



VeriStrat[®] classifier for survival and time to progression in non-small cell lung cancer (NSCLC) patients treated with erlotinib and bevacizumab

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ARTICLE INFO

Article history:

Received 26 October 2009

Received in revised form

23 November 2009

Accepted 29 November 2009

Keywords:

Lung cancer

Erlotinib

Bevacizumab

Serum biomarker

Proteomics

Personalized therapy

ABSTRACT

We applied an established and commercially available serum proteomic classifier for survival after treatment with erlotinib (VeriStrat[®]) in a blinded manner to pretreatment sera obtained from recurrent advanced NSCLC patients before treatment with the combination of erlotinib plus bevacizumab. We found that VeriStrat[®] could classify these patients into two groups with significantly better or worse outcomes and may enable rational selection of patients more likely to benefit from this costly and potentially toxic regimen.

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1. Introduction

Inhibition of the epidermal growth factor (EGFR) pathway with erlotinib improves survival compared to placebo for patients with advanced lung cancer [1], and antibodies against VEGF improve survival when combined with chemotherapy [2,3]. Response rates and progression-free survival (PFS) in unselected patients treated with both erlotinib and bevacizumab are much higher than those in unselected patients treated with erlotinib alone, suggesting significant activity for this combination [4]. However, many patients are exposed to toxicities without evident clinical benefit and these patients may be better served by earlier access to alternative therapies. This has encouraged an intense search for biomarkers of clinical significance for this and other targeted therapies. In the Iressa Non-small cell lung cancer Trial Evaluating REsponse and Survival against Taxotere (INTEREST) trial, in which the patients were randomized between gefitinib and docetaxel, no statistically significant prediction of survival benefit was seen for any of the biomarkers tested, including EGFR expression and mutation, EGFR gene amplification, or *ras* mutation [5]. These biomarkers are also

present only in a small minority of patients with NSCLC, and require the availability of a significant amount of fresh tumor tissue for analysis; thus none are adequate to practically stratify patients who can derive survival benefit from erlotinib-based therapy in a western population. Furthermore, there are no validated biomarkers for benefit from bevacizumab therapy [6,7]. Better predictive tools are thus needed to guide and optimize treatment decisions to maximize treatment benefits while minimizing cost and toxicity.

Recently, we reported a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) proteomic signature (VeriStrat[®]), comprised of eight protein features, that was able to classify patients for improved PFS and overall survival (OS) after treatment with EGFR-tyrosine kinase inhibitor (TKI) therapy but not with chemotherapy [8]. This signature was validated in two independent cohorts treated with gefitinib or erlotinib. In this study, we tested whether VeriStrat[®] could also predict outcome in an independent multi-institutional cohort of patients treated with erlotinib in combination with bevacizumab.

2. Materials and methods

Mass spectrometry was performed on 35 available pretreatment serum samples from an open-labeled, phase I/II study ($n = 40$)

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Table 1
Clinical characteristics of the patients analyzed.

Characteristics	Bevacizumab/erlotinib (n = 35 ^a)	Good	Poor
Sex (%)			
Male	14 (40)	11 (42)	3 (37.5)
Female	21 (60)	15 (58)	5 (62.5)
Age (years)			
Median	59	58	63
Range	36–72	36–72	49–72
Stage			
IIIB	6	5	0
VI	27	19	8
Recurrent			
RECIST (%)			
Partial response	8	8	0
Stable disease	21	14	6
<16 weeks	11	8	5
>16 weeks	10	6	1
Progressive disease	6	4	2

^a Stage was not available for two of the patients classified as “good”.

in which the patients were treated with erlotinib in combination with bevacizumab. All patients included in this study were diagnosed with NSCLC, were previously treated with chemotherapy, had good performance status (0–1), stage IIIB (with pleural effusion) or stage IV, and nonsquamous histology (Table 1). Additional details regarding patient demographics were described previously [9]. The previously developed algorithm was applied in a fully blinded manner to the patients’ serum samples. Serum aliquots were diluted 1:20 in a saturated sinapinic acid solution (35 mg/ml sinapinic acid (Sigma, St. Louis, MO); 50% acetonitrile (Burdick & Jackson, Muskegon, MI); and 0.1% trifluoroacetic acid (Sigma, St. Louis, MO)) and randomly spotted in triplicate on gold, 100-well, sample plates. Mass spectra for all samples were generated in a linear mode and in an automated manner using the Voyager-DE STR™ workstation. Results from 500 to 525 independent spectrum acquisitions for each sample were averaged to generate each spectrum. Raw spectra were coded and sent electronically to Bidesix (Steamboat Springs, CO). Spectral pre-processing was performed, which included background

(BG) and noise estimation, BG subtraction, normalization to partial ion current and alignment [8]. The classification algorithm (VeriStrat®) was based on eight distinct *m/z* features (5843, 11446, 11530, 11685, 11759, 11903, 12452 and 12580 Da) [8]. The identities of these features and their underlying biological significance are currently under investigation. The integrated intensities of these eight peaks were used as input for the fixed *k*NN classifier (*k* = 7), which then returned a label, either “good” or “poor”. The procedure was identical to the one described in Taguchi et al. [8]. The entire procedure was performed in a fully blinded manner, i.e. all clinical data were kept blinded until the classification into good and poor groups had been obtained. The log-rank test was used to determine whether progression-free survival and overall survival of the two groups (“good” and “poor”) were statistically different. All statistical calculations and graphs were generated using PRISM 5 (GraphPad Software, La Jolla, CA).

3. Results

From the available 35 samples with associated with clinical data, we generated 276 spectra, with 5–9 replicates per sample. A concordant classification among the replicates was obtained, except in one patient. In this sample, of the 7 spectra obtained, 3 were classified as “good” and 4 were classified as “poor”, likely due to tiny variations in the replicates in this borderline case. This patient was classified as “undefined” and excluded from the survival analyses.

Representative baseline corrected spectra for one “good”- (upper panel) and one “poor”-patient (lower panel) are shown in Fig. 1A. Fig. 1B displays the mean peak intensities of the eight mass spectral features used in the classifier from all 35 patients. Higher mean peak intensities are observed in the “poor” group when compared with the group that was classified as “good”. While this demonstrates that each feature is individually associated with the classification, classifiers based on only single features (peaks) lose substantial predictive power and robustness, as previously reported [8].

The group that was classified as “poor” had statistically significant lower overall survival and progression-free survival (log

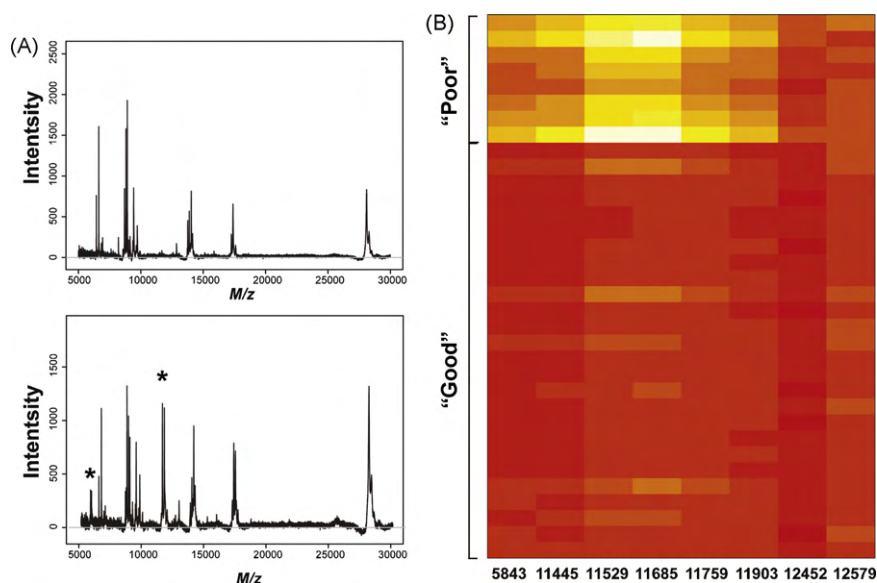


Fig. 1. (A) Representative spectra from a patient classified as “good” (upper panel) versus another patient who was classified as “poor”. A peak around 6000 Da and another cluster of peaks in the 11,000–12,000 Da range are observed in the spectrum from a patient who was classified as “poor”. (B) A heatmap is used to visually summarize peak intensities of the eight peaks in the VeriStrat algorithm from the cohort treated with bevacizumab and erlotinib. Differences can be observed between the patients who were classified as “poor” and those who were classified as “good”.

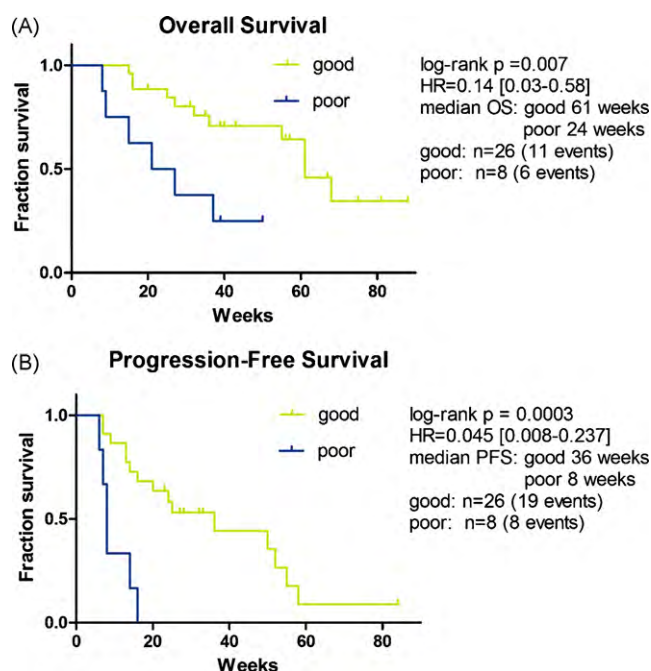


Fig. 2. Kaplan–Meier analysis is used to plot survival curves of patients treated with both bevacizumab and erlotinib according to “good” or “poor” classification. There was a statistically significant difference in progression-free survival and overall survival as determined by the log-rank test.

rank $p = 0.007$ and $p = 0.0003$, respectively) than the group that was classified as “good” (Fig. 2). For the “good” outcome group, the median OS time was 61 weeks compared to the 24 weeks for the group that classified as “poor” (hazard ratio (HR) = 0.14, 95% confidence interval (CI) = 0.03–0.58). Median PFS was 36 weeks for the “good” outcome group versus 8 weeks for the “poor” outcome group (HR = 0.045, 95% CI = 0.008–0.237).

4. Discussion

Identification of biomarkers is important to select the patients most likely to benefit from specific targeted therapies. It is also crucial for these biomarkers to be validated and be reproducible between independent studies and patient cohorts. We have shown in our current study that VeriStrat[®], which was developed on spectra from patients treated with single agent gefitinib, can accurately classify this new cohort of patients, who received both erlotinib and bevacizumab, into good and poor survival groups. We have previously shown that this classifier was not predictive of clinical outcome in two cohorts of patients treated with chemotherapy alone and another cohort treated with surgery alone, suggesting that VeriStrat’s predictive power is specific to treatment with EGFR TKIs. It is thus interesting that survival is accurately classified with the combination of erlotinib and bevacizumab, which yields a significantly improved response rate and progression-free survival compared to erlotinib alone. This observation may reflect the fact that the improved clinical outcome of the combination results from a synergy of these agents through a common mechanism (i.e. enhancement of erlotinib activity). This is supported by the fact that no clinical activity has been observed in lung cancer with bevacizumab alone. In our current study, the observed hazard ratio between “good” and “poor” groups for OS is 0.14, which is substantially smaller than the hazard ratio reported for EGFR-TKI monotherapy [8], though the current sample size ($n = 35$) is small and the confidence intervals for the two predictions have some overlap.

Confidence in our conclusions is increased by another study using the same samples in which an academic statistical group derived a classifier containing many of the same spectral features [10]. The present study, however, has significant immediate clinical implications, as VeriStrat is now performed in a CLIA-certified laboratory with strict quality control and is commercially available for clinical use.

5. Conclusions

We have demonstrated that an established proteomic classifier based on MALDI MS of pretreatment serum samples can accurately classify patients into two groups differing in their survival benefit from treatment with the clinically active combination of erlotinib and bevacizumab. The classifier has demonstrated no such ability on pretreatment samples in multiple cohorts treated with chemotherapy or surgery, suggesting that this classifier may be predictive and not prognostic. The biology underlying this classifier is unclear, but is under investigation and may reflect the activity of EGFR ligand-releasing proteases on abundant serum proteins. The study involves only a limited number of patients, and yet finds highly statistically significant differences. Further validation with larger cohorts and randomized prospective trials is needed, but our findings suggest that this mass spectrometry-based serum classifier may have clinical utility for selecting patients most likely to have improved survival after treatment with this costly and occasionally toxic but potentially effective, regimen.

Conflict of interest statement

David Carbone is an unpaid advisor to BioDesix, but holds no patents or ownership interest with them.

Heinrich Roder, Joanna Roder, and Maxim Tsybin are Biondesix employees who performed the proteomic classification.

Acknowledgements

This study was funded by the Vanderbilt Lung Cancer SPORE (P50 CA-90949) and SPECS (UO1 CA114771) grants.

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