Predictive value of a proteomic signature in patients with non-small-cell lung cancer treated with second-line erlotinib or chemotherapy (PROSE): a biomarker-stratified, randomised phase 3 trial

Vanesa Gregorc, Silvia Novello, Chiara Lazzari, Sandro Barni, Michele Aieta, Manlio Mencoboni, Francesco Grossi, Tommaso De Pas, Filippo de Marinis, Alessandra Bearz, Irene Floriani, Valter Torri, Alessandra Bulotta, Angela Cattaneo, Julia Grigorieva, Maxim Tsypin, Joanna Roder, Claudio Doglioni, Matteo Giaj Levra, Fausto Petrelli, Silvia Foti, Mariagrazia Viganò, Angela Bachi, Heinrich Roder

Summary

Background An established multivariate serum protein test can be used to classify patients according to whether they are likely to have a good or poor outcome after treatment with EGFR tyrosine-kinase inhibitors. We assessed the predictive power of this test in the comparison of erlotinib and chemotherapy in patients with non-small-cell lung cancer.

Methods From Feb 26, 2008, to April 11, 2012, patients (aged ≥18 years) with histologically or cytologically confirmed, second-line, stage IIIB or IV non-small-cell lung cancer were enrolled in 14 centres in Italy. Patients were stratified according to a minimisation algorithm by Eastern Cooperative Oncology Group performance status, smoking history, centre, and masked pretreatment serum protein test classification, and randomly assigned centrally in a 1:1 ratio to receive erlotinib (150 mg/day, orally) or chemotherapy (pemetrexed 500 mg/m², intravenously, every 21 days, or docetaxel 75 mg/m², intravenously, every 21 days). The proteomic test classification was masked for patients and investigators who gave treatments, and treatment allocation was masked for investigators who generated the proteomic classification. The primary endpoint was overall survival and the primary hypothesis was the existence of a significant interaction between the serum protein test classification and treatment. Analyses were done on the per-protocol population. This trial is registered with ClinicalTrials.gov, number NCT00989690.

Findings 142 patients were randomly assigned to chemotherapy and 143 to erlotinib, and 129 (91%) and 134 (94%), respectively, were included in the per-protocol analysis. 88 (68%) patients in the chemotherapy group and 96 (72%) in the erlotinib group had a proteomic test classification of good. Median overall survival was 9·0 months (95% CI 6·8–10·9) in the chemotherapy group and 7·7 months (5·9–10·4) in the erlotinib group. We noted a significant interaction between treatment and proteomic classification ($p_{interaction}$=0·017 when adjusted for stratification factors; $p_{interaction}$=0·031 when unadjusted for stratification factors). Patients with a proteomic test classification of poor had worse survival on erlotinib than on chemotherapy (hazard ratio 1·72 [95% CI 1·08–2·74], p=0·022). There was no significant difference in overall survival between treatments for patients with a proteomic test classification of good (adjusted HR 1·06 [0·77–1·46], p=0·714). In the group of patients who received chemotherapy, the most common grade 3 or 4 toxic effect was neutropenia (19 [15%] *vs* one [<1%] in the erlotinib group), whereas skin toxicity (one [<1%] *vs* 22 [16%]) was the most frequent in the erlotinib group.

Interpretation Our findings indicate that serum protein test status is predictive of differential benefit in overall survival for erlotinib versus chemotherapy in the second-line setting. Patients classified as likely to have a poor outcome have better outcomes on chemotherapy than on erlotinib.

Funding Italian Ministry of Health, Italian Association of Cancer Research, and Biodesix.

Lancet Oncol 2014; 15: 713–21

Published **Online** May 13, 2014 http://dx.doi.org/10.1016/S1470-2045(14)70162-7

See **Comment** page 671

Department of Medical Oncology, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale San Raffaele, Milan, Italy (V Gregorc MD, C Lazzari MD, A Bulotta MD, A Cattaneo, S Foti MD, M Viganò MD, A Bachi PhD); **Department of Oncology, University of Turin, Azienda Ospedaliera Universitaria San Luigi Orbassano, Turin, Italy** (S Novello MD, M Giaj Levra MD); **Division of Medical Oncology, Department of Medical Oncology, Azienda Ospedaliera Treviglio, Treviglio, Italy** (S Barni MD, F Petrelli MD); **Division of Medical Oncology, Centro di Riferimento Oncologico di Basilicata, Istituto di Ricovero e Cura a Carattere Scientifico, Rionero in Vulture, Italy** (M Aieta MD); **Oncology Unit, Villa Scassi Hospital, Azienda Sanitaria Locale 3, Genoa, Italy** (M Mencoboni MD); **Lung Cancer Unit, Istituto di Ricovero e Cura a Carattere Scientifico, Azienda Ospedaliera Universitaria San Martino, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy** (F Grossi MD); **Division of Thoracic Oncology, European Institute of Oncology, Milan, Italy** (T De Pas MD, F de Marinis MD); **1st Oncological Pulmonary Unit, San Camillo, High Specialization Hospital, Rome, Italy** (F de Marinis); **Department of Medical Oncology, National Cancer Institute of Aviano, Aviano, Italy** (A Bearz MD); **Department of Oncology, Istituto di Ricovero e Cura a**

Introduction

The selection of patients with advanced non-small-cell lung cancer who would benefit more from treatment with EGFR tyrosine-kinase inhibitors such as gefitinib, erlotinib, and afatinib has improved substantially with the establishment of the important role of *EGFR*-sensitising mutations, particularly in first-line treatment.[1–3] Although the use of EGFR tyrosine-kinase inhibitors in patients with an *EGFR*-activating mutation has become the standard of care, the role of EGFR tyrosine-kinase inhibitors in the second-line setting for patients with wild-type or unknown *EGFR* mutation status remains unclear.[4] In second or higher lines, treatment options are single-agent chemotherapy, such as docetaxel or pemetrexed,[5,6] or an oral EGFR tyrosine-kinase inhibitor.[7,8] In the NCIC BR.21 study,[9] the results of a subgroup analysis of erlotinib versus placebo in second and third lines showed that the EGFR tyrosine-kinase inhibitor is also active in patients with wild-type *EGFR* status and in those with advanced non-small-cell

Carattere Scientifico–Istituto di Richerche Farmacologiche Mario Negri, Milan, Italy (I Floriani PhD, V Torri MD); **Biodesix, Boulder, CO, USA** (J Grigorieva PhD, M Tsypin PhD, J Roder PhD, H Roder PhD); **and Università Vita-Salute San Raffaele, School of Medicine, Department of Pathology, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale San Raffaele, Milan, Italy** (Prof C Doglioni MD)

Correspondence to: Dr Vanesa Gregorc, Department of Medical Oncology, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale San Raffaele, Via Olgettina 60, Milan 20123, Italy **vanesa.gregorc@hsr.it**

lung cancer with unknown *EGFR* status. The results of the INTEREST[8] and TITAN[10] trials suggested similar overall survival with EGFR tyrosine-kinase inhibitors and standard second-line monochemotherapy, whereas the findings of the TAILOR trial[11] in patients with wild-type *EGFR* status showed that docetaxel was superior to erlotinib for progression-free survival (PFS) and overall survival.

Taguchi and colleagues[12] have developed a test in which mass spectrometry is used for the analysis of serum to identify patients likely to have good or poor survival on EGFR tyrosine-kinase inhibitors. This test, which is commercially available as VeriStrat (Biodesix, Boulder, CO, USA), is used to assign one of two classifications—good or poor—by comparison of the intensity of eight regions in the mass spectra obtained from patients' pretreatment serum samples with the intensity of those of a reference set.[12] The results of retrospective studies have shown that patients with proteomic test classification of good have a significantly better outcome than do those classified as poor when treated with EGFR tyrosine-kinase inhibitors.[12–17] The test classification is a significant predictor of outcome independent of clinical and molecular characteristics such as performance status and *EGFR* and *KRAS* mutation status.[15,16] The results of a retrospective analysis of samples from the placebo group of the NCIC BR.21 trial showed that the proteomic test has a prognostic role,[15] but no significant survival difference was noted between the two proteomic test classification groups when patients were given chemotherapy,[12] suggesting that the test might also be predictive of outcome between chemotherapy and EGFR tyrosine-kinase inhibitors. The predictive power of the test was reported in a study of elderly patients with non-small-cell lung cancer treated with erlotinib, erlotinib plus gemcitabine, and gemcitabine alone.[13] The available data indicate poor outcomes for patients with a proteomic test classification of poor who were given erlotinib.

The primary aim of this phase 3 trial was to assess the predictive power of the proteomic test in the comparison of two approved treatments—erlotinib and chemotherapy—in patients with non-small-cell lung cancer.

Methods

Study design and patients

For the **PROSE protocol** see http://www.highresearch.it/protocols/PROSE_HSRL-02-2007_protocol_v_20Mar2010.pdf

Patients were enrolled into PROSE, a biomarker-stratified, randomised phase 3 trial, between Feb 26, 2008, and April 11, 2012, in 14 centres in Italy. We designed the trial such that it not only provided information about the relative superiority of a treatment within each biomarker subgroup, but could also be used to ascertain whether the biomarker is prognostic, has predictive power in the comparison of treatments, or is both predictive and prognostic.[18]

Patients were eligible if they had histologically or cytologically documented advanced non-small-cell lung cancer (stage IIIB or IV), were aged 18 years and older, and had progressed on or were judged to be refractory to one previous platinum-based chemotherapy regimen—ie, patients must have had radiographic evidence of disease progression in the course of first-line platinum treatment or within 6 months from the last dose (only one line of treatment was allowed). Previous surgery or radiotherapy was permitted if completed at least 3 weeks before study enrolment. Additional inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, adequate haematological, renal, and hepatic functions, and at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST; version 1.0). Exclusion criteria were previous use of EGFR tyrosine-kinase inhibitors, evidence of uncontrolled brain metastases, clinically significant cardiac disease, renal failure or peripheral neuropathy, and concurrent other malignancies (with the exception of basal cell skin carcinoma).

The protocol was approved by institutional review boards and independent ethics committees at each site. The study was undertaken in accordance with the Declaration of Helsinki and with the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice. All patients provided written informed consent.

Randomisation and masking

Serum for proteomic analysis was obtained after failure of first-line treatment, but at a maximum of 7 days before study treatment was to be started. Serum samples were sent to Ospedale San Raffaele (Milan, Italy) from each centre for the generation of mass spectra. These spectra were sent electronically to Biodesix, where they were processed under masked conditions to generate proteomic test classifications,[12] which were entered directly into the customised, web-based study database within 5 working days from blood draw. Significant drifts in proportions of proteomic classifications were not noted during the course of the study.

After the proteomic classification was generated, and entered into the central database, patients were centrally randomised in a 1:1 ratio to the erlotinib or chemotherapy (pemetrexed or docetaxel) groups. Treatment was randomly allocated with a minimisation algorithm, which stratified treatment allocation by smoking history (never, former, or current smokers), ECOG performance status (0–1, or 2), proteomic test classification (poor or good), and centre. Investigators who did the mass spectrometry analyses and those who generated the proteomic classification were masked to treatment allocation, whereas physicians, who assessed outcome and provided treatment, and patients remained masked to the results of proteomic testing during the study, and were not masked to assigned treatment. Investigators who analysed results were masked to proteomic classification and treatment allocation until database lock for the final analysis.