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Research Article

CD155 expression impairs anti-PD1 therapy response in non-small cell lung cancer

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Abstract

CD155 is an immune checkpoint protein expressed in tumor cells that interacts with its ligand TIGIT, and inhibition of this point presents a new and novel way for cancer therapy. At present, whether the expression of CD155 affects the response to anti(α)-PD1 treatment in non-small cell lung cancer (NSCLC) patients is unclear. This observational study characterizes the expression of CD155 in NSCLC patients and its responses to PD1 inhibitors. We retrospectively detected the expression of CD155 and tumor-infiltrated lymphocyte (TIL) TIGIT by immunohistochemistry in advanced NSCLC patients who had received αPD1 therapy. The patients with CD155 positive had a significantly worse response to αPD1 therapy compared with CD155-negative patients (ORR: 25.6% vs 54.8%, $P < 0.01$; median PFS: 5.1 vs 7.1 months, HR = 2.322; 95% CI 1.396–3.861, $P = 0.001$). This effect is more prominent in PD-L1 positive patients. In PD-L1-positive patients, CD155 expression is associated with a poor response to αPD1 therapy in both LUAC (lung adenocarcinoma) and LUSC (lung squamous cell carcinoma); meanwhile, the expression of CD155 was associated with a poor response to the first-line αPD1 therapy, posterior-line αPD1 therapy, and αPD1 combination therapy. Furthermore, the expression of TIGIT was not correlated with the therapeutic effect of αPD1. Our pilot study suggests that CD155 expression attenuates the therapeutic effect of αPD1 therapy and is associated with a higher risk of progression. The CD155 pathway may be a promising immunotherapeutic target and simultaneously targeting CD155/TIGIT and PD1/PD-L1 can improve the effect of immunotherapy.

Keywords: NSCLC, CD155, TIGIT, immunotherapy

Abbreviations: ICIs: immune checkpoint inhibitors; LUAC: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; NSCLC: non-small cell lung cancer; ORR: objective response rate; PD: progressive disease; PD1: programmed cell death 1; PD-L1: programmed cell death ligand-1; PFS: progression-free survival; PR: partial response; SD: stable disease; TIL: tumor-infiltrated lymphocyte; TMB: tumor mutation burden

Introduction

Lung cancer is the most prevalent malignant cancer and the leading cause of cancer mortality worldwide [1]. Non-small cell lung cancer (NSCLC) is the major histological lung cancer subtype, accounting for approximately 80–85% of all lung cancer cases, with more than 60% of patients with NSCLC at the locally advanced or advanced stages at the time of diagnosis [2]. In the past, platinum-based doublet chemotherapy was the cornerstone of the treatment of NSCLC [3]. Recently, with the rapid development of immunotherapy based on immune checkpoints, the treatment of advanced NSCLC has been revolutionized. In contrast to chemotherapy, immune checkpoint inhibitors (ICIs) targeting programmed cell death 1(PD1) and programmed cell death ligand 1 (PD-L1) have demonstrated remarkable activity against advanced NSCLC [4–6]; however, only a few patients have achieved a response and durable clinical benefit from ICI therapy [7]. There are many predictive biomarkers for ICI therapy, such as PD-L1 and tumor mutation burden (TMB), but none of them can fully predict the response feature of αPD1/PD-L1 therapies [8]. The identification of alternative biomarkers and checkpoint therapies to complement or substitute the existing immunotherapies is a current priority.

CD155 is an adhesion molecule belonging to the nectin-like family, which functions as an immune checkpoint ligand of tumor cells [9]. CD155 has also been implicated in immune regulation. CD155 modifies the function of tumor-infiltrating lymphocytes via the interactions with the co-stimulatory immune receptor CD226 (DNAM-1) and the inhibitory checkpoint receptors TIGIT and CD96, which are expressed at the

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cell surface on T and NK cells [9]. The immunosuppressive effect induced by TIGIT plays a dominant role within the tumor microenvironment [10]. Blocking TIGIT promotes anti-tumor immunity in several mouse models [11]. Compared to the single blockade, the dual TIGIT and PD1/PD-L1 blockade induces complete tumor rejection and prolongs overall survival in mouse models [12, 13]. CD155 is often overexpressed on tumor cells across multiple solid tumors, including non-small-cell lung cancer; thus, promoting tumor progression, and is associated with poor patient outcomes. Previous studies have indicated that inhibited or knocked down CD155 effectively increase sensitivity to the combination of αPD1/CTLA4 therapy in mouse models [14, 15]. These studies suggest the clinical potential of co-targeting the PD1/PD-L1 axis and the TIGIT/CD155 axis.

At the annual ASCO meeting in 2020, the data of the CITYSCAPE study were updated. Compared with αPD-L1 plus a placebo, the dual PD-L1/TIGIT blockade (atezolizumab/tiragolumab) showed a clinically significant improvement in ORR (objective response rate) and PFS as the first-line therapy in PD-L1-positive patients with NSCLC, despite having a similar safety profile [16]. The results of this phase II clinical trial further validate the potential of co-targeting the PD1/PD-L1 axis and the CD155/TIGIT pathways.

Materials and methods

Patients and specimens

This retrospective analysis includes 81 pre-treated FFPE (formalin-fixed paraffin-embedded) samples from primary patients with NSCLC that have been radiologically confirmed as stage IV (AJCC 8 edition) and initiated αPD1 treatment between January 2017 and December 2020 at our institution. All cases were diagnosed with primary NSCLC based on histological features. The inclusion criteria include: the patients were ≥18 years of age and have at least one measurable disease defined by RECIST v1.1. The exclusion criteria were patients with other primary tumors, interstitial lung disease, pulmonary fibrosis, significant cardiovascular disease, and a history of autoimmune disease. Clinicopathological characteristics including age, sex, smoking, serum CEA level at first diagnosis, and radiographic assessment were extracted from the clinical records.

Immunohistochemistry

The expression of CD155 and TIGIT was detected via immunohistochemistry in FFPE tumors. Slides were dehydrated at 60 °C for 30 min, deparaffinized in three washes of xylene for 10 min each and rehydrated in graded ethanol. After rinsing with distilled water for 3 min, the slides were heated in a microwave with citrate at 95 °C for 20 min. Then, the slides were cooled to room temperature and washed three times with 1× TBST for 3 min each. The slides were incubated in an endogenous peroxidase blocker (PV-9001, Zhong Shan Golden Bridge Biotechnology) for 10 min and washed three times with 1× TBST for 3 min each. Slices were then incubated overnight with the primary anti-CD155 antibody (81254S, Cell Signaling Technology) at 1:100 and anti-TIGIT antibody (ab243903, Abcam) at 1:100. After washing 3 times for 3 min each in 1× TBST, the slides were incubated with a secondary antibody (PV-9001, Zhong Shan Golden Bridge Biotechnology) for 30 min. The DAB detection kit (ZLI-9018, Zhong Shan Golden Bridge Biotechnology) was used in accordance with the manufacturer's instructions. Finally, the slides were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and coverslipped.

PD-L1 was stained using the VENTANA PD-L1 (SP263) Assay on the BenchMark ULTRA platform according to the manufacturer's instructions. The SP263 antibody provided in this assay was already diluted at an unspecified ratio, and stained at the Department of Pathology at Beijing Chest Hospital.

Immunostaining assessment

The percentage of stained cells and the immunostaining intensity were used for semiquantitative assessment. The percent of staining was classified as 0 (1–10%), 1 (11–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The staining intensity was defined as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (dark staining). The total score was the product of the staining percentage × intensity scores. A final total score of 0 was regarded as negative, while a final total score of ≥1 was regarded as positive. PD-L1 expression was measured by the tumor proportion scores (TPS), irrespective of staining intensities. The proportion of staining was divided into three groups: <1% staining is defined as negative, staining ranging from 1% to 49% is considered low expression, and ≥50% staining is regarded as high expression.

The inflammatory infiltrations within all the samples were assessed and subclassified semiquantitatively into TIGIT negative (≤5%) and TIGIT positive (>5%), as previously reported [17].

Two pathologists scored the immunostaining separately; when the scoring was inconsistent between the two pathologists, a third senior pathologist will estimate the score of the immunohistochemistry test.

Statistical analysis

All statistical analyses were conducted using SPSS (Version.22.0) software. Correlations between the clinicopathological characteristics and CD155 expression were analyzed using Chi-square tests. The correlation between the PD-L1 expression and the CD155 immunostaining score was computed using a Spearman correlation test. The ORRs were compared between the subgroups using Chi-square tests. The Kaplan–Meier method was used to estimate the PFS; the stratified log-rank test was used to assess between-group differences within the PFS. Variable effects on the PFS were calculated using a Cox's regression model. $P < 0.05$ was considered statistically significant.

Results

Patient's clinicopathological characteristics and CD155/TIGIT expression distribution

A total of 81 cases diagnosed with advanced NSCLC were evaluated along with their CD155 and TIGIT expression. Patient demographics, characteristics of the primary tumor and therapy details are listed in Table 1. There were 42 (51.9%) cases of CD155 that were negative and 39 (48.1%) that were positive. The cases of TIGIT-negative patients were 48 (59.3%) and 33 (40.7%) were positive. The patient's ages ranged from 29 to 81 years old, and the median age was 63. There were 68 males and 13 females. Fifty-eight patients

Table 1: Specimen details

Specimen details	NSCLC
Gender	
Male	68
Female	13
Median age (range)	63(29–81)
Surgery type	
Excision	11
Biopsy	70
Histology	
Adenocarcinoma	49
Squamous-cell carcinoma	32
Therapy lines	
First-line therapy	37
Posterior-line therapy	44
Therapy patterns	
Anti-PD1 monotherapy	30
Anti-PD1+chemotherapy and (or) anti-angiogenic therapy	51
RECIST response	
PD	13
SD	35
PR	33
Progression summary	
censored subjects	16
Events (progression)	65
Median PFS (range)	5.6 (1.0–34.5)
PD-L1 expression	
0	23
1–49%	18
≥50%	40
CD155 expression	
Negative	42
Positive	39
TIGIT expression	
Negative	48
Positive	33

smoked, the other 23 were nonsmokers. There were 49 patients with adenocarcinoma and 32 patients with squamous carcinoma. PD-L1 negative, low expression, and high expression totaled 23, 18, and 40, respectively. Thirty-seven patients received first-line αPD1 therapy, the other 44 patients received posterior-line αPD1 therapy. The RECIST category partial response (PR), stable disease (SD), and progressive disease (PD) totaled 33, 35, and 13, respectively. As shown in Table 2, the expression of CD155 had no association with age, sex, smoking, histology, and serum CEA level at the first diagnosis; however, age was correlated with TIGIT expression.

The expression of CD155 correlates with PD-L1 expression in NSCLC

Representative photomicrographs of NSCLC tissues stained for CD155, PD-L1, and TIGIT are illustrated in Fig. 1A. To evaluate whether tumor CD155 expression correlates with PD-L1 expression, we conducted a correlation analysis which showed the higher the PD-L1 expression, the higher the CD155 immunostaining score (P = 0.027, Fig. 1B). This pilot study indicated that tumor CD155 expression might influence the αPD1 therapeutic response.

CD155 expression predicted poor prognosis in patients treated with αPD1 therapy

In this study, we initially explored the association between CD155 expression and the efficacy of αPD1 therapy. Patients were divided into two groups according to their CD155 status. The Kaplan–Meier survival curves revealed that patients with tumor CD155$^+$ showed poor PFS compared with CD155$^-$ patients (5.1 vs 7.1 months, HR = 2.322, 95% CI 1.396–3.861, P = 0.001, Fig. 1C). We also compared RECIST response categories with CD155 status for patients treated with αPD1 therapy. We found that patients with tumor CD155$^-$ demonstrated higher rates of PR, and lower rates of stable disease (SD) or progressive disease (PD), compared with patients whose tumor was CD155$^+$ (P = 0.008, Fig. 1D). In addition, we observed that patients with CD155$^-$ had a better 6-month response rate to αPD1 therapy, compared to CD155$^+$ patients (P = 0.002, Fig. 1E). A univariate Cox proportional hazards regression on the PFS was performed on the tumor and analyzed the CD155, TIGIT, and PD-L1 expression levels (Table 3). CD155$^+$ patients had a significantly increased risk of progression compared to CD155$^-$ patients (HR = 2.322, 95% CI: 1.396–3.861, P = 0.001). A multivariate analysis confirmed that CD155 expression was an independent risk factor for NSCLC patients (HR = 1.975, 95% CI 1.165–3.348, P = 0.011).

Furthermore, the expression of TIGIT was not correlated with the therapeutic effect of αPD1 on PFS (5.3 vs 6.8 months, HR = 1.513, 95% CI: 0.926–2.471, P = 0.092, Fig. 1F), ORR (33.3% vs 45.8%, P = 0.261, Fig. 1G), and the 6-month response rate (33.3% vs 50%, P = 0.137, Fig. 1H). Univariate and multivariate analysis shows that TIGIT was not an independent predictor of poor PFS in all patients.

CD155$^+$ tumor attenuates αPD1 efficacy in patients with either PD-L1 high or low expression

Eighty-one patients were divided into four groups: PD-L1$^+$/CD155$^+$ (n = 30); PD-L1$^+$/CD155$^-$ (n = 28); PD-L1$^-$/CD155$^+$ (n = 9), and PD-L1$^-$/CD155$^-$ (n = 14). When compared with the other three groups, patients whose tumor was PD-L1$^+$/CD155$^-$ had a better PFS (P = 0.003, Fig. 2A). Moreover, patients whose tumor was PD-L1$^+$/CD155$^-$ had a better ORR (P = 0.001, Fig. 2B) and a 6-month response rate (P = 0.011, Fig. 2C).

We then evaluated the effect of CD155 expression on αPD1 treatment in patients with different frequencies of PD-L1 expression, i.e. in PD-L1^{low} (TPS 1–49%) and PD-L1^{high} (TPS ≥50%). Patients whose tumors were CD155$^-$ had a better PFS, both in the PD-L1^{low} (HR = 0.22, 95% CI: 0.069–0.699, P = 0.008, Fig. 2D) and the PD-L1^{high} (HR = 0.319, 95% CI: 0.142–0.167, P < 0.001, Fig. 2G) groups. Furthermore, CD155$^-$ patients had a better ORR both in the PD-L1^{low} (P < 0.05, Fig. 2E) and the PD-L1^{high} (P < 0.05, Fig. 2H) groups. In the PD-L1^{high} group, CD155$^-$ patients also had a better 6-month response rate (P < 0.001, Fig. 2I). A similar trend in the 6-month response rate was observed in the PD-L1^{low} group; however, it did not reach statistical significance (Fig. 2F).

We also evaluated the effect of TIGIT expression on αPD1 treatment in patients with different frequencies of PD-L1

Table 2: Association between CD155 expression and patient characteristics NSCLC (n = 81)

Variable	Case, *n*	CD155		*P*	TIGIT		*P*
		Negative	Positive		Negative	Positive	
Age(year)				0.655			*0.023*
<60	29	16	13		22	7	
≥60	52	26	26		26	26	
Gender				0.952			0.425
Male	68	36	32		39	29	
Female	13	6	7		9	4	
Smoking				0.648			0.187
Yes	58	31	27		37	21	
No	23	11	12		11	12	
Histology				0.469			0.171
Adenocarcinoma	49	27	22		32	17	
Squamous-cell carcinoma	32	15	17		16	16	
CEA (ng/ml)				0.551			0.448
Normal (<6)	45	22	23		25	20	
Elevated (≥6)	36	20	16		23	13	

Statistical significance ($P < 0.05$) is shown in italic.

expression. The patients with PD-L1$^+$/TIGIT$^-$ did not shown a clinical benefit in PFS (Supplementary Fig. S1A), ORR (Supplementary Fig. S1B), and the 6-month response rate (Supplementary FIG. S1C) compared with PD-L1$^+$/TIGIT$^+$ patients. Whether in the PD-L1^{high} or PD-L1^{low} groups, the expression of TIGIT was not correlated with PFS, ORR, and the 6-month response rate (Supplementary Fig. S1D–I).

CD155$^+$ tumor responds poorly to αPD1 therapy in both LUAC and LUSC

We wanted to understand whether tumor expressed CD155 could affect the immunotherapy outcome for LUAC. Although there was no statistical significance in PFS and 6-month response rate, LUAC patients with CD155$^+$ had a worse trend in PFS (Fig. 3A left) and 6-month response rate (Fig. 3C left); furthermore, they had a poorer ORR ($P < 0.05$, Fig. 3B left). Next, we analyzed the influence of tumor CD155 on immunotherapy outcomes in PD-L1$^+$ LUAC. Compared to PD-L1$^+$/CD155$^-$ LUAC, PD-L1$^+$/CD155$^+$ LUAC had a shorter PFS (HR = 2.408, 95% CI: 1.035–5.601, P = 0.016, Fig. 3D left), a worse ORR ($P< 0.05$, Fig. 3E left) and 6-month response rate ($P < 0.05$, Fig. 3F left). We next wanted to understand the impact of CD155 on LUSC. We found that CD155$^+$ LUSC had a shorter PFS (HR = 3.07, 95% CI:1.366–6.9, P = 0.002, Fig. 3A right) and a worse 6-month response rate ($P<0.05$, Fig. 3C right). Even if it did not reach statistical significance, CD155$^+$ LUSC had a lower ORR (Fig. 3B right). We next compared tumor CD155 with the immunotherapy outcomes for PD-L1$^+$ LUSC. Similar to previous results, LUSC patients with PD-L1$^+$/CD155$^+$ also had a worse PFS (HR = 6.035, 95% CI: 2.133–17.09, $P < 0.001$, Fig. 3D right), ORR ($P < 0.05$, Fig. 3E right) and 6-month response rate ($P < 0.05$, Fig. 3F right).

We also explored whether TIGIT could affect the immunotherapy outcomes for LUAC. No statistical significance was observed in PFS, ORR, and 6-month response rate (Supplementary Fig. S2A–C left) between TIGIT$^+$ and TIGIT$^-$ LUAC patients. Even in PD-L1$^+$LUAC patients, the αPD1 therapeutic effect was not influenced by TIGIT expression (Supplementary Fig. S2D–F left). We next explored the impact of TIGIT on LUSC. Although TIGIT$^+$ LUSC patients had shorter PFS (HR = 2.283, 95% CI: 1.032–5.053, P = 0.024, Supplementary Fig. S2A right), the ORR and the 6-month response rate did not reach statistical significance (Supplementary Fig. S2C, D right). No significant differences were observed in LUSC patients with PD-L1$^+$ in PFS, ORR, and the 6-month response rate (Supplementary Fig. S2D–F right).

The CD155$^+$ tumor had a worse response to αPD1 therapy both in first-line and in posterior-line treatments

We applied hierarchical analysis according to the therapy line of the αPD1 therapy. A shorter PFS was correlated with CD155$^+$ tumor status in first-line (HR = 2.892, 95% CI:1.269–6.592, P = 0.003, Fig. 4A left) and posterior-line treatment (HR = 1.954, 95% CI: 1.001–3.815, P = 0.025, Fig. 4A right).

Patients with a CD155$^+$ tumor had a worse ORR in both the first-line αPD1 (Fig. 4B left) and posterior-line αPD1 groups (P <0.05, Fig. 4B right); however, the ORR in the first-line αPD1 group did not reach statistical significance. Moreover, the 6-month response rate was lower in both the first-line αPD1 group (P <0.01, Fig. 4C left) and the posterior-line αPD1 group (no statistical significance, Fig 4C right).

Furthermore, we selected PD-L1$^+$ patients who received first-line and posterior-line αPD1 treatment and analyzed the correlation between CD155 expression and αPD1 therapy response. Consistent with the previous result, patients whose tumor was CD155$^+$ had a shorter PFS, a worse ORR and a worse 6-month response rate, regardless of whether they received first-line or posterior-line αPD1 treatment (Fig. 4D–F). However, the ORR did not reach statistical significance in the first-line αPD1 therapy group.

Whether patients received first-line or posterior-line αPD1 therapy, the expression of TIGIT was no correlated with a αPD1 therapeutic effect (Supplementary Fig. S3A–C). In

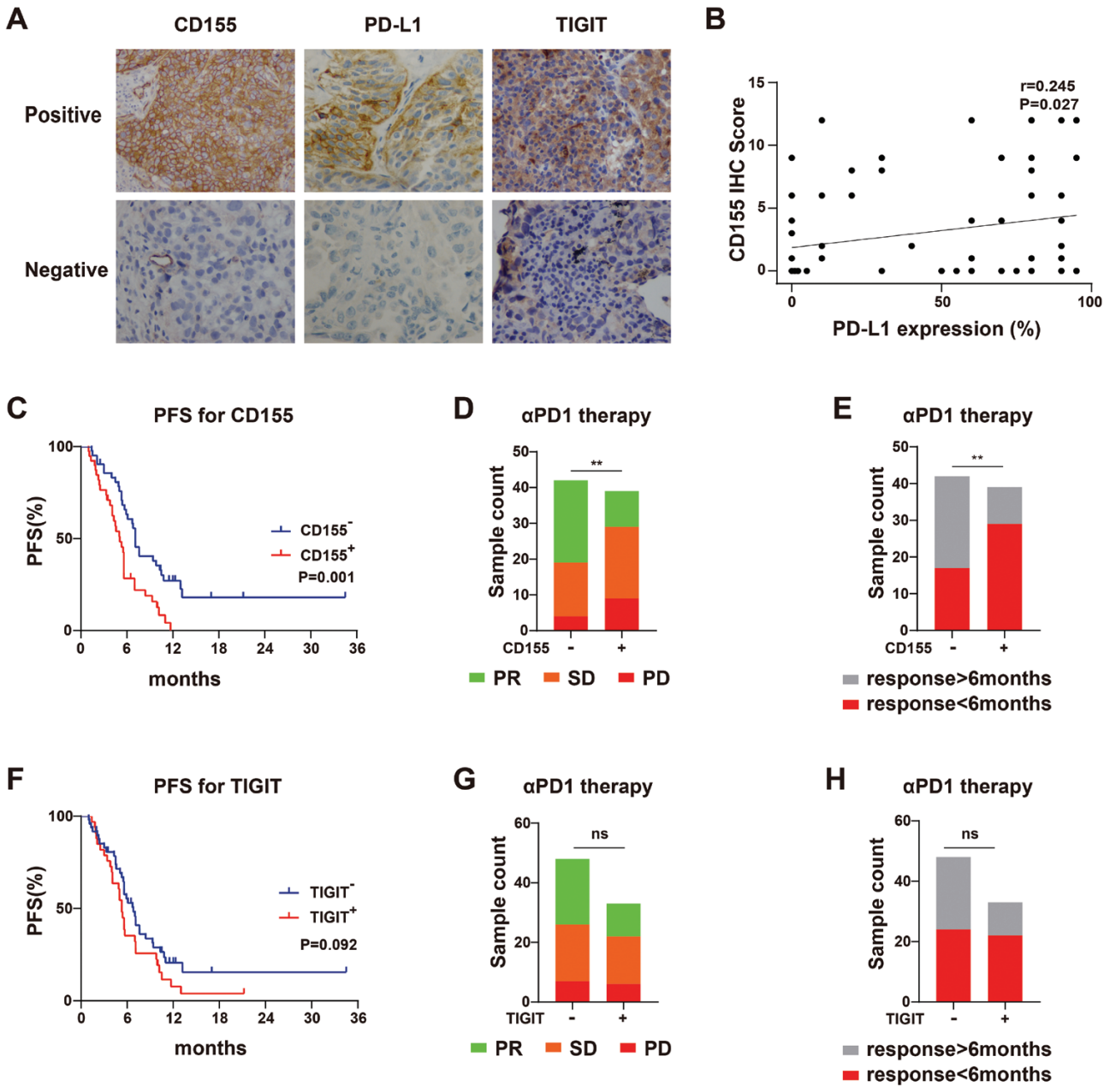

Figure 1: (A) Representative immunohistochemistry images of CD155, PD-L1, and TIGIT from NSCLC tumor specimens (magnification, ×400). (B) The correlation between CD155 immunostaining score and PD-L1 expression. (C) Association of tumor CD155⁻ vs CD155⁺ with PFS evaluated using the Kaplan–Meier method in NSCLC patients treated with αPD1 (n = 42, CD155⁻; n = 39, CD155⁺; P = 0.001). (D) Histograms of the RECIST response categories (partial response [PR], stable disease [SD], and progressive disease [PD]) from advanced NSCLC patients (n = 81) treated with αPD1 therapy. Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (E) The histograms of patients with progression free response to therapy > 6 months. Chi-square tests by response > 6 months vs response < 6 months and CD155⁻ vs CD155⁺. (F) Association of TIGIT⁻ vs TIGIT⁺ with PFS in patients with NSCLC treated with αPD1 therapy (n = 48, TIGIT⁻; n = 33, TIGIT⁺; P = 0.092). (G) Histograms of RECIST response categories (PR, SD, and PD) from advanced patients with NSCLC (n = 81) treated with αPD1 therapy. Chi-square tests by PR vs SD+PD and TIGIT⁻ vs TIGIT⁺. (H) The histograms of patients with progression-free response to therapy > 6 months. Chi-square tests by response > 6 months vs response < 6 months and CD155⁻ vs CD155⁺. **P < 0.01; ns, not significant.

PD-L1⁺ patients, the expression of TIGIT was not correlated with efficacy in the first-line and posterior-line therapies (Supplementary Fig. S3D–F).

Tumor CD155 limits response of NSCLC to αPD1 combination therapy

The Kaplan–Meier survival curves, the ORR and the 6-month response rate were used to evaluate the association between the CD155 expression and the response to αPD1-combination therapy (αPD1-combitherapy). In patients with CD155⁻, the median PFS was longer (HR = 0.32, 95% CI: 0.161–0.638, P < 0.001, Fig. 5A right), and the ORR and the 6-month response rate were better, compared to the CD155⁺ patients (Fig. 5B, C right). The 41 cases of PD-L1⁺ patients were divided into two groups: PD-L1⁺/CD155⁺ (n = 21), and PD-L1⁺/CD155⁻ (n = 20). Patients whose tumors were PD-L1⁺/CD155⁻ had a longer PFS (HR = 0.24, 95% CI: 0.104–0.552, P < 0.001, Fig. 5D right), a better ORR and a better 6-month response

Table 3: Univariate and multivariate analyses of prognostic factors in 81 NSCLC patients

Variable	*n*	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age			0.443		0.736
<60	29	0.818 (0.491–1.365)		1.107 (0.613–1.999)	
≥60	52	Reference		Reference	
Gender			*0.001*		0.115
Male	68	0.336 (0.177–0.639)		0.439 (0.158–1.223)	
Female	13	Reference		Reference	
Smoking			*0.002*		0.646
Yes	58	0.433 (0.253–0.740)		0.811 (0.331–1.984)	
No	23	Reference		Reference	
Histology			0.786		0.804
Adenocarcinoma	49	0.933 (0.567–1.535)		1.070 (0.626–1.830)	
Squamous-cell carcinoma	32	Reference		Reference	
CD155 expression			*0.001*		*0.011*
Positive	39	2.322 (1.396–3.861)		1.975 (1.165–3.348)	
Negative	42	Reference		Reference	
TIGIT expression			0.092		0.159
Positive	33	1.513 (0.926–2.471)		1.530 (0.846–2.767)	
Negative	48	Reference		Reference	
PD-L1 expression			*0.006*		0.087
Positive	58	0.477 (0.281–0.810)		0.605 (0.341–1.075)	
Negative	23	Reference		Reference	
Therapy patterns			0.140		0.530
Monotherapy	30	1.453 (0.884–2.390)		1.228 (0.647–2.330)	
Combitherapy	51	Reference		Reference	
Therapy line			*0.011*		*0.015*
First line	37	0.513 (0.308–0.857)		0.458 (0.244–0.862)	
Posterior line	44	Reference		Reference	

HR, hazard ratio; CI, confidence interval; combitherapy, combination therapy; statistical significance ($P < 0.05$) is shown in italic.

rate (Fig. 5E, F right). Moreover, the αPD1-monotherapy-treated patients, and the $CD155^-$ group did not have a better PFS, ORR, and 6-month response rate (Fig. 5A–C left). The $CD155^-$ patients treated with αPD1-monotherapy had a better trend within the PFS, PR rate, and 6-month response rate when the PD-L1 negative patients were excluded; however, none reached statistical significance (Fig. 5D–F left).

Similar to previous results, TIGIT expression did not affect the therapeutic outcome of αPD1-monotherapy and αPD1-combitherapy (Supplementary Fig. S4A–C). Consistent results were also observed within $PD\text{-}L1^+$ patients (Supplementary Fig. S4D–F).

Discussion

CD155 is an immune checkpoint protein belonging to the immunoglobulin superfamily and is barely or weakly expressed in normal human tissues; however, it is frequently overexpressed in numerous malignant tumors [17, 18]. CD155 has multiple biological functions, such as cell adhesion, proliferation, migration, and angiogenesis [19–21], which relates to tumor growth and invasion. Tumor cell deficiency of CD155 demonstrates defects in proliferation, cell cycle arrest [22]. In vitro assay, αCD155 treatment reduces tumor cell migration and invasion [23]. CD155 overexpression triggers cell-function disorders within the tumor microenvironment and eventually promotes tumor progression [24, 25].

Furthermore, as an immune checkpoint protein, CD155 plays a key role in tumor immunity. CD155 interacts with the inhibitory receptors TIGIT, CD96 and the (co)stimulatory receptor CD226; thus, regulating immune cells [26]. Multiple studies have shown that TIGIT binds to CD155 and contributes to immune suppression by mediating T and NK cells [27, 28]. Importantly, blocking the CD155/TIGIT/CD96 pathway reverses immune cell exhaustion and restore immunity against tumors [12, 15, 29, 30]. CD226 is primarily expressed on the surface of T and NK cells interacting with CD155 in order to increase the activation of T cells, NK cells and promotes cytokine (such as IFN-γ, TNFα) production. In mouse models, this interaction can inhibit tumor growth and decrease lung metastases [31, 32]. The expression of the inhibitory molecules TIGIT and CD96 is upregulated with a concomitant decrease in the expression of the (co)stimulatory molecule CD226. Eventually, the CD155/TIGIT/CD96 inhibitory signals dominate; therefore, contributing to immunosuppression and facilitating the immune escape of tumor cells [33].

Although previous reports in LUAC patients with CD155 expression had shorter PFS and overall survival (OS) [17, 25], similar results have been observed in small cell lung cancer (SCLC), pancreatic cancer, and breast cancer [18, 34, 35], the

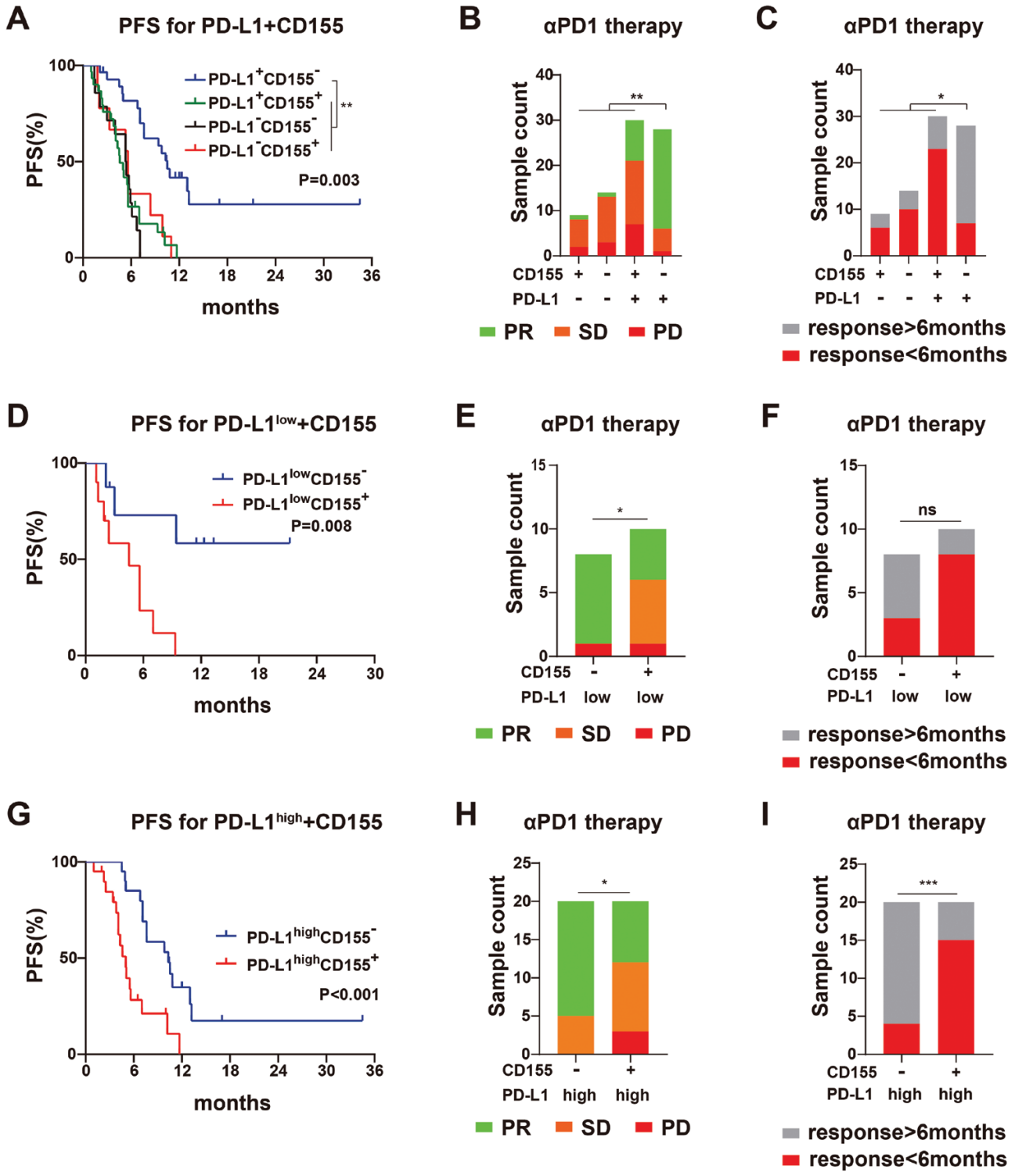

Figure 2: (A) The PFS of patients with NSCLC categorized by PD-L1 status and CD155 tumor expression. Association between PD-L1^{+}/CD155^{-} vs other groups evaluated using a Kaplan–Meier method. Patients were treated with αPD1 therapy (n = 30, PD-L1^{+}/CD155^{+}; n=28, PD-L1^{+}/CD155^{-}; n=9, PD-L1^{-}/CD155^{+}; n=14, PD-L1^{-}/CD155^{-}; P=0.003). (B) Histograms of the RECIST response categories (PR, SD, PD) in NSCLC by PD-L1 status and CD155 tumor expression from advanced NSCLC patients treated with αPD1 therapy (n = 81). Chi-square tests by PR vs SD+PD and PD-L1^{+}/CD155^{-} vs other groups. (C) The histograms of patients with response >6 months by PD-L1 status and CD155 tumor expression. Chi-square tests by response >6 months vs response <6 months and PD-L1^{+}/CD155^{-} vs other groups. (D) Association of CD155^{-} vs CD155^{+} with PFS evaluated using the Kaplan–Meier method in PD-L1^{low} patients treated with αPD1 therapy (n = 8, CD155^{-}; n = 10, CD155^{+}; P = 0.008). (E) Histograms of CD155 status by RECIST categories (PR, SD, PD) from PD-L1^{low} patients. Chi-square tests by PR vs SD+PD and CD155^{-} vs CD155^{+}. (F) The histograms of PD-L1^{low} patients with progression-free response to therapy >6 months. Chi-square tests by response >6 months vs response <6 months and CD155^{-} vs CD155^{+}. (G) Association of CD155^{-} vs CD155^{+} with PFS in PD-L1^{high} patients treated with αPD1 therapy (n = 20, CD155^{-}; n = 20, CD155^{+}; P < 0.001). (H) Histograms of CD155 status by RECIST categories (PR, SD, PD) from PD-L1^{high} patients. Chi-square tests by PR vs SD+PD and CD155^{-} vs CD155^{+}. (I) The histograms of PD-L1^{high} patients with progression-free response to therapy >6 months. Chi-square tests by response >6 months vs response <6 months and CD155^{-} vs CD155^{+}. *P < 0.05; ***P < 0.001; ns, not significant.

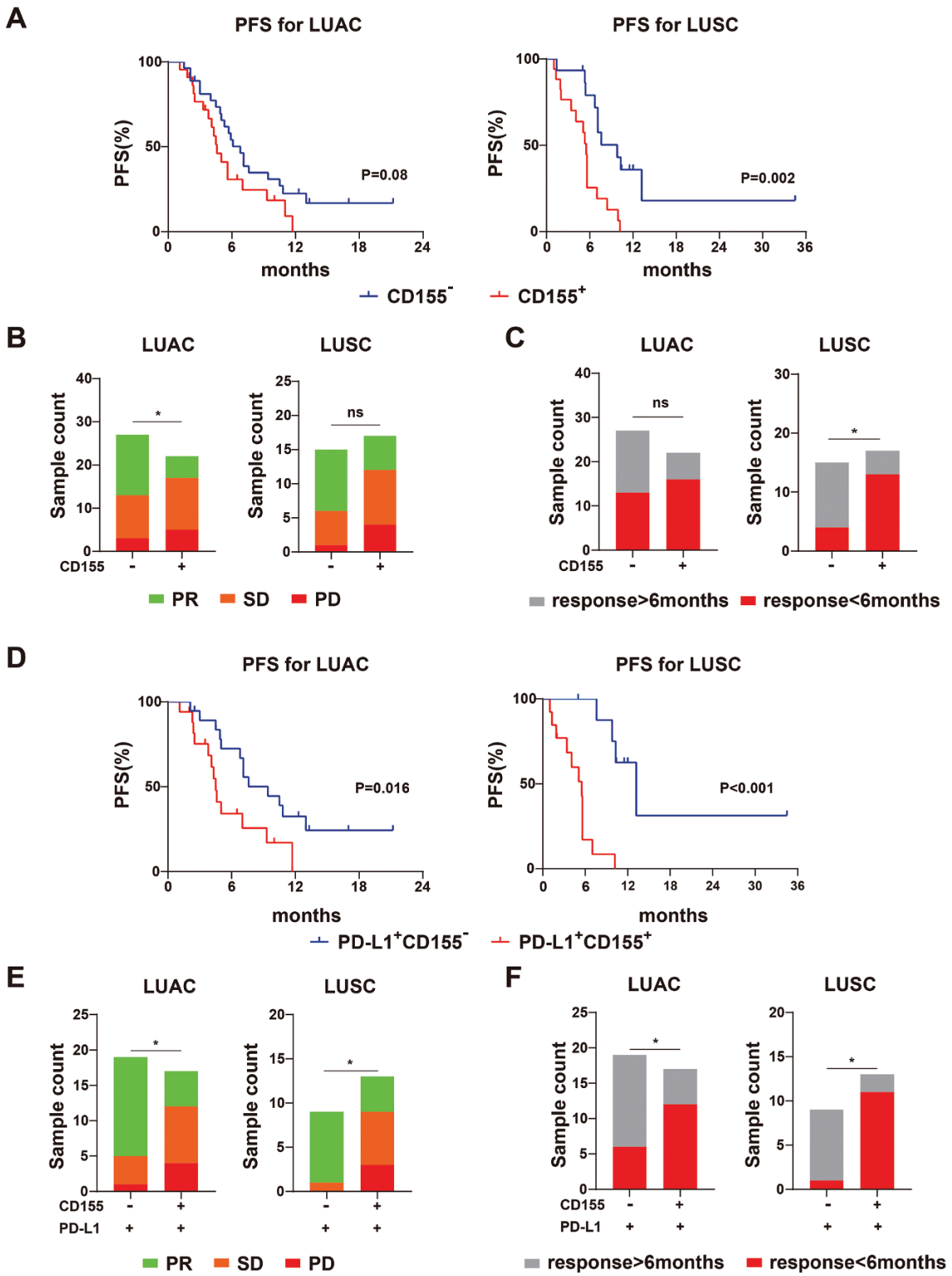

Figure 3: (A) Association of CD155⁻ vs CD155⁺ with PFS evaluated using the Kaplan–Meier method. LUAC patients (n = 27, CD155⁻; n = 22, CD155⁺; P = 0.08) or LUSC patients (n = 15, CD155⁻; n = 17, CD155⁺; P = 0.002). (B) Histograms for CD155 status by RECIST categories (PR, SD, PD) from advanced LUAC or LUSC. Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (C) The histograms of patients with progression free response to therapy > 6 months. Chi-square tests by response >6 months vs response <6 months and CD155⁻ vs CD155⁺. (D) Association of CD155⁻ vs CD155⁺ with PFS in PD-L1⁺ LUAC (n = 19, CD155⁻; n = 17, CD155⁺; P = 0.016) or LUSC (n = 9, CD155⁻; n = 13, CD155⁺; P < 0.001). (E) Histograms for CD155 status by RECIST categories (PR, SD, PD) from PD-L1⁺ LUAC or LUSC. Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (F) The histograms of PD-L1⁺ patients with progression-free response to therapy > 6 months. Chi-square tests by response >6 months vs response <6 months and CD155⁻ vs CD155⁺. *P < 0.05; ns, not significant.

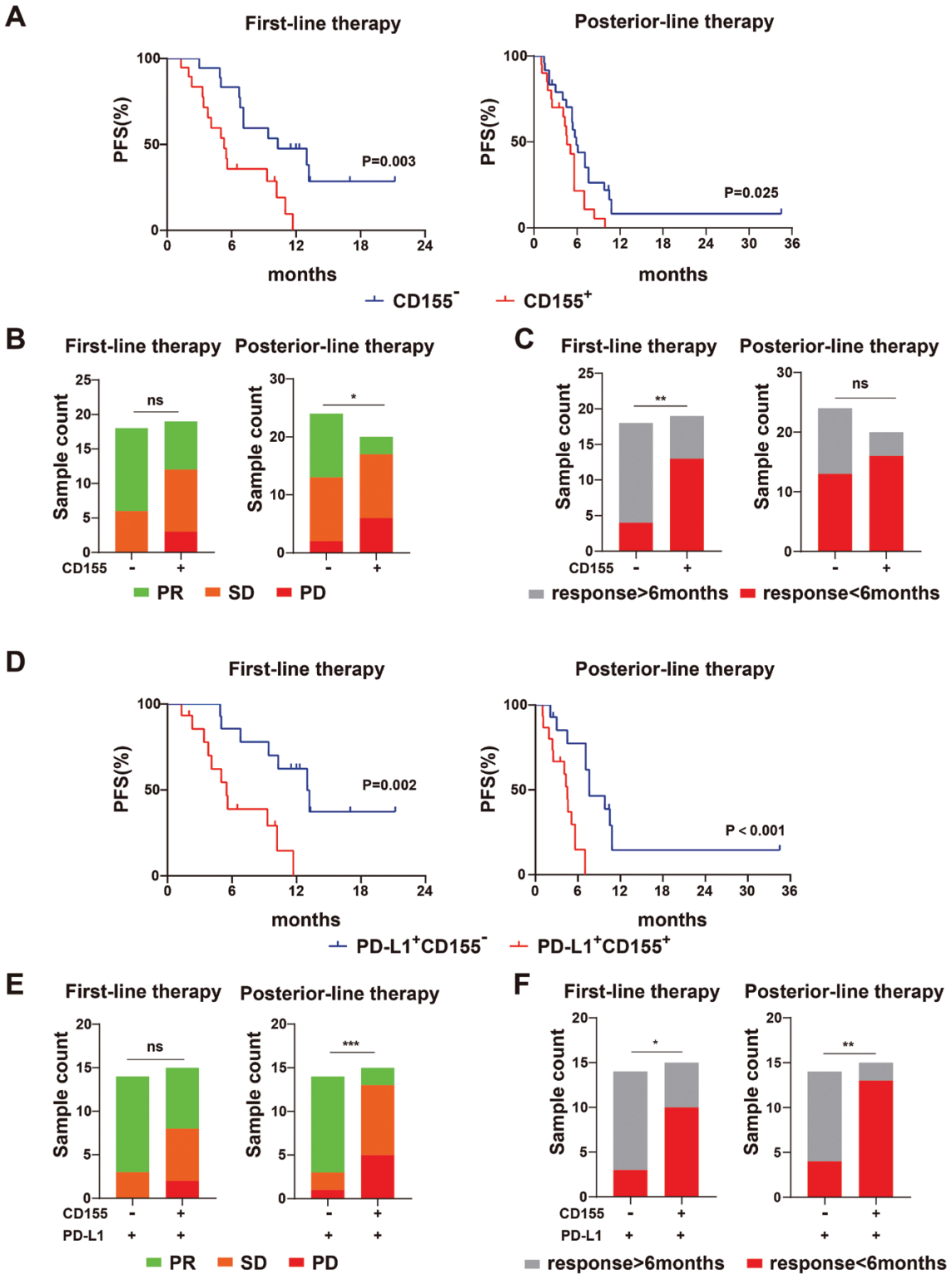

Figure 4: (A) Association of CD155⁻ vs CD155⁺ with PFS evaluated using the Kaplan–Meier method. Patients treated with first-line αPD1 therapy (n = 18, CD155⁻; n = 19, CD155⁺; P = 0.003) or posterior-line αPD1 therapy (n = 24, CD155⁻; n = 20, CD155⁺; P = 0.025). (B) Histograms for CD155 status by RECIST categories (PR, SD, PD) from advanced patients with NSCLC who received first-line αPD1 therapy (n = 37) or posterior-line αPD1 therapy (n = 44). Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (C) The histograms of patients with progression free response to therapy > 6 months. Chi-square tests by response >6 months vs response <6 months and CD155⁻ vs CD155⁺. (D) Association of CD155⁻ vs CD155⁺ with PFS in PD-L1⁺ NSCLC patients treated with first-line αPD1 therapy (n = 14, CD155⁻; n = 15, CD155⁺; P = 0.002) or posterior-line αPD1 therapy (n = 14, CD155⁻; n = 15, CD155⁺; P < 0.001). (E) Histograms for CD155 status by RECIST category (PR, SD, PD) from PD-L1⁺ NSCLC patients treated with first-line αPD1 therapy or posterior-line αPD1 therapy. Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (F) The histograms of PD-L1⁺ patients with progression free response to therapy >6 months. Chi-square tests by response >6 months vs response <6 months and CD155⁻ vs CD155⁺. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

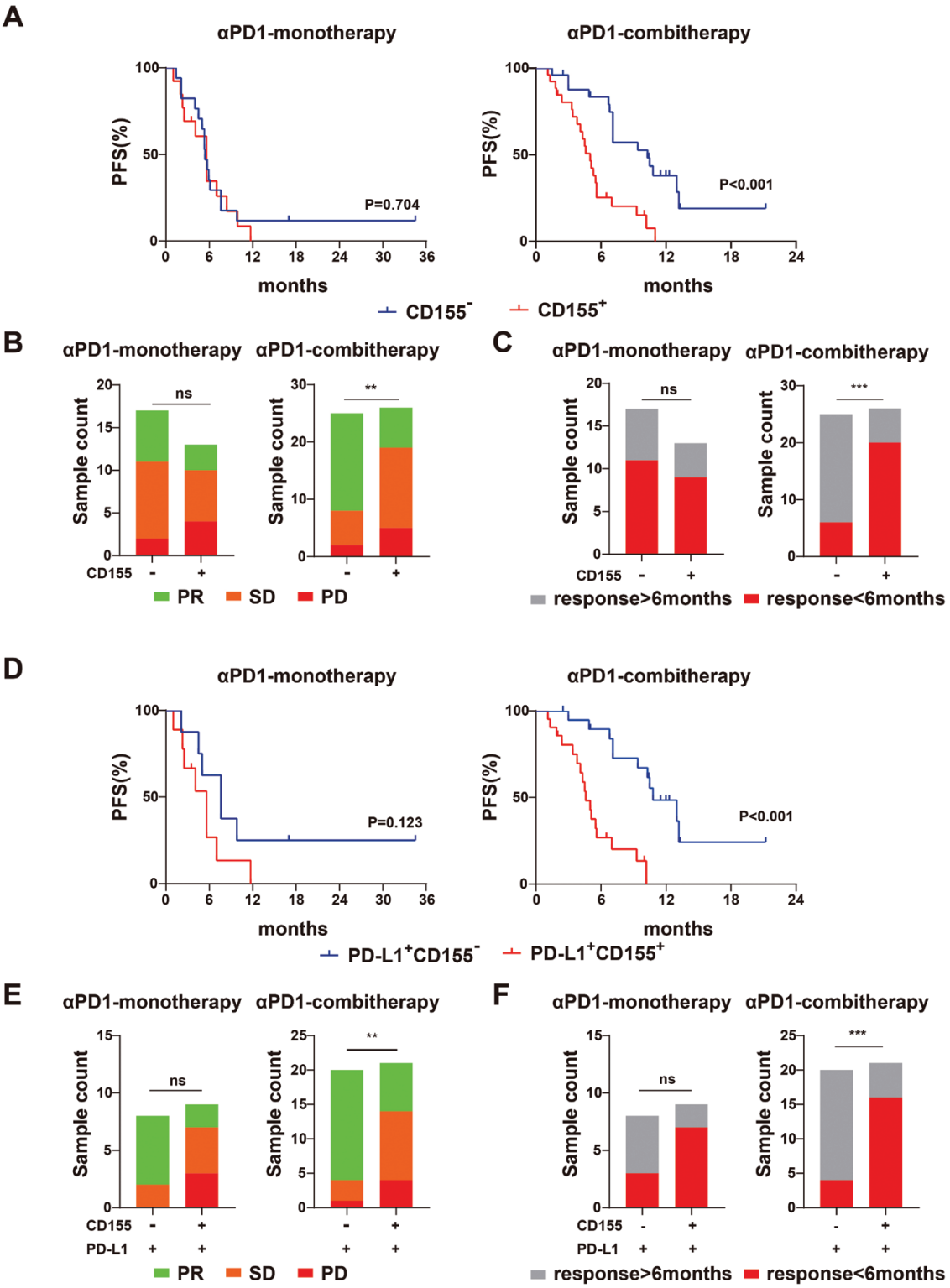

Figure 5: (A) Association of tumor CD155⁻ vs CD155⁺ with PFS evaluated using the Kaplan–Meier method in NSCLC patients treated with αPD1 monotherapy (n = 17, CD155⁻; n = 13, CD155⁺; P = 0.704), αPD1-combitherapy (n = 25, CD155⁻; n = 26, CD155⁺; P < 0.001). (B) Histograms for CD155 status by RECIST category (PR, SD, PD) from advanced patients NSCLC treated with αPD1-monotherapy(n = 30) or αPD1-combitherapy (n = 51). Chi-square tests by PR vs SD+PD and CD155⁻ vs CD155⁺. (C) The histograms of patients with progression free response to therapy > 6 months. Chi-square tests by response>6 months vs response<6 months and CD155⁻ vs CD155⁺. (D) PFS of NSCLC patients with PD-L1⁺ and categorized by CD155 tumor expression. Association between PD-L1⁺/CD155⁻ vs PD-L1⁺/CD155⁺ evaluated using Kaplan–Meier method. Patients were treated with αPD1-monotherapy (n = 8, PD-L1⁺/CD155⁻; n = 9, PD-L1⁺/CD155⁺; P = 0.123), αPD1-combitherapy (n = 20, PD-L1⁺/CD155; n = 21, PD-L1⁺/CD155⁺; P < 0.001). (E) Histograms for CD155 status by RECIST categories (PR, SD, PD) from patients with advanced NSCLC treated with αPD1-monotherapy (n = 17) or αPD1-combitherapy (n = 41). Chi-square tests by PR vs SD+PD and PD-L1⁺/CD155⁻ vs PD-L1⁺/CD155⁺. (F) The histograms of patients with response >6 months in PD-L1⁺/CD155⁻ and PD-L1⁺/CD155⁺ group. Chi-square tests by response >6 months vs response< 6 months and PD-L1⁺/CD155⁻ vs PD-L1⁺/CD155⁺. **P < 0.01; ***P <0.001; ns, not significant.

effect of CD155/TIGIT on recently administered αPD1 treatment is rarely reported.

In this study, we initially determined that 48.1% (39/81) of NSCLC patients expressed CD155 and the intensity of CD155 expression was correlated with PD-L1 expression levels. CD155 expression significantly affects the response to αPD1 treatment. In general, patients with $CD155^+$ had a shorter PFS, a lower PR rate and a lower 6-month response rate. Based on further subgroup analysis, $CD155^+$ patients showed a poor PFS, ORR, and a 6-month response rate in both LUAC and LUSC. In addition, for patients who received first-line or posterior-line αPD1 treatment, $CD155^+$ patients had a worse PFS, ORR, and a 6-month response rate; this trend was more discernible in PD-L1-positive patients. In the αPD1 combination therapy group, we see similar results as well as αPD1 combination therapy being more affected by the expression of CD155 than αPD1 treatment alone.

Exhausted T cells in the tumor microenvironment are major targets of immunotherapy. Exhausted T cells commonly co-express inhibitory receptors such as PD1, TIGT, TIM3, as well as expressing low levels of killer cytokines IFN-γ, IL-2, TNF-α [36]. In mouse models, the deletion of tumor CD155 significantly increased $CD8^+$ T and NK cells but reduced Tregs in tumors, and enhanced αPD-1 therapy sensitivity [37]. It has been reported that the $PD1^{hi}CD8^+$ T cells in cancer are dysfunctional and terminally differentiated; this was also considered to be the reason for the insensitivity to αPD1 therapy [36, 38]. Lepletier [39] reported that the expression of CD155 is associated with resistance to αPD1 immunotherapy in metastatic melanoma patients. Further research revealed that CD155 may increase PD1 expression on the surface of tumor-infiltrating $CD8^+$ T cells and decrease the expression of critical genes involved in T-cell function; therefore, promoting $CD8^+$ T-cell dysfunction and a terminally differentiated phenotype. Furthermore, the expression of CD155 correlated with an increase in the intratumor ratio of $PD1^+CD8^+/CD8^+$ T cells. $PD1^+CD8^+$ T cells were identified as $PD1^{hi}CD8^+$ T cells within Lepletier's study [39]. Co-stimulation via CD226 is required for anti-tumor $CD8^+$ T-cell response by PD1 blockade. However, CD226 is downregulated on the surface of $CD8^+$ TILs in mice and human tumor [40–42]. In tumor microenvironment, CD155 downregulates CD226 expression on $CD8^+$ TILs by triggering its internalization and proteasome degradation [40]. In addition to PD1, the co-inhibitory receptors TIGIT and CD96 expressed on the surface of $CD8^+$ T cells interact with CD155 and drives T-cell dysfunction. It has been demonstrated in existing studies that αTIGIT treatment is an effective strategy against tumors when used in combination with αPD1/PD-L1 treatment; thus, improving overall survival via modifications of T and NK cells in mouse models [12, 15, 43]. In addition, Smazynski [44] found that the CD155/TIGIT and the PD1/PD-L1 pathways represent non-redundant immunosuppressive mechanisms. In this study, TIGIT expression is not similar to CD155 as an independent prognostic factor for αPD-1 treatment, and is consistent with Patil's study [45]. This study demonstrated that CD155 is a prognostic marker and may play a vital role in promoting tumor immune suppression in the context of αPD1 therapy.

In summary, our results have shown that the intensity of CD155 is positively correlated with the expression levels of PD-L1. Furthermore, CD155 expression potentially attenuates the αPD1 therapeutic effect. The combination of CD155 and PD-L1 is a vital predictor of a group of patients who do not respond to αPD1 therapy. Moreover, co-targeting of the CD155/TIGIT and the PD1/PD-L1 pathways potentially improve the effect of immunotherapy.

There are some limitations in this study. First, this study's the limited sample size and retrospective design might have led to a degree of bias within the results. Meanwhile, the expression of CD96, and CD226 on T and NK cells were not detected, limiting the comprehensive analysis of the CD155-related signaling pathway. However, our findings are worthy of further investigation using a larger sample size.

Conclusion

CD155 expression attenuates the therapeutic effect of anti-PD1 therapy, especially in the PD-L1-positive patient population. Simultaneous targeting of the CD155/TIGIT and the PD-1/PD-L1 pathways might improve the effect of immunotherapy for patients with NSCLC.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

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Conflict of interest

None declared.

Author contributions

S.C.Z., H.T.Z., N.Y.C., and J.H.W. conceived the idea and designed the study. All authors contributed to data collection and analysis. C.J., H.T.Z., and S.C.Z. drafted the manuscript. All authors approved the final manuscript.

Ethical approval

This study was approved by the Ethics Committee of the Beijing Chest Hospital, Capital medical University (YJS-2020-011). No informed consent of patients due to this is a retrospective study.

Data availability

Not applicable.

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