The value of proteomics in lung cancer

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Abstract: Many studies have identified the prognostic and predictive value of proteins or peptides in lung cancer but most failed to provide strong evidence for their clinical applicability. The strongest predictive proteins seem to be fatty acid-binding protein heart (H-FABP), and the 8-peak mass spectrography signature of VeriStrat. When focusing on VeriStrat, a 'VeriStrat good' profile did not discriminate between chemotherapy and erlotinib. The 'VeriStrat poor' profile showed a better outcome to chemotherapy than to erlotinib. VeriStrat is a prognostic test and only the "poor profile" discriminates for the type of therapy that should be chosen. Whether it adds useful information in patients with advanced non-small cell lung cancer (NSCLC) and wild type EGFR mutations is still doubtful. The position of the VeriStrat test in clinical practice is still not clear and we are waiting for prospective studies where biomarker test are involved in clinical decision.

Keywords: Non-small cell lung cancer (NSCLC); proteomics; VeriStrat

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What do we know about proteomics in lung cancer?

Proteomics is the study of hundreds or even thousands of proteins and/or peptides in cells or organisms. Different studies have been performed to identify the prognostic and predictive value of proteins or peptides in lung cancer. Protein expression depends on transcriptional, translational and post-translational levels and can vary over a large range. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) and two-dimensional gel electrophoresis are commonly used techniques that detect hundreds of low-molecular weight and abundance proteins. Reproducibility and a large number of unidentified signals are known problems. More novel approaches with a better reproducibility is the high-throughput peptide sequence identification by multidimensional liquid chromatography tandem MS that can be used in tumour tissue, pleural fluid and plasma (1,2).

In a large set of blood derived proteins, acute phase reactant proteins are prominently present. For example macrophage migration inhibitory factor (MIF) and cyclophilin A (CyP-A) have been found in tissue (3), haptoglobine (HP) and α-1-antitrypsin (A1AT) have been identified amongst others as a diagnostic in serum (4).

Prognostic biomarkers have all been studied in tissue samples. Mostly factors predicting a poor prognosis have been found. Several markers for different types of lung carcinoma were identified. Examples are cytokeratines, heat shock proteins and annexins (5).

Predictive protein profiles have been identified as markers that can predict outcome on treatment in patients (6). In addition other markers have been identified as being predictive, e.g., fatty acid-binding protein heart (H-FABP), for patients treated with gefitinib (7). The other more known predictive proteomic assay is the 8-peak mass spectrography signature (VeriStrat) (8).

What do we learn from predictive proteomics in lung cancer?

Okano *et al*. found in plasma in advanced non-small cell lung cancer (NSCLC) nine spots using MS, which corresponded with nine gene products (Ig mu chain C region, Ig α-1 chain C region, SNX6, Cytoplasmic antiproteinase 3, Macrophage capping Protein, Sulfatase modifying factor 2, glutathione S-transferase P, ferritin

heavy chain, H-FABP), in a group of patients who responded to gefitinib treatment. However most of the patients that responded to gefitinib had an EGFR activating mutation, both in the study cohort and the validation group (7). Therefore it seems that the identified proteins found in this study, do not have any added value to mutation analysis.

Taguchi *et al*. identified eight peaks (5843, 11446, 11530, 11685, 11759, 11903, 12452 and 12580 Da) using MALDI MS, that are a predictive serum markers for a good or poor response to EGFR-TKI (8). This assay, also known as the VeriStrat essay, is under patent; therefore identification of the proteins involved is not publicly known. A single-arm phase II study of erlotinib in first-line advanced lung cancer (eastern cooperative oncology group 3503) showed that patients with a 'VeriStrat good' signature had a better overall survival (OS) than patients with a 'VeriStrat poor' signature (HR 0.36; 95% CI, 0.21-0.60; P=0.001) (9). However, in 155/239 patients mutational analysis on EGFR failed. Therefore, also this study may have been biased with activating EGFR mutations.

These results were confirmed in a study by Carbone *et al*., who treated patients with erlotinib and bevacizumab. Here also the patients with a MS outcome of 'VeriStrat good' had a better OS compared to the 'VeriStrat poor' group (HR 0.14; 95% CI, 0.03-0.58; P=0.007) (10). An Italian study showed comparable results (11).

In the NCIC BR.21 trial, patients with advanced NSCLC received either erlotinib or placebo. Retrospectively analysed the placebo group patients with 'VeriStrat good' signature had a far better outcome on OS compared to 'VeriStrat poor'. Both groups good and poor had benefited from treatment with erlotinib compared to placebo (12). This means that VeriStrat is a prognostic biomarker, rather than a predictive marker. The prognostic value of the VeriStrat test in advanced NSCLC has been observed in studies with combinations of targeted agents both for sorafenib or bevacizumab in combination with erlotinib (13,14). The prognostic test characteristics were further confirmed by a pooled analysis of two phase II trials (SAKK19/05 and NTR528) (15).

VeriStrat did not predict chemotherapy outcome. In a phase II study where gemcitabine was compared to erlotinib or gemcitabine/erlotinib in elderly patients, VeriStrat only was predictive for the groups who also received erlotinib in the treatment regimen (16).

A recent meta-analysis of the above mentioned studies concluded however, after pooling the data, that VeriStrat is a predictive factor for tumour response to EGFR-TKI (17).

The PROSE study, a biomarker stratified phase III trial comparing 2nd line chemotherapy to erlotinib, added some new findings regarding VeriStrat. An OS of 9.0 months (95% CI, 6.8-10.9) was found in the chemotherapy group compared to 7.7 months (95% CI, 5.9-10.4) in the erlotinib arm. Stratifying for 'VeriStrat good' showed comparable OS between chemotherapy and erlotinib (10.9 months; 95% CI, 8.4-15.1 *vs*. 11.0 months; 95% CI, 9.2-12.9). In the 'VeriStrat poor' group a far worse outcome on treatment has been found, especially for the erlotinib treated patients (6.4 months; 95% CI, 3.0-7.4 *vs*. 3.0 months; 95% CI, 2.0-3.8). According to the article OS results remained similar if the 14 patients with an activating EGFR mutation were excluded (18). Therefore we can conclude that the VeriStrat is a prognostic test and only a predictive test for the VeriStrat poor profile. These patients should be treated with chemotherapy. The EMPHASIS study of ETOP was designed to explore the predictive ability of the VeriStrat signature, by testing for interaction between erlotinib *vs*. docetaxel and VeriStrat status using progression-free survival as primary outcome. The study was prematurely closed.

How should we treat patients according to predictive blood-borne biomarker?

Summarizing the data, 'VeriStrat poor' patients should not be treated with an EGFR-TKI. Patients with a 'VeriStrat good' signature have better survival outcomes independent of treatment. This implies that we could test every wild type EGFR patient with VeriStrat and treat 'the poor' profile with chemotherapy. Until further validation studies have been performed with biomarkers as clinical decision tool, there is yet no place for these biomarker tests in clinical practice.

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