Prognostic Value of Serum Proteomic Test and Comorbidity Index in Diversified Population with Lung Cancer

MANI KESHTGARPOUR[1], WEI SEONG TAN[1], JACK ZWANZIGER[1], SARIA AWADALLA[1], FREDRICK G. LANGI[1] and ARKADIUSZ Z. DUDEK[2]

[1]School of Public Health, and [2]Division of Hematology/Oncology, College of Medicine, University of Illinois at Chicago, Chicago, IL, U.S.A.

Abstract. *Background: The usefulness of serum proteomic test (VeriStrat) in African-Americans with non-small cell lung cancer (NSCLC) as well as the relationship between comorbidity and test performance have not been studied. Materials and Methods: We reviewed records of patients with NSCLC in our practice for whom VeriStrat was performed to assist with the selection of therapy. We correlated survival with VeriStrat test classification, race, and comorbidity index using SAS software 9.4. Results: We identified 49 qualified patients; 33 with VeriStrat Good (VSG), 16 with VeriStrat Poor (VSP). When stratified by VSG vs. VSP, overall survival (OS) did not differ between African-Americans and Whites [hazard ratio (HR)$_{test\ (VSG/VSP)}$=0.78, 95% confidence interval (CI)=0.38-1.61; p=0.51]. OS adjusted for mean Charlson Comorbidity Index (CCI) was not different between erlotinib- and chemotherapy-treated groups in patients with non-squamous NSCLC (adjusted HR=0.91, 95% CI= 0.37-2.23; p=0.84), but was inferior in patients with squamous NSCLC treated with erlotinib (adjusted HR=10.6, 95% CI=1.28-87.8; p=0.029). Cox proportional hazard model for OS effect of VeriStrat test was estimated after adjusting for CCI. In both the VSG and VSP groups, a higher CCI value was associated with lower survival, and at any CCI value, the VSG group had better survival than the VSP group. Conclusion: Our study corroborates that race does not influence prognostic and predictive values of VeriStrat; however, comorbidities have a significant impact on survival in each proteomic stratum.*

Correspondence to: Arkadiusz Z. Dudek, Division of Hematology/Oncology, College of Medicine, University of Illinois at Chicago, 909 S. Wolcott Ave., MC 713, COMRB 5143, Chicago, IL 60612-3725, U.S.A. Tel.: +1 3124138878, Fax: +1 3129961309, e-mail: adudek@uic.edu

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Lung cancer is the leading cause of cancer death and the second most common cancer among both men and women in the United States (1). Although low-dose spiral computed tomography was recently approved for the high-risk population, most lung cancer cases are diagnosed at a more advanced and inoperable stage. National Comprehensive Cancer Network (NCCN) guidelines recommend treatment of metastatic non-small cell lung cancer (NSCLC) with systemic chemotherapy or targeted therapies based on the presence of a genetic mutation in the epidermal growth factor receptor (*EGFR*) gene or gene rearrangements in anaplastic lymphoma kinase (ALK) (2). While patients with metastatic disease who had treatment achieve an improvement in quality of life and survival, there is still a significant fraction of patients who are not offered therapy due to concerns of treatment toxicity, poor performance status or patient-physician choice (3).

Erlotinib, an EGFR tyrosine kinase inhibitor, is approved by the U.S. Food and Drug Administration for the treatment of locally advanced or metastatic NSCLC in those patients that i) have failed at least one prior chemotherapy regimen; ii) have a tumor that has mutations in the gene coding for the EGFR protein; iii) are candidates for maintenance therapy (based on results from the Sequential Erlotinib in Unresectable NSCLC study) (4); or iv) are otherwise ineligible for systemic chemotherapy (*e.g.* platinum-based doublet therapy) (2).

A multivariate, serum-based proteomic test, VeriStrat, has been clinically validated for patients with wild-type or unknown *EGFR* status advanced NSCLC to assist physicians when determining whether treatment should involve EGFR tyrosine kinase inhibitor (EGFRi) therapy. The test identifies patients with significantly better ('good') and those with significantly worse ('poor') outcomes following treatment with EGFRi. This test was developed based on a matrix-assisted laser desorption ionization mass spectrometry analysis (MALDI-TOF-MS) of sera from 139 patients with NSCLC from Italy (n=70) and Japan (n=69), and validated by sera from patients participating in Eastern

Cooperative Oncology Group protocol E3503, patients from Perugia Hospital, Perugia, Italy; Vanderbilt-Ingram Cancer Center, Nashville, TN; and the Medical University of Gdansk, Poland (5). Two cohorts of patients were treated with EGFRi, either gefitinib, or erlotinib. Patients classified as 'VeriStrat Good' (VSG) had better outcomes than patients classified as 'VeriStrat Poor' (VSP) [gefitinib cohort: hazard ratio (HR) of death 0.47, p=0.009; erlotinib cohort: HR of death 0.33, p=0.0007). In control cohorts, VeriStrat status did not significantly correlate with clinical outcome following chemotherapy (VSG: HR=0.74, p=0.42; and VSP: HR=0.81, p=0.54) or in the post-surgery setting (HR=0.90, p=0.79) (5). Several clinical validations of this proteomic test have been performed: in a subset of patients enrolled on the NCIC Clinical Trials Group BR.21 phase III trial of erlotinib *versus* placebo in previously treated advanced NSCLC patients (6), in a multicenter randomized phase II trial of gemcitabine, or erlotinib, or gemcitabine and erlotinib combination (7), in patients with NSCLC treated with erlotinib and bevacizumab (8), in patients treated with gefitinib (9), and in a prospective biomarker-stratified, randomized study (10). Based on the results derived from these studies, NCCN has recently recommended "proteomic testing for patients with NSCLC and wild-type *EGFR* or with unknown *EGFR* status" (2). "A patient with a 'poor' classification should not be offered erlotinib in the second-line setting." Recent quality-of-life and economic analysis of proteomic test-guided second- or third-line treatment for advanced NSCLC (the PROSE study) confirmed the value of this serum-based proteomic test in improving quality-adjusted life-years gained and in saving costs of management of metastatic NSCLC (11). However, it is important to point-out that all of the above studies analyzed plasma or serum from either White or Asian patients with NSCLC. African-Americans (AAs) have rates of NSCLC morbidity and mortality that are higher than in Whites or Asians (12). We therefore decided to perform this study to confirm that VSG and VSP serum signatures separate prognosis in AAs as they do in Whites.

VeriStrat is a proteomic algorithm based on eight MALDI TOF MS signals - four out of eight of these were identified as fragments of serum amyloid A protein 1 (13). This protein was found to be elevated in individuals with coronary disease (14, 15), lipid disorder (16), chronic obstructive pulmonary disease (17), and obesity (18). Charlson and others developed a comorbidity index that can be used in estimation of the risk of death from comorbidities (19). Since serum amyloid A protein 1 is associated with several comorbidities, in addition to analyzing effect of race in this study, we hypothesized that the Charlson Comorbidity Index (CCI) could have an impact on the VeriStrat test.

Materials and Methods

Patient population. This study is a retrospective cohort analysis of medical records of patients with advanced-stage NSCLC who visited a University of Illinois-affiliated cancer clinic between September 2009 and July 2014. The informed consent waiver was granted by the Institutional Review Board of the University of Illinois at Chicago, and the research data were collected and analyzed as an enumerated activity pursuant to the Health Insurance Portability and Accountability Act regulations. The patients' electronic health records were used as the main data source and the password-protected Research Electronic Data Capture under the grant support of the University of Illinois at Chicago Institute for Health Research and Policy-Center for Clinical and Translational Science (UL1RR029879) was used for data pool retention and analysis. As part of the inclusion criteria, only cohorts with available treatment status at both pre- and post-VeriStrat test time points were included in the study.

Measures. Demographic information included age, gender, race/ethnicity [White (defined as non-Hispanic Whites and Hispanic Whites) *vs*. AA], histopathological type of NSCLC [*e.g*. squamous, and non-squamous (including adenocarcinoma)], and line of therapy (first *vs*. second *vs*. third line). Covariates of age at diagnosis and past medical history were assigned scores of 1, 2 or 6 to build the final continuous variable of the CCI as the following: myocardial infarct (+1), congestive heart failure (+1), peripheral vascular disease (+1), cerebrovascular disease (except hemiplegia) (+1), dementia (+1), chronic pulmonary disease (+1), connective tissue disease (+1), ulcer disease (+1), mild liver disease (+1), diabetes (without complications) (+1), diabetes with end organ damage (+2), hemiplegia (+2), moderate or severe renal disease (+2), past history of other solid tumor (non-metastatic) (+2), leukemia (+2), multiple myeloma (+2), moderate or severe liver disease (+3), past history of other metastatic solid tumor (+6), and AIDS (+6). Each decade of age received the following CCI scores: 50-59 (+1); 60-69 (+2); 70-79 (+3); 80-89 (+4); 90-99 (+5).

Anti-neoplastic chemotherapy was grouped as 'chemotherapy followed by chemotherapy' allocated to patients who had received only conventional chemotherapy lines, and 'chemotherapy and erlotinib' if single-agent erlotinib was one of the therapy lines.

Statistical analysis. Using SAS statistics software version 9.4 (SAS Institute Inc., Cary, NC, USA), the product-limit survival estimates and Cox proportional hazard (PH) regression method were used to estimate overall survival (OS) days between the start of first-line treatment until the date of death, or at censoring for loss to follow-up. Statistical significance was calculated using log-rank test, Wilcoxon test, Chi square test statistics, HR, and 95% confidence interval (CI) for HR. The proportional hazard (PH) assumption was checked using log(−log) survival against time plot. Since erlotinib is mainly used as second-line treatment option (20), the significance of survival differences for second-line treatment (erlotinib *vs*. conventional chemotherapy) was tested controlling for each of the variables, serum VeriStrat status (VSG *vs*. VSP), race (AA *vs*. White), and histopathology (squamous *vs*. non-squamous) by adjusting or non-adjusting for CCI.

Results

Demographics. From the original 62 potential patients with available VeriStrat test results, 49 were qualified for inclusion in our study; among excluded cases, clinical

Table I. *Patients' demographics (n=49).*

Variable	Covariate, no. of patients
Gender	Male, 30; female, 19
Race	White, 38; Hispanic, 5; African–American, 6
Histopathology	Squamous, 14; non-squamous, 35
Charlson Comorbidity Index (past medical history and age)	Score range=3-12; median=8; mean=7.92
VeriStrat test result*	VSG, 31; VSI, 1; VSP, 17
VSG by race**	Non-Hispanic White, 25; Hispanic White, 4; African–American, 3
VSP by race**	Non-Hispanic White, 13; Hispanic White, 1; African–American, 3
Timing of VeriStrat test	Before first-line therapy: VSG, 20, VSI, 1, VSP, 9; After first-line therapy***: VSG, 11; VSP, 8;
Survival status	Died, 32; alive or lost to follow-up, 17 Died, 32; alive or lost to follow-up, 17
First-line treatment	Erlotinib, 13; chemotherapy, 36
Second-line treatment and test status (total cases: 33)	Erlotinib/VSG, 10; erlotinib/VSP, 4; chemotherapy/VSG, 11; chemotherapy/VSP, 8
Second-line survival (days)	Min=9; max=710; median=166; mean=233; sd=191; mode=251
Third-line treatment	Erlotinib, 6; chemotherapy, 27
Treatment type (number of cases)	Chemotherapy followed chemotherapy, 16; chemotherapy and erlotinib, 33

VeriStrat test VSG: Good; VSI: indeterminate; VSP: poor. *VSI added to VSG pool for survival analysis. **In analyses involving race, African–Americans were compared to non-Hispanic and Hispanic Whites. ***All had received chemotherapy.

records were not available for six, another six patients had only one oncology visit, and the diagnosis in another one was stage I NSCLC. From these 49 patients, 31 cases had the VeriStrat test done prior to receiving any treatment, and 18 had the VeriStrat test done during or after receiving first-line chemotherapy. Due to the small sample size, the decision was made to pool both VeriStrat test groups into one larger sample cohort for survival analysis. Among the 49 patients, 32 had a VSG status, one had an indeterminate result (VSI) (added to VSG for analysis), and 16 had a VSP status. In the survival analysis, the VSI case was added to the VSG data pool. Patient demographics are shown in Table I.

Statistical model validation. The PH assumption for survival effect of second-line treatments, by plotting log(−log) survival estimate against log of the time, showed nearly parallel curves in favor of a PH assumption. The PH null hypothesis was tested for the second-line survival hazard using interaction of different variables by time, *i.e*. treatment type, histopathology type, VeriStrat status, and race. Only histopathology type squamous *vs*. non-squamous, after controlling for second-line erlotinib therapy and age-adjusted CCI, had a near-significant interaction with time (p=0.071), in favor of a time-dependent non-proportional survival hazard for tumor histopathology. In this regression model, the effect of CCI on histopathology hazard was significant (p=0.03). Multivariate Cox PH model for OS, including serum VeriStrat status (VSG *vs*. VSP), race (AA *vs*. White), tumor histopathology (squamous *vs*. non-squamous), treatment type (chemotherapy followed by chemotherapy *vs*. chemotherapy and erlotinib), and CCI (low *vs*. high), was estimated; the global null hypothesis failed to reject a PH assumption (Wald p=0.043).

Effect of race (AA vs. White) and CCI on OS. Using the Cox PH model, we calculated effect of race (AA *vs*. White) on OS. The CCI-unadjusted HR for survival of AA *vs*. White was 0.38 (95% CI=0.09-1.58; p=0.18), and the CCI-adjusted HR was 0.59 (95% CI=0.14-2.58; p=0.48). CCI adjustment had a significant effect on race survival hazard (p=0.02). A trend towards better survival in AA compared to White patients was seen. The small sample size of the AA group (n=6) was possibly an important factor in why statistical significance was not reached.

Then we evaluated the effect of treatment type, chemotherapy and erlotinib *vs*. chemotherapy followed by chemotherapy, stratified by tumor histopathology (squamous *vs*. non-squamous NSCLC) and adjusted for mean CCI value (5.57 in our series, without age effect) on OS. In the non-squamous group (n=35; events=23; censored=12), the CCI-unadjusted treatment had an OS HR of 0.95 (95% CI=0.39-2.32; p=0.91), while the CCI-adjusted HR was 0.91 (95% CI= 0.37-2.23; p=0.84) (Figure 1A). Effect of CCI on treatment survival hazard was significant (p=0.01). However in the squamous cell group (n=14; events=9; censored=5), the CCI-unadjusted treatment had an OS HR of 9.2 (95% CI=1.1-75.5; p=0.039), and the CCI-adjusted HR was 10.6 (95% CI=1.28-87.8; p=0.029), demonstrating better outcomes when sequential chemotherapy regimens were used rather than chemotherapy in sequence with erlotinib (Figure

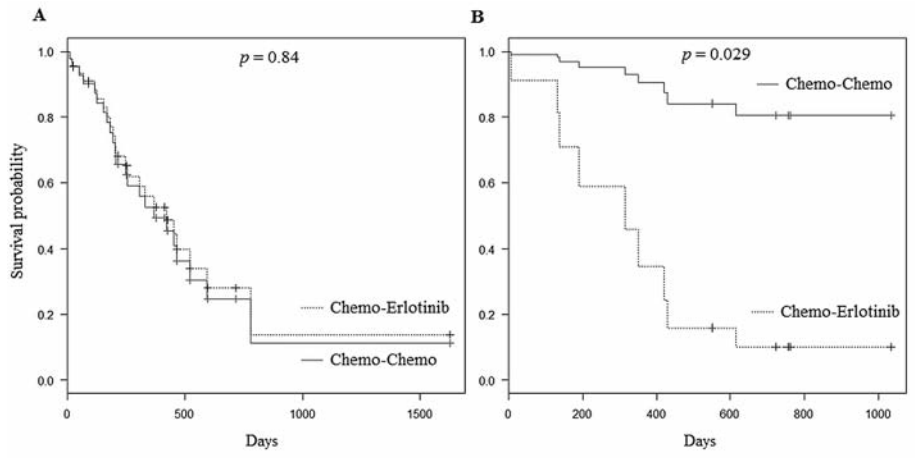

Figure 1. *Cox proportional hazard regression model for effect of treatment type on overall survival for chemotherapy followed by chemotherapy (Chemo-Chemo) vs. chemotherapy and erlotinib (Chemo-Erlotinib) in patients with non-squamous (A) and squamous (B) non-small-cell lung cancer histopathological types and adjusted for mean Charlson Comorbidity Index value.*

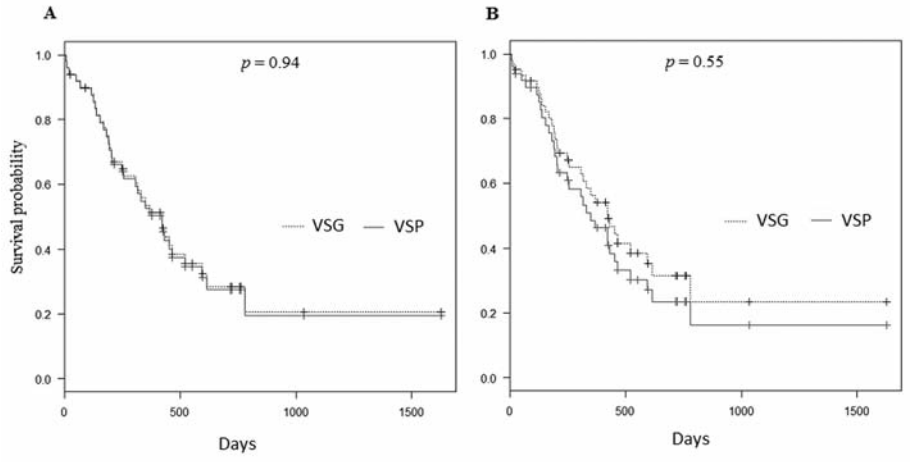

Figure 2. *Cox proportional hazard regression model for effect of VeriStrat test on overall survival hazard comparing good (VSG) vs. poor (VSP) signatures when unadjusted (A) and adjusted (B) for the Charlson Comorbidity Index.*

1B). Effect of CCI on treatment survival hazard was non-significant (p=0.18).

Using Cox PH model, the CCI-unadjusted survival hazard effect of second-line treatment erlotinib (n=14) *vs.* chemotherapy (n=19) had a HR of 2.16 (95% CI=0.90-5.17; p=0.085), with worse survival in the erlotinib-treated group. CCI-adjusted second-line treatment effect on survival had a significant HR of 2.41 (95% CI=0.99-5.87; p=0.054); the effect of CCI adjustment on second-line treatment hazard was borderline significant (p=0.07).

The second-line treatment (n=33; event=22; censored=11) progression-free survival hazard controlled for tumor histopathology (squamous *vs.* non-squamous NSCLC) and adjusted for mean CCI value was estimated. In the non-squamous cell group (n=22; events=14;

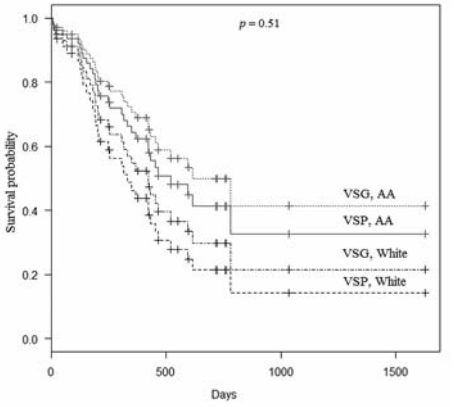

Figure 3. *Multivariate Cox proportional hazard regression model for interaction effects of VeriStrat test [good (VSG) vs. poor (VSP)] and race [African-American (AA) vs. White] on overall survival after controlling for age-unadjusted mean Charlson Comorbidity Index.*

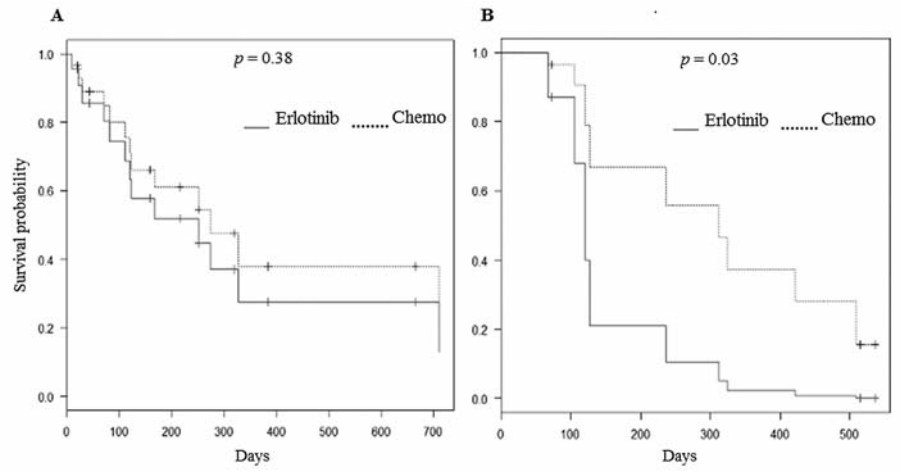

Figure 4. *Cox proportional hazard regression model for the effect of second-line treatment type erlotinib vs. conventional chemotherapy on progression-free survival in patients with good (VSG) (A) and poor (VSP) (B) VeriStrat test adjusted for the mean Charlson Comorbidity Index.*

censored=8), the unadjusted survival HR was 1.74 (95% CI=0.57-5.32; p=0.33); after adjustment for CCI, the treatment survival HR was 2.18 (95% CI=0.68-7.05; p=0.19). Thus outcome of patients with non-squamous cell histology treated with erlotinib was not different from patients treated with chemotherapy in second-line setting. Effect of CCI adjustment on second-line treatment hazard in non-squamous group was significant (p=0.03). In the squamous cell group, the second-line treatment (n=11; events=8; censored=3), had unadjusted survival HR of 7.1 (95% CI=1.2-43.5; p=0.03), and adjusted HR of 7.82 (95% CI=1.19-51.2; p=0.03). Thus outcome of patients with squamous cell histology treated with erlotinib in the second-line setting was inferior to that of patients treated with chemotherapy. The effect of CCI value adjustment on second-line treatment hazard in the squamous cell group was not significant (p=0.58).

Prognostic and predictive value of VeriStrat test status. Cox PH model for OS effect of serum VeriStrat test status, VSG *vs*. VSP, (n=49; events=32; censored=17) demonstrated CCI-unadjusted HR of 0.97 (95% CI=0.48-1.97; p=0.94) (Figure 2A), and a CCI-adjusted HR of 0.80 (95% CI=0.39-1.64; p=0.55) (Figure 2B). CCI adjustment had a significant effect on survival hazard according to VeriStrat test status (p=0.007).

In a multivariate Cox PH model for OS of combined effects of VeriStrat test status (VSG *vs*. VSP) and race (AA *vs*. White), after controlling for age-unadjusted mean CCI, the VeriStrat test survival hazard for AAs was not different than that for Whites (HR=0.78, 95% CI=0.38-1.61; p=0.51), but a trend for better survival with the combined effect of VSG result and AA race was seen (Figure 3). In this model, the effect of age-unadjusted CCI was statistically significant (p=0.019).

Cox PH model for OS effect of VeriStrat test, VSG *vs*. VSP, was estimated both before and after adjusting for CCI without inclusion of age index. In the unadjusted model, there was no survival difference between VSG and VSP groups (HR=0.97, 95% CI=0.48-1.97; p=0.94); the CCI-adjusted model also showed no significant trend towards better survival in the VSG group (HR=0.80, 95% CI=0.39-1.64; p=0.54); CCI adjustment was statistically significant (p=0.007). In both the VSG and VSP groups, CCI value was inversely associated with survival; at any CCI value, the VSG group had better survival than the VSP group.

Stratified by VSG status (n=21; events=13; censored=8), second-line treatment with erlotinib (n=10) *vs*. conventional chemotherapy (n=11) had a non-significant progression-free survival HR of 1.71 (95% CI=0.53-5.45; p=0.37); the CCI-adjusted treatment effect also had a non-significant survival HR of 1.69 (95% CI=0.52-5.43; p=0.38) (Figure 4A). Effect of CCI adjustment on second-line treatment survival hazard was non-significant (p=0.10). However, when stratified by the VSP (n=12; events=9; censored=3), the second-line conventional chemotherapy (n=8) had a trend of providing better survival compared to erlotinib (n=4), with HR=3.78 (95% CI=0.92-15.54; p=0.07), and CCI-adjusted treatment HR of 9.48 (95% CI=1.27-70.81; p=0.03) (Figure 4B); CCI adjustment for the second-line treatment survival hazard was non-significant (p=0.13).

Discussion

In a randomized study of erlotinib *versus* conventional chemotherapy of docetaxel or pemetrexed in second-line treatment of NSCLC, erlotinib performed equally well to chemotherapy, with different toxicities (21, 22). In patients

with unknown status or wild-type *EGFR,* erlotinib in the second-line setting produced 8.9% response rate and progression-free survival of only 2.2 months (23). In an attempt to identify individuals who will benefit from EGFRi therapy, a proteomic signature VeriStrat test was developed. The predictive value for benefit from EGFRi and prognostic value of this test was confirmed in the PROSE study, in which a significant interaction was seen between treatment and proteomic classification (11). Similarly in our study, patients with a VSP status had worse survival on erlotinib than on chemotherapy (HR=9.48, 95% CI=1.27-70.81; p=0.03; in CCI adjusted analysis), however, there was no significant difference in OS between conventional chemotherapy or erlotinib therapy for patients with a VSG status (HR=1.69, 95% CI=0.52-5.43; p=0.38).

The results of our study extend findings previously established in Whites and Asians to another race with significant incidence of NSCLC, AAs, and confirm predictive and prognostic value of the VeriStrat test in assessment of survival benefit in AAs as was previously described in Whites.

In a Cox PH model of test and race, the CCI-adjusted OS effect of race (AA *vs*. White) had a race HR of 0.57 (95% CI=0.13-2.54; p=0.46). To determine if combining Hispanic Whites with non-Hispanic Whites altered the finding of lack of an effect of race on the test predictive value, we employed another race model in which only non-Hispanic Whites were compared to AAs. The race HR was 1.38 (95% CI=0.31-6.15; p=0.68) and was still not significant.

CCI was previously evaluated in lung cancer and was not helpful in providing guidance as a predictive tool to estimate a patient's prognosis in 514 patients with NSCLC histology, although 53% of them had early-stage disease (24). In contrast, in a study of 82 patients with inoperable NSCLC treated with radiofrequency ablation, an increasing CCI score was associated with an increased risk of death (HR=1.3); CCI score ≥5 was associated with an OS of 10.43 months (95% CI=7.61-19.85 months) and patients had significantly (p<0.001 in all cases) increased mortality, compared to those with CCI scores 1 to 2 with OS of 55.5 months (95% CI=39.46-64.02 months) or CCI scores 3 to 4 with OS of 36.62 months (95% CI=25.54- 58.29 months) (25).

In an analysis of 20,511 veterans with NSCLC, advancing age, but not comorbidities, was a strong negative predictive factor of treatment outcomes (26).

In our study, interestingly, only when the analysis was adjusted for CCI did OS separate individuals with VSG and VSP (Figure 2B). Thus, in our analysis the prognostic value of the VeriStrat test for survival was dependent on inclusion of comorbidities. On the other hand, separation of survival of individuals with VSG proteomic pattern *vs*. individuals with VSP pattern was seen regardless of low or high CCI. Thus, VeriStrat based on four out of eight MALDI-TOF-MS signals associated with serum amyloid A protein 1 (13), which is elevated in a number of comorbidities (14-18), could be dependent on comorbidities, as suggested by our analysis. Our unique observation of an effect of CCI on proteomic signatures and ensuing separation of survival requires further testing.

In conclusion, our study showed that VeriStrat testing results maintained its predictive and prognostic value regardless of race, but this was significantly influenced by CCI. The effect of CCI on VeriStrat should be evaluated in previously reported studies.

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Conflicts of Interests

None.

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