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Journal

Translational Lung Cancer Research, 9(4)

ISSN

2218-6751

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Publication Date

2020-08-01

DOI

10.21037/tlcr-20-148

Peer reviewed



Mass spectrometry-based serum proteomic signature as a potential biomarker for survival in patients with non-small cell lung cancer receiving immunotherapy

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Background: VeriStrat test is a serum assay which uses a mass spectrometry (MS)-based proteomic signature derived from machine learning. It is currently used as a prognostic marker for patients with non-small cell lung cancer (NSCLC) receiving chemotherapy. However, little is known about its role for NSCLC patients receiving immune checkpoint inhibitors (ICIs).

Methods: This is a retrospective study that includes 47 patients with advanced stage NSCLC without an activating EGFR mutation, who underwent the VeriStrat test from 2016 to 2018. Spectra from blood samples were evaluated to assign patients into the VeriStrat ‘Good’ (VS-G) or VeriStrat ‘Poor’ (VS-P) risk group. The clinical outcomes of 32 patients who received programmed cell death 1 (PD-1) inhibitors nivolumab or pembrolizumab were analyzed by VeriStrat status.

Results: The VS-G group demonstrated significantly higher progression-free survival (PFS) and overall survival (OS) compared to the VS-P group among overall NSCLC patients regardless of treatment (median PFS of 7.1 *vs.* 4.2 months, $P=0.013$, and median OS, not reached *vs.* 17.2 months, $P=0.012$). Among NSCLC patients treated with ICIs, VS-G classification was associated with significantly increased PFS in comparison to VS-P classification (median PFS of 6.2 *vs.* 3.0 months, $P=0.012$), while the differences in OS trended towards significance (median OS, not reached *vs.* 16.5 months $P=0.076$). Multivariate analysis showed that the VeriStrat status was significantly correlated with PFS and OS in NSCLC patients treated with ICIs ($P=0.017$, $P=0.034$, respectively).

Conclusions: MS-based serum proteomic signature has potential as a biomarker for survival outcome in NSCLC patients receiving immunotherapy.

Keywords: Non-small cell lung cancer (NSCLC); VeriStrat test; serum proteomic test; programmed death-1 (PD-1); immunotherapy

Submitted Jan 10, 2020. Accepted for publication Mar 13, 2020.

doi: [10.21037/tlcr-20-148](https://doi.org/10.21037/tlcr-20-148)

View this article at: <http://dx.doi.org/10.21037/tlcr-20-148>

Introduction

Lung cancer is the leading cause of cancer related death worldwide (1). The majority of patients with non-small cell lung cancer (NSCLC) is diagnosed in advanced stage with the historical five-year survival rate being less than 5%. Except for patients who have actionable targets mutations with clearly defined drugs, patients receive either immunotherapy or a combination of immunotherapy and chemotherapy as first-line treatment (2-4).

Evidence has emerged demonstrating better survival outcomes and durable responses for patients using immune checkpoint inhibitors (ICIs) (2,5-12). Pembrolizumab, a PD-1 inhibitor, in combination with carboplatin and pemetrexed is approved as first-line treatment for patients with metastatic or advanced non-squamous NSCLC, regardless of PD-L1 expression (13,14). While ICIs have shown promising results, a benefit in survival was only seen in a small subset of patients (15–25% of all NSCLC patients) (15). There is a strong clinical need for biomarkers that can predict which patients will respond to ICIs.

Programmed cell death-ligand 1 (PD-L1) expression status on tumor cells is a widely accepted biomarker that has been used to predict the efficacy of anti-PD-1 and anti-PD-L1 antibodies (6-8). However, a significant survival benefit has been observed even in PD-L1 negative patients and, on the other hand, not all patients with high PD-L1 expression status are responsive to PD-1/PD-L1 inhibitors (16). These results can be explained by intratumoral heterogeneity of PD-L1 expression, changes in PD-L1 expression depending on the timing of biopsy collection, and different IHC assay methods (4). For these reasons, PD-L1 expression status on tumor cells may not be sufficient for patient stratification. Furthermore, tumor mutational burden (TMB), quantification of the mutations that result in new peptides or protein sequences in tumor cells, has also been correlated with response to PD-1/PD-L1 inhibitors. Greater PFS was demonstrated in NSCLC patients with high TMB when treated with a combination of nivolumab and ipilimumab compared to those treated with chemotherapy in the first-line setting (17). However, many challenges remain including a lack of standard method for calculating TMB and controversies on the definition of the cut-off for different tumor types (9,16). To improve the selection of patients who will most likely respond to immunotherapy as a single agent or in combination regimens, the development of new biomarkers is essential.

The VeriStrat test, a serum-based proteomic assay, is

a multivariate tool that provides information regarding expected response to treatment as well as overall prognosis. It uses matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) to analyze serum samples of the patients to define eight mass spectra features (i.e., peaks). Peaks correspond to ions formed from abundant species such as peptides and proteins. Algorithms along with optimizations using machine learning are then performed to assign each spectrum to a VeriStrat ‘Good’ (VS-G) or VeriStrat ‘Poor’ (VS-P) classification (18). Based on this classification, the test can be used to predict which patients will experience longer survival in response to specific treatments. In comparison to PD-L1 expression status and TMB, the VeriStrat test has a standardized analysis procedure and only involves a peripheral blood draw. Furthermore, different MS tests with different cutoffs can be developed and validated for different subpopulations of immunotherapy treated NSCLC patients to predict survival outcomes more accurately.

The VeriStrat test’s utility in predicting patient response has been validated in NSCLC patients treated with chemotherapy and targeted therapy in various conditions (18-26). In one study, seventy-six non-squamous lung cancer patients treated with standard first-line chemotherapy demonstrated that the VS-G patients had longer progression-free survival (PFS) and overall survival (OS) than VS-P patients (6.5 *vs.* 1.6 months, $P < 0.0001$, and 10.8 *vs.* 3.4 months, $P < 0.0001$, respectively). The VeriStrat status was found to be a statistically significant predictor of OS (25). In the phase III Lux-Lung 8 study in which a serum protein test was used to predict differential clinical outcomes with afatinib versus erlotinib, the OS was significantly longer in VS-G *vs.* VS-P patients, both in the overall population (HR 0.41, 95% CI: 0.35–0.49) and in afatinib-treated patients (HR 0.40, 95% CI: 0.31–0.51) (20). Furthermore, the prospective randomized phase III PROSE study demonstrated that patients classified as VS-P had better outcomes when receiving chemotherapy rather than erlotinib (23).

In addition to predicting survival outcomes, accurate prognostic measurements provided by the VeriStrat test help avoid ineffective, costly overtreatment and improve patient quality of life by precisely determining the appropriate time for optimal support and hospice care (27-30). A study that utilized a cost-effectiveness model concluded that the VeriStrat test reduced the use of active treatment by 9.1%, changed the choice of active treatment in 20% of patients, improved OS by 0.7 months and 0.5 quality-adjusted

months, and resulted in a net saving of \$1,050 per patient after factoring in the cost of the VeriStrat test (30). Patients who changed from erlotinib to chemotherapy on second-line treatment after using the proteomic test achieved an increase in quality-adjusted life years (QALYs) and saved costs (29). Among VS-P patients, the benefit was more substantial (27).

However, the VeriStrat classification has not been well studied in patients treated with immunotherapy. We hypothesize that the VeriStrat status has the potential to be a novel biomarker that can help determine which lung cancer patients will derive benefit from immunotherapy. The objective of this retrospective analysis was to evaluate whether there were differences in survival outcomes based on VeriStrat classification in patients treated with ICIs.

Methods

Study design and end points

This study was approved by The Institutional Review Board (IRB) of Northwestern University Feinberg School of Medicine (No. STU00205299). For this retrospective study, the written informed consent from patients was waived per the IRB, and the study was performed following the Health Insurance Portability and Accountability Act and the Declaration of Helsinki (as revised in 2013).

Seventy patients with advanced stage (III/IV) NSCLC at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University underwent the VeriStrat test from 2016 to 2018. The median follow-up period was 17 months. Patients with *EGFR* mutation (n=8), patients lost to follow-up (n=9), patients who received combination treatment of chemotherapy and immunotherapy (n=5), and a patient whose result was determined to be VeriStrat-Indeterminate (VS-I) were not included in the study, resulting in the final sample size of 47 patients. For this study, patients were grouped into three sets for analyses: overall NSCLC patients (n=47), NSCLC patients treated with ICIs (n=32) and NSCLC patients treated with chemotherapy (n=35). There was an intersection between the ICIs and chemotherapy groups because some patients received both treatments at different lines. This explains why the sum of the two groups do not add up to (n=47). In this study, ICIs consisted of either the PD-1 inhibitor, pembrolizumab or nivolumab.

The survival outcome was assessed by analyzing PFS and OS for all three sets of patients. PFS was calculated

from the start of first-line systemic treatment, ICI, or chemotherapy to disease progression or death in the overall NSCLC patients, NSCLC patients treated with ICIs and NSCLC patients treated with chemotherapy, respectively. OS was calculated from the start of first-line systemic treatment to the date of death, or in the case of NSCLC patients initially treated with ICIs, the start of ICIs to the date of death. In NSCLC patients treated with chemotherapy as first-line therapy, OS was calculated from the start of the chemotherapy to the date of death. Patients were censored if they were event-free at the final clinical assessment. Additionally, PFS and OS of patients treated with ICIs as first-line treatment (n=8) were compared to those of patients treated with chemotherapy as first-line treatment (n=35) to assess the predictive property of ICIs in comparison to standard chemotherapy.

VeriStrat analysis

Serum samples were collected from 2016 to 2018 and were frozen at -80°C until their use for mass spectrum generation for the VeriStrat test. The VeriStrat test was conducted by Biodesix (Boulder, CO, USA) according to the standard protocol previously described (18,19). MALDI-TOF spectrometer was used to attain spectra of samples, which were then assigned a VS-G or VS-P risk group classification if all the replicates from the same sample resulted in the same classification; VS-I classification was assigned in the event of inconsistent classification.

Statistical analysis

Baseline characteristics of patients are presented as counts and percentages for categorical variables, and as mean and range for continuous variables. Comparison of continuous variables between the VeriStrat groups was performed using unpaired Student's *t*-test. Comparison of categorical variables was calculated using Fisher's exact test. To assess whether the VeriStrat classification was associated with clinical outcomes, the hazard ratios of PFS and OS were compared with forest plot, Microsoft Office Excel (Microsoft, Redmond, WA). Cox regression model was used to verify the VeriStrat test's prognostic value on PFS and OS while adjusting for other baseline factors, including gender, age, smoking status, ECOG performance status, histology, and prior treatment. These analyses were performed using SPSS Version 23 (SPSS Inc., Armonk, NY, USA).

For survival analyses, Kaplan-Meier curves were

generated using the log-rank test. Time-to-event outcomes were analyzed using the data from patients who received at least one dose of first-line systemic therapy and were classified as VS-G or VS-P. These analyses were performed using GraphPad Prism 7 (La Jolla, CA, USA).

For this study, the same statistical analyses were performed in the main three subsets of patients: overall NSCLC patients (n=47), NSCLC patients treated with ICIs (n=32) and NSCLC patients treated with chemotherapy (n=35). Additionally, a similar analysis was tried for NSCLC patients who underwent the VeriStrat test before ICIs (n=12), and NSCLC patients who underwent the VeriStrat test before chemotherapy (n=10).

Results

Patient baseline characteristics

Forty-seven patients were included in the study. Thirty-two patients were classified as VS-G, and fifteen patients were classified as VS-P. *Table 1* shows the distribution of clinical characteristics by the VeriStrat test classification. The patient population had a mean age of 65.7 years (range, 30–91 years), 25 patients (53.2%) were female, and 22 patients (46.8%) were male. By the time of the analysis, 17 patients (36.2%) had died (nine and eight in VS-G and VS-P group, respectively). The majority of patients (85.1%) were former or current smokers and most (76.6%) had stage IV NSCLC. There were significantly more squamous cell carcinoma cases among VS-P than among VS-G patients (60.0% vs. 25.0%, respectively, $P=0.027$, *Table 1*). Thirty-two patients (68.1%) had undergone treatment with ICIs at the time of analyses, whereas 15 patients (31.9%) had not. Of the overall 47 analyzed patients, 16 had undergone other treatment modalities such as radiation and surgery before chemotherapy or ICIs. Of the 32 NSCLC patients treated with ICIs, 12 were subject to the VeriStrat test before ICIs (*Table S1*). Of the 35 NSCLC patients treated with chemotherapy, 10 were subject to the VeriStrat test before chemotherapy.

The majority of patients received ICIs as second or third-line treatment (n=24) after receiving first-line treatment such as chemotherapy (n=35) (*Table 2*). Furthermore, more NSCLC patients treated with ICIs were subject to the VeriStrat test after the start of treatment with ICIs (62.5%). The percentage of VS-P patients was higher among patients who were subject to the VeriStrat test after the start of treatment with ICIs (50.0%) than for those who were

subject to it before the start of ICI treatment (33.3%), but the difference was not significant ($P=0.471$, *Table S1*).

Survival in overall NSCLC patients by serum proteomic signature

VS-G patients in the overall NSCLC population had a 65% lower risk of progression (HR =0.35, 95% CI: 0.15–0.82) and 78% lower risk of death (HR =0.22, 95% CI: 0.07–0.72) compared to VS-P patients (*Figure 1*). The median PFS was 7.1 months for VS-G patients, significantly longer than the 4.2 months for VS-P patients ($P=0.013$) (*Figure 2A*). VS-G did not reach median OS as survival exceeded 50% at the time of analysis, which was significantly longer than 17.2 months by VS-P patients ($P=0.012$) (*Figure 2B*). Multivariate analysis showed that the VeriStrat status was significantly correlated with PFS, after adjusting for clinical factors such as age (HR =0.38, 95% CI: 0.16–0.88, $P=0.024$) (*Table S2*). Also, the VeriStrat status was significantly correlated with OS (HR =0.21, 95% CI: 0.05–0.89, $P=0.035$) (*Table S3*).

Survival in NSCLC patients treated with ICIs by serum proteomic signature

VS-G patients treated with ICIs had a 72% lower risk of progression compared to VS-P patients (HR =0.28, 95% CI: 0.11–0.76, $P=0.012$, *Figure 1*). The median PFS was 6.2 months for VS-G patients, significantly longer than the 3.0 months for VS-P patients (*Figure 2C*). The median OS of VS-G in NSCLC patients treated with ICIs was longer than VS-P with a trend toward significance ($P=0.076$) (*Figure 2D*). Multivariate analysis determined that the VeriStrat status was significantly correlated with PFS (HR =0.26, 95% CI: 0.08–0.79, $P=0.017$) and OS (HR =0.16, 95% CI: 0.03–0.87, $P=0.034$) among patients treated with ICIs (*Tables 3 and 4*, respectively).

Survival in NSCLC patients treated with chemotherapy by serum proteomic signature

VS-G patients in the NSCLC patients treated with chemotherapy had a 61.1% lower risk of PFS (HR =0.39, 95% CI: 0.15–0.99) and an 81.0% lower risk of OS (HR =0.19, 95% CI: 0.05–0.76) compared to VS-P patients (*Figure 1*). In the NSCLC patients treated with chemotherapy, VS-G patients had significantly longer median PFS than VS-P patients (*Figure 2E*). A multivariate

Table 1 Baseline characteristics of patients by the VeriStrat test classification

Characteristic	Overall (n=47)	VeriStrat-classified population		P value
		VS-G (n=32)	VS-P (n=15)	
Living status				0.114
Alive	30 (63.8)	23 (71.9)	7 (46.7)	
Deceased	17 (36.2)	9 (28.1)	8 (53.3)	
Age				
Mean	65.7	66.9	63.3	0.332
Range	30–91	30–90	43–91	
Mean (alive)	66.6	68	62.7	0.256
Mean (deceased)	64.1	64.4	63.8	0.923
Gender				0.999
Female	25 (53.2)	18 (56.2)	7 (46.7)	
Male	22 (46.8)	14 (43.8)	8 (53.3)	
ECOG PS				0.999
≤2	40 (0.85)	27 (0.84)	13 (0.87)	
3, 4	7 (0.15)	5 (0.16)	2 (0.13)	
Smoking				0.552
Never	7 (14.9)	6 (18.7)	1 (6.7)	
Former	29 (61.7)	19 (59.4)	10 (66.7)	
Current	11 (23.4)	7 (21.9)	4 (26.6)	
Histology				0.027 [†]
Poorly differentiated	2 (4.3)	1 (3.1)	1 (6.7)	
Adenocarcinoma	26 (55.3)	22 (68.8)	4 (26.6)	
Squamous	17 (36.1)	8 (25.0)	9 (60.0)	
Large cell	2 (4.35)	1 (3.1)	1 (6.7)	
Stage				0.999
III	11 (23.4)	8 (25)	3 (20)	
IV	36 (76.6)	24 (75)	12 (80)	
ICI user				0.017
Yes	32 (68.1)	18 (56.3)	14 (93.3)	
No	15 (31.9)	14 (43.7)	1 (6.7)	
Prior surgery or radiation therapy				0.528
Yes	16 (34.0)	12 (37.5)	4 (26.7)	
No	31 (66.0)	20 (62.5)	11 (73.3)	

Data given as No. (%) unless otherwise indicated. [†], squamous vs. non-squamous. ECOG PS, Eastern Cooperative Oncology Group Performance Status; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ICI, immune-checkpoint inhibitor.

Table 2 Status of ICI treated patients by the VeriStrat test classification

Line of treatment	Overall (n=32)	VeriStrat-classified population		P value
		VS-G (n=18)	VS-P (n=14)	
ICI				0.204
1st line	8 (25.0)	4 (22.2)	4 (28.6)	
2nd line	17 (53.1)	8 (44.5)	9 (64.3)	
3rd line	7 (21.9)	6 (33.3)	1 (7.1)	

Data given as No. (%) unless otherwise indicated. ICI, immune-checkpoint inhibitor; VS-G, VeriStrat Good; VS-P, VeriStrat Poor.

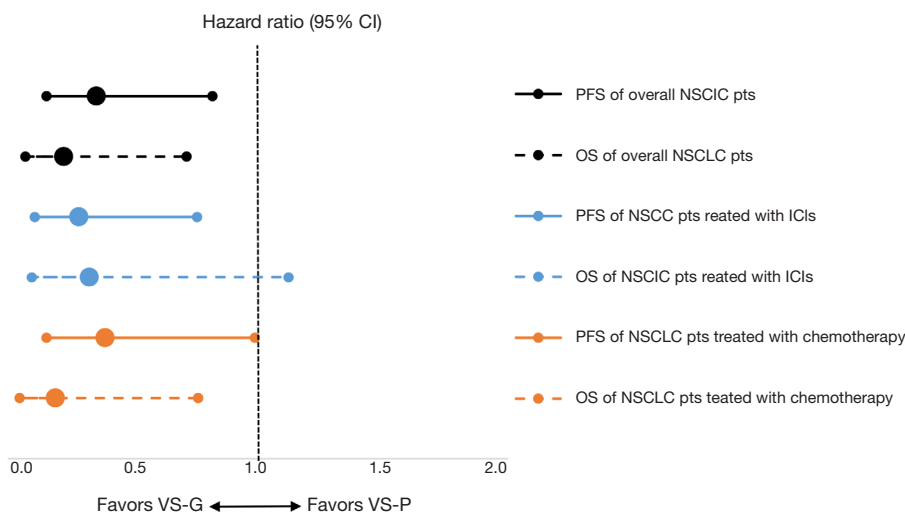


Figure 1 Forest plot by the VeriStrat test classification. Hazard ratio of 1 suggests no difference in survival outcomes between patients with VS-G and VS-P classification. Hazard ratio towards the left of the dotted line favors VS-G, whereas a hazard ratio greater than 1 favors VS-P. CI, confidence interval; PFS, progression-free survival; OS, overall survival; pts, patients; NSCLC, non-small cell lung cancer; ICIs, immune checkpoint inhibitors; VS-G, VeriStrat Good; VS-P, VeriStrat Poor.

analysis displayed a trend toward significance for VeriStrat status being correlated with PFS for these patients, while adjusting for other covariates (HR =0.38, 95% CI: 0.12–1.15, P=0.087) (Table S4). Furthermore, VS-G was associated with longer median OS when compared with VS-P in the overall NSCLC patients treated with chemotherapy (Figure 2F). A multivariate analysis displayed a trend for VeriStrat status being significantly correlated with OS (HR =0.18, 95% CI: 0.03–1.12, P=0.065) (Table S5).

Survival in NSCLC patients who received the VeriStrat test before the treatment

The median PFS and OS were not significantly different

between VS-G and VS-P patients who were subject to the VeriStrat test before the start of ICIs (Figure 3A,B, respectively). In contrast, VS-G was associated with longer median PFS and OS when compared with VS-P in patients who were subject to the VeriStrat test before the start of chemotherapy (Figure 3C,D, respectively).

Survival in NSCLC patients treated with ICIs versus chemotherapy as first-line treatments

When projecting the survival curves for VS-G patients, PFS distribution was not significantly different between the ICIs and chemotherapy treated groups (Figure 4A). The median PFS of patients treated with ICIs was 7.4 months while that of patients treated with chemotherapy was 7.0 months

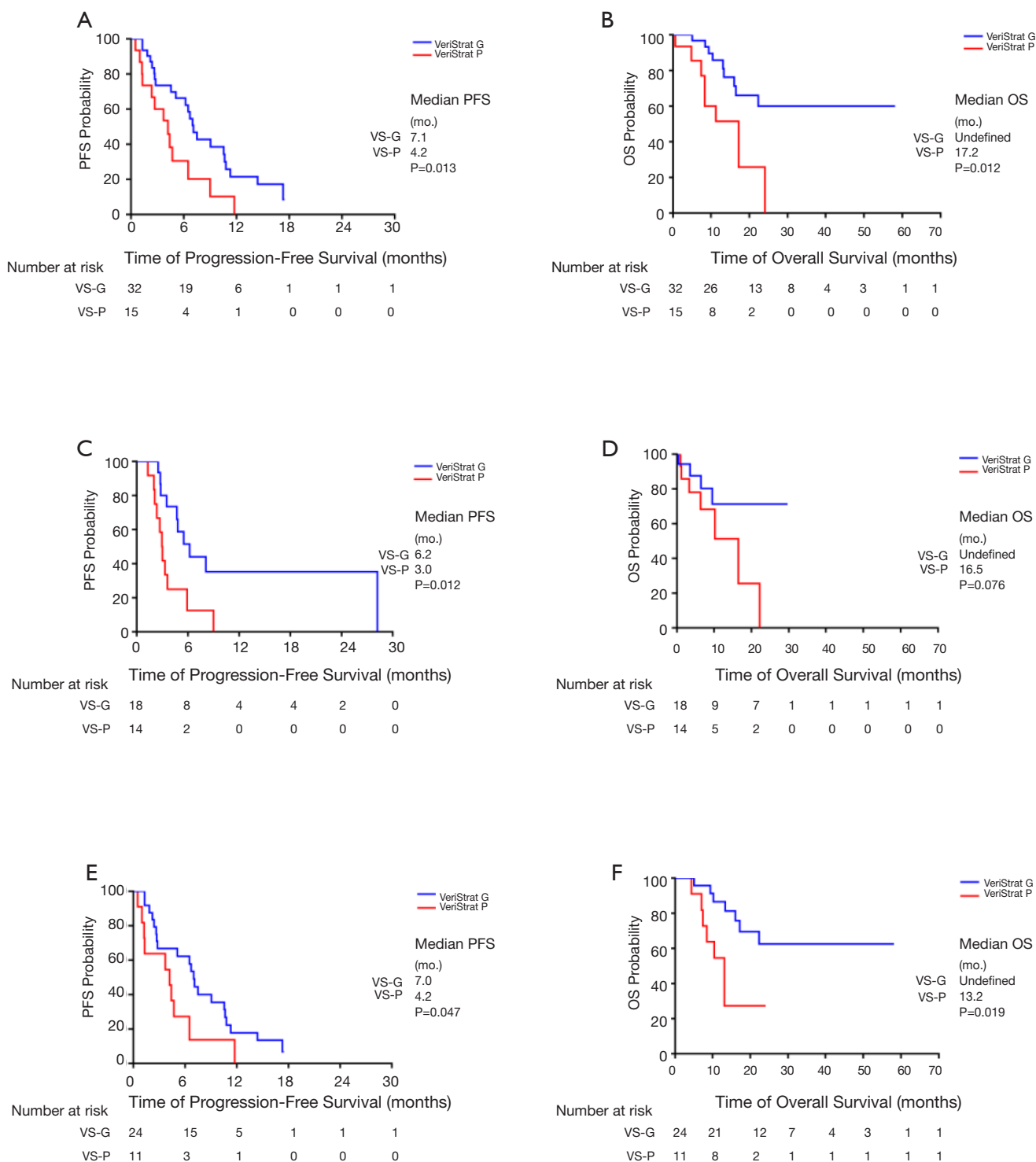


Figure 2 Survival curves of NSCLC patients by the VeriStrat classification. (A) PFS of the overall NSCLC population; (B) OS of the overall NSCLC population; (C) PFS of NSCLC population treated with ICIs; (D) OS of NSCLC population treated with ICIs; (E) PFS of NSCLC population treated with chemotherapy; (F) OS of NSCLC population treated with chemotherapy. PFS, progression-free survival; OS, overall survival; NSCLC, non-small cell lung cancer; ICIs, immune checkpoint inhibitors; VS-G, VeriStrat Good; VS-P, VeriStrat Poor.

Table 3 Cox proportional hazard models of progression-free survival by NSCLC patients treated with immune checkpoint inhibitors

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
VeriStrat				
P	1		1	
G	0.33 (0.13–0.81)	0.016	0.26 (0.08–0.79)	0.017
Gender				
Male	1		1	
Female	1.68 (0.66–4.23)	0.274	1.24 (0.38–4.05)	0.722
Age				
30–60	1		1	
61–91	0.24 (0.08–0.72)	0.011	0.38 (0.11–1.37)	0.141
Smoking status				
Never	1		1	
Ever	0.42 (0.13–1.33)	0.139	0.39 (0.11–1.41)	0.149
ECOG PS				
≤2	1		1	
3, 4	1.13 (0.25–5.06)	0.875	0.41 (0.06–2.77)	0.360
Histology				
Squamous	1		1	
Non-squamous	0.67 (0.27–1.67)	0.389	0.84 (0.22–3.19)	0.799
Prior surgery or radiation therapy				
Yes	1		1	
No	0.93 (0.35–2.45)	0.876	0.79 (0.21–3.03)	0.730

NSCLC, non-small cell lung cancer; CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

(HR 0.62, 95% CI: 0.13–3.06, $P=0.556$). There was also no significant difference in PFS between treatment groups for the VS-P patients (HR 0.83, 95% CI: 0.23–3.01, $P=0.783$). Similarly, OS was not significantly different between the ICI and chemotherapy-treated VS-G patients (*Figure 4B*). The median OS of the patients treated with ICIs was 22.3 months while that of patients treated with chemotherapy was not reached (HR 1.71, 95% CI: 0.14–21.19, $P=0.675$). There was no significant difference in OS between the treatment groups in the VS-P patients (HR 0.61, 95% CI: 0.13–2.89, $P=0.534$).

Discussion

The VeriStrat test is a serum-based proteomic test that has

been shown to have potential application in guiding decision making for patients undergoing chemotherapy and targeted therapy but has been studied to a limited extent in NSCLC patients undergoing immunotherapy. In this study, we assessed whether the VeriStrat status could be a prognostic and predictive biomarker test in NSCLC patients treated with ICIs. In a retrospective analysis of NSCLC patients, we found that patient classifications based on the VeriStrat test were prognostic for PFS in advanced NSCLC patients treated with ICIs. Our results show that the VeriStrat test, which was developed initially as a biomarker for NSCLC patients treated with *EGFR* TKIs, has the potential to be used as a biomarker for NSCLC patients treated with ICIs.

Numerous studies support the clinical utility of the VeriStrat test but the mechanistic connection between

Table 4 Cox proportional hazard models of overall survival by NSCLC patients treated with immune checkpoint inhibitors

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
VeriStrat				
P	1		1	
G	0.34 (0.10–1.18)	0.089	0.16 (0.03–0.87)	0.034
Gender				
Male	1		1	
Female	1.42 (0.41–4.92)	0.578	1.33 (0.24–7.34)	0.743
Age				
30–60	1		1	
61–91	0.38 (0.09–1.68)	0.204	0.63 (0.09–4.62)	0.653
Smoking status				
Never	1		1	
Ever	1.34 (0.17–10.74)	0.784	0.93 (0.10–8.44)	0.947
ECOG PS				
≤2	1		1	
3, 4	0.99 (0.12–7.86)	0.988	0.42 (0.03–5.64)	0.514
Histology				
Squamous	1		1	
Non-squamous	1.79 (0.53–6.03)	0.350	3.36 (0.74–15.36)	0.118
Prior surgery or radiation therapy				
Yes	1		1	
No	0.95 (0.34–3.74)	0.946	1.68 (0.37–7.67)	0.505

CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

VeriStrat poor classification and poor prognosis in untreated and previously treated patients still remains an area for active research. Multiple isoforms of serum amyloid A1 (SAA1), acute phase proteins, were identified as the main proteins that generate 4 out of 8 mass signals composing the proteomic algorithm of VeriStrat (31). In fact, a study that collected samples from 57 patients receiving salvage chemotherapy and 70 non-EGFR mutated patients receiving erlotinib demonstrated that VeriStrat poor classification was significantly associated with proinflammatory/acute phase reactants including higher C-reactive protein, serum amyloid A, Cyfra 21-1, ferritin, osteopontin, and lower IGF-II (32). These proinflammatory reactants are known to be associated with carcinogenesis and poor prognosis in

cancer patients (33). Similarly, we anticipate that *vs.* poor patients may receive less benefit from ICI therapy due to presence of more molecules and pathways associated with tumor progression and treatment resistance. More studies need to identify mechanisms at molecular level to validate VeriStrat test's prognostic and predictive power to improve outcome in NSCLC patients classified as VeriStrat poor.

According to the FDA-NIH Biomarker working group, a prognostic biomarker provides information about the likelihood of a clinical event, disease recurrence, or disease progression, regardless of therapy, while a predictive biomarker measures the effect of a medical therapy (34). The VeriStrat status has proven to be both prognostic and predictive in patients treated with chemotherapy and

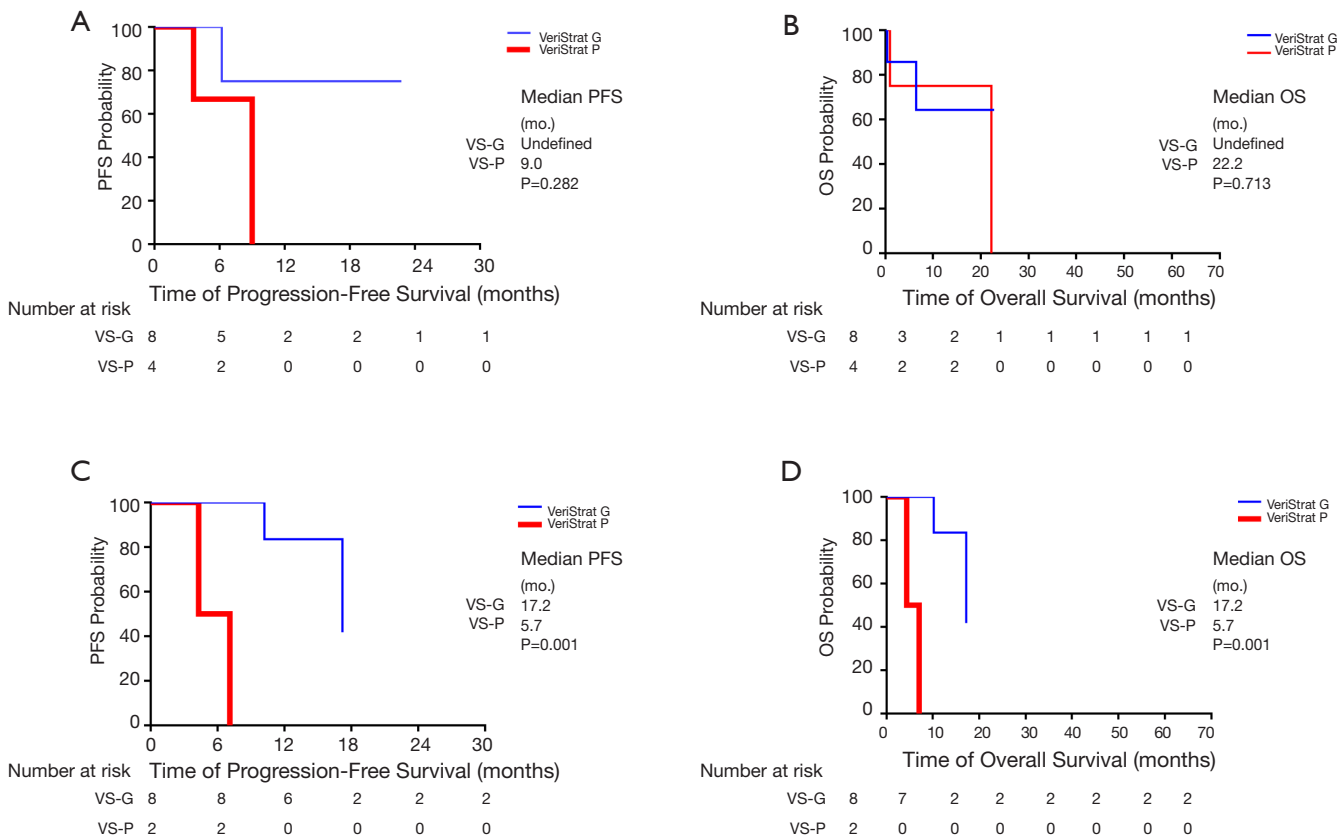


Figure 3 Survival curves of NSCLC patients who received the VeriStrat test before the treatment. (A) PFS of NSCLC population who received the VeriStrat test before the start of ICIs; (B) OS of NSCLC population who received the VeriStrat test before the start of ICIs; (C) PFS of NSCLC population who received the VeriStrat test before the start of chemotherapy; (D) OS of NSCLC population who received the VeriStrat test before the start of chemotherapy. PFS, progression-free survival; OS, overall survival; NSCLC, non-small cell lung cancer; ICI, immune checkpoint inhibitor; VS-G, VeriStrat Good; VS-P, VeriStrat Poor.

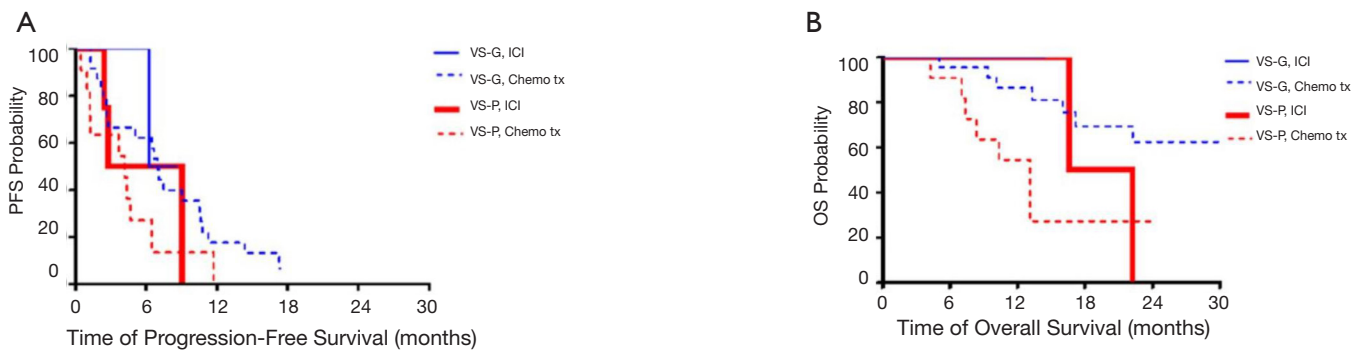


Figure 4 Survival curves of NSCLC patients treated with ICIs versus chemotherapy as first-line treatments. (A) Progression-free survival curves; (B) overall survival curves. NSCLC, non-small cell lung cancer; ICI, immune checkpoint inhibitor.

targeted therapy (19,21-26). Studies including the NExUS trial verified that the VeriStrat test could identify patients who would go on to have better clinical outcome from first-line platinum doublet therapy. These studies demonstrated that patients with the VS-G classification were likely to derive benefit from a first-line platinum-based therapy, while those with a VS-P classification were less likely to respond to platinum-based therapy (24,25). Additionally, the BR.21 phase III trial, which examined erlotinib versus placebo in previously treated advanced NSCLC patients, demonstrated that VS-G patients had a significantly longer OS as compared with VS-P patients in erlotinib-treated NSCLC patients, independent of clinical covariates (19). In agreement with these previous studies, our study demonstrated that the VS-G classification strongly correlated with longer PFS and OS compared to the VS-P classification in the overall pool of NSCLC patients (n=47), which includes patients treated with ICIs. The VeriStrat classification was an independent prognostic indicator for PFS and OS in the multivariate analysis.

The prognostic property of the serum-proteomic test for NSCLC patients treated with ICIs was studied for the first time in a study of NSCLC patients treated with second-line nivolumab (35). The study comprised of sixty previously treated advanced NSCLC patients and the VeriStrat test of pre-treatment patients demonstrated that the VeriStrat status was prognostic for OS. Similarly, our study was also able to identify that patients with advanced NSCLC treated with ICIs and a VS-G classification were likely to have a trend towards increased OS when compared with VS-P patients. Furthermore, our study demonstrated that the VeriStrat status is prognostic for PFS among NSCLC patients treated with ICIs. VeriStrat may still hold its prognostic features for ICIs-treated NSCLC patients.

A previous study on the VeriStrat test in the course of *EGFR* TKIs therapy suggested that about one third of baseline VS-G classifications will change to the VS-P classification during the course of treatment (36). We ran a separate analysis on survival outcomes for patients who received the VeriStrat test before the start of ICIs, but the small number of patients in each VeriStrat classification subgroup made the results exploratory. Our results could not demonstrate that the VeriStrat test, when obtained pre-immunotherapy, is a significant prognostic indicator of PFS or OS for NSCLC patients treated with ICIs, though the inadequate sample size interferes with drawing conclusions. Another concern was that lack of sufficient follow-up time could harm the visualization of differences in long-term

survival between the VeriStrat classification groups. The median follow-up period of the VeriStrat test was seventeen months, and over half of the VS-G patients were alive at the point of analysis. A longer follow-up period could reveal significant differences between VS-G and VS-P patients regarding OS, in place of the current results.

The predictive property of the VeriStrat test has been demonstrated in various studies, including the phase III PROSE study, and the BR.21 trial, the latter of which verified that erlotinib should be used in VS-G classified previously treated advanced NSCLC patients (19,21,23). The median survival was 10.5 months on erlotinib *vs.* 6.6 months for placebo in VS-G patients (HR 0.63, 95% CI: 0.47–0.85, P=0.002). The VeriStrat status was predictive for objective response to erlotinib (P=0.002). Furthermore, the prospective randomized phase III PROSE study, which was comprised of stage IIIB or IV NSCLC patients, demonstrated that patients classified as VS-P should select chemotherapy over erlotinib as second-line therapy. VS-P patients had worse survival on erlotinib than on chemotherapy (HR 1.72, 95% CI: 1.08–2.74, P=0.022), whereas VS-G patients had similar OS with either regimen. In our study, the VeriStrat status could not predict differences in survival outcomes in ICIs relative to chemotherapy, as the difference in PFS and OS for VS-G patients were not significant between those receiving ICIs and those receiving chemotherapy as first-line treatments. However, our result on the predictive power of the VeriStrat status was limited, as there were only eight patients who received ICIs as first-line treatment compared to thirty-five patients who received chemotherapy.

Inherent limitations of our study include the retrospective nature of this analysis and the relatively small number of patients (n=47). We would have favored in limiting our analysis to patients who were subject to the VeriStrat test before the start of systemic therapy, as previous treatment lines may influence results. Unfortunately, those patients were the minority in our cohort.

In addition to NSCLC patients, a study on melanoma patients has provided a proof of concept that serum proteomic test like the VeriStrat test can potentially serve as a prognostic biomarker test for patients with other malignancies treated with ICIs (37,38). A study on stage IV melanoma patients treated with nivolumab (n=119) reported protein signatures that predicted survival outcome using MS analysis and machine-learning. Similar to these efforts, optimized serum proteomic tests can be developed to predict the NSCLC patients who will likely respond to

ICIs. First however, the VeriStrat test needs to be evaluated on whether it is a prognostic test of survival outcomes in NSCLC patients treated with ICIs.

Future trials are warranted that stratify patients according to serum proteomic signature in order to identify durable responders for ICI-treated NSCLC patients. From those results, new treatment strategies could be attempted for poor prognosis patients.

Conclusions

There has been limited research assessing the clinical utility of serum-based proteomic testing in NSCLC patients treated with immunotherapy. This retrospective study evaluated the prognostic and predictive properties of the VeriStrat test and demonstrated that the VS-G status is associated with a favorable PFS compared to VS-P classification in NSCLC patients treated with ICIs. The VeriStrat status could serve as a potential biomarker for patients undergoing treatment with ICIs and may therefore improve treatment decision-making for advanced NSCLC patients.

Acknowledgments

Funding: None.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tlcr-20-148>). YKC would like to disclose advisory and consulting for AstraZeneca, Genentech, Takeda Pharmaceuticals, Guardant Health and Foundation Medicine. YKC is participating in research projects with Abbvie, BMS, Lexent Bio, Freenome, and Biodesix. 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. This study was approved by The Institutional Review Board (IRB) of Northwestern University Feinberg School of Medicine (No. STU00205299). For this retrospective study, the written informed consent from patients was waived per the IRB, and the study was performed following the Health Insurance Portability and Accountability Act and the Declaration of

Helsinki (as revised in 2013).

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Cite this article as: Chae YK, Kim WB, Davis AA, Park LC, Anker JF, Simon NI, Rhee K, Song J, Cho A, Chang S, Ko T, Oh M, Bhave M, Viveiros P. Mass spectrometry-based serum proteomic signature as a potential biomarker for survival in patients with non-small cell lung cancer receiving immunotherapy. *Transl Lung Cancer Res* 2020;9(4):1015-1028. doi: 10.21037/tlcr-20-148

Supplementary

Table S1 Time of the VeriStrat test relative to the treatment with ICI

Variable	Overall (n=32)	VeriStrat-classified population		P value
		VS-G (n=18)	VS-P (n=14)	
Time of the VeriStrat test				0.471
After ICI	20 (62.5)	10 (55.6)	10 (71.4)	
Before ICI	12 (37.5)	8 (44.4)	4 (28.6)	

Data given as No. (%) unless otherwise indicated. ICI, immune-checkpoint inhibitor; VS-G, VeriStrat Good; VS-P, VeriStrat Poor.

Table S2 Cox proportional hazard models of progression-free survival by the overall NSCLC patients

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR ^a (95% CI)	P value
VeriStrat				
P	1		1	
G	0.43 (0.21–0.87)	0.018	0.38 (0.16–0.88)	0.024
Gender				
Male	1		1	
Female	1.30 (0.65–2.59)	0.462	1.13 (0.49–2.60)	0.779
Age				
30–60	1		1	
61–91	0.50 (0.24–1.03)	0.060	0.61 (0.24–1.57)	0.303
Smoking status				
Never	1		1	
Ever	0.81 (0.33–1.95)	0.632	0.89 (0.29–2.77)	0.846
ECOG PS				
≤2	1		1	
3, 4	1.41 (0.54–3.66)	0.484	1.26 (0.40–3.92)	0.693
Histology				
Squamous	1		1	
Non-squamous	1.16 (0.59–2.28)	0.663	1.55 (0.70–3.43)	0.284
Prior surgery or radiation therapy				
Yes	1		1	
No	1.20 (0.59–2.45)	0.615	1.07 (0.48–2.41)	0.868

^a, adjusted for other six covariates. CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table S3 Cox proportional hazard models of overall survival by the overall NSCLC patients

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR ^a (95% CI)	P value
VeriStrat				
P	1		1	
G	0.34 (0.13–0.90)	0.030	0.21 (0.05–0.89)	0.035
Gender				
Male	1		1	
Female	0.80 (0.31–2.08)	0.647	0.57 (0.16–2.04)	0.386
Age				
30–60	1		1	
61–91	0.54 (0.18–1.60)	0.264	0.63 (0.13–3.03)	0.563
Smoking status				
Never	1		1	
Ever	1.10 (0.32–3.84)	0.881	2.80 (0.32–24.79)	0.356
ECOG PS				
≤2	1		1	
3, 4	1.80 (0.58–5.53)	0.309	2.63 (0.53–13.12)	0.238
Histology				
Squamous	1		1	
Non-squamous	2.04 (0.72–5.80)	0.182	5.01 (1.21–20.66)	0.026
Prior surgery or radiation therapy				
Yes	1		1	
No	0.61 (0.23–1.61)	0.317	0.70 (0.25–1.96)	0.491

^a, adjusted for other six covariates. CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table S4 Cox proportional hazard models of progression-free survival by NSCLC patients treated with chemotherapy

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR ^a (95% CI)	P value
VeriStrat				
P	1		1	
G	0.46 (0.21–1.01)	0.053	0.38 (0.12–1.15)	0.087
Gender				
Male	1		1	
Female	1.10 (0.51–2.38)	0.800	0.83 (0.29–2.41)	0.729
Age				
30–60	1		1	
61–91	0.53 (0.24–1.14)	0.102	0.75 (0.22–2.59)	0.654
Smoking status				
Never	1		1	
Ever	0.99 (0.38–2.59)	0.982	1.00 (0.31–3.27)	0.997
ECOG PS				
≤2	1		1	
3, 4	1.41 (0.49–4.09)	0.523	1.62 (0.37–7.08)	0.519
Histology				
Squamous	1		1	
Non-squamous	1.68 (0.80–3.54)	0.174	2.19 (0.91–5.30)	0.081
Prior surgery or radiation therapy				
Yes	1		1	
No	1.77 (0.75–4.22)	0.196	1.20 (0.43–3.38)	0.730

^a, adjusted for other six covariates. CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table S5 Cox proportional hazard models of overall survival by NSCLC patients treated with chemotherapy

Variable	Univariate		Multivariate	
	Unadjusted HR (95% CI)	P value	Adjusted HR ^a (95% CI)	P value
VeriStrat				
P	1		1	
G	0.27 (0.08–0.86)	0.027	0.18 (0.03–1.12)	0.065
Gender				
Male	1		1	
Female	0.61 (0.20–1.82)	0.374	0.36 (0.07–2.02)	0.247
Age				
30–60	1		1	
61–91	0.47 (0.15–1.51)	0.204	0.49 (0.08–2.91)	0.434
Smoking status				
Never	1		1	
Ever	0.76 (0.21–2.75)	0.672	1.20 (0.16–8.87)	0.859
ECOG PS				
≤2	1		1	
3, 4	0.84 (0.11–6.45)	0.863	1.96 (0.20–19.46)	0.565
Histology				
Squamous	1		1	
Non-squamous	2.94 (0.81–10.70)	0.102	6.21 (1.34–28.84)	0.020
Prior surgery or radiation therapy				
Yes	1		1	
No	1.01 (0.31–3.27)	0.992	0.78 (0.15–4.07)	0.765

^a, adjusted for other six covariates. CI, confidence interval; HR, hazard ratio; VS-G, VeriStrat Good; VS-P, VeriStrat Poor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.