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# 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

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## 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.

## 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

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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.

### Procedures

Patients randomly assigned to receive erlotinib received the drug at 150 mg/day, orally, until disease progression, unacceptable toxicity, death, or withdrawal of consent. Those assigned to receive chemotherapy received up to six cycles of pemetrexed 500 mg/m² every 21 days, intravenously, or docetaxel 75 mg/m² every 21 days, intravenously, according to the investigators' choice.

Standard baseline clinical and biochemical assessments were done within 4 weeks before study entry and at day 1 of each cycle during treatment. Adverse events were assessed according to the Common Terminology Criteria for Adverse Events (version 3.0) throughout the study. National or international guidelines were followed for the occurrence of grade 3 or 4 adverse events: treatment was temporarily interrupted for a maximum of 3 weeks, and if the adverse event persisted the patient was withdrawn from the study. At recovery, erlotinib dose was adjusted to 100 mg daily, whereas chemotherapy dose was reduced by a maximum of 25%. The concomitant use of a haematopoietic growth factor as primary or secondary prophylaxis was allowed. Tumour response or disease progression was assessed with thorax–abdomen CT scans at 8 week intervals according to RECIST. No independent radiological review was done. Available tissue was obtained at the time of diagnosis and stored as fresh frozen and as paraffin blocks for *EGFR* and *KRAS* mutation testing.

### Outcomes

The primary efficacy endpoint was overall survival, calculated as months from randomisation to whichever occurred first—death from any cause or the date of patient's last contact—and the primary hypothesis was the existence of significant interaction between the proteomic test classification and treatment for the primary efficacy endpoint.

Secondary efficacy endpoints were PFS, calculated as months from the date of randomisation to whichever occurred first—progression or death from any cause or the date of last on-study tumour assessment—and the proportion of patients with an objective response. Secondary analysis of differential treatment effect on response rate between the two proteomic test classification groups was done with logistic regression analysis.

### Statistical analysis

Assuming median overall survival of 7 months for patients given chemotherapy,[6,8] irrespective of proteomic test classification, the median overall survival in patients treated with erlotinib would be 4·2 months for proteomic test classification of poor and 9·9 months for good outcome, respectively.[12] The expected difference in efficacy for erlotinib versus chemotherapy in these subgroups translates into hazard ratios (HRs) of 1·67 for people with a proteomic test classification of poor and 0·71 for good test classification. 220 events were needed to detect a HR for interaction of 2·35, with a two-sided 5% significance level and a power of 90%.[19] Sample size was calculated based on a 65%:35% ratio of good to poor proteomic test classification. With a recruitment period of 18 months, a minimum 12-month follow-up, and a dropout rate of 10%, the number of patients needed was about 275. An interim analysis was planned at accrual of 120 patients to check accuracy of the assumed ratio of proteomic test classifications, without accessing any outcome data. Any differential treatment effect in the proteomic test classification groups was assessed with a Cox proportional hazard model including treatment, proteomic classification, and first-order interaction term between treatment and proteomic classification.

The primary analysis population was defined as all patients who were randomly assigned to receive study treatment, without any limitation on treatment duration, and did not have any major protocol violations (eg, receiving treatments not permitted by protocol or important violations of exclusion or inclusion criteria related to stage or histology) by clinical review before the analysis.

Survival curves were calculated with Kaplan-Meier methods and the difference between groups in time-to-event analysis was assessed with Cox proportional HRs, 95% CI, and p values. Patients' characteristics at study entry are presented as median and range for continuous variables, and counts and percentages for discrete variables. Analyses were done with SAS (version 9.2).

This trial is registered with ClinicalTrials.gov (NCT00989690).

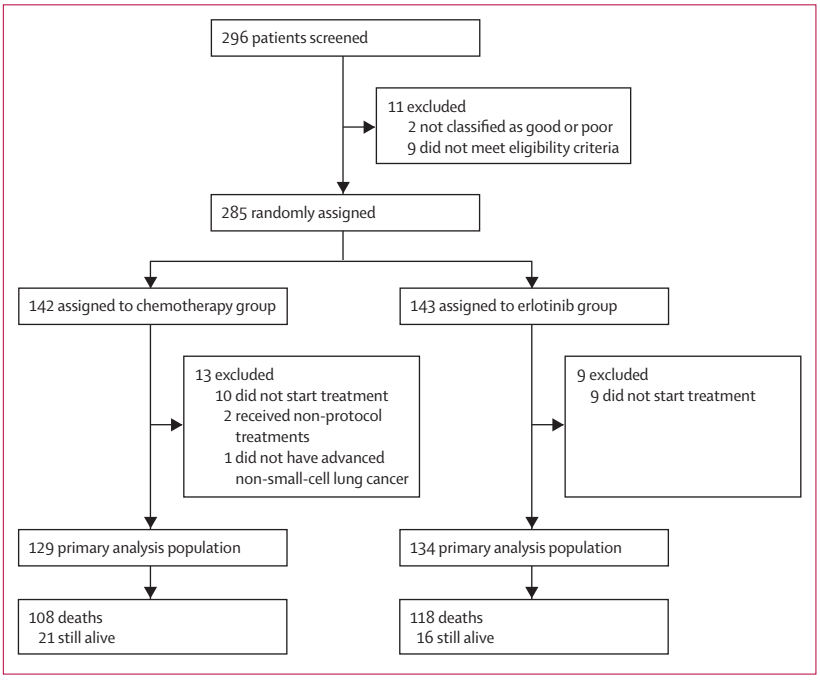


*Figure 1:* **Trial profile**

## Role of the funding source

The grant providers had no role in study design, data gathering and interpretation, or writing the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit this report for publication.


## Results

Between Feb 26, 2008, and April 11, 2012, 285 patients were accrued to the study from 14 medical centres in Italy (appendix). The primary analysis population was 263 patients who met the trial eligibility criteria and were receiving treatment according to randomisation: 129 were assigned to chemotherapy (74 received docetaxel only, 55 pemetrexed only) and 134 to erlotinib (figure 1). Three patients with major protocol violations (one receiving gemcitabine instead of a study drug, one receiving radical radiotherapy, and one did not have stage IIIB or IV disease), and 19 patients who did not start any treatment were excluded from the primary analysis (figure 1). *EGFR* and *KRAS* mutation analyses were done on samples from 192 (73%) of 263 and 163 (62%) patients, respectively; 14 (5%) patients had sensitising *EGFR* mutations (table 1).

See Online for appendix

Table 1 shows the baseline characteristics of the primary analysis population by treatment group and table 2 shows them by proteomic test classification. 184 (70%) of 263 patients in the primary analysis had a proteomic text classification of good and 79 (30%) had a proteomic text classification of poor. Treatment groups were balanced for clinical characteristics, with the exception of histology—the erlotinib group had more patients with squamous cell carcinoma than did the chemotherapy group. A proteomic test classification of good was associated with ECOG PS 0–1 (p=0·002) and female sex (p=0·007; table 2).

At the time of the primary analysis, 226 deaths had occurred—118 (88%) of 134 patients in the erlotinib group and 108 (84%) of 129 in the chemotherapy group (figure 1). Median follow-up was 32·4 months (IQR 22·3–44·5) for the whole study population. Overall survival was not significantly different in the two treatment groups according to the results of the unadjusted and adjusted analyses (HR 1·14 [95% CI 0·88–1·49], p=0·313, and 1·22

| | Chemotherapy group (n=129) | Erlotinib group (n=134) |
|---|---|---|
| Proteomic test classification | | |
| Good | 88 (68%) | 96 (72%) |
| Poor | 41 (32%) | 38 (28%) |
| ECOG performance status | | |
| 0 | 65 (50%) | 73 (54%) |
| 1 | 56 (43%) | 53 (40%) |
| 2 | 8 (6%) | 8 (6%) |
| Smoking | | |
| Never | 17 (13%) | 21 (16%) |
| Former | 75 (58%) | 77 (57%) |
| Current | 37 (29%) | 36 (27%) |
| Sex | | |
| Male | 91 (71%) | 99 (74%) |
| Female | 38 (29%) | 35 (26%) |
| Histology | | |
| Adenocarcinoma | 91 (71%) | 76 (57%) |
| Squamous | 16 (12%) | 31 (23%) |
| Other | 22 (17%) | 27 (20%) |
| Stage | | |
| IIIB | 17 (13%) | 12 (9%) |
| IV | 110 (85%) | 121 (90%) |
| Not available | 2 (2%) | 1 (<1%) |
| *EGFR* status | | |
| Mutated | 6 (5%) | 8 (6%) |
| Wild type | 84 (65%) | 79 (59%) |
| Not available | 39 (30%) | 47 (35%) |
| Age (years) | 64 (39–77) | 66 (33–85) |

Data are number (%) or median (range). ECOG=Eastern Cooperative Oncology Group.

***Table 1:*** **Baseline characteristics of patients by treatment group**

| | Good (n=184) | Poor (n=79) | p value |
|---|---|---|---|
| Treatment | | | .. |
| Chemotherapy | 88 (48%) | 41 (52%) | .. |
| Erlotinib | 96 (52%) | 38 (48%) | .. |
| ECOG performance status | | | 0·002 |
| 0 | 108 (59%) | 30 (38%) | .. |
| 1 | 69 (38%) | 40 (51%) | .. |
| 2 | 7 (4%) | 9 (11%) | .. |
| Smoking | | | 0·052 |
| Never | 31 (17%) | 7 (9%) | .. |
| Former | 109 (59%) | 43 (54%) | .. |
| Current | 44 (24%) | 29 (37%) | .. |
| Sex | | | 0·007 |
| Male | 124 (67%) | 66 (84%) | .. |
| Female | 60 (33%) | 13 (16%) | .. |
| Histology | | | 0·295 |
| Adenocarcinoma | 122 (66%) | 45 (57%) | .. |
| Squamous | 29 (16%) | 18 (23%) | .. |
| Other | 33 (18%) | 16 (20%) | .. |
| Stage | | | 0·196* |
| IIIB | 17 (9%) | 12 (15%) | .. |
| IV | 165 (90%) | 66 (84%) | .. |
| Not available | 2 (1%) | 1 (1%) | .. |
| *EGFR* status | | | 0·384 |
| Mutated | 12 (7%) | 2 (3%) | .. |
| Wild type | 114 (62%) | 49 (62%) | .. |
| Not available | 58 (32%) | 28 (35%) | .. |
| Age (years) | 65 (39–85) | 65 (33–84) | .. |

Data are number (%) or median (range), unless otherwise indicated. ECOG=Eastern Cooperative Oncology Group. *Stage IIIB versus IV.

***Table 2:*** **Baseline characteristics of patients by proteomic test classification**

[0·93–1·59], p=0·148, respectively; figure 2A), and PFS was not significantly different in the two treatment groups in the unadjusted analysis (1·27 [0·99–1·62], p=0·060), but was significantly better for chemotherapy-treated patients in the adjusted analysis (1·35 [1·05–1·73], p=0·020; figure 2B). 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. By combining treatment groups, patients with a classification of good had better overall survival and PFS than did those with a classification of poor (HR 2·50 [95% CI 1·88–3·31], p<0·0001, and 1·75 [1·34–2·29], p<0·0001, respectively). Median overall survival was 11·0 months (95% CI 9·3–12·6) and 3·7 months (2·9–5·2) for good and poor classifications, respectively. Median PFS was 3·4 months (95% CI 2·4–4·6) and 2·0 (1·6–2·4) for good and poor classification groups, respectively. Overall results were similar in the intention-to-treat and per-protocol populations (appendix).

In the primary endpoint analysis, we noted a significant interaction between proteomic classification and treatment for overall survival ($p_{interaction}$=0·017 when adjusted for stratification factors, and $p_{interaction}$=0·031 when unadjusted for stratification factors). Furthermore, even after adjustment for other clinical characteristics and *EGFR* mutation status, the proteomic test classification by treatment interaction remained significant ($p_{interaction}$=0·022), along with other independent

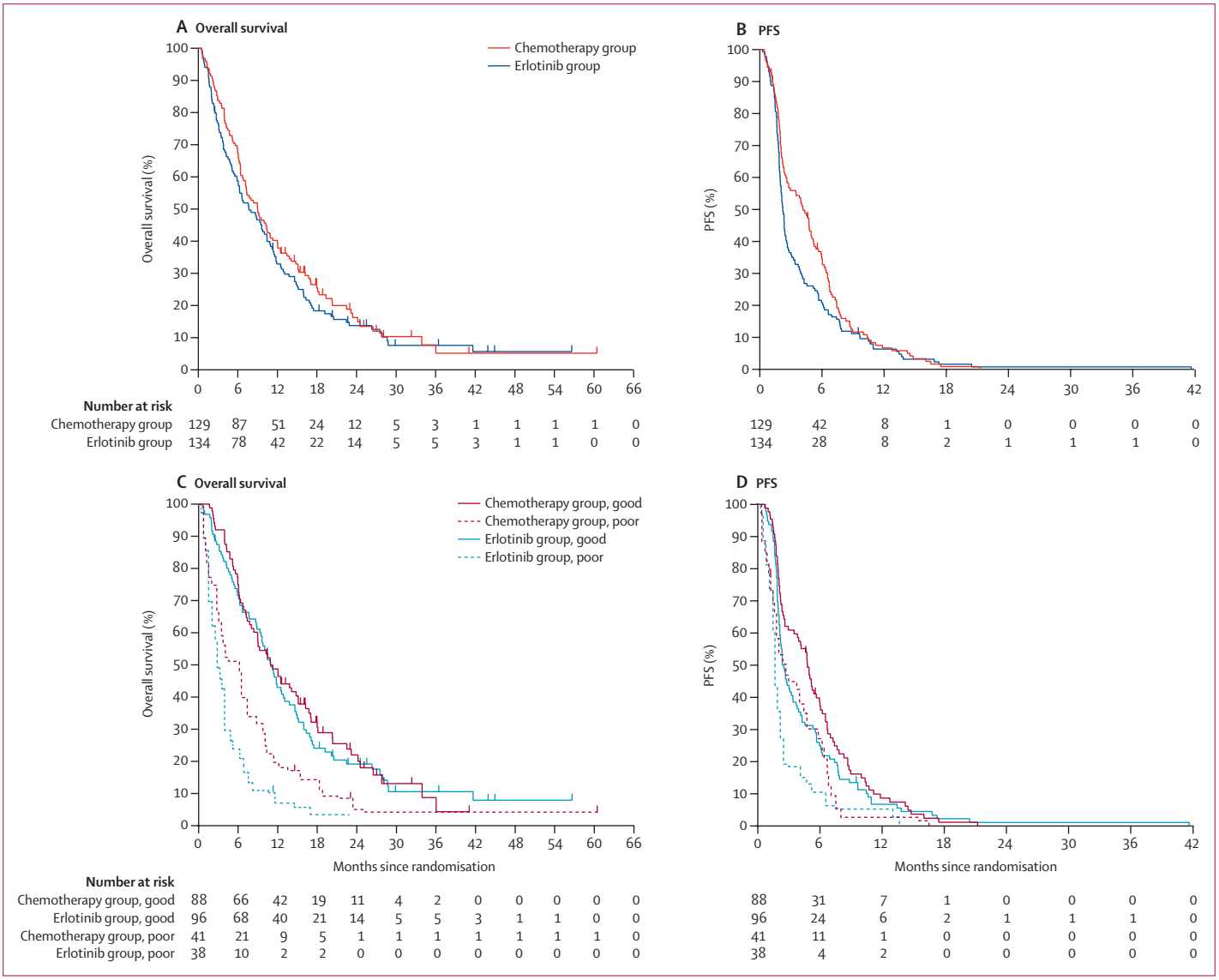


***Figure 2:*** **Kaplan-Meier curves of overall survival and PFS**
(A) Overall survival by treatment group. (B) PFS by treatment group. (C) Overall survival by treatment group and proteomic test classification. (D) PFS by treatment group and proteomic test classification. PFS=progression-free survival.

| | Hazard ratio (95% CI) | p value |
|---|---|---|
| Proteomic test classification (poor *vs* good) | 1·88 (1·25–2·84) | 0·003 |
| Treatment (erlotinib *vs* chemotherapy) | 1·15 (0·83–1·59) | 0·405 |
| Proteomic test classification by treatment interaction | 1·98 (1·10–3·57) | 0·022 |
| Performance status (2 *vs* 0 and 1) | 2·67 (1·57–4·53) | 0·0003 |
| Smoking history (ever *vs* never) | 1·25 (0·79–1·98) | 0·337 |
| Histology (squamous *vs* non-squamous) | 1·08 (0·75–1·57) | 0·672 |
| Sex (female *vs* male) | 0·90 (0·64–1·27) | 0·557 |
| Age (years) | 0·99 (0·97–1·01) | 0·155 |
| *EGFR* status (mutation *vs* wild-type or squamous) | 0·66 (0·34–1·27) | 0·212 |
| *EGFR* status (not available *vs* wild-type or squamous) | 1·25 (0·90–1·73) | 0·180 |

***Table 3:*** **Adjusted interaction analysis of overall survival**

predictors of outcome—proteomic test classification (p=0·003) and performance status (p=0·0003; table 3), showing that the test is predictive of differential survival benefit between chemotherapy and erlotinib groups independently of other clinical factors. In the good classification group, there was no significant difference in overall survival between the treatment groups—median overall survival was 10·9 months (95% CI 8·4–15·1) in the chemotherapy group and 11·0 months (9·2–12·9) in the erlotinib group (HR 1·06 [95% CI 0·77–1·46], p=0·714; figure 2C, figure 3). By contrast, patients with a poor proteomic test classification had significantly shorter overall survival when treated with erlotinib than did those given chemotherapy (median 3·0 months [95% CI 2·0–3·8] *vs* 6·4 months [3·0–7·4]; HR 1·72, [95% CI 1·08–2·74], p=0·022; figure 2C; figure 3).

The difference in PFS in the erlotinib and chemotherapy groups was not significant when analysed by proteomic test results. In patients with a classification of good, median PFS was 4·8 months (95% CI 3·0–6·0) in the chemotherapy group and 2·5 months (2·1–3·4) in the erlotinib group (HR 1·26 [95% CI 0·94–1·69], p=0·129); in patients with a classification of poor, PFS was 2·8 months (95% CI 1·8–4·3) in the chemotherapy group and 1·7 months (1·4–2·1) in the erlotinib group (HR 1·51 [95% CI 0·96–2·38], p=0·078; figure 2D, figure 3). The proteomic test classification by treatment interaction was not significant for PFS in either unadjusted ($p_{interaction}$=0·445) or adjusted ($p_{interaction}$=0·268) analyses.

No complete responses were noted during the trial (table 4); no difference was noted between treatment groups in the proportion of patients who had a partial response to treatment: 13 (12%) of 108 patients in the chemotherapy group and ten (9%) of 110 in the erlotinib group had partial responses and 95 (88%) and 100 (91%) patients, respectively, had no objective response; there were no differences in response rate by proteomic test status (table 4) and there was no significant interaction between proteomic test classification and treatment (p=0·519).

Third-line treatments are shown in table 5. 34 (26%) of 129 patients in the chemotherapy group received erlotinib in the third line, and 65 (49%) of 134 in the erlotinib group received third-line chemotherapy (table 5). 76 (59%) of 129 patients in the chemotherapy group and 65 (49%) of 134 in the erlotinib group did not receive third-line treatment.

The results of an exploratory subgroup analysis of the population excluding the 14 patients with *EGFR* sensitising mutations were qualitatively similar to those obtained for the primary analysis population (data not shown). For overall survival, the interaction between proteomic classification and treatment remained significant ($p_{interaction}$=0·024), confirming the predictive properties of the test in this clinically relevant group of patients for whom biomarkers for treatment selection are lacking. A forest plot of *EGFR* status, sex, ECOG performance status, histology, and smoking status subgroups by treatment is provided in the appendix.

Grade 3 or 4 adverse events that were judged to be related to treatment were reported in 36 (28%) of 129 patients in the chemotherapy group and 31 (23%)

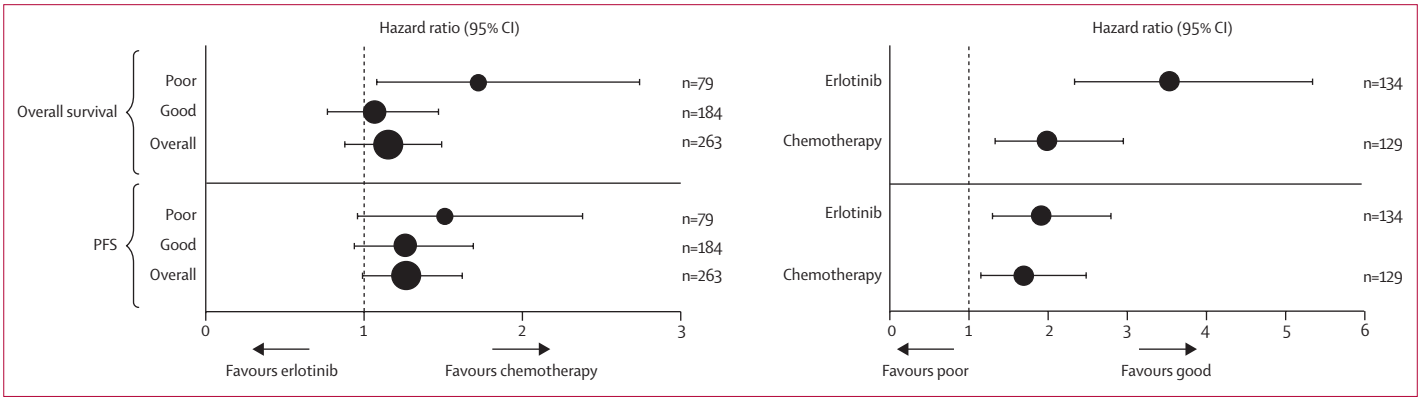


***Figure 3:*** **Forest plots by treatment group and proteomic test classification**
PFS=progression-free survival.

of 134 in the erlotinib group. The most common adverse events in the chemotherapy group were neutropenia, nausea, neurotoxicity, alopecia, and asthenia; rash and diarrhoea were the most common adverse events in the erlotinib group (table 6). Serious adverse events were reported in three (2%) patients in the chemotherapy group and four (3%) in the erlotinib group. Adverse events led to treatment discontinuation in four (3%) patients in the chemotherapy group and two (1%) in the erlotinib group. 23 patients in the chemotherapy group and 76 in the erlotinib group had dose reductions; 16 and 29, respectively, due to toxicity. There were no drug-related deaths.

| | Chemotherapy group, good (n=88) | Chemotherapy group, poor (n=41) | Erlotinib group, good (n=96) | Erlotinib group, poor (n=38) |
|---|---|---|---|---|
| Complete response | 0 | 0 | 0 | 0 |
| Partial response | 10 (11%) | 3 (7%) | 9 (9%) | 1 (3%) |
| Stable disease | 39 (44%) | 13 (32%) | 30 (31%) | 3 (8%) |
| Progressive disease | 30 (34%) | 13 (32%) | 46 (48%) | 21 (55%) |
| Not available or evaluable | 9 (10%) | 12 (29%) | 11 (11%) | 13 (34%) |

Data are number (%). RECIST=Response Evaluation Criteria in Solid Tumors (version 1.0).

*Table 4:* **Best RECIST response by treatment and proteomic test classification**

| | Chemotherapy group, good (n=88) | Chemotherapy group, poor (n=41) | Erlotinib group, good (n=96) | Erlotinib group, poor (n=38) |
|---|---|---|---|---|
| Erlotinib | 24 (27%) | 10 (24%) | 3 (3%) | 1 (3%) |
| Pemetrexed | 0 | 0 | 11 (11%) | 4 (11%) |
| Chemotherapy (other) | 17 (19%) | 1 (2%) | 40 (42%) | 10 (26%) |
| Crizotinib | 1 (1%) | 0 | 0 | 0 |

Data are number (%).

*Table 5:* **Third-line therapy by treatment group and proteomic test classification**

| | Chemotherapy group (n=129) | | | Erlotinib group (n=134) | | |
|---|---|---|---|---|---|---|
| | All grades | Grade 3 | Grade 4 | All grade | Grade 3 | Grade 4 |
| Diarrhoea | 19 (15%) | 2 (2%) | 0 | 30 (22%) | 4 (3%) | 1 (<1%) |
| Alopecia | 22 (17%) | 0 | 0 | 1 (<1%) | 0 | 0 |
| Asthenia | 38 (29%) | 10 (8%) | 0 | 27 (20%) | 3 (2%) | 0 |
| Neurological events | 15 (12%) | 3 (2%) | 0 | 4 (3%) | 0 | 0 |
| Nausea | 17 (13%) | 1 (<1%) | 0 | 2 (1%) | 0 | 0 |
| Dermatological events | 11 (9%) | 1 (<1%) | 0 | 69 (51%) | 21 (16%) | 1 (<1%) |
| Neutropenia | 46 (36%) | 10 (8%) | 9 (7%) | 4 (3%) | 1 (<1%) | 0 |

Data are number (%).

*Table 6:* **Drug-related adverse events**

## Discussion

We noted a significant interaction between treatment and proteomic test classification for overall survival ($p_{interaction}$=0·017 when adjusted for stratification factors, and $p_{interaction}$=0·031 when unadjusted for stratification factors), indicating differential overall survival benefit for patients in the chemotherapy and erlotinib groups according to proteomic classification. Patients with a test classification of poor had better overall survival when treated with chemotherapy than with erlotinib; by contrast, no significant differences were noted between treatments in patients with a test classification of good.

An increasing number of patients with advanced non-small-cell lung cancer can be treated after progression on first-line treatment because of improvements in the use of concomitant supportive medications together with better tolerability of the newer treatment regimens for first-line. Patients with non-small-cell lung cancer with *EGFR* mutations or *EML4-ALK* rearrangements who have not been given the appropriate targeted drug in the first-line setting should be treated with EGFR tyrosine-kinase inhibitors or crizotinib, respectively, in the second line. However, about 80–85% of white patients do not have mutations that can be targeted by drugs and, in at least 30% of cases, their molecular status cannot be ascertained because of the lack of adequate tissue.[20] The standard treatment options in the second-line setting for this patient population are still single-agent chemotherapy (docetaxel or pemetrexed) or erlotinib, but there are no standard recommendations or guidelines for which treatment to use (panel).

When the study was designed in 2007, the predictive role of *EGFR* activating mutations was still unclear, and, consequently, the presence of *EGFR* mutations was not an exclusion criterion. However, after the results from IPASS[1] and WJTOG3405[21] were reported, clinical practice changed radically, and EGFR tyrosine-kinase inhibitors have become the standard of care in patients with *EGFR* mutations. As a result, only 7% of the patients enrolled in PROSE had an *EGFR* activating mutation because previous treatment with an EGFR inhibitor was an exclusion criteria of the study. The results from TAILOR—a study of docetaxel versus erlotinib in the second-line—showed that unselected patients with wild-type *EGFR* status treated with docetaxel had superior outcomes compared with those treated with erlotinib. The results of PROSE are complementary to the TAILOR trial. PROSE was designed to address the question of whether an established serum protein test is predictive for chemotherapy versus erlotinib and not to directly compare the treatments in the unselected population, which was the aim in the TAILOR study. Additionally, only patients with confirmed *EGFR* wild-type tumours were enrolled in the TAILOR trial, whereas in the PROSE study patients did not have to have a known *EGFR* status and so participation of patients whose *EGFR* status could not be ascertained was allowed. Furthermore, in TAILOR erlotinib and docetaxel were compared directly, whereas in PROSE physicians were allowed to select between pemetrexed and docetaxel for patients randomly assigned to the chemotherapy group.

***Panel:* Research in context**

**Systematic review**

We searched PubMed up to January, 2007, for English-language reports of clinical trials of EGFR tyrosine-kinase inhibitors in patients with non-small-cell lung cancer, with the search terms "EGFR-TKIs", "NSCLC", "second line therapy", and "proteomic". We identified phase 3 randomised clinical trials of the comparison of EGFR tyrosine-kinase inhibitors and second-line chemotherapy or placebo in unselected patients when there was no web search evidence for proteomic or biomarker selection at that time. Our study was designed on the basis of Taguchi and colleagues' report of the development and initial validation of a proteomic test.[12] The results of subsequent retrospective studies have shown that the test has both prognostic[15] and predictive power.[13,14]

**Interpretation**

Although EGFR tyrosine-kinase inhibitors are now standard of care for patients with sensitising *EGFR* mutations, their role in the treatment of patients with wild-type or unknown *EGFR* mutation status is equivocal. The results of this study have shown that a proteomic test can be used to predict differential survival benefit between chemotherapy and erlotinib in the overall population with non-small-cell lung cancer. In particular, patients who are classified as poor by the proteomic test derive greater benefit in overall survival from chemotherapy than from erlotinib. This study has identified the subgroup of patients with non-small-cell lung cancer who should not receive EGFR tyrosine-kinase inhibitors.

Results of a post-hoc exploratory analysis of the comparison of treatments in the unselected intention-to-treat population and *EGFR* wild-type subgroup in the PROSE study are provided in the appendix.

The results of PROSE show that an established proteomic test is a predictive marker of differential benefit of chemotherapy versus erlotinib in overall survival and that patients with a proteomic test classification of poor (30%) should not receive erlotinib. Our results do not show superiority of either treatment within the population of patients with a good classification; however, it should be noted that this study was not originally designed to detect survival benefit of erlotinib versus chemotherapy within the good proteomic classification group. In hindsight, this is a limitation of the trial, and the study design could have been improved by enlarging the study to increase its power to address this additional analysis. Additionally, the results confirmed the prognostic role of the test, as shown by the better outcomes in patients with a test classification of good than in those with a classification of poor in the overall study population. Although the results of this study corroborated previous data showing the significant association of proteomic test classification with specific prognostic factors, such as performance status, the results of the multivariate analyses showed that the test's predictive and prognostic power is retained when adjusted for these factors in accord with findings from previous studies.[12–16]

No significant interaction was noted between proteomic classification and treatment in terms of PFS. Several factors might have contributed to this discrepancy between PFS and overall survival. First, CT scans were scheduled every 8 weeks (longer than the median PFS for patients with a poor test classification in the erlotinib group), which might have overestimated the PFS in this group of patients. Second, PFS is not always a perfect surrogate for overall survival in advanced non-small-cell lung cancer, and it is possible that PFS did not capture the full treatment effect within the different test classification groups.[22] Alternatively, although the proportion of patients who received third-line therapy was similar in both good and poor classifications according to the proteomic test (table 5), the treatment effect measured with PFS might have been diluted by higher-line treatment in a way that was dependent on both second-line treatment and proteomic test classification.

The biological rationale for differential outcome according to proteomic test status is being investigated. Four of the eight spectral regions in the signature have been shown to contain proinflammatory proteins.[23] A notable hypothesis is that patients who have a poor proteomic test classification have a systemic inflammatory response to their tumours that promotes tumour growth and apoptotic resistance, either through direct effects on tumour cells or secondary effects mediated by tumour stromal cells. These additional growth and antiapoptotic signals could enhance the aggressiveness of the tumours (leading to poorer prognosis), and provide them with a means to bypass EGFR blockade.

In conclusion, our results suggest that a serum protein test is clinically useful for the identification of patients with non-small-cell lung cancer who should not be treated with erlotinib—chemotherapy should be the preferred option for patients with a proteomic test classification of poor. However, without a clear indication supporting the choice of erlotinib or chemotherapy for patients with a classification of good according to the test, a clinical decision for treatment for this group of patients remains open.

**Contributors**

VG designed the trial and was the principal investigator. IF and VT defined the statistical plan and procedures, and analysed the data. ABa and AC did the mass spectrometry analysis. HR was involved in trial design. JR, JG, and MT did the classification of the analysed spectra. CD was responsible for the molecular analysis of the enrolled patients. All the other authors were responsible for patients' enrolment and data gathering. VG, HR, IF, and VT wrote the manuscript, which was approved by the all the authors.

**Declaration of interests**

JG, MT, JR, and HR are employees of Biodesix. The other authors declare that they have no competing interests.

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