Lung Cancer 79 (2013) 59–64

Contents lists available at SciVerse ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

VeriStrat® has a prognostic value for patients with advanced non-small cell lung cancer treated with erlotinib and bevacizumab in the first line: Pooled analysis of SAKK19/05 and NTR528☆

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ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form 27 August 2012
Accepted 5 October 2012

Keywords:
Biomarker
Chemotherapy
EGFR inhibitor
Lung cancer
Personalized medicine
Proteomics

ABSTRACT

Background: VeriStrat® is a serum proteomic test used to determine whether patients with advanced non-small cell lung cancer (NSCLC) who have already received chemotherapy are likely to have good or poor outcomes from treatment with gefitinib or erlotinib. The main objective of our retrospective study was to evaluate the role of VS as a marker of overall survival (OS) in patients treated with erlotinib and bevacizumab in the first line.
Patients and methods: Patients were pooled from two phase II trials (SAKK19/05 and NTR528). For survival analyses, a log-rank test was used to determine if there was a statistically significant difference between groups. The hazard ratio (HR) of any separation was assessed using Cox proportional hazards models.
Results: 117 patients were analyzed. VeriStrat classified patients into two groups which had a statistically significant difference in duration of OS ($p = 0.0027$, HR = 0.480, 95% confidence interval: 0.294–0.784).
Conclusion: VeriStrat has a prognostic role in patients with advanced, nonsquamous NSCLC treated with erlotinib and bevacizumab in the first line. Further work is needed to study the predictive role of VeriStrat for erlotinib and bevacizumab in chemotherapy-untreated patients.

1. Introduction

VeriStrat (VS), a pre-treatment blood-based test correlated with clinical outcome after EGFR-TKI therapy in non-small cell lung cancer (NSCLC) patients, was developed and validated in a multi-institutional study of advanced NSCLC patients treated with gefitinib [1]. The VS algorithm was developed using a training set of pre-treatment serum samples from patients who experienced either long term stable disease or early progression on gefitinib therapy. Mass spectra from these patients' serum samples were used to define eight mass spectral features (i.e. peaks), differentiating these two outcome groups. An algorithm (VS) utilizing these features and based on a k-nearest neighbors (kNN) classification scheme was created, its parameters were optimized using additional spectra from the training cohort. All aspects of VS were frozen after development. VS assigns each test sample a label of "good" (VSG) or "poor" (VSP). It was validated in a blinded fashion on two independent patient cohorts treated with gefitinib or erlotinib. These studies confirmed that patients classified as VSG had better outcome than those classified as VSP (hazard ratio (HR)

☆ The results of this manuscript have been presented in part as an oral presentation at the European Lung Cancer Conference (ELCC) in Geneva on April 20, 2012 (abstract 800).

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http://dx.doi.org/10.1016/j.lungcan.2012.10.006

of death = 0.47, $p = 0.0094$ in one cohort; HR = 0.33 and $p = 0.0007$ in the other) [2]. VS demonstrated outstanding reproducibility, confirmed in the comparative analysis between data obtained in two different institutions (Vanderbilt University and University of Colorado, USA), the overall concordance of the classification results was 97% [1]. Concordance between classifications obtained using the identical VS test on matched serum and plasma samples has also been demonstrated [1]. Retrospective VS analysis performed on available plasma samples of a subset from the randomized NCIC CTG BR.21 trial confirmed the above results within the erlotinib treatment arm [3,4]. In the BR.21 substudy, there was no significant benefit of treatment with erlotinib over placebo for VSP, however there was for VSG. Separation in outcome between VS groups in the placebo arm demonstrated that VS has a significant prognostic component, indicating that VS measures an innate property of the disease. Furthermore, the BR.21 substudy showed a strong statistically significant correlation of VSG with objective response rate (ORR) and disease control rate (DCR) in the treatment arm (Fisher's exact test 2-sided $p = 0.0022$ for ORR and $p = < 0.001$ for DCR). This substudy also indicated independence of VS from EGFR FISH and mutation status. While these studies demonstrated the test's prognostic power and validated its ability to identify patients likely to have better or worse outcomes when treated with EGFR-TKIs, more recent studies have shown that the test also has predictive power to determine differential treatment benefit between VS groups [5,6].

Two published studies have investigated the utility of VS in patients treated with the combination of erlotinib with the anti-VEGF antibody bevacizumab. One study involved 35 advanced NSCLC patients treated in second-line [7] and the other 32 patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (SSCHN) [8]. The NSCLC study found that the group of patients classified as VSG had better OS and PFS than those classified as VSP (log-rank $p = 0.007$ for OS and 0.0003 for PFS). Only OS data were available for the SCCHN study and these also demonstrated improved outcomes for the VSG group compared with the VSP group (log-rank $p = 0.02$). The objective of our retrospective study was to evaluate in univariate and multivariate analyses the role of VS as a marker of overall survival (OS) and progression-free survival (PFS) in patients with untreated, advanced nonsquamous NSCLC receiving front-line therapy with erlotinib and bevacizumab.

An exploratory subproject of the phase II trial SAKK19/05 demonstrated a borderline significant difference ($p = 0.0844$) between VSG and VSP for OS (unpublished data on file at SAKK). SAKK19/05 tested erlotinib and bevacizumab first-line therapy in patients with untreated, advanced nonsquamous NSCLC [9]. To explore this preliminary finding, a pooled VS analysis of SAKK19/05 and NTR528 (a parallel Dutch phase II trial of first-line therapy with erlotinib and bevacizumab in patients with untreated, advanced nonsquamous NSCLC [10]) was performed.

2. Materials and methods

2.1. Patients and protocols

Frozen pretreatment serum samples and individual patient data from two comparable single-arm, multicenter, phase II trials, SAKK19/05 (NCT00354549) and NTR528, were combined for pooled analysis [9,10]. Both trials tested first-line therapy with erlotinib 150 mg daily and bevacizumab 15 mg/kg every 3 weeks in patients with chemotherapy-untreated advanced, nonsquamous NSCLC. Overall, 158 evaluable patients were enrolled (NTR528: 47 from 2006–2007; SAKK19/05: 101 from 2006–2009). The primary endpoint of NTR528 was rate of non-progression at 6 weeks, whereas it was the rate of disease stabilization (DS) at week 12 under erlotinib and bevacizumab for SAKK19/05. DS was measured every 6 weeks by computed tomography (CT) scan according to the Response Evaluation Criteria In Solid Tumors (RECIST version 1.0). Both trials included PFS (for SAKK19/05 the definition of time to progression (TTP) was equivalent to PFS, henceforth in this publication it will be referred to as PFS) and OS as secondary endpoints. PFS was calculated from registration until progression or death. OS was calculated from registration until death. Adjacent translational research projects included MRI and PET in NTR528 [11], and fresh-frozen tumor biopsies for gene expression arrays in SAKK19/05 [12,13]. Patient consent for biobanking and translational research was obtained at the time of enrollment. The current subproject was approved by the ethical committee of St. Gallen, Switzerland.

2.2. VeriStrat® testing

Aliquots of pre-treatment serum samples were shipped to Biodesix Inc. (USA) for VS testing. Samples were removed from the freezer and thawed on ice. A 10 μl aliquot of each sample was spotted onto a Whatman Human ID Bloodstain Card BFC180 (GE Healthcare Biosciences Corp.) and dried at room temperature for 1 h. Afterwards, a 3 mm punch of each sample was extracted in 50 μl of HPLC-grade water. The extracts were vortexed for 3 min. A 10 μl aliquot of each extract was transferred to a fresh 0.5 ml tube and combined 1:1 with sinapinic acid (25 mg/ml in 50% acetonitrile (Burdick & Jackson)); 0.1% trifluoroacetic acid (EMD Biosciences) and randomly spotted in triplicate on polished steel MALDI target plates (Bruker Daltonics). Mass spectra for all samples were generated in linear, positive-ion mode in an automated manner using the autoflex III MALDI-TOF spectrometer (Bruker Daltonics). Results from 2000 transient acquisitions were summed to generate each sample spectrum.

Spectral pre-processing was performed, including background (BG) and noise estimation, BG subtraction, normalization to partial ion current and alignment. The classification algorithm (VS) was based on eight distinct m/z features (5843, 11,446, 11,530, 11,685, 11,759, 11,903, 12,452 and 12,580 Da). The integrated intensities of these eight peaks were used as input for the fixed kNN classifier ($k = 7$), which returned a label of "good/VSG", "poor/VSP" or, in a few (<3%) equivocal cases, "indeterminate". This procedure was performed in a blinded manner by Biodesix Inc., VS results were sent to the SAKK Coordinating Center, where the prespecified statistical analyses were performed.

2.3. Statistical analyses

For survival analyses, a log-rank test was used to determine if there was a statistically significant difference between groups. The HR of any separation was assessed using Cox proportional hazards (CPH) models in the univariate and multivariate setting. The multivariate analysis was explored further using the stepwise selection method (criteria used: entry = 0.25; stay = 0.15). For continuous variables, a t-test was used to determine if there was a statistically significant difference between groups and for categorical variables, chi-square test or Fisher's exact test. In addition to the analyses stated above, a stratified log-rank test or Cox regression and stratified chi-squared test were also considered for the pooled analysis. All data analysis was performed using SAS 9.2 (SAS Institute Inc.) and R 2.11.1.

3. Results

3.1. Patient characteristics and VS results

Based on the availability of frozen pretreatment serum, 117 patients were evaluable for this analysis (SAKK19/05, $n = 88$; NTR528, $n = 29$) (Table 1). There was no selection bias