Medical Policy

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Title: Proteomic Testing for Targeted Therapy in Non-Small-Cell Lung Cancer (NSCLC), e.g., VeriStrat®

Description/Background

Non-Small-Cell Lung Cancer

Lung cancer is the leading cause of cancer death in the United States, with an estimated 234,580 new cases and 125,070 deaths due to the disease in 2024.[1] NSCLC accounts for more than 80% of lung cancer cases and includes nonsquamous carcinoma (adenocarcinoma, large cell carcinoma, other cell types) and squamous cell carcinoma.

Diagnosis

The stage at which lung cancer is diagnosed has the greatest impact on prognosis.[2] Localized disease confined to the primary site has a 55.6% relative 5-year survival but accounts for only 16% of lung cancer cases at diagnosis. Mortality increases sharply with advancing stage. Metastatic lung cancer has a relative 5-year survival of 4.5%. Overall, advanced disease, defined as regional involvement and metastatic, accounts for approximately 80% of cases of lung cancer at diagnosis. These statistics are mirrored for the population of NSCLC, with 85% of cases presenting as advanced disease and up to 40% of patients with metastatic disease. In addition to tumor stage, age, sex, and performance status are independent prognostic factors for survival particularly in early-stage disease.

In addition to tumor stage, age, sex, and performance status are independent prognostic factors for survival particularly in early-stage disease. Wheatley-Price et al (2010) reported on a retrospective pooled analysis of 2349 advanced NSCLC patients from 5 randomized chemotherapy trials.[3,] Women had a higher response rate to platinum-based chemotherapy than men. Additionally, women with adenocarcinoma histology had greater overall survival than men. A small survival advantage exists for squamous cell carcinoma over non-bronchiolar nonsquamous histology.[4,]

The oncology clinical care and research community use standard measures of performance status: Eastern Cooperative Oncology Group scale and Karnofsky Performance Scale.

Treatment

Treatment approaches are multimodal and generally include surgery, radiotherapy, and chemotherapy (either alone or in combination with another treatment, depending on disease stage and tumor characteristics). Per the National Comprehensive Cancer Network (NCCN) guidelines, the clinical management pathway for stage I or II NSCLC is dependent on surgical findings and may involve resection, radiotherapy, chemotherapy, or chemoradiation. First-line chemotherapy regimens for neoadjuvant and adjuvant therapy utilize platinum-based agents (e.g., cisplatin, carboplatin) in combination with other chemotherapeutics and/or radiotherapy. Treatment recommendations are based on the overall health or performance status of the patient, presence or absence of metastases, as well as the presence or absence of a treatment-sensitizing genetic variant. These aspects inform the selection of targeted and systemic therapies.[1]

For patients who experience disease progression following initial systemic therapy, subsequent treatment regimens are recommended, mainly featuring novel programmed death-ligand 1 (PD-L1) inhibitors. For patients with sensitizing epidermal growth factor receptor (*EGFR)*mutations, recommendations include first-line therapy with EGFR tyrosine kinase inhibitors (TKIs) afatinib, erlotinib, dacomitinib, gefitinib, or osimertinib and subsequent therapy with osimertinib. The NCCN does not make any recommendations for the use of EGFR TKIs in the absence of a confirmed sensitizing *EGFR* mutation. For patients with progression on TKIs other than osimertinib, testing for T790M is recommended, however, switching to osimertinib can be considered regardless of mutational status. Osimertinib carries a Category 1 recommendation for T790M+ patients with disease progression on an alternative EGFR TKI. For progression on osimertinib with limited and/or isolated lesions, a continuation of osimertinib and definitive local therapy via surgery, stereotactic ablative radiotherapy, or stereotactic radiosurgery is recommended. Initial systemic therapy recommendations can be considered for multiple, symptomatic, systemic lesions.[1]

Genomic Alterations

Several common genetic alterations in NSCLC have been targets for drug therapy, the most well-established of which is the use of TKIs targeting the EGFR and crizotinib targeting the anaplastic lymphoma kinase (ALK) gene rearrangement.

EGFR Variants in NSCLC

The EGFR, a receptor TK, is frequently over expressed and activated in NSCLC. Drugs that inhibit EGFR signaling either prevent ligand binding to the extracellular domain (monoclonal antibodies) or inhibit intracellular TK activity (small molecule TKIs). These targeted therapies dampen signal transduction through pathways downstream to the EGF receptor, such as the RAS/RAF/MAPK cascade. RAS proteins are G-proteins that cycle between active and inactive forms in response to stimulation from cell surface receptors such as EGFR, acting as binary switches between cell surface EGFR and downstream signaling pathways. These pathways are important in cancer cell proliferation, invasion, metastasis, and stimulation of neovascularization.

Variants in 2 regions of the *EGFR* gene, including small deletions in exon 19 and a point mutation in exon 21 (L858R) appear to predict tumor response to TKIs such as erlotinib. The

prevalence of *EGFR* variants in NSCLC varies by population, with the highest prevalence in nonsmoking, Asian women, with adenocarcinoma, in whom *EGFR* variants have been reported to be up to 30% to 50%. The reported prevalence of *EGFR* variants in lung adenocarcinoma patients in the United States is approximately 15%.[5]

ALK Variants

In about 2% to 7% of NSCLC patients in the United States, tumors express a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene (*EML4-ALK*), which is created by an inversion on chromosome 2p.[6] The *EML4* fusion leads to ligand-independent activation of *ALK,* which encodes a receptor TK whose precise cellular function is not completely understood. *EML4-ALK* variants are more common in never-smokers or light smokers and tend to be associated with younger age of NSCLC onset, and typically do not occur in conjunction with *EGFR* variants.

Testing for the *ALK-EML4* fusion gene in patients with adenocarcinoma-type NSCLC is used to predict response to the small molecule TKI crizotinib.

Other Genetic Variants

Other genetic variants have been identified in subsets of patients with NSCLC. The role of testing for these variants in selecting targeted therapies for NSCLC is less well established than for *EGFR* variants.

Targeted Treatment Options

EGFR-Selective Small Molecule TKIs

Orally administered EGFR-selective small molecule TKIs have been approved by the US FDA for use in treating NSCLC: gefitinib erlotinib, afatinib, dacomitinib, and osimertinib. Although the FDA approved gefitinib in 2004, a phase 3 trial has suggested gefitinib was not associated with a survival benefit. In 2003, the FDA revised gefitinib labeling, further limiting its use to patients who had previously benefited or were currently benefiting from the drug; no new patients were to be given gefitinib. However, in 2015, the FDA approved gefitinib as a first-line treatment for patients with metastatic, sensitizing *EGFR*-variant positive NSCLC.

In 2016, osimertinib (Tagrisso; AstraZeneca), an irreversible selective EGFR inhibitor that targets *T790M* variant-positive NSCLC, received FDA approval for patients with *T890M*-variant-positive NSCLC who have progressed on an EGFR TKI.

A 2013 meta-analysis of 23 trials of erlotinib, gefitinib, and afatinib in patients with advanced NSCLC reported improved progression-free survival (PFS) in *EGFR* mutation-positive patients treated with EGFR TKIs in the first- and second-line settings and for maintenance therapy.[7] Comparisons were with chemotherapy, chemotherapy and placebo, and placebo in the first-line, second-line, and maintenance therapy settings, respectively. Among EGFR mutation-negative patients, PFS was improved with EGFR TKIs compared with placebo for maintenance therapy but not in the first- and second-line settings. Overall survival (OS) did not differ between treatment groups in either mutation-positive or mutation-negative patients. Statistical heterogeneity was not reported for any outcome. The authors concluded that EGFR mutation testing is indicated to guide treatment selection in NSCLC patients.

On the basis of the results of 5 phase 3 randomized controlled trials, the American Society of Clinical Oncology recommends that patients with NSCLC who are being considered for first-line therapy with an EGFR TKI (patients who have not previously received chemotherapy or an EGFR TKI) should have their tumor tested for *EGFR* variants to determine whether an EGFR TKI or chemotherapy is the appropriate first-line therapy.[5]

The primary role for TKIs in NSCLC is for *EGFR* mutation-positive patients with advanced NSCLC. The use of TKIs in NSCLC in *EGFR* mutation-negative patients is controversial. The TITAN trial demonstrated no significant differences in OS between erlotinib and chemotherapy as second-line treatment for patients unselected on the basis of *EGFR* mutation status, with fewer serious adverse events in erlotinib-treated patients.[8] Karampeazis et al reported similar efficacy between erlotinib and standard chemotherapy (pemetrexed) for second-line therapy in patients unselected based on *EGFR* mutation status.[9] In contrast, in the TAILOR trial, standard chemotherapy was associated with longer OS than erlotinib for second-line therapy in patients with wild-type *EGFR*.[10] Auliac et al compared sequential erlotinib plus docetaxel with docetaxel alone as second-line therapy among patients with advanced NSCLC and *EGFR* wild-type or unknown status.[11] Based on a Simon's optimal 2-stage design, the erlotinib plus docetaxel strategy was rejected, with 18 of 73 patients in the erlotinib plus docetaxel arm achieving PFS at 15 weeks compared with 17 of 74 patients in the docetaxel arm.

In 2016, Cicenas et al reported results of the IUNO RCT, which compared maintenance therapy with erlotinib followed by second line chemotherapy if progression occurred to placebo followed by erlotinib if progression occurred in 643 patients with advanced NSCLC with no known *EGFR* variant.[12] Because there were no significant differences between groups in terms of PFS, objective response rate, or disease control rate, maintenance therapy with erlotinib in patients without *EGFR* variants was not considered efficacious.

Exon 19 deletions and p.L858R point mutations in exon 21 are the most commonly described sensitizing *EGFR* mutations, or mutations in *EGFR* that are associated with responsiveness to EGFR TKI therapy. According to the NCCN, most recent data indicate that NSCLC tumors that do not harbor a sensitizing *EGFR* mutation should not be treated with an EGFR TKI in any line of therapy.[1]

Proteomics Testing in Selecting Targeted Treatment for NSCLC

The term *proteome* refers to the entire complement of proteins produced by an organism or cellular system, which may vary over time and in response to selected stressors, and proteomics refers to the large-scale comprehensive study of a specific proteome. A cancer cell's proteome is related to its genome and to genomic alterations, but may not be static over time. The proteome may be measured with mass spectrometry or protein microarray. For cancer, proteomic signatures in the tumor or in bodily fluids (i.e., pleural fluid or blood) other than the tumor have been investigated as a biomarker for cancer activity.

A commercially available serum-based test (VeriStrat) has been developed and proposed to be used as a prognostic tool to predict expected survival for standard therapies used in the treatment of NSCLC. [13,] The test uses matrix-assisted laser desorption ionization MS analysis, and a classification algorithm was developed on a training set of pretreatment sera from 3 cohorts (Italian A, Japan A, Japan B) totaling 139 patients with advanced NSCLC who were treated with second-line gefitinib.[14,] The classification result is either "good" or "poor". Two validation studies using pretreatment sera from 2 cohorts of patients (Italian B, Eastern

Cooperative Oncology Group 3503) totaling 163 patients have been reported (see Tables 2 and 3).

This assay uses an 8-peak proteomic signature; 4 of the 8 have been identified as fragments of serum amyloid A protein 1.[15,] This protein has been found to be elevated in individuals with a variety of conditions associated with acute and chronic inflammation.[16-20,] The specificity for malignant biologic processes and conditions has not been determined.[21,] With industry support, Fidler et al (2018) used convenience biorepository samples to investigate 102 analytes for potential correlations between the specific peptide and protein biomarkers and VeriStrat classification.[22,] The VeriStrat test is currently marketed as a tool to measure a patient's "immune response to lung cancer." Biodesix indicates that a VeriStrat "Good" result indicates "a disease state that is more likely to respond to standard of care treatment," whereas a VeriStrat "Poor" rating indicates a chronic inflammatory disease state associated with aggressive cancer and patients that "may benefit from an alternative treatment strategy." [13,]

Although the VeriStrat matrix-assisted laser desorption ionization MS-based predictive algorithm has the largest body of literature associated with it, other investigators have used alternative MS methods, such as surface-enhanced laser desorption ionization/time-of-flight MS, and alternative predictive algorithms, to assess proteomic predictors of lung cancer risk.[23,] Best practices for peptide measurement and guidelines for publication of peptide and protein identification have been published for the research community.[24,]

Regulatory Status

No U.S. Food and Drug Administration (FDA)-cleared proteomic tests were identified. The available commercial proteomic tests to characterize protein content in pre-treatment serum samples of patients with NSCLC are offered as laboratory-developed tests. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA) of 1988.

Medical Policy Statement

The use of proteomic testing such as VeriStrat® is considered experimental/investigational. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Inclusionary and Exclusionary Guidelines

N/A

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

N/A

Other codes (investigational, not medically necessary, etc.):

81538 84999

Rationale

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

NON-SMALL-CELL LUNG CANCER

Clinical Context and Test Proposed

The purpose of proteomic testing in individuals with non-small-cell lung cancer (NSCLC) who are epidermal growth factor receptor (*EGFR*)-negative or *EGFR*-status unknown NSCLC with disease progression after first-line treatment is to predict response to EGFR tyrosine kinase inhibitors (TKIs). Testing could impact the decision point of second-line treatment (i.e., whether patients should receive *EGFR* treatment or chemotherapy). That is, those with VeriStrat “poor” findings might be less likely to respond to EGFR-TKIs, and thus chemotherapy would be a better choice.

The following **PICOs** were used to select literature to inform this review.

Populations

The relevant population of interest is individuals with EGFR-negative or EGFR-status unknown NSCLC with disease progression after first-line treatment.

Intervention

The intervention of interest is management with a serum proteomic test to select second-line therapy. The test is available commercially through a single laboratory.

Comparator
The comparator of interest is standard medical management.

Outcomes
The outcomes of interest are overall survival (OS) and progression-free survival (PFS). The timing of testing is prior to treatment following a new diagnosis of NSCLC or with disease progression after first-line systemic therapy.

Review of Evidence

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Proteomic Testing in NSCLC for Disease Prognosis

Prospective and Retrospective Studies
The largest body of evidence on the clinical validity of proteomic testing for NSCLC relates to its ability to predict disease outcomes.

No published studies were identified that assessed the use of VeriStrat proteomic testing in newly diagnosed stage I or II NSCLC.

For individuals with newly diagnosed advanced NSCLC without prior systemic therapy, multiple studies (Taguchi et al [2007],[14,] Amann et al [2010],[25,] Kuiper et al [2012],[26,] Akerley et al [2013],[27,] Gautschi et al [2013],[28,] Stinchcombe et al [2013],[29,] Grossi et al [2017][30,], Grossi et al [2018][31,], Lee et al [2019][32,]) have assessed the use of VeriStrat score (good or poor) as a prognostic test to discriminate between OS (primary outcome) and PFS (secondary outcome) outcomes. Most studies were retrospective and intended to validate the extent to which the VeriStrat proteomic classification correlated with OS or PFS. Grossi et al (2017) was an observational nonrandomized study with prospective sample collection for proteomic testing before NSCLC treatment and reported PFS as the primary outcome.[30,] This is the only study that included a first-line treatment consistent with current guidelines-based recommendations; platinum-doublet-based chemotherapy with cisplatin or carboplatin in combination with pemetrexed.

The VeriStrat classification was not used to direct the selection of treatment in any of the clinical trials from which the validation samples were derived. Testing for the presence of a sensitizing variant (*EGFR*) for targeted therapy with TKIs was variably performed in these studies. When testing was performed and results known as wild-type (negative) or positive, the analysis of OS and PFS was variably adjusted for variant status. The relationship between VeriStrat classification and OS and PFS in populations with unknown variant status, when reported, was not analyzed. Disposition of populations with variant status “not reported” was generally not clear and could not be construed as “unknown” when wild-type or positive variant status was reported.

For individuals with advanced NSCLC who had recurrent disease or who had failed prior systemic therapy, multiple studies assessed the use of VeriStrat as a prognostic test to discriminate between good and poor survival outcomes (Taguchi et al [2007],[14,] Carbone et al

[2010],[33,] Keshtgarpour et al [2016],[15,] Spigel et al [2018][31,]). All studies were retrospective and intended to validate the extent to which VeriStrat proteomic classification correlated with OS or PFS. The VeriStrat classification was not used to direct the selection of treatment in any of the clinical trials from which the validation samples were derived. None of the trials from which the samples for VeriStrat proteomic classification were derived used a therapy consistent with current guidelines-based recommendations. The populations in all studies were unselected for *EGFR*-variant status.

Grossi et al (2018) conducted a retrospective study that combined samples from 3 separate cohorts of treatment-naive recurrent or advanced NSCLC patients who received platinum-based chemotherapy.[34,] One cohort, identified as Italian, is duplicative of the population reported in Grossi et al (2017).[30,] The NExUS and eLung cohorts reported data that is only referenced in abstracts in Grossi et al (2018) and, thus, is of limited value to the evidentiary appraisal of VeriStrat classification. The data imported into the publication for the PFS outcome showed that the median PFS of 5.7 months for VeriStrat "good" is included in the outer bound of the confidence interval (CI) for VeriStrat "poor" in the NExUS cohort. The median PFS of 5.1 months for VeriStrat "good" is included within the CI of VeriStrat "poor" in the eLung cohort. A summary of the study characteristics and results of this study is presented in Tables 2 and 3. Appendix Table 1 summarizes the treatment regimens used in Grossi et al (2018). As noted, only the Italian cohort included from Grossi et al (2017) represents current approaches to treatment. Cetuximab does not have an established role in the treatment of NSCLC either as a component of initial therapy or as second-line therapy.

While most of the literature has focused on the use of matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) techniques and predictive algorithms similar to those used in the VeriStrat assay, other MS techniques, and predictive algorithms have been investigated. Jacot et al (2008) used surface-enhanced laser desorption ionization/time-of-flight MS technology in combination with a predictive algorithm to discriminate between malignant and benign disease and between good and poor outcomes.[23,] Using data from a population of 87 patients with stage III or IV NSCLC receiving conventional first-line chemotherapy and with at least 1-year follow-up available, the authors developed a predictive survival classifier to differentiate between poor prognosis (n=33; OS <12 months) and good prognosis (n=54; OS >12 months). In the multivariate analysis, the proteomic-based predictor was significantly associated with OS (hazard ratio [HR], 3.45; 95% CI, 1.22 to 6.13; p<.001).

Table 1: Clinical Validity of Proteomic Testing in NSCLC for Diagnosis Prognosis

Study	Study Type	N	Population	Selection Criteria	Participant Disposition
VeriStrat-specific studies					
Taguchi et al (2007)[14,,b] Italian B validation set	Retrospective	67	Sequential cohort of late-stage or recurrent NSCLC treated with single-agent gefitinib used as VS algorithm validation set. • Stage IIIA: 2 (3%) • Stage IIIB: 5 (7.4%) • Stage IV: 58 (86.6%) • Postoperative recurrence: 0	• ECOG PS: 29.8% grade 0; 46.3% grade 1; 23.9% grade 2 • Histology: 56.7% adeno; 22.4% squamous; 20.9% NOS	2 (3%) had stage IIA disease

			Previous Chemotherapy[a]	*n (%)*		
			0	13 (19.4)		
			1	26 (38.9)		
			2	15 (22.4)		
			≥3	4 (6.0)		
Taguchi et al (2007)[14]·ECOG 3503 validation set	Retrospective	96	ECOG 3503 single-arm phase 2 trial of first-line erlotinib in patients with stage IIIB or IV or recurrent NSCLC used as VS algorithm validation set. • Stage IIIA: 0 • Stage IIIB: 9 (9.4%) • Stage IV: 67 (69.8%) • Postoperative recurrence: 20 (20.8%)		• ECOG PS: 30.2% grade 0; 43.8% grade 1; 26.0% grade 2 • Histology: 64.6% adeno; 11.5% squamous; 1% LCC; 22.9% NOS	20 (20.8%) had postoperative occurrence
			Previous Chemotherapy[a]	*n (%)*		
			0	96 (100)		
Amann et al (2010)[25],[b]	Retrospective	88	Sample of ECOG 3503 trial patients (enrolled 137) with stage IIIB or IV or recurrent NSCLC in phase 2 single-arm treatment with first-line erlotinib		• ECOG PS: 28.4% grade 0; 46.1% grade 1; 25.5% grade 2 • Histology: 64.7% adeno; 10.8% squamous; 1% LCC; 16.7% NOS; 6.9% other	• 102 analyzable pretreatment biologic samples • Missing values: 14 (16%) VS score • EGFR exon 19 status: 61 (60%) • EGFR exon 21 status: 61 (60%) • No EGFR exon 19-positive samples
Carbone et al (2010)[33],[b]; Herbst et al (2005)[35],	Retrospective	35	• Sample of phase 1/2 stage IIIB or IV (n=40): phase 1 (n=12), phase 2 (n=28) recurrent, nonsquamous NSCLC treated with open-label erlotinib and bevacizumab • 22 (55%) had ≥2 prior chemotherapy regimens		• KPS: 7.5% KPS 70%; 47.5% KPS 80%; 45% KPS 90% • Histology: 75% adeno; 22.5% NOS; 2.5% other	35 available pretreatment samples with associated clinical data
Kuiper et al (2012)[26],[b]	Retrospective	50	Sample of chemotherapy-naive patients (n=50) with pathologically documented, inoperable, locally advanced,		• ECOG PS: 40% grade 0; 60% grade 1 • Histology: 68% adeno; 32% other	• VS score not available or indeterminate (n=2) • *EGFR* status: (31) 62% WT; (7) 14% variant

			recurrent, or metastatic NSCLC; single-arm phase 2 treated with erlotinib and sorafenib		positive; 12 (24%) unknown
Akerley et al (2013)[27,,b]	Retrospective	42	Sample of stage IIIB or IV or recurrent nonsquamous NSCLC, with no prior chemotherapy for metastatic disease (n=40), treated with erlotinib and bevacizumab; PET and serum biomarker ancillary study (n=10)	• ECOG PS: 26% grade 0; 74% grade 1 • Histology: 48% adeno; 48% NOS; 4% other	• Previously treated brain metastases allowed in expanded cohort • Participant accrual (n=20) prior to interim safety analysis; additional 20 participants accrued after safety threshold of PFS at 6 mo exceeded • 42 VS assays performed on pretreatment sera • 28 patients received cytotoxic chemotherapy after study therapy
Gautschi et al (2013)[28,,b]	Retrospective	117	Pooled analysis of patients (158 enrolled) from SAKK19/05 (n=101) and NTR528 trials (n=47): untreated, advanced nonsquamous NSCLC, treated with first-line therapy using erlotinib and bevacizumab	• ECOG PS: 52.9% grade 0; 42.5% grade 1; 4.6% grade 2 • Histology: 89.7% adeno; 10.2% other	• 117 pretreatment frozen serum available for VS (SAKK19/05, n=88; NTR528, n=29) • SAKK19/05: *EGFR* variant status: positive identification but data NR • NTR528: *EGFR* variant status: NR
Stinchcombe et al (2013)[29,,b]	Retrospective	98	Sample from noncomparative randomized phase 2 trial of first-line treatment for stage IIIB or IV NSCLC: • Arm A (gemcitabine) • Arm B (erlotinib) or • Arm C (gemcitabine and erlotinib)	• Age: ≥70 y • ECOG PS: 0-2 • Histology: unselected	• Treatment arm assignments stratified for sex, smoking history (never or light vs current or former use), and PS • 146 eligible patients received protocol therapy • 124 samples available for VS • 14 samples unevaluable • 110 samples assayed
Keshtgarpour et al (2016)[15,]	Retrospective	49	• Advanced-stage squamous and nonsquamous NSCLC medical record review at a single clinic (62 patients identified). • Determine use of VS in African Americans • Determine relation between of VS and comorbidities using CCI	• Baseline histology and PS not reported	• 49 cases qualified for inclusion • VS pretreatment: 31 • VS during or after first-line chemotherapy
Grossi et al (2017)[30,,b]	Prospective	76	• Clinically based stage IIIB NSCLC with supraclavicular lymph node	• ECOG PS: 26% grade 0; 71% grade 1; 3% grade 2 • Histology: 100% nonsquamous	• 105 participants enrolled • 89 with nonsquamous histology included • 15 with squamous histology and 1 with small cell lung cancer excluded

			metastases, or stage IV or recurrent NSCLC, chemotherapy-naive • To be treated with platinum doublet chemotherapy: pemetrexed plus carboplatin or cisplatin		• 6 additional patients ineligible (no treatment, consent, had surgery) • 83 eligible for VS • 7 did not receive VS • Choice of chemotherapy regimen at physician discretion based on age, ECOG PS, creatinine clearance
Grossi et al (2018)[34,,b]	Retrospective	481	• 3 cohorts (NExUS, Italian, eLung) of treatment-naive recurrent or advanced NSCLC patients who received platinum-based chemotherapy • NExUS cohort: prospective RCT of gemcitabine plus cisplatin and sorafenib vs gemcitabine plus cisplatin and placebo • Italian: clinically-based cohort treated with platinum-doublet chemotherapy • eLung: multicenter randomized phase 2b study of cetuximab plus platinum-based chemotherapy as first-line treatment. ○ Arm A: carboplatin plus paclitaxel and cetuximab then maintenance cetuximab ○ Arm B: carboplatin or cisplatin (investigator choice) plus gemcitabine and cetuximab then	• NExUS: stage IIIB or IV NSCLC ○ ECOG PS: 0/1 ○ Histology: NR • Italian: stage IIIB NSCLC with supraclavicular lymph node metastases, or stage IV or recurrent NSCLC ○ Histology:100 % nonsquamous (Grossi et al [2017]) • eLung ○ ECOG PS: 0/1 ○ Histology: nonsquamous and squamous	• NExUS: Baseline plasma samples 419 of 722 nonsquamous participants available for VS assay • Italian: 105 participants enrolled • 89 with nonsquamous histology included • 15 with squamous histology and 1 with small cell lung cancer excluded • 6 additional patients ineligible (no treatment, consent, had surgery) • 83 eligible for VS • 7 did not receive VS • eLung: 206 of 601 participants had serum available for VS • 203 VS performed

			maintenance cetuximab ○ Arm C: carboplatin or cisplatin (investigator choice) plus pemetrexed and cetuximab then maintenance cetuximab ○ Arm C limited to squamous histology ○ Delivery of 4, 5, or 6 cycles of chemotherapy at investigator discretion		
			Previous Chemotherapy [a]	n (%)	
			1	119 (62%)	
			2	73 (38%)	
Spigel et al (2018)[31,]	Retrospective	192	Sample from RCT of treatment for stage IV NSCLC following 1-2 chemotherapy regimens • Arm A (erlotinib plus pazopanib) or • Arm B (erlotinib plus placebo)	Age: 35-88 y ECOG PS: 0-2 Histology: nonsquamous and squamous	Treatment arm assignments stratified for histology and prior exposure to bevacizumab • 190 eligible patients received protocol therapy • 93 samples available for VS • 2 samples unevaluable • 88 samples assayed

adeno: adenocarcinoma; CCI: Charleston Comorbidity Index; ECOG: European Cooperative Oncology Group; *EGFR*: epidermal growth factor receptor; KPS: Karnofsky Performance Status; LCC: large cell carcinoma; NOS: not otherwise specified; NR: not reported; NSCLC: non-small-cell lung cancer; PET: positron emission tomography; PFS: progression-free survival; PS: Performance Status; RCT: randomized controlled trial; VS: VeriStrat; WT: wild-type.

[a] Number of prior chemotherapy regimens.

[b] Industry sponsorship or collaboration.

Table 2. Clinical Validity Study Results of Proteomic Testing in NSCLC for Disease Prognosis

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
VeriStrat-specific studies					
Taguchi et al (2007)[14], Italian B validation set	Retrospective	67	Sequential cohort of late-stage or recurrent NSCLC treated with single-agent gefitinib: • VS "good": 39 (58.3%) • VS "poor": 27 (40.3%) • VS undefined: 1	Unadjusted • HR of death, 0.50 (0.24 to 0.78; p=.005) Adjusted[a] • HR of death, 0.74 (0.55 to 0.99; p=.048)	Unadjusted • TTP: HR=0.56 (0.28 to 0.89; p=.02)
Taguchi et al (2007)[14], ECOG 3503 validation set	Retrospective	96	ECOG 3503 single-arm, phase 2 trial of first-line erlotinib in patients with stage IIIB or IV or recurrent NSCLC: • VS "good": 69 (71.9%) • VS "poor": 27 (28.1%) • VS undefined: 0	Unadjusted • HR of death, 0.4 (0.24 to 0.70; p<.001) Adjusted[b] • HR of death, 0.53 (0.30 to 0.94; p=.03)	Unadjusted • TTP: HR=0.53 (0.33 to 0.85; p=.007)
Amann et al (2010)[25],		88	VS "good" (n=64),VS “poor” (n=24) • *EGFR* exon 19 WT: 41 • *EGFR* exon 19-positive: none identified • *EGFR* exon 21 WT: 38 • *EGFR* exon 21-positive: 3 • *EGFR* exon 21-positive and VS “good”: 2 • *EGFR* exon 21-positive and VS “poor”: 1	Unadjusted • HR of death, 0.36 (0.21 to 0.60; p=.001) Adjusted (for *EGFR* status) • HR of death, 0.26 (0.06 to 1.16; p=.08)	Unadjusted • TTP: HR=0.51 (0.28 to 0.90; p=.02)
Carbone et al (2010)[33],	Retrospective	35	Treatment-experienced recurrent stage IIIB or IV, nonsquamous NSCLC treated with erlotinib and bevacizumab enrolled in a phase 1 dose-finding and phase 2 efficacy and tolerability study: • VS "good": 26 • VS “poor”: 8	Unadjusted • HR of death (61 wk vs 24 wk), 0.14 (0.03 to 0.58)	Unadjusted • PFS (36 wk vs 8 wk): HR=0.045 (0.008 to 0.237)
Kuiper et al (2012)[26],	Retrospective	50	• Chemotherapy-naive patients with pathologically documented, inoperable, locally advanced, recurrent, or metastatic NSCLC, treated with erlotinib and sorafenib • VS classification was performed at 3 time points (pretreatment, 1	Unadjusted using pretreatment classification only • HR for OS=0.30 (0.12 to 0.74; p=.009) • Median OS=13.7 mo (12 mo to undefined) for VS “good” and	Unadjusted using pretreatment classification only • PFS: HR=0.40 (0.17 to 0.94; p=.035) • Median PFS=5.5 mo (3.0 to 6.9 mo) for VS

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
			and 3 wk after initiation therapy) • Pretreatment VS "good" (n=33), VS "poor" (n=15): ○ *EGFR* WT: 31 ○ *EGFR*-positive: 7 ○ *EGFR* unknown: 12	5.6 mo (1.6 to 7.6 mo) for VS "poor"	"good" vs and 2.7 mo (1.4 to 5.6 mo) for VS "poor"
Akerley et al (2013)[27,]	Retrospective	42	Stage IIIB or IV or recurrent nonsquamous NSCLC, with no prior chemotherapy for metastatic disease, treated with erlotinib and bevacizumab: • VS "good": 32 (76%) • VS "poor": 9 (21%) • VS indeterminate: 1 (2%)	Unadjusted on study therapy • HR for OS=0.27 (0.11 to 0.64) • Median OS=71.4 wk vs "good" and 19.9 wk for VS "poor" (p=.002)	Unadjusted on study therapy • Median PFS=18.9 wk VS "good" vs 6.3 wk VS "poor" (p=.004) Study therapy plus chemotherapy • Median PFS=43.9 wk for VS "good" and 6.3 wk for VS "poor" (p<.001)
Gautschi et al (2013)[28,]	Retrospective	117	Pooled analysis from SAKK19/05 and NTR528 trials: untreated, advanced nonsquamous NSCLC, treated with first-line therapy with erlotinib and bevacizumab: • VS "good": 87 (SAKK19/05, n=70; NTR528, n=17) • VS "poor": 27 (SAKK19/05, n=16; NTR528, n=11) • SAKK19/05: *EGFR* variant status: positive identification but data NR • NTR528: *EGFR* variant status: NR	Unadjusted • HR=0.48 (0.29 to 0.78; p=.003) • Median OS=13.4 mo for VS "good" and 6.2 mo for VS "poor"	Unadjusted • PFS: HR=0.768 (0.482 to 1.22; p=.253) • Median PFS=4 mo for VS "good" vs 3.2 mo for VS "poor"
Stinchcombe et al (2013)[29,]	Retrospective	98	• 110 samples VS assayed: ○ VS "good": 64 ○ VS "poor": 39 ○ VS Indeterminate: 7 ○ (5 samples could not be	Unadjusted Arm A • HR=0.82 (0.35 to 1.90; p=.64) • Median OS=201 d for VS "good" vs 197 d for VS "poor"	Unadjusted Arm A • HR=1.21 (0.51 to 2.88; p=.67 • Median PFS=133 d for VS "good"

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
			matched with clinical data VS "good": 1 and VS "poor": 4) • VS results matched with clinical data: ○ VS "good": 63 ○ VS "poor": 35 • Arm A (gemcitabine): ○ VS "good": 20 ○ VS "poor": 8 ○ 12 of 28 also received erlotinib as second-line therapy on protocol in absence of disease progression or unacceptable toxicity • Arm B (erlotinib): ○ VS "good": 26 ○ VS "poor": 12 ○ 14 of 38 received second-line therapy (type NR) off protocol • Arm C (gemcitabine and erlotinib): ○ VS "good": 17 ○ VS "poor": 15 ○ 13 of 32 received second-line therapy (type NR) off protocol	Unadjusted Arm B • HR=0.40 (0.19 to 0.86; p=.014) • Median OS=255 d for VS “good” vs 51 d for VS “poor” Unadjusted Arm C • HR=0.48 (0.23 to 1.02; p=.051) • Median OS=302 d for VS “good” vs 106 d for VS “poor” Adjusted [e] • HR=0.53 (0.32 to 0.90; p=.017)	vs 137 d for VS “poor” Unadjusted Arm B • HR=0.33 (0.16 to 0.70; p=.002) • Median PFS=89 d for VS “good” vs 22 d for VS “poor” Unadjusted Arm C • HR=0.42 (0.19 to 0.93; p=.027) • Median PFS=122 d for VS “good” vs 89 d for VS “poor” Adjusted [e] • HR=0.51 (0.30 to 0.86; p=.011)
Keshtgarpour et al (2016)[15,]	Retrospective	49	Advanced-stage squamous and nonsquamous NSCLC seen at a single clinic: • VS "good": 32 • VS "poor": 16 • VS indeterminate: 1	Unadjusted for CCI • HR=0.97 (0.48 to 1.97; p=.94) CCI adjusted model • HR=0.80 (0.39 to 1.64; p=.54) VS “poor” on erlotinib vs chemotherapy, CCI adjusted • HR=9.48 (1.27 to 70.81; p=.03)	
Grossi et al (2017)[30,]	Prospective	76	• Stage IIIB NSCLC with supraclavicular lymph	Unadjusted secondary outcome in study	Unadjusted primary outcome in study

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
			node metastases, or stage IV or recurrent NSCLC, chemotherapy-naive treated with platinum doublet chemotherapy • Carboplatin plus pemetrexed (n=43; median age, 57 y) • Cisplatin plus pemetrexed (n=33; median age, 70 y) • VS “good”: 50 ○ VS "good": carboplatin/pemetrexed: 28 ○ VS "good": cisplatin/pemetrexed: 22 ○ VS "poor": 26 ○ VS "poor": carboplatin/pemetrexed:15 ○ VS "poor": cisplatin/pemetrexed: 11 • TKI-sensitizing variant status results: ○ *EGFR* WT: 67 (88%) ○ *EGFR*-negative: 2 (3%) ○ *EGFR* unknown: 7 (9%) ○ *ALK* translocation negative: 54 (71%) ○ *ALK* translocation positive: 1 (1%) ○ *ALK* translocation unknown: 21 (28%) ○ *KRAS* WT: 31 (41%) ○ *KRAS*-positive: 29 (38%) ○ *KRAS* unknown: 16 (21%)	• HR=0.26 (0.15 to 0.47; p<.001) • Median OS=10.8 mo for VS “good” vs 3.4 mo for VS “poor” Unadjusted <u>secondary</u> outcome based on treatment-defined group • Carboplatin plus pemetrexed vs cisplatin plus pemetrexed: ○ HR=1.64 (0.96 to 2.82; p=.070) ○ Median OS carboplatin plus pemetrexed, 6.0 mo (954.2 to 10.0 mo) vs cisplatin plus pemetrexed 10.3 mo (6.6 to 17.9 mo) • Carboplatin plus pemetrexed VS “good” vs “poor”: ○ HR=0.26 (0.12 to 0.55; p<.001) ○ Median OS=9.4 mo (5.0 to 15.3 mo) for VS “good”	• HR=0.36 (0.22 to 0.61; p<.001) • Median PFS=6.5 mo for VS “good” vs 1.6 mo for VS “poor” Unadjusted <u>primary</u> outcome based on treatment-defined group • Carboplatin plus pemetrexed vs cisplatin plus pemetrexed: ○ HR=1.59 (0.97 to 2.61; p=.063) ○ Median PFS carboplatin plus pemetrexed, 2.8 mo (2.0 to 4.0 mo) vs cisplatin plus pemetrexed 5.7 mo (3.8 to 8.8 mo) • Carboplatin plus pemetrexed VS “good” vs VS “poor”: ○ HR=0.30 (0.14 to 0.62; p<.001) ○ Median PFS=3.8 mo (2.7 to 8.7 mo) for VS “good” vs 1.6 mo (1.0 to 2.5 mo) for VS “poor • Cisplatin plus pemetrexed VS “good” vs VS “poor”:

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
				vs 3.4 mo (1.0 to 4.3 mo) for VS "poor • Cisplatin plus pemetrexed VS "good" vs "poor": ○ HR=0.25 (0.10 to 0.62; p=.001) ○ Median OS=17.7 mo (9.9 to 24.19 mo) for VS "good" vs 4.2 mo (2.6 to 8.9 mo) for VS "poor" Adjusted[c] • HR=0.23 (0.12 to 0.44; p<.001) Adjusted[d] • HR=0.23 (0.11 to 0.46; p<.001)	○ HR=0.39 (0.18 to 0.85; p=.014) ○ Median PFS=7.9 mo (5.2 to 13.1 mo) for VS "good" vs 1.7 mo (1.1 to 3.9 mo) for VS "poor Adjusted[c] • HR=0.32 (0.18 to 0.58; p<.001) Adjusted[d] • HR=0.39 (0.22 to 0.71; p=.002)
Grossi et al (2018)[34,]		481	NExUS: VS assay: 202 patients in gemcitabine/cisplatin/placebo arm: • VS "good": 136 • VS "poor": 66 Italian: VS assay: 76 patients pemetrexed plus carboplatin or cisplatin: • VS "good": 50 • VS "good": carboplatin plus pemetrexed: 28 • VS "good": cisplatin plus pemetrexed: 22 • VS "poor": 26 • VS "poor": carboplatin plus pemetrexed: 15 • VS "poor": cisplatin plus pemetrexed: 11 eLung: VS assay: 203	Unadjusted secondary outcome in NExUS study • HR=0.41 (0.30 to 0.58; p<.001) • Median OS=14.7 mo (12.5 to 16.9 mo) for VS "good" vs 6.3 mo (5.6 to 8.1 mo) for VS "poor" Unadjusted secondary outcome in Italian study • HR=0.26 (0.15 to 0.47; p<.001) • Median OS=10.8 mo (7.8 to 17.7 mo)	Unadjusted primary outcome in NExUS study • HR=0.51 (0.37 to 0.71; p<.001) • Median PFS=5.7 mo (5.5 to 6.9 mo) for VS "good" vs 4.6 mo (4.1 to 5.7 mo) for VS "poor" Unadjusted primary outcome in Italian study

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
			• VS “good”: 142 • VS “good”: carboplatin plus paclitaxel and cetuximab: 52 • VS “good”: carboplatin or cisplatin plus gemcitabine and cetuximab: 56 • VS “good”: carboplatin or cisplatin plus pemetrexed and cetuximab :34 • VA “poor”: 61 • VS “poor”: carboplatin plus paclitaxel and cetuximab:27 • VS “poor”: carboplatin or cisplatin plus gemcitabine and cetuximab: 26 • VS “poor”: carboplatin or cisplatin plus pemetrexed and cetuximab: 8	for VS “good” vs 3.4 mo (2.4 to 4.3 mo) for VS “poor” Unadjusted secondary outcome in eLung study • HR=0.51 (0.37 to 0.71; p<.001) • Median OS=10.9 mo (9.5 to 12.9 mo) for VS “good” vs 6.4 mo (4.0 to 9.0 mo) for VS “poor”	• HR=0.36 (0.22 to 0.61; p<.001) • Median PFS=6.5 mo (3.9 to 8.8 mo) for VS “good” vs 1.6 mo (1.1 to 2.5 mo) for VS “poor” Unadjusted primary outcome in eLung study • HR=0.72 (0.53 to 0.97) • Median PFS=5.1 mo (4.2 to 5.7 mo) for VS “good” vs3.6 mo (2.7 to 5.3 mo) for VS “poor”

Spigel et al (2018)[31],	Retrospective	88	Stage IV NSCLC, with prior chemotherapy
			• VS “good”: 63
			• VS “good”: erlotinib plus placebo: 23
			• VS “good”: erlotinib plus pazopanib: 40
			• VS “poor”: 25
			• VS “poor”: erlotinib plus placebo: 8
			• VS “poor”: erlotinib plus pazopanib: 17
				Unadjusted secondary outcome
				• HR=0.42 (0.26 to 0.69; p<.001)
				• Median OS=8.6 mo (6.6 to 11.6 mo) for VS “good” vs 2.8 mo (1.4 to 4.9 mo) for VS “poor”
				Unadjusted secondary outcome based on VS-defined groups
				• VS “good”
				  ○ HR=1.02 (0.58 to 1.81; p=.934)
				  ○ Median PFS: erlotinib plus pazopanib, 8.2 mo (5.4 to 12.4 mo) vs erlotinib
					Unadjusted primary outcome
					• HR=0.44 (0.26 to 0.73; p <.001)
					• Median PFS=2.1 mo (1.8 to 3.6 mo) for VS “good” vs 1.8 mo (1.4 to 2.2 mo) for VS “poor”
					Unadjusted primary outcome based on VS-defined groups
					• VS “good”
					  ○ HR=0.47 (0.26 to 0.86; p=.010)
					  ○ Median PFS: erlotinib

Study	Study Type	N	Patient Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
				plus placebo, 8.6 mo (5.1 to 13.9 mo) • VS "poor" ○ HR=2.10 (0.83 to 5.26; p=.1089) ○ Median PFS: erlotinib plus pazopanib, 2.8 mo (1.2 to 4.7 mo) vs erlotinib plus placebo, 7.5 mo (0.9 to 16.8 mo)	plus pazopanib, 3.6 mo (1.8 to 4.1 mo) vs erlotinib plus placebo, 1.8 mo (1.7 to 2.5 mo) • VS "poor" ○ HR=0.87 (0.37 to 2.05; p=.745) ○ Median PFS: erlotinib plus pazopanib, 1.8 mo (1.0 to 2.5 mo) vs erlotinib plus placebo, 1.7 mo (0.8 to 2.8 mo)

ALK: anaplastic lymphoma kinase; CI: confidence interval; CCI: Charleston Comorbidity Index; ECOG: European Cooperative Oncology Group; *EGFR*: epidermal growth factor receptor; HR: hazard ratio; NR: not reported; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival; TKI: tyrosine kinase inhibitor; TTP: time to progression; VS: VeriStrat; WT: wild-type.

[a] Adjusted based on age, performance status, sex, histology, smoking history, and MALDI-MS classification.

[b] Adjusted based on age, number of involved sites, prior weight loss, histology, and MALDI-MS classification.
[c] Adjusted based on clinical characteristics: VS classification, sex, smoking status (ever vs never), ECOG PS (≥1 vs 0), *KRAS* status (mutant vs WT or unknown), *KRAS* (known vs unknown), maintenance (yes vs no).
[d] Adjusted based on clinical characteristics and treatment: VS classification, sex, cisplatin/pemetrexed *vs* carboplatin/pemetrexed smoking status (ever vs never), ECOG PS (≥1 vs 0), *KRAS* status (mutant vs WT or unknown), *KRAS* (known vs unknown), maintenance (yes vs no).
[e] Adjusted for VS status, histology (other histologies vs adenocarcinoma), race (nonwhite vs white), sex (female vs male), treatment arm (erlotinib vs gemcitabine), treatment arm (gemcitabine/erlotinib vs gemcitabine), smoking history (never vs ever), PS (2 vs 0 or 1), stage IV vs IIIB.

Table 3. Clinical Validity - Study Relevance Limitations for Proteomic Testing in NSCLC for Disease Prognosis

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of FU[e]
Taguchi et al (2007)[14,] Italian B validation set	1. Population unselected for *EGFR* variant status	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	
Taguchi et al (2007)[14,] ECOG 3503 validation set	1. Population unselected for *EGFR* variant status 2. 20 (20.8%) of participants had postoperative recurrence, which may be an indicator of earlier stage at diagnosis	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	
Amann et al (2010)[25,]	1. *EGFR v*ariant status unknown excluded 4. Use of erlotinib (or other TKIs) in *EGFR* variant-negative population no longer accepted treatment approach 5. 90 (88.2%) with multisite metastatic disease; 55 (54%) had prior radiotherapy or surgery	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	
Carbone et al (2010)[33,]	1. No determination of *EGFR v*ariant status 4. Study population participating in phase 1/2 study 4. Use of erlotinib (or other TKIs) in *EGFR* variant-negative or -unknown population no longer accepted treatment approach 4. Use of	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of FU[e]
	combination *EGFR* (erlotinib) and VEGF inhibition (bevacizumab) not currently accepted treatment approach				
Kuiper et al (2012)[26,]	4. Use of erlotinib (or other TKIs) in *EGFR* variant-negative or -unknown population no longer accepted treatment approach 4. Use of combination *EGFR* (erlotinib) and VEGF inhibition (sorafenib) not currently accepted treatment approach	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. A typical clinical assessment tool used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway No outcome reported for *EGFR* variant status unknown	
Akerley et al (2013)[27,]	Participants might have received prior adjuvant chemotherapy 4. Use of combination *EGFR* (erlotinib) and VEGF inhibition (bevacizumab) not currently accepted treatment approach	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy 3.Survival of participants without VeriStrat assay reported as not different but no data provided	
Gautschi et al (2013)[28,]	4. Use of combination *EGFR* (erlotinib) and VEGF inhibition (bevacizumab) not currently accepted treatment approach	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	
Stinchcombe et al (2013)[29,]	1. Population unselected for *EGFR* variant status2. Participants in 2 arms received treatment off protocol 4.Use of erlotinib (or other TKIs) in *EGFR* variant-negative or -unknown population no longer accepted treatment approach	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1.VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway	
Keshtgarpour et al (2016)[15,]	1. No determination of *EGFR* variant status 1. Participants may have received prior first-line chemotherapy	Other related: Identity of proteins that make up the MALDI-MS	3. Clinical assessment of prognosis not used	Other related: Decision model based on outdated clinical pathway	

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of FU[e]
	4. Use of erlotinib (or other TKIs) in *EGFR* variant-negative or -unknown population no longer accepted treatment approach	features still being investigated at time of publication			
Grossi et al (2017)[30,]	3. Median age (57 y) of patients in cisplatin plus pemetrexed arm significantly younger than median age (70 y) in carboplatin plus pemetrexed arm	Other related: Identity of proteins that make up the MALDI-MS features still being investigated at time of publication	3. Clinical assessment of prognosis not used	1. VeriStrat classification not used to direct therapy 2. Inclusion of *KRAS* variant/exclusion of *EGFR* and *ALK* testing results in adjusted analyses appears to be potential new decision model Other related: No outcome reported for *EGFR* variant status unknown No outcomes reported for *EGFR* wild-type No outcomes reported for *ALK* variant status Range of values for median OS and PFS not reported in this publication but reported in Grossi et al (2018)	
Grossi et al (2018)[34,]	1.NExUS cohort reference is abstract only 1.eLung cohort reference is abstract only 2.NExUS cohort reference is abstract only 2.eLung cohort reference is abstract only 4.eLung cohort results based on treatment (cetuximab) not currently used for first- or second-line NSCLC	Other related: Identity of the proteins that make up the MALDI-MS features still being investigated at the time of publication		1. VeriStrat classification not used to direct therapy Other related: Decision model based on outdated clinical pathway in NExUS and eLung cohorts	
Spigel et al (2018)[31,]	1.No determination of *EGFR v*ariant status 4. Use of erlotinib (or other TKIs) in *EGFR* variant - negative or -unknown population no longer accepted treatment approach	Other related: Identity of the proteins that make up the MALDI-MS features still being investigated at the time of publication		1. VeriStrat classification not used to direct therapy	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
ALK: anaplastic lymphoma kinase; *EGFR*: epidermal growth factor receptor; FU: follow-up; MALDI-MS: matrix-assisted laser desorption ionization mass spectrometry; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival; TKI: tyrosine kinase inhibitor; VEGF: vascular endothelial growth factor.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 4. Clinical Validity - Study Design and Conduct Limitations for Proteomic Testing in NSCLC for Disease Prognosis

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Taguchi et al (2007)[14], Italian B validation set	2. Selection not random or consecutive (ie, convenience)				Other related: • Variable response assessment times and intervals	Other related: • Sample sizes small • Impacts test of difference in multivariate analysis
Taguchi et al (2007)[14], ECOG 3503 validation set	2. Selection not random or consecutive (ie, convenience)					Other related: • Sample sizes small • Impacts test of difference in multivariate analysis
Amann et al (2010)[25],	2. Selection not random nor consecutive (i.e., convenience)		Other related: • Proteomic testing not applied to EGFR variant status unknown population	•	Other related: • Variable response assessment times and intervals	Other related: • Confidence that the proteomic classifier is independent of *EGFR* variant status is limited by very small number of positive variants • Small sample sizes • Unadjusted for demographic and histologic characteristics associated with prognosis • Small sample sizes
Carbone et al (2010)[33],	2.Selection not random or				Other related: • Variable response	1. p-value not reported. Other related:

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Herbst et al (2005)[35],	consecutive (i.e., convenience)				assessment times and intervals	• Sample sizes small • Unadjusted for demographic and histologic characteristics associated with prognosis
Kuiper et al (2012)[26],	2. Selection not random or consecutive (i.e., convenience)		3. VeriStrat classification performed at 3 time points (pretreatment, 1 and 3 wk after initiation therapy)		Other related: • Variable response assessment times and intervals	Other related: • Sample sizes small • Unadjusted for demographic and histologic characteristics associated with prognosis
Akerley et al (2013)[27],	2. Selection not random or consecutive (i.e., convenience)				Other related: • Variable response assessment times and intervals	Other related: • Small sample sizes
Gautschi et al (2013)[28],	2. Selection not random or consecutive (i.e., convenience)				Other related: • Variable response assessment times and intervals	Other related: • Small sample sizes • OS (primary outcome) and PFS (secondary outcome) data not shown for reported multivariate analysis or stratification by trial • Adjusted analysis (sex, age, histology, disease stage, PS, smoking status) reported as no significant association between VeriStrat and tumor variant status; data not shown

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Stinchcombe et al (2013)[29,]	2.Selection not random or consecutive (i.e., convenience)				Other related: • Variable response assessment times and intervals	Other related: • Small sample sizes
Keshtgarpour et al (2016)[15,]	2.Selection not random or consecutive (i.e., convenience)		Other related • Pre- and posttreatment VeriStrat scores used		Other related: • Variable response assessment times and intervals	Other related: • Small sample sizes • VeriStrat indeterminate case added to VeriStrat "good" data pool
Grossi et al (2017)[30,]	2. Participant recruitment not random from single lung cancer treatment unit				Other related: • Variable response assessment times and intervals	Other related: • Adjusted analyses for PFS and OS did not include age or other sensitizing variants (*EGFR*, *ALK*) although data reported • Overall sample sizes small • Slow accrual • Number of *EGFR* variant-positive and *ALK* translocation findings too small to assess correlation with VeriStrat classification
Grossi et al (2018)[34,]	2. Participant selection differs between and among cohorts			2. VeriStrat classification results for 2 of 3 cohorts imported from abstract sources	Other related: • Variable response assessment times and intervals	Other related: • Small sample sizes
Spigel et al (2018)[31,]	2.Selection not random					Other related: Unadjusted for

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
	or consecutive (i.e., convenience)					demographic and histologic characteristics associated with prognosis

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
ALK: anaplastic lymphoma kinase; *EGFR*: epidermal growth factor receptor; OS: overall survival; PFS: progression-free survival; PS: performance status.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Table 5. Clinical Validity Results of Proteomic Testing in NSCLC for Disease Prognosis Non-VeriStrat Assays

Study	Study Type	N	Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
Salmon et al (2009)[36], Erlotinib/ bevacizumab generation set[c]	Retrospective	35	Stage IIIB or IV, recurrent, nonsquamous NSCLC treated with erlotinib and bevacizumab	Adjusted [a] • HR of death, 1.024 (1.009 to 1.040; p=.003)	
Salmon et al (2009) ECOG 3503 validation set[c]	Retrospective	82	ECOG 3503 trial patients with stage IIIB or IV or recurrent NSCLC treated with first-line erlotinib	Adjusted [b] • HR of death, 1.012 (1.003 to 1.021; p=.012)	
Wu et al (2013)[37], Validation set[d]	Retrospective	44	Stage IIIB or IV NSCLC failed or intolerant to chemotherapy, treated with gefitinib or erlotinib • Histology: 79.2% adeno; 20.8% squamous	OS (predicted "good" vs predicted "poor"): HR=0.357 (0.186 to 0.688; p=.002)	PFS (predicted "good" vs predicted "poor"): HR=0.06 (0.022 to 0.016; p<.001)
Yang et al (2015)[38], Validation set[e]	Retrospective	123	Stage IIIB or IV NSCLC with a known *EGFR* variant status • Variant status: 42.3% with EGFR TKI-sensitive variant; 57.7% with *EGFR* WT • Previous EGFR treatment: 67.5% (30.9% as first-	Following EGFR TKI treatment (81 patients in validation set): OS=29.0 mo for assay "mutant" and 28.0 mo for assay "wild" (p=*NS*)	Following EGFR TKI treatment (81 patients in validation set): PFS=10.0 mo for assay "mutant" and 2.3 mo for assay "wild" (p<.001)

Study	Study Type	N	Population	Summary of Outcomes: OS for "Good" vs "Poor" Assay (95% CI)	Summary of Outcomes: PFS for "Good" vs "Poor" Assay (95% CI)
			line, 26.8% as second-line, 9.8% as third-line or greater)		

adeno: adenocarcinoma; CI: confidence interval; ECOG: European Cooperative Oncology Group; *EGFR*: epidermal growth factor receptor; HR: hazard ratio; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival; TKI: tyrosine kinase inhibitor; WT: wild-type.
[a] Adjusted based on age, sex, histology.
[b] Adjusted based on metastatic site and performance status.
[c] Test based on 11 *m/z* features.
[d] Test based on 3 peptides/proteins.
[e] Test based on 5 peptides/proteins.

Proteomic Testing in NSCLC to Predict Response to Therapy

No studies were identified that used VeriStrat proteomic testing to inform therapeutic options for patients with stage I or II NSCLC if surgery or surgery plus radiotherapy had been completed or who were upstaged as a result of surgical findings.

No studies were identified that used VeriStrat proteomic testing to inform therapeutic options for patients with stage I or II NSCLC who were considered medically inoperable.

Based on the association between VeriStrat status and outcomes in patients treated with EGFR TKIs, it was postulated that VeriStrat testing might predict response to EGFR TKIs.

No studies were identified that used VeriStrat proteomic testing to predict response to first-line targeted therapies or first-line chemotherapy in patients with newly diagnosed advanced NSCLC.

Randomized Controlled Trials

In the PROSE trial, Gregorc et al (2014) prospectively evaluated the VeriStrat test in a randomized controlled trial (RCT) comparing erlotinib with chemotherapy as a second-line treatment for patients with stage IIIB or IV NSCLC, stratified by performance status, smoking history, treatment center, and (masked) pretreatment VeriStrat classification.[39,]

In a multivariate model to predict OS, which included clinical characteristics and *EGFR*-variant status, VeriStrat classification was significantly associated with OS (HR for VeriStrat "good" vs "poor," 1.88; 95% CI, 1.25 to 2.84; p=.003).

In the entire analysis cohort, the median OS was 9.0 months in the chemotherapy group and 7.7 months in the erlotinib group; OS did not differ significantly by treatment group in adjusted or unadjusted analyses. Moreover, PFS did not differ significantly by treatment group in the unadjusted analysis but was improved for the chemotherapy group in adjusted analysis (HR=1.35; 95% CI, 1.05 to 1.73; p=.020). Stratification of patients by VeriStrat classification changed the estimate of the effect of chemotherapy. In the VeriStrat "good" group, there was no significant difference in OS between the 2 treatment groups, whereas, in the VeriStrat "poor" group, OS was shorter for patients treated with erlotinib.

The authors of the PROSE trial concluded that the VeriStrat proteomic test predicted differential benefit for erlotinib compared with chemotherapy as second-line treatment of NSCLC, suggesting that patients classified as VeriStrat “poor” would have better outcomes with chemotherapy than erlotinib.

Peters et al (2017) published a randomized phase 2, open-label (EMPHASIS) trial exploring the differential effect of second-line erlotinib vs docetaxel in VeriStrat “good” vs VeriStrat “poor” patients.[40,] Patients had stage IIIB or IV squamous cell NSCLC and had failed first-line platinum-based doublet chemotherapy. Recruitment for the trial ended early due to low enrollment and the release of results from other trials (e.g., PROSE). The EMPHASIS investigators analyzed trial findings and conducted an exploratory analysis combining EMPHASIS results with those from the squamous cell NSCLC cohort in the PROSE trial. Eighty patients were randomized, of whom 58 (72.5%) were categorized as VeriStrat “good.” The primary endpoint was PFS and was analyzed on an intention-to-treat basis. After a median follow-up of 20.5 months, 73 patients had experienced disease progression (median PFS, 2.7 months). Median PFS was 1.6 months in the erlotinib group and 3.0 months in the docetaxel group; the difference between groups was not statistically significant (p=.37). PFS did not differ significantly by VeriStrat status, and there was no significant interaction between treatment and VeriStrat status (p=.80). These trial characteristics and results, as well as results for the secondary outcome OS, are presented in Tables 7 and 8. This trial was restricted to squamous NSCLC histology, and the treatment decision model is not representative of current guideline recommendations.

Lee et al (2019) published results from a randomized, double-blind trial (TOPICAL) in patients (n=527) with previously untreated advanced-stage IIIB/IV NSCLC who were considered unfit for platinum doublet chemotherapy due to poor performance status (PS 2: 56%; PS 3: 27%) and/or the presence of multiple comorbidities.[32,] Patients were unselected for *EGFR* status and randomized for treatment with erlotinib or placebo and active supportive care. This treatment approach is not consistent with current guidelines that cite recent data indicating that NSCLC tumors that do not harbor a sensitizing *EGFR* mutation should not be treated with an EGFR TKI in any line of therapy. For patients with comorbidities and PS 0-1, carboplatin-based regimens are often used. For patients with PS 2, several alternative systemic therapy regimens not involving platinum-based agents are also available, including paclitaxel, albumin-bound paclitaxel, docetaxel, gemcitabine, gemcitabine/docetaxel, gemcitabine/vinorelbine, and pemetrexed.[1,] Fifty-five percent of patients were categorized as VeriStrat 'good,' which includes 164 patients in the erlotinib arm and 124 patients in the placebo arm. Forty-five percent of patients were classified as VeriStrat 'poor,' which includes 115 patients in the erlotinib arm and 124 patients in the placebo arm. For patients with VeriStrat 'good' vs 'poor' scores, median OS was 4.6 months vs 2.9 months in the placebo group (HR=0.54; 95% CI, 0.41 to 0.78; p0.001) and 4.9 months vs 3.1 months in the erlotinib group (HR=0.60; 95% CI, 0.47 to 0.77; p<.001). The difference between groups was not statistically significant in the unadjusted analysis (HR=0.93; 95% CI, 0.87 to 1.11; p=.41). *EGFR*-variant status was known in 41.2% of patients, which includes *EGFR*-variant positive status in 21/288 (7.3%) with a VeriStrat 'good' score and 6/239 (2.5%) with a VeriStrat 'poor' score. were *EGFR*-variant positive. Both VeriStrat "good" vs "poor" classification and *EGFR*-variant positive vs wild-type status were found to have prognostic value for OS. Only VeriStrat classification was found to have prognostic value for PFS. VeriStrat classification did not have predictive value for response to erlotinib vs placebo. The authors indicate that the VeriStrat assay was able to

stratify patients within ECOG PS grades 0-1 and 2-3, however, CIs for these groups were not reported. *EGFR*-variant status was not reported according to respective treatment groups.

Retrospective Studies

Several retrospective analyses of data from RCTs evaluating the efficacy of TKIs have examined VeriStrat as a prognostic and/or predictive test. Carbone et al (2012) investigated the prognostic and predictive effects of VeriStrat classification on response to treatment and survival in a subset of patients enrolled in a phase 3 trial of erlotinib vs placebo.[41,] BR.21, a randomized, placebo-controlled study of erlotinib, enrolled 731 previously treated patients with advanced NSCLC. In the primary study, PFS and OS were prolonged by erlotinib. *EGFR* variants were prognostic for OS, but not predictive of erlotinib benefit, while increased *EGFR* copy number variants were both prognostic and predictive of erlotinib benefit. For the present trial, plasma from 441 patients was tested with the VeriStrat test, of which 436 (98.9%) could be classified as “good” or “poor.”

Among the 144 placebo patients, VeriStrat test results were prognostic, with “good” patients (median OS=6.6 months; 95% CI, 4.4 to 8.2 months) surviving significantly longer than “poor” patients (median OS=3.1 months; 95% CI, 2.2 to 3.7 months; HR=0.44, 95% CI, 0.31 to 0.63; p<.001). Similar results were seen for PFS, with VeriStrat “good” patients having longer PFS than “poor” patients (HR=0.59; 95% CI, 0.42 to 0.86; p=.002). Median survival was 10.5 months for VeriStrat “good” patients treated with erlotinib and 6.6 months for those on placebo (HR=0.63; 95% CI, 0.47 to 0.85; p=.002), while for VeriStrat “poor” patients, the median survival for erlotinib was 3.98 months and 3.09 months for placebo (HR=0.77; 95% CI, 0.55 to 1.06; p=.11). For 252 erlotinib-treated patients with data available to evaluate for objective response, VeriStrat “good” patients (n=157 [62%]) had a significantly higher response rate (11.5%) than VeriStrat “poor” patients (1.1%; p=.002). In a Cox multivariate regression model to predict OS, the interaction between VeriStrat status and treatment type was not statistically significant, indicating that both “good” and “poor” cohorts derived a similar survival benefit from erlotinib. The authors concluded that VeriStrat status predicted response to erlotinib but did not predict differential benefit from erlotinib for OS or PFS.

Gadgeel et al (2017) retrospectively analyzed data from the LUX-Lung 8 trial, which compared second-line treatment with 1 of 2 TKIs (erlotinib, afatinib) in patients with advanced-stage IIIB or IV squamous NSCLC.[42,]*EGFR*-variant status was not considered in study eligibility. Blood samples for VeriStrat analysis were available for 691 (87%) of 795 randomized patients; of these, 12 were indeterminate results, and 4 could not be analyzed. The primary objective of the analysis was to evaluate whether VeriStrat status pretreatment is associated with OS and in the afatinib vs erlotinib groups. In the cohort with VeriStrat results (n=675), OS was significantly longer in the afatinib group (median, 7.8 months) than in the erlotinib group (median, 6.9 months; p=.03). When stratified by VeriStrat status, OS was significantly longer with afatinib than with erlotinib in the VeriStrat “good” group (median, 11.5 months vs 8.9 months; HR=0.79; 95% CI, 0.63 to 0.98) but not the VeriStrat “poor” group (median, 4.7 months vs 4.8 months; HR=0.90; 95% CI, 0.70 to 1.16). In the VeriStrat stratified analysis, findings were similar for PFS. The study lacked a group receiving chemotherapy with which to compare the efficacy of TKIs.

Buttigliero et al (2018)[43,] retrospectively examined VeriStrat as a prognostic and/or predictive test in a randomized controlled phase 3 RCT (MARQUEE trial[44,]) of previously treated patients with advanced nonsquamous NSCLC who were given erlotinib plus tivantinib or

placebo. *EGFR*-variant status was not considered in trial eligibility, and patients previously treated with EGFR inhibitors were excluded from the trial. Of the 1048 patients assigned to treatment protocols, 976 (93%) patients discontinued treatment by protocol (duration of therapy, 0.1-92 weeks), which was discontinued for futility at an interim analysis. In this cohort, no significant difference was seen between the treatment arms for OS. Intention-to-treat analysis of VeriStrat pretreatment status was performed on data for 996 patients.

When stratified by VeriStrat status, PFS and OS were significantly longer for patients in the VeriStrat "good" group than the VeriStrat "poor" group for both treatment arms (p<.01); no direct comparison of treatment arms within the VeriStrat "good" or "poor" groups was performed. A prespecified Cox multivariate regression analysis of OS for the cohort demonstrated that there was a statistically significant difference between VeriStrat "good" and "poor" groups (p<.001). There was a significant correlation between treatment and VeriStrat status (p=.037) in multivariate analysis considering *EGFR* variant status; this interaction was no longer significant (p=.068) when *KRAS* variant status was entered into the analysis. For patients who were *EGFR* wild-type (n=895 [90%]), OS was higher for both treatment arms in the VeriStrat "good" group (tivantinib arm median, 10.3 months; 95% CI, 8.9 to 11.5 months; placebo arm median, 9.2 months; 95% CI, 7.8 to 10.2 months) than in the VeriStrat "poor" group (tivantinib arm median, 3.9 months,;95% CI, 3.1 to 4.3 months; placebo arm median, 3.8 months; 95% CI, 2.9 to 5.4 months). The trial was restricted to nonsquamous NSCLC and lacked a group receiving chemotherapy with which to compare the efficacy of TKIs.

Tables 6 and 7 summarize study relevance, design, and conduct limitations analyses for proteomic testing in NSCLC to predict response to therapy.

Table 6. Clinical Validity Study Characteristics of Proteomic Testing in NSCLC to Predict Response to Therapy

Study	Study Type	N	Population	Selection Criteria	Participant Disposition
Gregorc et al (2014)[39] (PROSE)[a]	Prospective multicenter	263	Stage IIIB or IV NSCLC progressed on or were judged to be refractory to 1 prior platinum-based chemotherapy regimen randomized 1:1 to erlotinib or chemotherapy (single-agent pemetrexed or docetaxel investigator choice) • Erlotinib arm: 134 ○ *EGFR* WT: 79 ○ *EGFR* positive: 8 ○ *EGFR* unknown: 47 • Chemotherapy arm: 129 (74 docetaxel only, 55 pemetrexed only) ○ *EGFR* WT: 84 ○ *EGFR* positive: 6 ○ *EGFR* unknown: 39	• ECOG PS: 0-2 (93.9% grade 0-1) • Histology: 63.5% adeno; 17.8% squamous ; 18.6% other	• 296 patients screened • 285 randomized (2/11 exclusions due to "not classified as good or poor") • 142 assigned to chemotherapy • 129 primary analysis population in chemotherapy group (13 exclusions) • 143 assigned to erlotinib • 134 primary analysis population in

					erlotinib arm (9 exclusions) • Total: 19 (7.2%) exclusions due to not starting treatment • Patients with controlled brain metastases could be included
Peters et al (2017)[40.] (EMPHASIS-lung Trial)[a]	Prospective multicenter	80	Randomized phase 3 trial of second-line erlotinib vs docetaxel in VS "good" vs VS "poor" • Stage IIIB or metastatic stage IV NSCLC patients with documented progression during or after a previous line of chemotherapy (including platinum-doublet therapy) • Erlotinib arm: 38 • Docetaxel arm: 42 Combined with Gregorc (2014) PROSE squamous cell population	• ECOG PS: 0-2 • Histology: squamous cell	Stage IIIB patients not amenable to radical radiotherapy were eligible: • 94 assessed for eligibility • 81 randomized (1 randomized by mistake) Intention-to-treat cohort: • Erlotinib arm: 38 • Docetaxel arm: 42
Lee et al (2019)[45.] (TOPICAL)	Prospective multicenter	527	Randomized trial of active supportive care plus erlotinib vs placebo for previously untreated stage IIIB or IV NSCLC considered unfit for first-line platinum-based chemotherapy based on presence of comorbidities or poor ECOG PS • Erlotinib + active supportive care arm: 279 • Placebo + active supportive care arm: 248	• ECOG PS: 0-3 (17% grade 0-1; 56% • Histology: squamous cell	670 patients were randomized from original cohort, of which: • 350 assigned to erlotinib • 329 received erlotinib • 320 assigned to placebo • 311 received placebo • 527/535 VeriStrat samples collected and available, due to 8 indeterminate classifications • *EGFR* status: known (n=310/527), wild-type (283/310,

					91.3%), positive (27/310, 8.7%) • *EGFR* status for VeriStrat 'good': positive (n=21); wild-type (n=145) • *EGFR* status for VeriStrat 'poor': positive (n=6); wild-type (n=138)

adeno: adenocarcinoma; ECOG: European Cooperative Oncology Group; *EGFR*: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; PS: performance status; VS: VeriStrat; WT: wild-type.
[a] Industry sponsor or collaborator.

Table 7. Clinical Validity Results of Proteomic Testing in NSCLC to Predict Response to Therapy

Study	Median (95% CI), mo	Median (95% CI), mo	HR (95% CI)	HR (95% CI)
Gregorc et al (2014)[39] (PROSE)	VeriStrat "Good" (n=184)	VeriStrat "Poor" (n=79)	VeriStrat "Good" vs "Poor"	Chemotherapy vs Erlotinib
OS	11.0 (9.3 to 12.6) Chemotherapy (n=88): 10.9 (8.4 to 15.1) Erlotinib (n=96):11.0 (9.2 to 12.9)	3.7 (2.9 to 5.2) Chemotherapy (n = 41): 6.4 (3.0 to 7.4) Erlotinib (n =38): 3.0 (2.0 to 3.8)	2.5 (1.88 to 3.31; p<.001)	• Unadjusted HR=1.14 (0.88 to 1.49; p=.313) • Adjusted HR=1.22 (0.93 to 1.59; p=.148) • For VeriStrat 'Good': 1.05 (0.77 to 1.46, p=.714) • For VeriStrat 'Poor': 1.72 (1.08 to 2.74, p=.022)
PFS	3.4 (2.4 to 4.6)	2.0 (1.6 to 2.4)	1.75 (1.34 to 2.29; p<.001)	• Unadjusted HR=1.27 (0.99 to 1.62; p=.60) • Adjusted HR=1.35 91.05 to 1.73; p=.20) • Median OS=9.0 mo (6.8 to 10.9 mo) vs 7.7

				mo (5.9 to 10.4 mo)
Peters et al (2017)[40.](EMPHASIS-lung Trial)	VeriStrat “Good” (n=58)	VeriStrat “Poor” (n=22)	VeriStrat 'Good' vs 'Poor'	Erlotinib and Docetaxel
OS	8.2 (6.7 to 10.6)	5.2 (3.1 to 7.1)	0.49 (0.28 to 0.86; p=NR)	Median OS=7.1 mo for both erlotinib and docetaxel
PFS	NR (87% experienced a progression-defining event)	NR (100% experienced a progression defining event)	0.73 (0.44 to 1.22; p=NR)	
Lee et al (2019)[45.] (TOPICAL)	VeriStrat 'Good' (n=288)	VeriStrat 'Poor' (n=239)	VeriStrat 'Good' vs 'Poor'	Erlotinib + ASC vs Placebo + ASC
OS	Median OS unadjusted for treatment NR Erlotinib (n=164): 4.9 (NR) Placebo (n=124): 4.6 (3.3 to 6.9)	Median OS unadjusted for treatment NR Erlotinib (n=115): 3.1 (NR) Placebo (n=124): 2.9 (2.3 to 3.5)	0.58 (0.48 to 0.70; p<.001) For erlotinib: 0.60 (0.47 to 0.77; p<.001) For placebo: 0.54 (0.41 to 0.71; p<.001)	0.93 (0.87 to 1.11; p=.41) For *EGFR*-variant positive vs wild-type: 0.53 (0.33 to 0.83; p=.006)
PFS	Median PFS unadjusted for treatment NR Erlotinib (n=164): 2.9 (NR) Placebo (n=124): 2.8 (NR)	Median PFS unadjusted for treatment NR Erlotinib (n=115): 2.2 (NR) Placebo (n=124): 2.2 (NR)	0.67 (0.56 to 0.81; p<.001) For erlotinib: 0.70 (0.55 to 0.89; p=.004) For placebo: 0.66 (0.51 to 0.85; p=.001)	0.85 (0.71 to 1.02; p=.51) For *EGFR*-variant positive vs wild-type: 0.65 (0.42 to 1.01; p=.06)

ASC: active supportive care; CI: confidence interval; HR: hazard ratio; NR: not reported; NSCLC: non-small-cell lung cancer; OS: overall survival; PFS: progression-free survival.

Table 8. Clinical Validity - Study Relevance Limitations for Proteomic Testing in NSCLC to Predict Response to Therapy

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Gregorc et al (2014)[39.] (PROSE)	2.Table 5 reports other drug interventions used as third-line treatment without protocol information 4.Use of erlotinib (or other TKIs) in *EGFR*-variant wild-type or unknown population is not consistent	Other related: • Identity of proteins that make up the MALDI-MS features still being investigated at the time of publication		1. VeriStrat assay not used to direct clinical management. Other related: • Decision model based on outdated clinical pathway • Variable response assessment	

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
	with published treatment guidelines			times and intervals	
Peters et al (2017)[40] (EMPHASIS-lung Trial)	1. Accrual terminated 3. PROSE (Gregorc et al [2014]) squamous cell cohort not described	Other related: • Identity of proteins that make up the MALDI-MS features still being investigated at the time of publication		1. VeriStrat assay not used to direct clinical management. Other related: • Decision model based on outdated clinical pathway for treatment of squamous cell histology • Variable response assessment times and intervals • Incomplete data on PROSE squamous cell cohort	
Lee et al (2019)[45] (TOPICAL)	4. Use of erlotinib in *EGFR*-variant wild-type or unknown population is not consistent with published treatment guidelines, including patients with poor performance status or comorbidities			1. VeriStrat assay not used to direct clinical management. Other related: • Decision model based on outdated clinical pathway • Response assessment times and intervals unclear	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
EGFR: epidermal growth factor receptor; MALDI-MS: matrix-assisted laser desorption ionization mass spectrometry; NSCLC: non-small-cell lung cancer; TKI: tyrosine kinase inhibitor.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 9. Clinical Validity - Study Design and Conduct Limitations for Proteomic Testing in NSCLC to Predict Response to Therapy

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Gregorc et al (2014)[39.] (PROSE)						Other related: • Included variables not explicit for adjusted PFS comparing treatment groups
Peters et al (2017)[40.] (EMPHASIS-lung Trial)				Other related: Incomplete data on PROSE squamous cell cohort		1. Confidence intervals and/or p values not reported
Lee et al (2019)[45.] (TOPICAL)				1-2. Referenced study registry number does not describe published study.	Other related: • Unadjusted median OS for VeriStrat 'Good" vs "Poor" independent of treatment group not provided • Known *EGFR*-variant status characteristics not described according to treatment group	1. Confidence intervals and/or p values not reported. Other related: • Confidence that the VeriStrat classification is independent of *EGFR* variant status is limited by trend toward higher number of EGFR variant positive patients with VeriStrat 'Good" score among those with known mutation status

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
EGFR: epidermal growth factor receptor; OS: overall survival; PFS: progression-free survival.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Section Summary: Clinical Validity

No published studies were identified that assessed the prognostic use of VeriStrat proteomic testing in newly diagnosed stage I or II NSCLC.

For individuals with newly diagnosed advanced NSCLC without prior systemic therapy, 5 retrospective studies assessed the use of VeriStrat ("good" or "poor") as a prognostic test to discriminate between OS (primary outcome) and PFS (secondary outcome) using available samples from previously conducted clinical trials as validation of the classification. Classification based on proteomic testing (i.e., VeriStrat "good" vs "poor") was associated with survival outcomes in analyses that were primarily unadjusted for clinical and patient factors known to be associated with disease survival. The evidence is limited by heterogeneity in the patient population characteristics such as histology and the treatment regimens used. The treatment regimens using EGFR TKIs represent an outdated clinical decision model. The populations studied were unselected for *EGFR*-sensitizing variants or unknown variant status was excluded. The use of erlotinib (or other TKIs) in *EGFR* variant-negative or unknown population is no longer an accepted treatment approach. Combination EGFR plus VEGF inhibition therapy is not an accepted treatment approach. The disposition of indeterminate proteomic test results varied, and sample sizes in the classification groups were small. There is a single observational, nonrandomized study with prospective sample collection for proteomic testing before NSCLC treatment; it reported PFS as the primary outcome. This is the only study that included a first-line treatment consistent with current guidelines-based recommendations (platinum-doublet-based chemotherapy with cisplatin or carboplatin in combination with pemetrexed). Participant recruitment was nonrandom from a single lung cancer treatment unit. Adjusted analyses for PFS and OS did not include age or other sensitizing variants (*EGFR*, *ALK*), although data were reported. Overall, sample sizes in classification groups were small and limited generalizability.

For individuals with advanced NSCLC that was recurrent or had advanced on prior systemic therapy, retrospective studies have assessed the use of VeriStrat ("good" or "poor") as a prognostic test to discriminate between OS (primary outcome) and PFS (secondary outcome) using available samples from previously conducted clinical trials as validation of the classification. None of the trials from which the samples for VeriStrat proteomic classification were derived used a therapy consistent with current guidelines-based recommendations. The populations in all studies were unselected for *EGFR*-variant status. One study used pre- and posttreatment proteomic test scores and added an indeterminate result to the "good" result data pool.

One additional retrospective study (Grossi et al [2018]) has limited evidentiary value. It combined the previously reported single prospective study cohort with results from 2 cohorts that are only referenced in abstract form.

No published studies were identified that assessed the use of VeriStrat proteomic testing to inform treatment options in newly diagnosed stage I or II NSCLC.
No published studies were identified that assessed the use of VeriStrat proteomic testing to inform treatment options for newly diagnosed advanced NSCLC patients who had not received prior systemic therapy.

The literature on the predictive value of proteomic testing consists of 2 RCTs in patients with advanced NSCLC who failed first-line chemotherapy. The 2 RCTs demonstrated that classification based on proteomic testing (i.e., VeriStrat "good" vs "poor") is associated with

survival outcomes. The evidence is limited by heterogeneity in the treatment regimens used and patient population characteristics. In the PROSE RCT, for patients classified as VeriStrat "good," there were no significant differences in OS between the erlotinib and chemotherapy groups; however, for patients classified as VeriStrat "poor," there was a significantly longer median OS in patients in the erlotinib group. In the EMPHASIS trial, there were no significant differences in PFS or OS among patients with VeriStrat "good" status receiving erlotinib or chemotherapy or among patients with VeriStrat "poor" status receiving erlotinib or chemotherapy. Moreover, in both the PROSE and EMPHASIS RCTs, there were no significant benefits to PFS or OS with erlotinib treatment compared with chemotherapy overall, making the application of VeriStrat in this population uncertain.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

The proposed clinical utility of VeriStrat is for use by physicians to predict expected survival for standard therapies in the treatment of patients with NSCLC. Clinical utility is also proposed for physicians to use VeriStrat to select patients for systemic therapy based on the presence or absence of *EGFR*-sensitizing variants. Direct evidence from studies that demonstrate improved outcomes for patients managed with a strategy that includes proteomic testing compared with a strategy that does not, is not available for use of proteomic testing to select targeted therapy or other systemic therapy for NSCLC. Confidence that the proteomic classifier is independent of *EGFR*-variant status, as well as other tumor and patient characteristics, has not been demonstrated and, thus, VeriStrat lacks clinical validity. The identity of the proteins that make up the MALDI-MS features was still being investigated at the time of publication of the studies for both prognostic and predictive uses, further challenging the specificity for malignant biologic processes and conditions.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Absent direct evidence, a chain of evidence could be used to support the use of VeriStrat to select patients for EGFR TKI therapy. If EGFR TKI therapy were used as a standard of care in patients with unknown or negative *EGFR* status in the first-, second-, or third-line settings, proteomic testing could be used to select patients who are least likely to benefit. However, the IUNO trial did not find that erlotinib was efficacious in patients with NSCLC with no known *EGFR* variant, and the PROSE and EMPHASIS trials found that OS did not differ significantly for patients with advanced NSCLC treated with second-line erlotinib or chemotherapy. There were mixed findings on PFS in the PROSE and EMPHASIS trials. Due to study findings and the lack of support from guidelines for EGFRTKIs in this setting, EGFR TKI therapy is no longer standard therapy for any *EGFR*-negative or -unknown patients.

Platinum-based chemotherapy and immunotherapy (based on programmed death-ligand 1 testing) are the guidelines-based options for previously untreated advanced *EGFR*-negative or -unknown patients with NSCLC or those with recurrent NSCLC or who have progressed on prior systemic therapy.

The available evidence does not demonstrate that the addition of a VeriStrat proteomic classification of "good" or "poor" to the standard clinical assessment of prognosis would influence treatment or define a treatment pathway. Similarly, there is no evidence to demonstrate the impact of the substitution of a VeriStrat proteomic classification in the standard of care treatment pathways. The negative predictive value of a VeriStrat "poor" score has not been demonstrated; there has been no validation in patients who received no or surgical therapy only.

Although studies of physician decision making using VeriStrat proteomic testing have been reported; they did not evaluate patient outcomes and did not evaluate the impact of *EGFR* testing on treatment recommendations (the number of patients who had previously received *EGFR* tests was not reported). Thus, these studies are insufficient to demonstrate clinical utility.

Two studies have evaluated the impact of VeriStrat testing on physician treatment recommendations. Akerley et al (2013) reported on 226 physicians who provided pre- and post-test treatment plan information for 403 VeriStrat tests.[46,] In the 262 cases where pretreatment recommendations were for erlotinib only, for those patients who were classified as VeriStrat "poor," physicians recommended erlotinib in 13.3%. In a larger study, Akerley et al (2017) reported on 2411 physicians who received 14327 VeriStrat test results.[47,] The investigators only included tests that were ordered for NSCLC, were ordered as the sole test, were not indeterminate, and were not ordered in patients with known *EGFR*-variant status. VeriStrat findings were a classification of "good" for 1950 (78.2%) patients and "poor" for 544 (21.8%) patients. After receiving the test results, physicians changed their treatment recommendations in 28.2% of the cases; within this group, 13.2% were classified as VeriStrat "good" and 81.6% as VeriStrat "poor." Physicians initially considered treatment with an EGFR TKI in 484 (89.0%) of 544 classified as VeriStrat "poor"; after receiving test results only, 49 (10%) were actually recommended EGFR TKI treatment.

Section Summary: Clinically Useful

No direct evidence for a serum proteomic test for the selection of an NSCLC treatment strategy was identified. In the absence of direct evidence, a chain of evidence could be developed to support the use of VeriStrat to select patients for EGFR TKI therapy. If EGFR TKI therapy were used as a standard of care in patients with EGFR-unknown or wild-type status in the first-, second-, or third-line settings, proteomic testing could be used to identify patients who are least likely to benefit. However, given the evidence from the available trials and the lack of support from guidelines for EGFR TKIs in this setting, EGFR TKI therapy is no longer standard therapy for any patient with wild-type or unknown EGFR-variant status. There are no studies that have directly evaluated the use of the proteomic classification to inform treatment selection based on current treatment pathways that consider other targeted therapy, chemotherapy, or immunotherapy options. Two studies by the same research group evaluated changes in treatment recommendations before and after receiving VeriStrat test results; patient outcomes were not reported.

SUMMARY OF EVIDENCE

For individuals with newly diagnosed NSCLC and wild-type *EGFR*-variant status who receive management with a serum proteomic test to predict survival and select treatment, the evidence includes retrospective studies and a prospective nonrandomized study. Relevant outcomes are overall survival (OS), disease-specific survival, and treatment-related mortality and morbidity. No published studies were identified that assessed the prognostic use of VeriStrat proteomic testing in newly diagnosed stage I or II NSCLC. For individuals with newly diagnosed advanced NSCLC and *EGFR*-negative variant status without prior systemic therapy, 5 studies have assessed the use of VeriStrat ("good" or "poor") as a prognostic test to discriminate between OS (primary) and progression-free survival (PFS) (secondary) outcomes. All studies were retrospective and intended to validate the extent to which the VeriStrat proteomic classification correlated with OS or PFS. Only 1 of the 5 studies reported the percentage of participants who were *EGFR*-negative, but it did not report outcomes based on variant status. One observational, nonrandomized study with prospective sample collection for proteomic testing before NSCLC treatment reported the percentage of participants who were *EGFR*-negative, but it did not report outcomes based on variant status. This was also the only study that included a first-line treatment consistent with current guideline-based recommendations -- platinum-doublet-based chemotherapy plus cisplatin or carboplatin plus pemetrexed. The VeriStrat classification was not used to direct the selection of treatment in any of the clinical trials from which the validation samples were derived. Disposition of populations with variant status "not reported" was generally not clear and could not be construed as "unknown" when wild-type or positive were reported. No studies were identified that used VeriStrat proteomic testing to inform therapeutic options for patients with stage I or II NSCLC if surgery or surgery plus radiotherapy have been completed or who were upstaged as a result of surgical findings. No studies were identified that used VeriStrat proteomic testing to inform therapeutic options for patients with stage I or II NSCLC who were considered medically inoperable. No studies were identified that used VeriStrat proteomic testing to predict response to first-line targeted therapies or first-line chemotherapy in patients with newly diagnosed advanced NSCLC. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with newly diagnosed NSCLC and unknown *EGFR*-variant status who receive management with a serum proteomic test to predict survival and select treatment, the evidence includes a randomized controlled trial (RCT), 4 retrospective studies, and a prospective study. Relevant outcomes are OS, disease-specific survival, and treatment-related mortality and morbidity. All study populations were either unselected for *EGFR*-variant status or status was expressly reported as unknown in conjunction with negative or positive status reports. None of the studies that reported unknown *EGFR*-variant status reported outcomes for the proteomic score based on unknown *EGFR*-variant status. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with NSCLC and wild-type *EGFR*-variant status and disease progression after first-line systemic therapy who receive management with a serum proteomic test to predict survival and select treatment, the evidence includes a RCT and a retrospective analysis. Relevant outcomes are OS, disease-specific survival, and treatment-related mortality and morbidity. No studies were identified that reported or analyzed outcomes using the proteomic test as a prognostic tool in *EGFR*-negative variant status populations. The evidence includes

an RCT (PROSE) using proteomic testing to predict response to erlotinib compared with chemotherapy as a second-line treatment for patients with stage IIIB or IV NSCLC, stratified by performance status, smoking history, treatment center, and (masked) pretreatment VeriStrat classification. In a multivariate model to predict OS, which included clinical characteristics and *EGFR*-variant status, VeriStrat classification was significantly associated with OS (hazard ratio for VeriStrat "good" vs "poor," 1.88; 95% confidence interval, 1.25 to 2.84; p=.003). However, 62% of the combined study population was *EGFR*-negative. A retrospective analysis was also performed on the MARQUEE trial, a phase 3 RCT in patients with stage IIIB or IV nonsquamous NSCLC, comparing the patient response to erlotinib in conjunction with either tivantinib or a placebo; patients were stratified by *EGFR* and *KRAS* variant status, sex, smoking history, and treatment history. Protocol treatments were subsequently discontinued by 93% of patients, and the trial discontinued after prespecified interim futility analysis. In a multivariate model to predict OS, which included clinical characteristics and *EGFR*-variant status, VeriStrat classification was significantly associated with OS (hazard ratio for VeriStrat "good" vs "poor," 0.52; 95% confidence interval, 0.40 to 0.67; p<.001). Ninety percent of the combined study population was *EGFR*-negative. An interaction between treatment and VeriStrat status was significant for multivariate analysis including *EGFR* status (p=.036) but not significant for multivariate analysis including both *EGFR* and *KRAS* variant status (p=.068). Currently, the use of erlotinib in patients unselected for the presence or absence of an *EGFR*-sensitizing variant is not standard clinical practice. It is recommended that variant status be determined, if not previously ascertained, before selecting treatment after progression or recurrence. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with NSCLC and unknown *EGFR*-variant status with disease progression after first-line systemic therapy who receive management with a serum proteomic test to predict survival and select treatment, the evidence includes 2 RCTs and 3 retrospective studies. Relevant outcomes are OS, disease-specific survival, and treatment-related mortality and morbidity. The use of VeriStrat as a prognostic test to discriminate between good and poor survival outcomes was assessed in 3 retrospective studies intended to validate the extent to which VeriStrat proteomic classification correlates with OS or PFS. The VeriStrat classification was not used to direct treatment selection in any of the trials from which the validation samples were derived. None of the clinical trials from which the samples for VeriStrat proteomic classification were derived used a therapy consistent with current guidelines-based recommendations. The populations in all 3 studies were unselected for *EGFR*-variant status. In the PROSE RCT, using a multivariate model to predict OS, which included clinical characteristics and *EGFR*-variant status, VeriStrat classification was significantly associated with OS (hazard ratio for VeriStrat "good" vs "poor," 1.88; 95% confidence interval, 1.25 to 2.84; p=.003). However, 32.6% of the combined study population had unknown *EGFR* status. In the EMPHASIS RCT, there were no significant differences in PFS or OS among patients with VeriStrat "good" status receiving erlotinib or chemotherapy or among patients with VeriStrat "poor" status receiving erlotinib or chemotherapy. The results of the EMPHASIS RCT were restricted to squamous NSCLC histology. Currently, the use of erlotinib in patients unselected for the presence or absence of an *EGFR*-sensitizing variant is not standard clinical practice. It is recommended that variant status be determined, if not previously ascertained, before selecting treatment after progression or recurrence. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 10.

Table 10. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03289780[a]	An Observational Study Assessing the Clinical Effectiveness of VeriStrat and Validating Immunotherapy Tests in Subjects With Non-Small Cell Lung Cancer	5,000	Dec 2025 (active, not recruiting)

NCT: national clinical trial
[a] Denotes industry sponsorship or co-sponsorship.

SUPPLEMENTAL INFORMATION

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network Guidelines

The National Comprehensive Cancer Network (v.2.2025) guidelines on the management of non-small-cell lung cancer (NSCLC) recommend routine testing for epidermal growth factor receptor (EGFR) variants in patients with metastatic nonsquamous NSCLC (category 1 recommendation) and consideration for EGFR-variant testing in patients with metastatic squamous NSCLC who were never smokers or with small biopsy specimens or mixed histology (category 2A recommendation).[1] Recommendations for first-line treatment for EGFR-positive patients with advanced or metastatic NSCLC, and EGFR-negative or -unknown patients as well as for patients in either category who have progressed on therapy are provided in the background section of this policy.

American Society of Clinical Oncology

The American Society of Clinical Oncology (2023) updated its clinical practice guidelines on systemic therapy for stage IV NSCLC.[48,49] The recommendation states: All patients with nonsquamous NSCLC should have the results of testing for potentially targetable mutations (alterations) before implementing therapy for advanced lung cancer, regardless of smoking status recommendations, when possible, following other existing high-quality testing guidelines. Most patients should receive targeted therapy for these alterations: Targeted therapies against ROS-1 fusions, BRAF V600e mutations, RET fusions, MET exon 14 skipping mutations, and NTRK fusions should be offered to patients, either as initial or second-line therapy when not given in the first-line setting. New or revised recommendations include the following: Osimertinib is the optimal first-line treatment for patients with activating epidermal growth factor receptor mutations (exon 19 deletion, exon 21 L858R, and exon 20 T790M); alectinib or brigatinib is the optimal first-line treatment for patients with anaplastic lymphoma kinase fusions. For the first time, to our knowledge, the guideline includes recommendations regarding RET, MET, and NTRK alterations. Chemotherapy is still an option at most stages.

The society also updated guidelines (2023) for therapy of stage IV NSCLC without driver alterations. The recommendations state: Recommendations apply to patients without driver alterations in epidermal growth factor receptor or ALK. For patients with high programmed death ligand 1 (PD-L1) expression (tumor proportion score [TPS] ≥ 50%) and non–squamous

cell carcinoma (non-SCC), the Expert Panel recommends single-agent pembrolizumab. Additional treatment options include pembrolizumab/carboplatin/pemetrexed, atezolizumab/carboplatin/paclitaxel/bevacizumab, or atezolizumab/carboplatin/nab-paclitaxel. For most patients with non-SCC and either negative (0%) or low positive (1% to 49%) PD-L1, the Expert Panel recommends pembrolizumab/carboplatin/pemetrexed. Additional options are atezolizumab/carboplatin/nab-paclitaxel, atezolizumab/carboplatin/paclitaxel/bevacizumab, platinum-based two-drug combination chemotherapy, or non–platinum-based two-drug therapy. Single-agent pembrolizumab is an option for low positive PD-L1. For patients with high PD-L1 expression (TPS ≥ 50%) and SCC, the Expert Panel recommends single-agent pembrolizumab. An additional treatment option is pembrolizumab/carboplatin/(paclitaxel or nab-paclitaxel). For most patients with SCC and either negative (0%) or low positive PD-L1 (TPS 1% to 49%), the Expert Panel recommends pembrolizumab/carboplatin/(paclitaxel or nab-paclitaxel) or chemotherapy. Single-agent pembrolizumab is an option in select cases of low positive PD-L1. Recommendations are conditional on the basis of histology, PD-L1 status, and/or the presence or absence of contraindications.[40]

Government Regulations
National/Local:

L35396, effective on or after 12/13/2020.

Novitas Solutions has established a local Medicare coverage determination for the VeriStrat in June 2013, which serves as a national coverage determination since the test is only offered at a single lab within the local carrier's coverage region. The coverage determination document notes that "The VeriStrat® assay (81538*)* is a mass spectrophotometric, serum-based predictive proteomics assay for NSCLC patients, where "first line" EGFR mutation testing is either wild-type or not able to be tested (e.g., if tissue might not be available)."[52]

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

N/A

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through January 2025, the date the research was complete.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
5/1/14	2/18/14	2/28/14	Joint policy established
7/1/15	4/21/15	5/8/15	Policy revamped BCBSA policy; title changed to mirror the BCBSA policy title. No change in policy status. Updated Medicare section to indicate coverage for Medicare advantage members effective June 2013.
5/1/16	2/16/16	2/16/16	Routine policy maintenance, updated references, added code 81538, effective 1/1/16.
11/1/16	9/29/16	9/28/16	Routine policy maintenance, updated references, policy status changed to established.
11/1/17	8/15/17	8/15/17	Updated rationale section. Added references # 10 and 24. No change in policy status.
3/1/18	2/20/18	2/20/18	Routine policy maintenance. No change in status.
5/1/19	2/19/19		Routine policy maintenance. Added references 38-40. No change in policy status.
5/1/20	2/18/20		Updated rationale, added references 41 and 42. No change in policy status.
5/1/21	2/16/21		Routine policy maintenance. No change in policy status.
5/1/22	3/11/22		Discussed information received from Biodesix and current NCCN guidelines. Status changed to E/I based on NCCN guidelines. Rationale section rewritten.
5/1/23	2/21/23		Routine policy maintenance, no new references added. No change in policy status. (ds)

5/1/24	2/20/24		Routine policy maintenance, no change in status. Vendor managed: N/A (ds)
5/1/25	2/18/25		Routine policy maintenance, no change in status. Vendor managed: N/A (ds)

Next Review Date: 1st Qtr. 2026

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: PROTEOMIC TESTING FOR TARGETED THERAPY IN NON-SMALL-CELL LUNG CANCER (NSCLC), E.G., VERISTRAT®

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Not Covered.
BCNA (Medicare Advantage)	See government section.
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

N/A