patients with 22C3 (C) 1–49% and <1%, 21 (24%) and 4 (9%) had positive expression of PD-L1 in 22C3 (TMA), respectively (*Figure 2H,2I*).

Regarding the evaluation of PD-L1 expression in tumor cells, almost all patients with SP142 TC positivity had 22C3 (TMA) ≥1%, whereas 58% of patients with 22C3 (TMA) ≥1% had SP142 TC positivity (*Figure 3*).

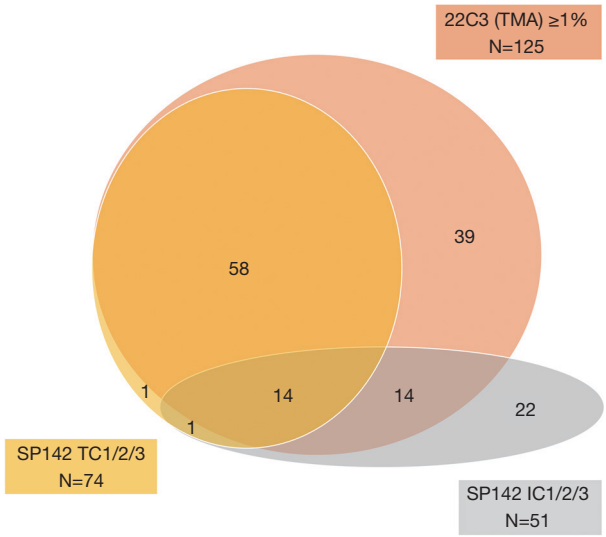

Figure 3 Venn diagram of 22C3 (TMA), SP142 TC and SP142 IC. 22C3 (TMA): PD-L1 tumor proportion score determined in tissues from the tissue microarray stained with the 22C3 antibody; SP142 TC1/2/3: PD-L1 expression on at least 1% of tumor cells stained with the SP142 antibody; SP142 IC1/2/3: PD-L1 expression on at least 1% of tumor-infiltrating immune cells stained with the SP142 antibody. TMA, tissue microarray; PD-L1, programmed death-ligand 1.

With regard to the correlation of TC and IC results in the SP142 assay, only 14% of SP142 TC1/2/3 or IC1/2/3 patients exhibited both TC and IC positivity. Twenty-two patients showed PD-L1 expression only in immune cells and not in tumor cells with both 22C3 (TMA) and SP142 TC (*Figure 3*).

Analysis of the response rate to ICI monotherapy based on the SP142 results

We analyzed the ORR with ICI monotherapy according to two SP142 cutoff levels (TC3 or IC3 *vs.* TC0/1/2 and IC0/1/2, and TC1/2/3 or IC1/2/3 *vs.* TC0 and IC0). For patients in whom the PD-L1 expression level was assessed using the 22C3 assay, the ORR of ICI monotherapy was higher in the SP142 TC3 or IC3 group than in the SP142 TC0/1/2 and IC0/1/2 group (*Figure 4A*). The ORR of ICI monotherapy was also higher in the SP142 TC1/2/3 or IC1/2/3 group than in the SP142 TC0 and IC0 group, except among patients with 22C3 (C) <1% (*Figure 4B*). In particular, the ORR was significantly higher in the SP142 TC1/2/3 or IC1/2/3 group than in the SP142 TC0 and IC0 group (54% *vs.* 29%, P=0.040) among patients with 22C3 (C) ≥50% (*Figure 4B*).

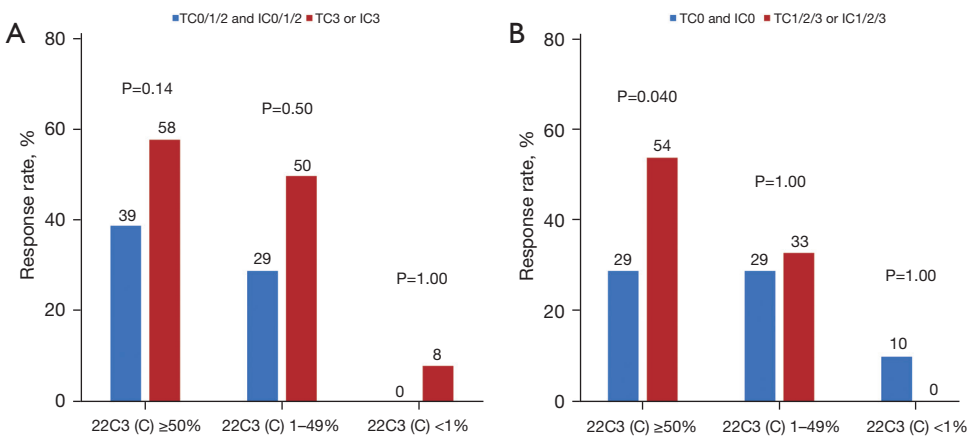

Figure 4 Analysis of the objective response rate of immune checkpoint inhibitor monotherapy based on SP142 results at each 22C3 (C) level. Comparison of the objective response rate between patients with SP142 TC0/1/2 and IC0/1/2 and patients with SP142 TC3 or IC3 among patients with 22C3 (C) ≥50%, 22C3 (C) 1–49%, 22C3 (C) <1%. (A) Comparison of the objective response rate between patients with SP142 TC0 and IC0 and patients with SP142 TC1/2/3 or IC1/2/3 among patients with 22C3 (C) ≥50%, 22C3 (C) 1–49%, 22C3 (C) <1% (B). TC0/1/2 and IC0/1/2: PD-L1 expression on less than 50% of tumor cells and less than 10% of tumor-infiltrating immune cells, TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells, 22C3 (C): PD-L1 TPS reviewed from medical records evaluated by 22C3 in clinical practice, TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells, TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells. PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

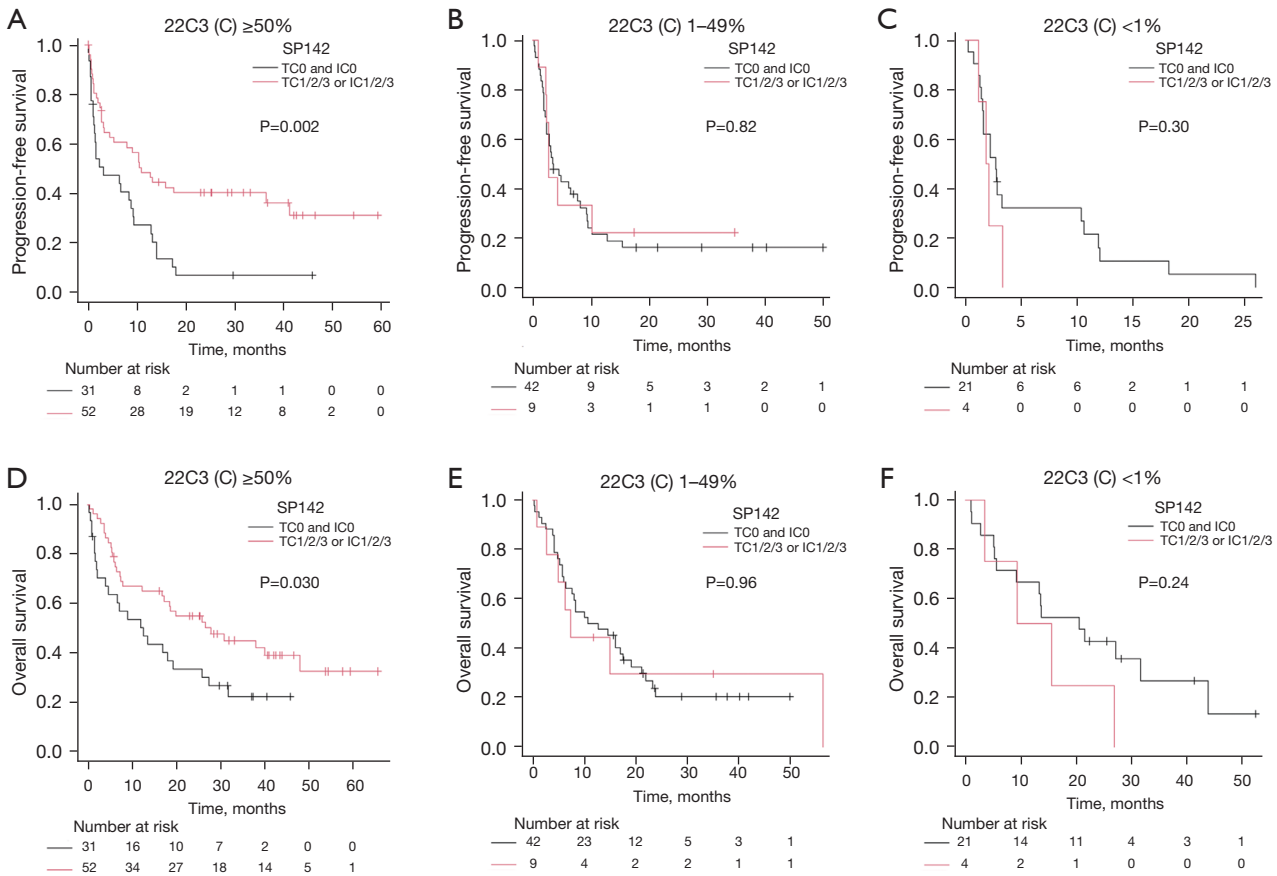

Figure 5 PFS and OS analyses compared by stratifying the SP142 results at each 22C3 (C) level. Comparison of Kaplan-Meier curves of PFS between patients with SP142 TC0 and IC0 and patients with SP142 TC1/2/3 or IC1/2/3 among patients with 22C3 (C) ≥50% (A), 22C3 (C) 1–49% (B), 22C3 (C) <1% (C). Comparison of Kaplan-Meier curves of OS between patients with SP142 TC0 and IC0 and patients with SP142 TC1/2/3 or IC1/2/3 among patients with 22C3 (C) ≥50% (D), 22C3 (C) 1–49% (E), 22C3 (C) <1% (F). TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells, TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells, 22C3 (C): PD-L1 tumor proportion score reviewed from medical records evaluated by 22C3 in clinical practice. PFS, progression-free survival; OS, overall survival; PD-L1, programmed death-ligand 1.

PFS and OS evaluated for PD-L1 expression levels measured using each PD-L1 assay

The median duration of follow-up (defined as the time from ICI administration to death or the date of data cutoff for those who were alive) in all patients was 29.6 months (IQR, 22.1–39.9 months). The median PFS of patients treated with ICI monotherapy with 22C3 (C) ≥50%, 1–49% and <1% were 8.8, 3.3 and 2.2 months (P=0.011), and that of patients with SP142 TC3 or IC3, TC1/2 and/or IC1/2 and TC0 and IC0 were 7.9, 3.3, and 3.1 months (P=0.028), respectively (Figure S1A,S1B). The median OS of patients treated with ICI monotherapy with 22C3 (C) ≥50%, 1–49% and <1% were 18.7, 10.7 and 15.5 months (P=0.16), and that of patients with SP142 TC3 or IC3, TC1/2 and/or IC1/2 and TC0 and IC0 were 18.6, 15.0 and 13.7 months (P=0.49), respectively (Figure S1C,S1D).

PFS and OS analyses compared based on SP142 results for each group divided by 22C3 results

We compared PFS and OS with ICI monotherapy based on SP142 results (TC1/2/3 or IC1/2/3 *vs.* TC0 and IC0) in patients with each 22C3 (C) level (≥50%, 1–49% and <1%) (*Figure 5*). The SP142 TC1/2/3 or IC1/2/3 group showed significantly longer PFS and OS than the TC0 and IC0 group among patients with 22C3 (C) ≥50% (median =11.0 *vs.*

Table 2 Univariate and multivariate analyses of PFS for patients with 22C3 (C) ≥50% receiving ICI monotherapy

Factor	N	Median PFS (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	P value	HR	95% CI	P value
SP142								
TC1/2/3 or IC1/2/3	52	11.0						
TC0 and IC0	31	3.2	2.23	1.32–3.73	0.003	2.60	1.51–4.48	0.001
Sex								
Female	18	2.6						
Male	65	9.2	0.78	0.44–1.47	0.42	1.13	0.50–2.88	0.78
Smoking status								
Current or former smoker	70	9.2						
Never smoker	12	1.2	1.76	0.84–3.35	0.13	1.81	0.66–4.96	0.25
ECOG PS								
0–1	64	10.4						
≥2	19	1.4	2.12	1.14–3.74	0.020	2.51	1.31–4.55	0.006
Histological type								
Squamous cell carcinoma	30	10.4						
Nonsquamous cell carcinoma	53	5.4	1.33	0.79–2.32	0.29	1.34	0.77–2.39	0.31
ICI treatment line								
1st line	61	10.4						
2nd or later line	22	3.9	1.38	0.78–2.35	0.26	1.60	0.88–2.79	0.12

PFS, progression-free survival; ICI, immune checkpoint inhibitor; 22C3 (C): PD-L1 TPS reviewed from medical records and evaluated using the 22C3 assay in clinical practice; TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells; HR, hazard ratio; CI, confidence interval, ECOG PS, Eastern Cooperative Oncology Group Performance Status.

3.2 months, P=0.002, median =27.9 *vs.* 12.6 months, P=0.030, respectively), however, no significant differences were observed among patients with 22C3 (C) 1–49% and <1%. Next, we conducted similar analysis using another SP142 cutoff (TC3 or IC3 versus TC0/1/2 and IC0/1/2), and there were no significant differences in each group (Figure S2).

The multivariate analysis revealed that the status of SP142 TC0 and IC0 was an independent prognostic factor for PFS and OS in patients with 22C3 (C) ≥50% treated with ICI monotherapy (HR 2.60, 95% CI: 1.51–4.48, P=0.001, HR 1.94, 95% CI: 1.08–3.46, P=0.027, respectively) (*Tables 2,3*).

PFS and OS analyses compared based on 22C3 results in groups divided by SP142 results

We compared PFS and OS outcomes of ICI monotherapy based on 22C3 results (≥1% *vs.* <1%) in patients with different SP142 levels (TC3 or IC3, TC1/2 or IC1/2 and TC0 and IC0), and there were no significant differences in

Table 3 Univariate and multivariate analyses of OS for patients with 22C3 (C) ≥50% receiving ICI monotherapy

Factor	N	Median OS (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	P value	HR	95% CI	P value
SP142								
TC1/2/3 or IC1/2/3	52	27.9						
TC0 and IC0	31	12.6	1.82	1.04–3.16	0.036	1.94	1.08–3.46	0.027
Sex								
Female	18	9.7						
Male	65	19.9	0.72	0.39–1.39	0.31	1.30	0.53–3.53	0.58
Smoking status								
Current or former smoker	70	19.9						
Never smoker	12	6.1	1.77	0.81–3.48	0.14	2.58	0.85–7.83	0.093
ECOG PS								
0–1	64	27.9						
≥2	19	6.5	2.83	1.52–5.08	0.002	3.31	1.72–6.18	0.001
Histological type								
Squamous cell carcinoma	30	19.9						
Non-squamous cell carcinoma	53	12.3	1.09	0.63–1.94	0.75	1.15	0.64–2.09	0.65
ICI treatment line								
1st line	61	18.6						
2nd or later line	22	19.3	0.77	0.39–1.42	0.42	0.89	0.44–1.68	0.72

22C3 (C): PD-L1 TPS reviewed from medical records and evaluated using the 22C3 assay in clinical practice; TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells. OS, overall survival; ICI, immune checkpoint inhibitor; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

each group (Figure S3).

PFS and OS analyses based on SP142 results between immune checkpoint inhibitor monotherapy and combination with chemotherapy in first-line treatment among patients with 22C3 (C) ≥50%

We compared the difference in PFS and OS between ICI monotherapy and ICI concurrent with chemotherapy as the first-line treatment among patients with 22C3 (C) ≥50% and SP142 TC0 and IC0 (Figure S4). The ICI concurrent with chemotherapy group showed a tendency toward a longer PFS and OS than ICI monotherapy group (median =13.7 *vs.* 2.3 months, P=0.054, median =12.0 months *vs.* not estimable, P=0.064, respectively), whereas there was no significant difference in PFS and OS among patients with 22C3 (C) ≥50% and SP142 TC1/2/3 or IC1/2/3 (median =23.3 *vs.* 15.9 months, P=0.78, median = not estimable *vs.* 26.6 months, P=0.13, respectively).

PFS and OS analyses compared based on SP142 TC results at each PD-L1 TPS level obtained with the 22C3 assay

We compared PFS and OS for patients receiving ICI monotherapy between the SP142 TC0 group and the SP142 TC1/2/3 group based on the PD-L1 TPS level determined with the 22C3 assay (Figure S5). Among patients with 22C3 (C) ≥50%, the SP142 TC1/2/3 group experienced a significantly longer PFS and a tendency toward a longer OS than the SP142 TC0 and IC0 group (median =12.8 *vs.* 3.4 months, P=0.029, median =27.9 *vs.* 9.0 months, P=0.063, respectively).

PFS and OS analyses stratified by PD-L1 expression on immune cells

We analyzed PFS and OS for ICI monotherapy in patients with and without PD-L1 expression in immune cells and did not observe significant differences in PFS and OS between the SP142 IC1/2/3 group and the SP142 IC0 group among patients with 22C3 (C) ≥1%, SP142 TC1/2/3, 22C3 (C) <1%, and SP142 TC0 (Figure S6).

Discussion

Among patients treated with ICI monotherapy with 22C3 (C) ≥50%, the SP142 TC1/2/3 or IC1/2/3 group showed significantly better ORR, PFS and OS than the SP142 TC0 and IC0 group. Furthermore, multivariate analysis revealed that SP142 TC0 and IC0 was an independent unfavorable prognostic factor for PFS and OS in patients with 22C3 (C) ≥50% treated with ICI monotherapy. To the best of our knowledge, this is the first report to compare ICI efficacy by stratifying 22C3 plus SP142 in patients with NSCLC.

This result suggests that measuring PD-L1 by the SP142 assay improves the accuracy of predicting ICI treatment response for patients with high PD-L1 expression assessed by 22C3; hence, the addition of SP142 evaluation might help in determining the treatment regimen and ICI indication for NSCLC highly expressing PD-L1. The OAK trial, which compared atezolizumab versus docetaxel in previously treated NSCLC patients, reported subgroup analysis of the predictive value of 22C3 and SP142 for the outcome (9,19). In that study, a remarkably good hazard ratio (HR) of 0.39 for OS was found for patients with 22C3 <50% and SP142 TC3 or IC3 with atezolizumab versus docetaxel, whereas the HR for OS was relatively high at 0.73 in patients with 22C3 ≥50% and SP142 TC0/1/2 or IC0/1/2. Similarly, in our study, low PD-L1 expression in the SP142 assay, especially TC0 and IC0, was associated with worse outcomes of ICI monotherapy, despite 22C3(C) ≥50%. These results suggest that even if PD-L1 TPS ≥50% assessed by the 22C3 assay, the efficacy of ICI monotherapy may be limited if PD-L1 evaluation by the SP142 assay is negative. Researchers have not clearly determined whether ICI monotherapy or combination therapy is better for patients with PD-L1 TPS ≥50%, and a report of no significant difference in OS between ICI monotherapy and combination therapy in these patients has been published (27). However, our study showed a trend toward a longer OS in patients treated with combination therapy than with monotherapy as a first-line ICI treatment who had a 22C3 (C) ≥50% and SP142 TC0 and IC0; hence, if PD-L1 expression using the SP142 assay is negative, ICI combination therapy may be the better choice. On the other hand, the good ICI monotherapy efficacy observed in patients with 22C3 (C) ≥50% and SP142 TC1/2/3 or IC1/2/3 might encourage ICI monotherapy treatment for these patients.

Regarding the concordance rate between 22C3 (TMA) and SP142 results, 60% of 22C3 (TMA) ≥50% showed SP142 TC3 or IC3, which is similar to that reported in the IMpower110 study and supports the validity of the PD-L1 evaluation in this study (12). The SP142 assay is reported to have a lower tumor cell PD-L1 positivity rate than the 22C3 assay, and it is unclear whether SP142 is less sensitive or more specific for predicting ICI efficacy, as previous studies investigating concordance between 22C3 and SP142 assays did not evaluate a direct relationship with PD-L1 expression results and clinical outcomes of ICI treatment (20,21,28). In the present study, the SP142 TC0 group experienced a shorter PFS and OS than the SP142 TC1/2/3 group among patients with 22C3 (C) ≥50%, suggesting that ICI effectiveness may be limited in patients with positive results only obtained using the 22C3 assay. Regarding PD-L1 expression on immune cells, no significant differences in PFS and OS were observed between the SP142 IC1/2/3 group and the SP142 IC0 group, regardless of tumor cell PD-L1 expression; therefore, PD-L1 expression on immune cells is of limited significance for predicting ICI efficacy in patients with NSCLC.

In total, 9% of patients were negative for 22C3 (C) but positive for 22C3 (TMA), including a small number of 22C3 (TMA) ≥50% cases. Tumor heterogeneity may be one of the reasons, and testing using the SP142 assay may provide an opportunity to cover such false-negative cases in the first PD-L1 evaluation by the 22C3 assay.

This study has several important limitations. First, we found a lower positivity rate for PD-L1 by TMA than that measured by the 22C3 assay in clinical practice. Because there was not much difference in the PD-L1 expression rate between 22C3 (TMA) and SP142, the low PD-L1 expression may be attributed to the TMA method rather than the type of PD-L1 antibody used. This may be due to the limited amount of tissue used with the microarray method and using residual tissue after examining PD-L1 expression and driver oncogene mutations in clinical practice, even though there was no significant difference in the expression rate between surgical and biopsy specimens by TMA. Many reports evaluating PD-L1 expression using

microarrays have shown lower PD-L1 positivity rates than in prospective clinical trials, such as the KEYNOTE189 and PACIFIC trials (14,24,29-32). The low rate of positivity detected using the TMA led to a low number of patients with SP142 TC1/2/3 or IC1/2/3 and SP142 TC3 or IC3 in the present study, especially among patients with PD-L1 TPS <50% assessed using 22C3 staining, which was the second limitation. Hence, we cannot yet draw conclusions about the significance of SP142 positivity for predicting treatment response to ICI in patients with PD-L1 TPS <50% assessed by 22C3. Larger-scale trials are warranted to compare ICI treatment efficacy according to SP142 results in patients with PD-L1 TPS <50% assessed by 22C3. Third, the analysis in this study included patients receiving different treatment lines, which may lead to bias. However, no extreme differences in treatment lines were observed between the comparison groups, and the status of SP142 TC0 and IC0 was a significant poor prognostic factor in the 22C3 (C) ≥50% group, even after adjusting for the treatment line in the multivariate analysis. Fourth, this study involved an evaluation by a single pathologist. Although tumor cell PD-L1 expression has been reported to have a good interevaluator concordance rate, an assessment using immune cells has been reported to have low interevaluator agreement, and careful interpretation is needed for this study (20,33). Regardless, the concordance rate between 22C3 (TMA) and SP142 in this study was similar to that previously reported (13), and the results obtained using each PD-L1 evaluation showed a significant association with the therapeutic effect of ICI. Thus, the PD-L1 evaluation in this study was highly likely to be appropriate. Finally, we did not evaluate the type of ICI applied, such as anti-PD-1 or anti-PD-L1 antibodies.

Conclusions

Evaluation of PD-L1 expression by SP142, in addition to 22C3, contributes to predicting treatment response to ICIs for patients with PD-L1 TPS ≥50% assessed by 22C3. Even in patients with PD-L1 TPS ≥50%, the efficacy of ICI monotherapy may be limited among patients with SP142 TC0 and IC0. Measurement with the SP142 assay can assist in determining treatment strategies for advanced NSCLC patients with high PD-L1 expression assessed by 22C3.

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Footnote

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Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-496/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-496/coif). HK reports personal fees from MSD, Ono Pharmaceutical Co., Ltd., and Bristol-Myers Squibb K.K. Chugai Pharmaceutical Co., Ltd., outside the submitted work. SM reports personal fees from MSD K.K., during the conduct of the study; and personal fees from Ono Pharmaceutical Co., Ltd., Bristol-Myers Squibb K.K., Taiho Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Lilly, Chugai Pharmaceutical Co., Ltd., and AstraZeneca K.K., outside the submitted work. TK reports personal fees from MSD, Novartis, and Pfizer; grants and personal fees from Ono Pharmaceutical Company, Chugai Pharmaceutical Company, AstraZeneca, Taiho Pharmaceutical Company, Boehringer Ingelheim Pharmaceutical, Bristol Myers Squibb, and Lilly; and grants from Kyowa Kirin, outside the submitted work. KS reports personal fees from Chugai Pharmaceutical Co., Ltd, Nippon Boehringer Ingelheim Co., Ltd., and DAIICHI SANKYO COMPANY, outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The protocol was approved by the institutional review boards or ethics committees of all participating institutions (approval numbers: Osaka Metropolitan University Hospital 2020-177, Ishikiriseiki Hospital 20-26, and Bell Land General Hospital 2020-020). Because the application of the opt-out method in this

research is permitted under Japan's most preferential law governing clinical research, informed consent was obtained in the form of an opt-out option on the website.

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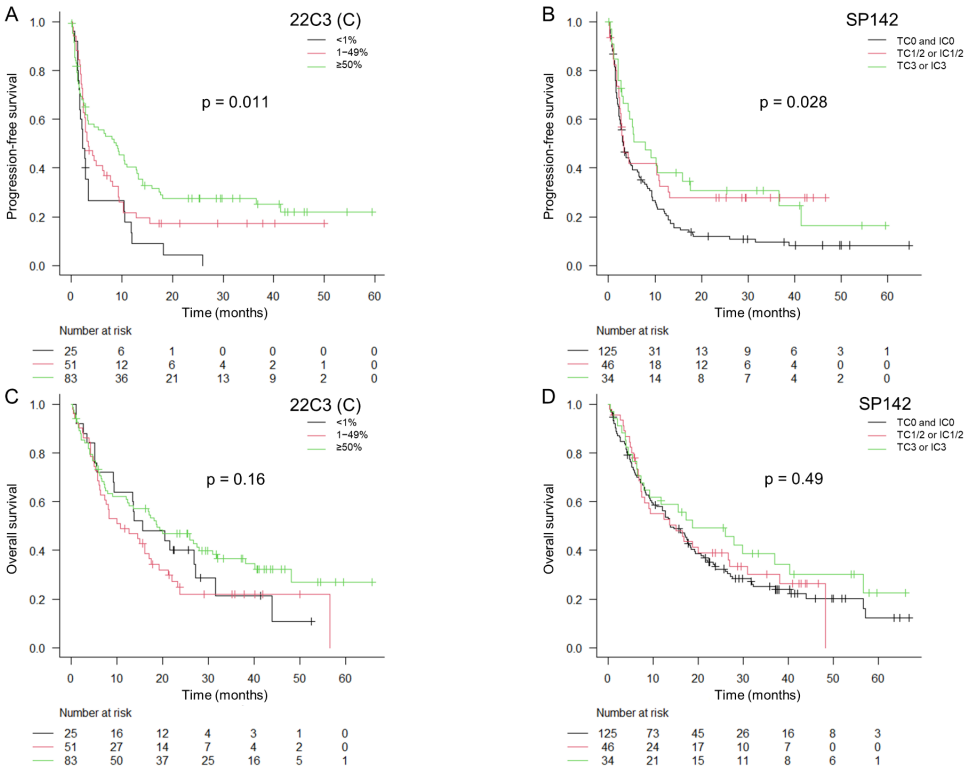

Figure S1 Kaplan-Meier curves of PFS and OS in patients evaluated for PD-L1 expression levels using each PD-L1 assay. The Kaplan-Meier curves of PFS in patients treated with ICI monotherapy with 22C3 (C) ≥50%, 1–49% and <1% (A) and with SP142 TC3 or IC3, TC1/2 or IC1/2 and TC0 and IC0 (B). Kaplan-Meier curves of OS in patients treated with ICI monotherapy with 22C3 (C) ≥50%, 1–49% and <1% (C) and with SP142 TC3 or IC3, TC1/2 or IC1/2 and TC0 and IC0 (D). 22C3 (C): PD-L1 TPS reviewed from medical records and evaluated by performing 22C3 staining in clinical practice; TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC1/2 or IC1/2: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells and less than 50% of tumor cells or less than 10% of tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells.

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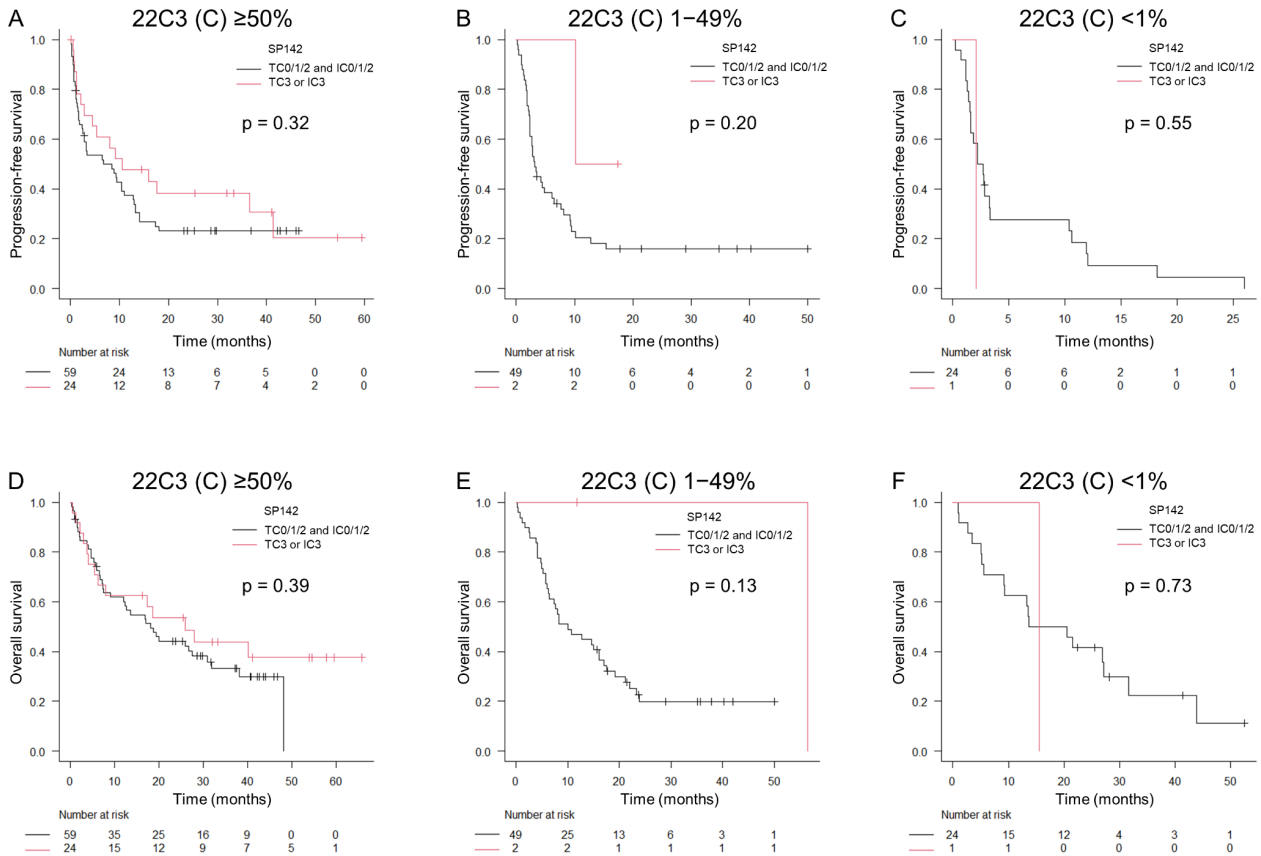

Figure S2 PFS and OS analyses compared based on the SP142 results at each 22C3 (C) level. The comparison of Kaplan-Meier curves for PFS between patients with SP142 TC0/1/2 and IC0/1/2 and patients with SP142 TC3 or IC3 among patients with 22C3 (C) ≥50% (A), 22C3 (C) 1–49% (B), 22C3 (C) <1% (C). The comparison of Kaplan-Meier curves for OS between patients with SP142 TC0/1/2 and IC0/1/2 and patients with SP142 TC3 or IC3 among patients with 22C3 (C) ≥50% (D), 22C3 (C) 1–49% (E), 22C3 (C) <1% (F). TC0/1/2 and IC0/1/2: PD-L1 expression on less than 50% of tumor cells and less than 10% of tumor-infiltrating immune cells; TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; 22C3 (C): PD-L1 TPS reviewed from medical records and evaluated by performing 22C3 staining in clinical practice.

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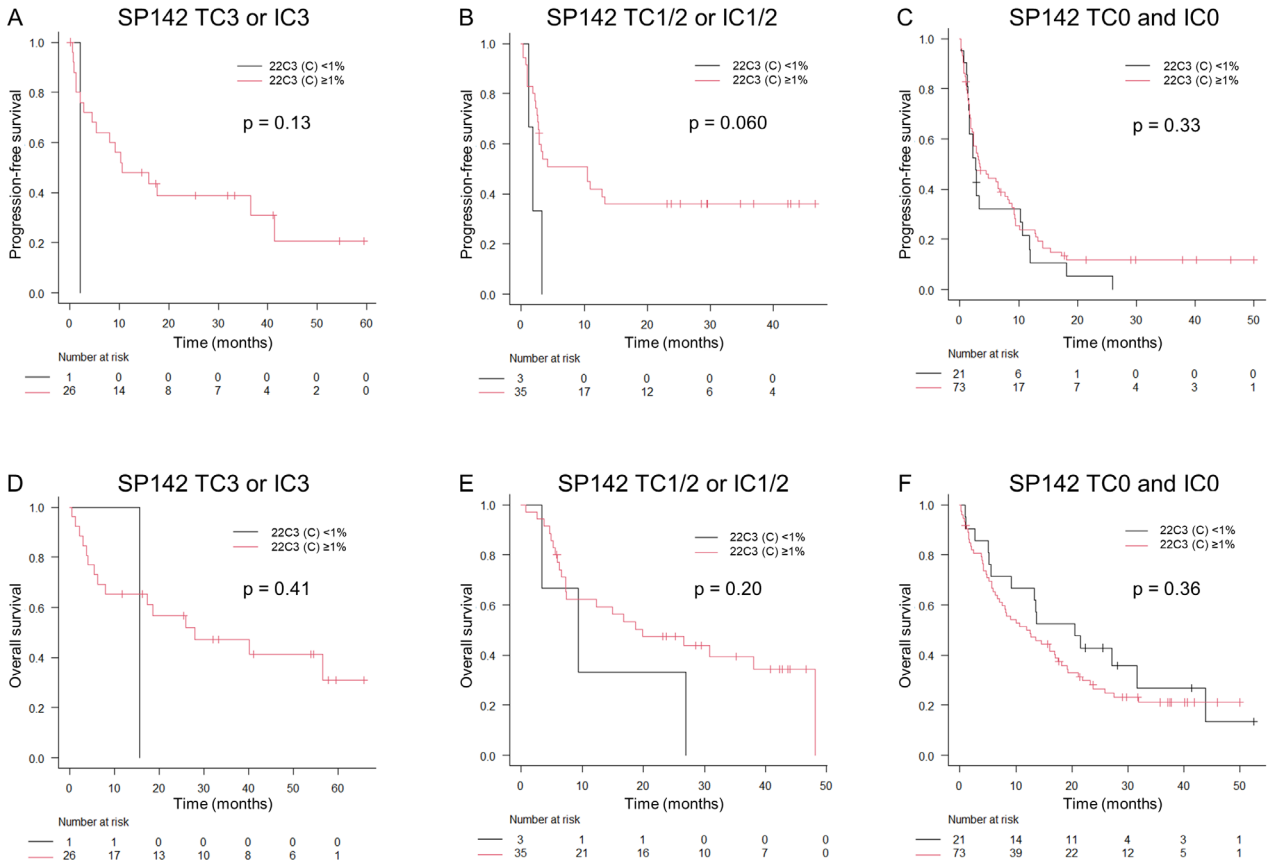

Figure S3 Progression-free survival (PFS) and overall survival (OS) analyses compared based on 22C3 results in groups divided by SP142 results. The comparison of Kaplan-Meier curves for PFS between patients with 22C3 (C) <1% and 22C3 (C) ≥1% among patients with SP142 TC3 or IC3 (A), TC1/2 or IC1/2 (B), TC0 and IC0 (C). The comparison of Kaplan-Meier curves for OS between patients with 22C3 (C) <1% and 22C3 (C) ≥1% among patients with SP142 TC3 or IC3 (D), TC1/2 or IC1/2 (E), TC0 and IC0 (F). 22C3 (C): programmed death-ligand 1 (PD-L1) tumor proportion score reviewed from medical records evaluated by 22C3 in clinical practice, TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC1/2 or IC1/2: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells and less than 50% of tumor cells or less than 10% of tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells.

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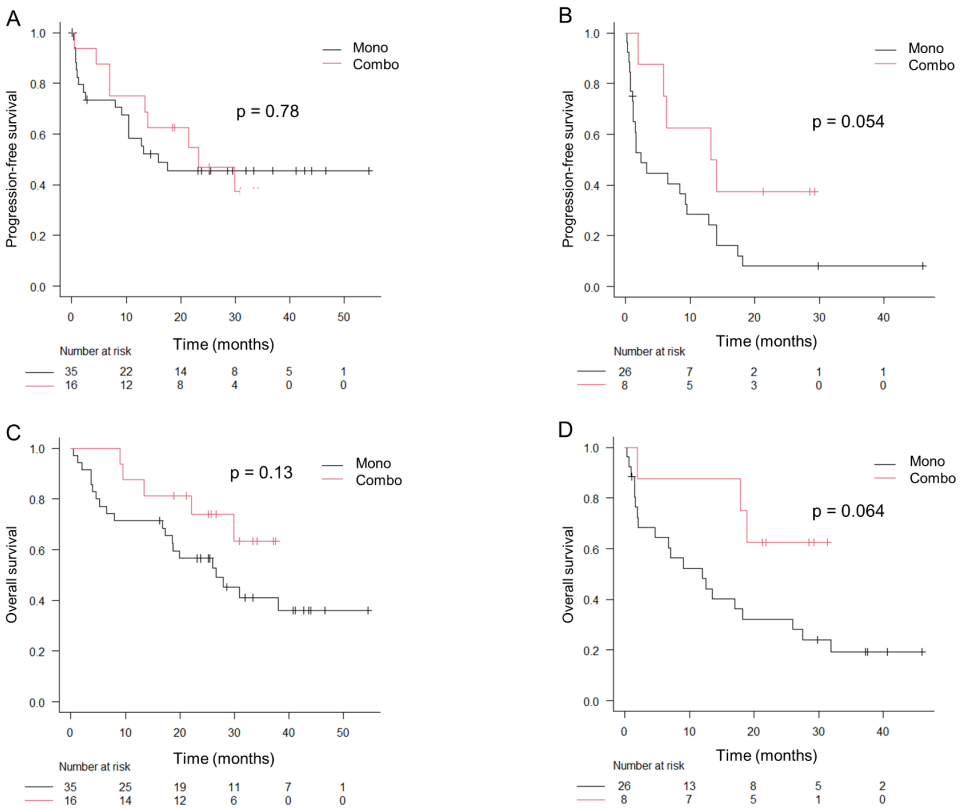

Figure S4 Progression-free survival (PFS) and overall survival (OS) analyses based on SP142 results between immune checkpoint inhibitor monotherapy and combination with chemotherapy in first-line treatment among patients with 22C3 (C) ≥50%. Comparison of Kaplan-Meier curves for PFS (A) and OS (C) between ICI monotherapy and combination with chemotherapy as a first-line treatment among patients with 22C3 (C) ≥50% and SP142 TC1/2/3 or IC1/2/3, and for PFS (B) and OS (D) between ICI monotherapy and combination with chemotherapy as a first-line treatment among patients with 22C3 (C) ≥50% and SP142 TC0 and IC0. 22C3 (C): programmed death-ligand 1 (PD-L1) tumor proportion score reviewed from medical records evaluated by 22C3 in clinical practice, TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells, TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells, Mono: immune checkpoint inhibitor monotherapy, Combo: combination of immune checkpoint inhibitors and cytotoxic chemotherapy.

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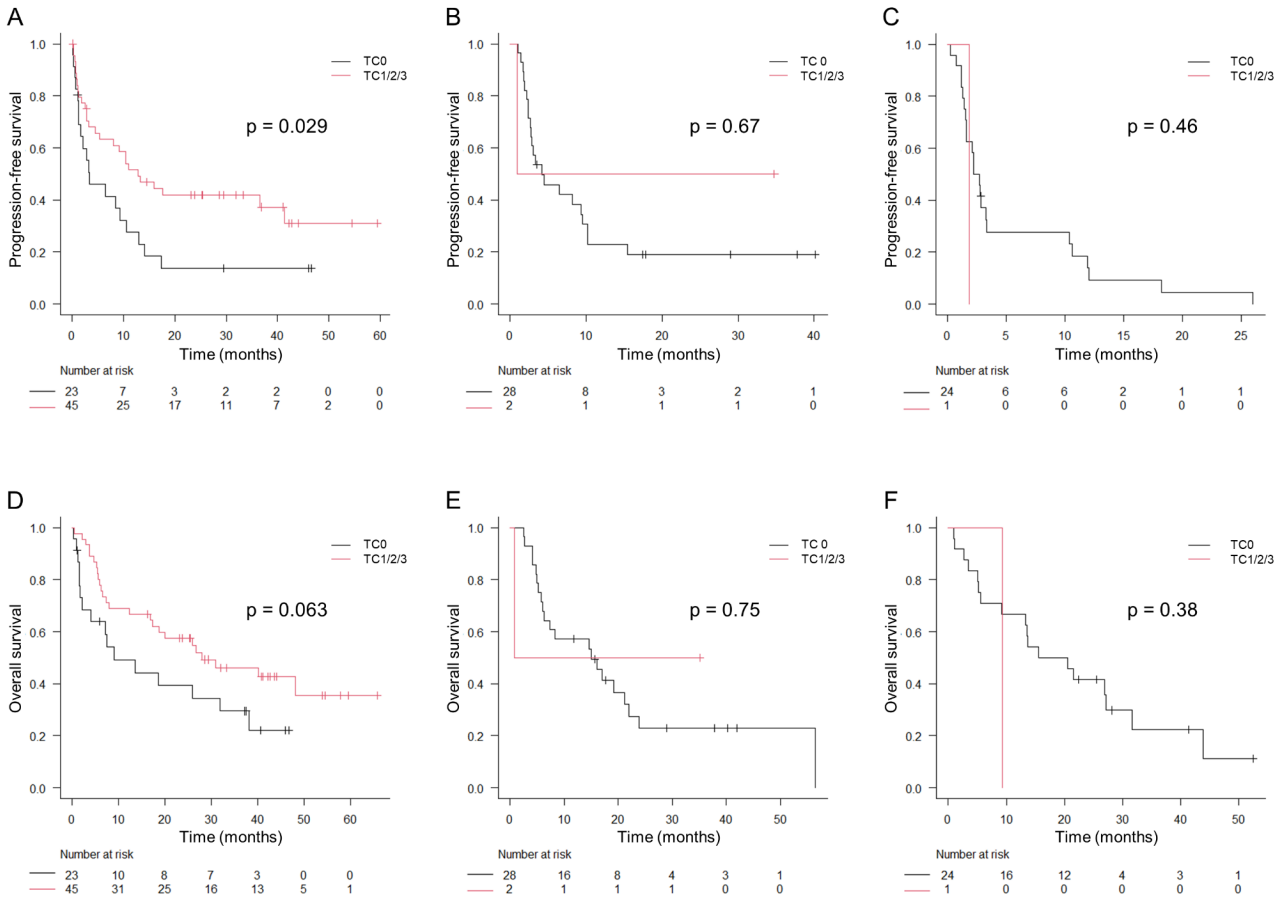

Figure S5 PFS and OS analyses compared based on SP142 TC results at each PD-L1 TPS level obtained with the 22C3 assay. Comparison of Kaplan-Meier curves for PFS between patients treated with ICI monotherapy presenting with SP142 TC0 and TC1/2/3 among patients with 22C3 (C) ≥50% (A), 1–49% (B), <1% (C). Comparison of Kaplan-Meier curves for OS between patients treated with ICI monotherapy presenting with SP142 TC0 and TC1/2/3 among patients with 22C3 (C) ≥50% (D), 1–49% (E), <1% (F). PD-L1: programmed death-ligand 1; TPS: tumor proportion score; TC0: PD-L1 expression on less than 1% of tumor cells; TC1/2/3: PD-L1 expression on at least 1% of tumor cells; 22C3 (C): PD-L1 TPS reviewed from medical records evaluated by performing the 22C3 assay in clinical practice.

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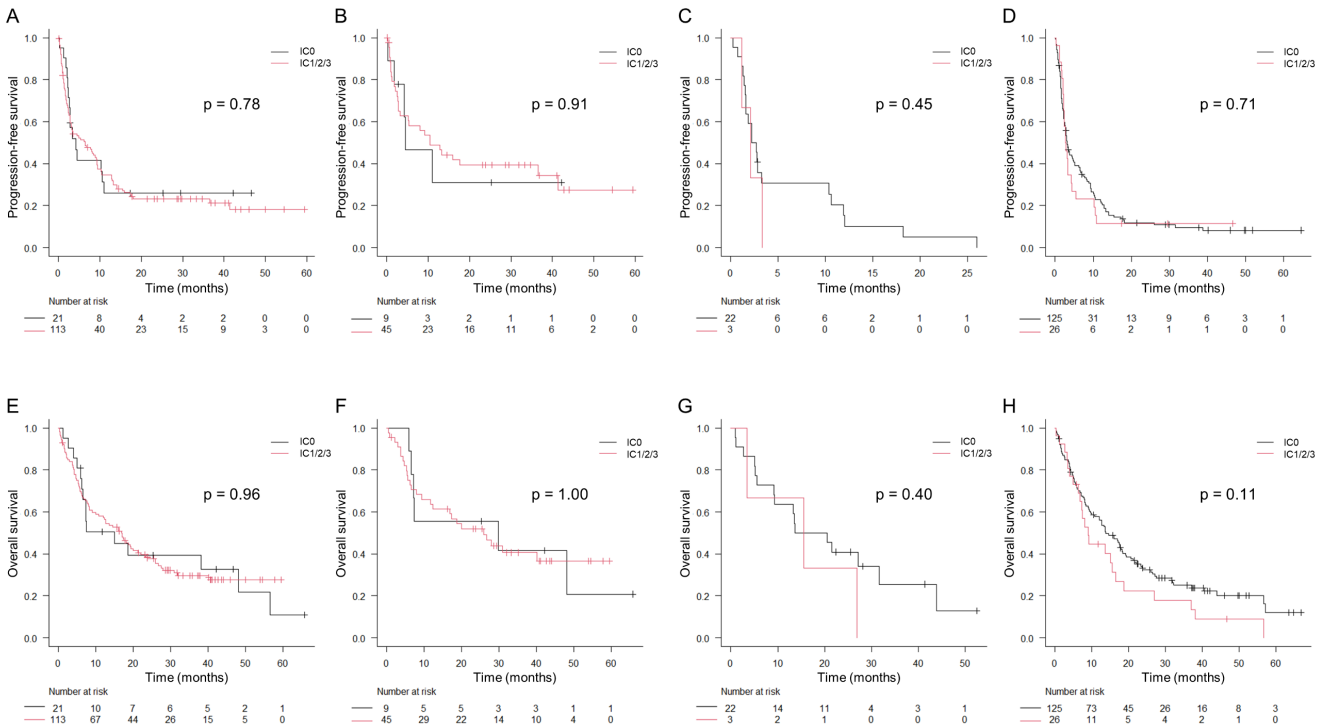

Figure S6 PFS and OS analyses of patients with PD-L1 expression in immune cells. Comparison of Kaplan-Meier curves for PFS (A) and OS (E) between patients treated with ICI monotherapy presenting with SP142 IC0 and SP142 IC1/2/3 among patients with 22C3 (C) ≥1%. Comparison of Kaplan-Meier curves for PFS (B) and OS (F) between patients treated with ICI monotherapy presenting with SP142 IC0 and SP142 IC1/2/3 among patients with SP142 TC1/2/3. Comparison of Kaplan-Meier curves for PFS (C) and OS (G) between patients treated with ICI monotherapy presenting with SP142 IC0 and SP142 IC1/2/3 among patients with 22C3 (C) <1%. Comparison of Kaplan-Meier curves for PFS (D) and OS (H) between patients treated with ICI monotherapy presenting with SP142 IC0 and SP142 IC1/2/3 among patients with SP142 TC0. 22C3 (C): PD-L1 TPS reviewed from medical records and evaluated by performing the 22C3 assay in clinical practice; IC0: PD-L1 expression on less than 1% of immune cells; IC1/2/3: PD-L1 expression on at least 1% of immune cells; TC1/2/3: PD-L1 expression on at least 1% of tumor cells.

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Table S1 The positive rate of PD-L1 expression in the tissue microarray between resection and biopsy samples based on 22C3 and SP142 assays

	Resection (N=83)	Biopsy (N=205)
22C3 (TMA) ≥50%	17 (20)	33 (16)
22C3 (TMA) 1–49%	23 (28)	52 (25)
22C3 (TMA) <1%	43 (52)	120 (59)
SP142 TC3 or IC3	13 (16)	30 (15)
SP142 TC1/2 or IC1/2	22 (27)	45 (22)
SP142 TC0 and IC0	48 (58)	130 (63)

Data presented as No (%). 22C3 (TMA): PD-L1 TPS for the tissue microarray stained with the 22C3 antibody; TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC1/2 or IC1/2: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells and less than 50% of tumor cells or less than 10% of tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells.

Table S2 Details of the ICI treatment lines in each group, divided by 22C3 and SP142 results

	1st line	2nd or later line
22C3 (C) ≥50%		
SP142 TC3 or IC3	16 (67)	8(33)
SP142 TC1/2/3 and IC1/2/3	35(67)	17 (33)
SP142 TC0/1/2 and IC0/1/2	45 (76)	14 (24)
SP142 TC0 and IC0	26(84)	5 (16)
22C3 (C) 1–49%		
SP142 TC3 or IC3	0 (0)	2 (100)
SP142 TC1/2/3 and IC1/2/3	2 (22)	7 (78)
SP142 TC0/1/2 and IC0/1/2	18 (37)	31 (63)
SP142 TC0 and IC0	16 (38)	26 (62)
22C3 (C) <1%		
SP142 TC3 or IC3	0 (0)	1 (100)
SP142 TC1/2/3 and IC1/2/3	0 (0)	4 (100)
SP142 TC0/1/2 and IC0/1/2	1 (4)	23 (96)
SP142 TC0 and IC0	1 (5)	20 (95)

Data presented as No (%). 2C3 (C): PD-L1 TPS reviewed from medical records and evaluated using 22C3 staining in clinical practice; TC3 or IC3: PD-L1 expression on at least 50% of tumor cells or 10% of tumor-infiltrating immune cells; TC1/2/3 or IC1/2/3: PD-L1 expression on at least 1% of tumor cells or tumor-infiltrating immune cells; TC0/1/2 and IC0/1/2: PD-L1 expression on less than 50% of tumor cells and less than 10% of tumor-infiltrating immune cells; TC0 and IC0: PD-L1 expression on less than 1% of tumor cells and tumor-infiltrating immune cells.

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