Erlotinib and bevacizumab in patients with advanced non-small-cell lung cancer and activating *EGFR* mutations (BELIEF): an international, multicentre, single-arm, phase 2 trial

Rafael Rosell, Urania Dafni, Enriqueta Felip, Alessandra Curioni-Fontecedro, Oliver Gautschi, Solange Peters, Bartomeu Massutí, Ramon Palmero, Santiago Ponce Aix, Enric Carcereny, Martin Früh, Miklos Pless, Sanjay Popat, Athanasios Kotsakis, Sinead Cuffe, Paolo Bidoli, Adolfo Favaretto, Patrizia Froesch, Noemí Reguart, Javier Puente, Linda Coate, Fabrice Barlesi, Daniel Rauch, Michael Thomas, Carlos Camps, Jose Gómez-Codina, Margarita Majem, Rut Porta, Riyaz Shah, Emer Hanrahan, Roswitha Kammler, Barbara Ruepp, Manuela Rabaglio, Marie Kassapian, Niki Karachaliou, Rachel Tam, David S Shames, Miguel A Molina-Vila, Rolf A Stahel, for the BELIEF collaborative group

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Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain (Prof R Rosell MD, E Carcereny MD); **Frontier Science Foundation-Hellas & National and Kapodistrian University of Athens, Athens, Greece** (Prof U Dafni ScD); **Vall d'Hebron University Hospital, Institute of Oncology, Barcelona, Spain** (E Felip MD); **University Hospital Zurich, Clinic of Oncology, Zurich, Switzerland** (A Curioni-Fontecedro MD, R A Stahel MD); **Cantonal Hospital Lucerne, Medical Oncology, Lucerne, Switzerland** (O Gautschi MD); **Centre Hospitalier Universitaire Vaudois, Département d'Oncologie, Lausanne, Switzerland** (S Peters MD); **Hospital General Universitario Alicante, Oncología Médica, Alicante, Spain** (B Massutí MD); **Catalan Institute of Oncology, Hospital Duran i Reynals, Bellvitge, Spain** (R Palmero MD); **Hospital Universitario 12 de Octubre, Oncología Médica, Madrid, Spain** (S Ponce Aix MD); **Cantonal Hospital St Gallen, Oncology and Hematology, St Gallen, Switzerland** (M Früh MD); **Cantonal Hospital Winterthur, Medical Oncology, Winterthur, Switzerland** (M Pless MD); **Medical Oncology, Royal Marsden Hospital, London, UK** (S Popat FRCP); **University General Hospital of Heraklion, Medical Oncology, Heraklion, Crete, Greece** (A Kotsakis MD); **Cancer Trials Ireland and St James's Hospital, Medical Oncology, Dublin, Ireland** (S Cuffe MD); **Ospedale San Gerardo, Oncologia Medica,**

Summary

Background **The tyrosine kinase inhibitor erlotinib improves the outcomes of patients with advanced non-small-cell lung carcinoma (NSCLC) harbouring epidermal growth factor receptor (*EGFR*) mutations. The coexistence of the T790M resistance mutation with another *EGFR* mutation in treatment-naive patients has been associated with a shorter progression-free survival to EGFR inhibition than in the absence of the T790M mutation. To test this hypothesis clinically, we developed a proof-of-concept study, in which patients with *EGFR*-mutant NSCLC were treated with the combination of erlotinib and bevacizumab, stratified by the presence of the pretreatment T790M mutation.**

Methods **BELIEF was an international, multicentre, single-arm, phase 2 trial done at 29 centres in eight European countries. Eligible patients were aged 18 years or older and had treatment-naive, pathologically confirmed stage IIIB or stage IV lung adenocarcinoma with a confirmed, activating *EGFR* mutation (exon 19 deletion or L858R mutation). Patients received oral erlotinib 150 mg per day and intravenous bevacizumab 15 mg/kg every 21 days and were tested centrally for the pretreatment T790M resistance mutation with a peptide nucleic acid probe-based real-time PCR. The primary endpoint was progression-free survival. The primary efficacy analysis was done in the intention-to-treat population and was stratified into two parallel substudies according to the centrally confirmed pretreatment T790M mutation status of enrolled patients (T790M positive or negative). The safety analysis was done in all patients that have received at least one dose of trial treatment. This trial was registered with ClinicalTrials.gov, number NCT01562028.**

Findings **Between June 11, 2012, and Oct 28, 2014, 109 patients were enrolled and included in the efficacy analysis. 37 patients were T790M mutation positive and 72 negative. The overall median progression-free survival was 13·2 months (95% CI 10·3–15·5), with a 12 month progression-free survival of 55% (95% CI 45–64). The primary endpoint was met only in substudy one (T790M-positive patients). In the T790M-positive group, median progression-free survival was 16·0 months (12·7 to not estimable), with a 12 month progression-free survival of 68% (50–81), whereas in the T790M-negative group, median progression-free survival was 10·5 months (9·4–14·2), with a 12 month progression-free survival of 48% (36–59). Of 106 patients included in the safety analysis, five had grade 4 adverse events (one acute coronary syndrome, one biliary tract infection, one other neoplasms, and two colonic perforations) and one died due to sepsis.**

Interpretation **The BELIEF trial provides further evidence of benefit for the combined use of erlotinib and bevacizumab in patients with NSCLC harbouring activating *EGFR* mutations.**

Funding **European Thoracic Oncology Platform, Roche.**

Introduction

Activating mutations in the epidermal growth factor receptor (EGFR) gene have been identified in 10–40% of the patients with non-small-cell lung cancer (NSCLC), and EGFR inhibition leads to suppression of downstream signalling pathways such as mitogen-activated protein kinase (MAPK) and AKT protein kinase pathways.[1] *EGFR* mutations are associated with longer progression-free survival with EGFR tyrosine kinase inhibitors as compared with chemotherapy; however, the duration of progression-free survival is limited to an average of 1 year.[2–4] Previous studies suggest that the T790M mutation pre-exists in the cis configuration (on the same allele) with the primary EGFR-activating mutation in a small population of

Monza, Italy (P Bidoli MD); **Istituto Oncologico Veneto, IRCCS, Padova, Italy** (A Favaretto MD); **Instituto Oncologica Della Svizzera Italiana, Bellinzona, Switzerland** (P Froesch MD); **Hospital Clínic, Medical Oncology & Genomics and Targeted Therapeutics in Solid Tumors, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain** (N Reguart MD); **Thoracic, Urologic & Melanoma Cancer Unit Medical Oncology Department Hospital Clinico Universitario San Carlos, Madrid, Spain** (J Puente MD); **University Hospital Limerick and Cancer Trials Ireland, Limerick, Ireland** (L Coate MD); **Aix Marseille University; Assistance Publique Hôpitaux de Marseille, Marseille, France** (Prof F Barlesi MD); **Okologiezentrum Thun-Berner Oberland, Thun, Switzerland** (D Rauch MD); **Internistische Onkologie der Thoraxtumoren, Thoraxklinik im Universitätsklinikum Heidelberg, Translational Lung Research Center Heidelberg, Member of the German Center for Lung Research, Heidelberg, Germany** (Prof M Thomas MD); **University Hospital Valencia, Valencia, Spain** (C Camps MD); **Hospital La Fe, Valencia, Spain** (J Gómez-Codina MD); **Hospital de la Santa Creu i Sant Pau, Barcelona, Spain** (M Majem MD); **Insitut Catalan d'Oncologia and University of Girona, Girona, Spain** (R Porta MD); **Kent Oncology Centre, Maidstone, UK** (R Shah FRCP); **Cancer Trials Ireland and St Vincent's University Hospital, Dublin, Ireland** (E Hanrahan MRCPI); **European Thoracic Oncology Platform Coordinating Office, Bern, Switzerland** (R Kammler BA, B Ruepp PharmD, M Rabaglio MD); **Frontier Science Foundation-Hellas, Athens, Greece** (M Kassapian MSc); **Institute of Oncology Rosell, University Hospital Sagrat Cor, Barcelona, Spain** (N Karachaliou MD); **Oncology Biomarker Development, Genentech, South San Francisco, CA, USA** (R Tam BSc, D S Shames PhD); **Pangaea Biotech S L, Quirón Dexeus University Hospital, Barcelona, Spain**

Research in context

Evidence before this study

Pretreatment *EGFR* T790M resistance mutation occurs in a proportion of patients with non-small-cell lung cancer (NSCLC) and activating *EGFR* mutations. In preclinical studies, bevacizumab as a single drug or in combination with erlotinib inhibited tumour growth of H1975 xenografts (bearing *EGFR* L858R and T790M mutations). We searched MEDLINE for studies published in English between Jan 1, 2006, and Dec 31, 2011, using the terms "erlotinib and bevacizumab" AND "gefitinib and bevacizumab" AND "non-small cell lung cancer" OR "NSCLC" AND "*EGFR* mutations" AND "pre-treatment T790M" OR "de novo T790M". We did not identify any clinical trials addressing this combination.

Added value of this study

This study was designed to provide evidence for the combined activity of erlotinib and bevacizumab as first-line therapy for patients with activating epidermal growth factor receptor (*EGFR*) mutations, according to the absence or the presence of pretreatment T790M resistance mutation. Pretreatment T790M mutation was detected in 37 (34%) of the 109 patients enrolled in the study. We noted that the combination of erlotinib plus bevacizumab had substantial antitumour activity in patients with *EGFR*-mutant NSCLC and pretreatment T790M. The 12 month progression-free survival was 68% for patients who were T790M positive and 48% for patients who were T790M negative. Furthermore, responses were durable, independent of the status of pretreatment T790M, and the safety profile of combined drug treatment was tolerable.

Implications of all the available evidence

To our knowledge, this trial is the first to assess the combination of EGFR and VEGFR inhibition in patients with *EGFR*-mutant NSCLC according to the presence or absence of the pretreatment T790M mutation as determined by a highly sensitive method. Notably, the median progression-free survival with the combination of erlotinib plus bevacizumab was higher in the T790M-positive group compared with the T790M-negative group. These data support the need for the development of a sensitive screening method for the pretreatment T790M mutation, and the implementation of first-line therapies for this subpopulation of *EGFR*-mutant NSCLCs that are more efficient than EGFR tyrosine kinase inhibitors alone. The BEVERLY study is the only ongoing phase 3 study in Europe comparing bevacizumab plus erlotinib versus erlotinib alone as first-line treatment of patients with *EGFR*-mutant NSCLC. However, in the BEVERLY study, patients with the pretreatment T790M mutation are excluded. Similarly, the detection of the pretreatment T790M mutation is an exclusion criterion in the Chinese ARTEMIS and the Japanese NEJ026 phase 3 clinical trials that are assessing the combination of bevacizumab with erlotinib versus erlotinib alone in patients with *EGFR*-mutant NSCLC. The randomised phase 2 clinical trial ACCRU RC1126 (NCT01532089) is evaluting erlotinib with or without bevacizumab as first-line therapy for patients with *EGFR*-mutant NSCLC. A secondary objective of the ACCRU RC1126 study is to estimate the prevalence of the pretreatment T790M mutation and to investigate progression-free survival of patients with *EGFR*-mutant NSCLC with and without pretreatment T790M mutation. The ACCRU RC1126 study is ongoing and the results are awaited to confirm the efficacy and safety shown in our study.

patients and is positively selected during EGFR tyrosine kinase inhibitor therapy.[5]

The coexistence of activating *EGFR* and pretreatment T790M resistance mutations has been underappreciated despite accumulating evidence that the pretreatment T790M mutation occurs in approximately 35–60% of patients with *EGFR*-mutant NSCLC, depending on the detection method.[6,7] Identification of this mutation by conventional direct sequencing might be made difficult by allelic dilution; however, our findings suggest that a low frequency of pretreatment T790M mutant allele expression is sufficient to confer shorter progression-free survival.[8,9] Rosell and colleagues[8] have developed methods to detect pretreatment T790M mutations.[9] Many resistance-associated mutations might be enriched from a small and undetectable pre-existing population, as has been seen with the T798M mutation in the HER2 kinase domain in breast cancer cells.[10] The H1975 cell line was derived from a female never smoker patient with pulmonary adenocarcinoma carrying exon 21 missense mutation (L858R) and exon 20 missense mutation (T790M).[11] The H1975 cell line is resistant to EGFR tyrosine kinase inhibition, but the combination of gefitinib with the vascular endothelial growth factor (VEGF) inhibitor bevacizumab was previously shown to inhibit tumour growth in H1975 xenograft tumours.[12,13]

Preclinical evidence suggests that NSCLCs with both activating *EGFR* and T790M mutations exhibit elevated levels of phosphorylated signal transducer and activator of transcription 3 (STAT3), which is not inhibited by gefitinib.[14] STAT3 levels were increased almost immediately after starting erlotinib treatment in *EGFR*-mutant NSCLC cells.[15] Experimental results have shown that STAT3 upregulates VEGF expression; STAT3, but not MAPK kinase or AKT-dependent pathways, was noted to be essential for interleukin 6 induced expression of VEGF in cervical cancer and VEGF was found to be inhibited by blocking STAT3 or by treatment with an anti-VEGF antibody.[16] Interleukin 6 concentrations are elevated in *EGFR* mutant cell lines and mechanistically correlate with STAT3 levels;[14] as such, a combined treatment with EGFR tyrosine kinase

inhibitors and VEGF-neutralising antibodies might attenuate the development of resistance driven by the interleukin 6–STAT3–VEGF pathway in *EGFR*-mutant NSCLC.

Additionally, we previously reported that low mRNA levels of breast cancer-related gene 1 (*BRCA1*) correlate with prolonged progression-free survival in patients with *EGFR*-mutant NSCLC treated with erlotinib.[8] We were prompted to investigate the potential effect of astrocyte elevated gene-1 (AEG-1) because it is involved in regulating the nuclear factor kappa B signalling pathway,[17] which is associated with resistance to EGFR tyrosine kinase inhibitor in *EGFR*-mutant NSCLC.[18]

To test this hypothesis clinically, the Spanish Lung Cancer Group and the European Thoracic Oncology Platform developed the BELIEF trial, a proof-of-concept study, in which patients with *EGFR*-mutant NSCLC were treated with the combination of erlotinib and bevacizumab, stratified by the presence of the pretreatment T790M allele (ie, T790M positive or negative).

Methods

Study design and participants

BELIEF was an international, multicentre, single-arm, phase 2 trial to test the efficacy of the combination of erlotinib and bevacizumab in treatment-naive patients with NSCLC positive for an activating *EGFR* mutation (exon 19 deletion or L858R mutation), with or without T790M, according to centralised assessment. The trial was done at 29 centres in eight European countries (Spain, Switzerland, UK, Greece, Italy, Ireland, France, and Germany).

Eligible patients were aged 18 years or older, had measurable or assessable stage IIIB or IV lung adenocarcinoma, documented and centrally confirmed activating *EGFR* mutation (exon 19 deletion or L858R mutation), Eastern Cooperative Oncology Group performance status 0–2, adequate haematological, hepatic, and renal function, and a life expectancy of 2 months or longer at the time of registration. Patients with symptomatic brain metastases, increased risk of bleeding, or coagulation disorders were excluded.

Two substudies, one for each centrally confirmed pretreatment T790M mutation status, each with its own statistical design, were run in parallel. To address the biological hypothesis of the BELIEF study that the combination of erlotinib plus bevacizumab can benefit patients who are T790M positive, we chose a Simon's two-stage design for substudy one, allowing us to interrupt the study early in case of futility. Substudy two was a companion substudy aiming to show that the 12 month progression-free survival in patients who are T790M negative receiving erlotinib plus bevacizumab would be similar, or slightly higher, than that obtained in previous studies of erlotinib only treatment in T790M-negative patients. No early study interruption for futility was of interest for this substudy, and we used a simple one-arm design (Fleming's single-stage design). The inclusion of all patients with *EGFR* mutations in the study, not only the patients who were T790M positive, allowed for the assessment of the overall response to erlotinib and bevacizumab and the response by T790M status. Each substudy was run as an independent study with patients receiving the same treatment and with the same primary endpoint—namely 12 month progression-free survival.

All patients provided written informed consent before study entry, and the institutional review boards of all participating institutions approved the trial protocol.

Procedures

Patients received erlotinib 150 mg per day orally and bevacizumab 15 mg/kg intravenously on day 1 of each 21 day cycle. Patients remained on trial treatment until documented disease progression according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 or unacceptable toxicity. For the analysis, the patients were considered as on trial treatment as long as at least one of the study drugs could be continued. We allowed a maximum of 6 weeks of repeated dose interruptions. The main tolerability criterion was the occurrence of adverse events graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Activating *EGFR* and T790M mutations were centrally assessed, as previously described,[8,19] with a peptide

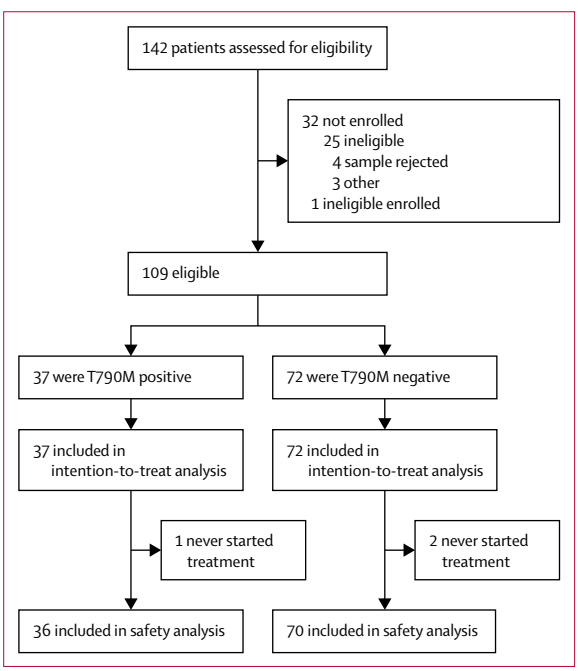

***Figure 1*: Trial profile**

(M A Molina-Vila PhD); **and Swiss Group of Clinical Cancer Research, Bern, Switzerland** (A Curioni-Fontecedro, R A Stahel, O Gautschi, S Peters, M Früh, M Pless, P Froesch, D Rauch)

Correspondence to: Dr Rolf A Stahel, University Hospital Zurich, Clinic of Oncology, Zurich, CH-8091, Switzerland
rolf.stahel@usz.ch

See Online for appendix

nucleic acid (PNA) probe-based 5′-nuclease real-time PCR (PNA probe-based TaqMan assay; appendix p 2).[20] Detailed information on the validation process of the PNA probe-based TaqMan assay is provided in the appendix (pp 5–8).

We sent tumour material that was remaining after the per-protocol central analysis in Barcelona to a third party research organisation where DNA was extracted and examined with the COBAS EGFR assay and droplet digital PCR (both of which are approved by the US Food and Drug Administration). Further details about the analysis of *EGFR* mutations are provided in the appendix (pp 3–4).

Outcomes

The primary endpoint was progression-free survival, which was defined as the time from enrolment until an investigator-documented progression of disease according to RECIST 1.1 or death if no documented progression occurred. We deemed patients who died without a documented progression to have progressed on the date of their death. Patients who had not progressed or died were censored on the date of their last tumour assessment.

Secondary endpoints were overall survival, time-to-treatment failure, proportion of patients achieving an objective response (defined as best overall response [complete response or partial response] according to RECIST 1.1 criteria), proportion achieving disease control (defined as objective response or stable disease for at least 6 weeks) according to RECIST 1.1, duration of response, and safety profile. A secondary objective of the trial was to investigate whether the T790M status and the amount of *BRCA1* expression and *AEG1* expression affect progression-free survival in patients given erlotinib and bevacizumab.

	T790M positive (n=37)	T790M negative (n=72)	All patients (N=109)
Age* (years)	69·5 (62·3–74·0)	63 (53·4–71·2)	66·1 (57·1–72·4)
Sex			
Female	25 (68%)	42 (58%)	67 (61%)
Male	12 (32%)	30 (42%)	42 (39%)
Smoking status			
Current smoker	0 (0%)	7 (10%)	7 (6%)
Former smoker	10 (27%)	20 (28%)	30 (28%)
Never smoked	27 (73%)	45 (63%)	72 (66%)
Histological diagnosis			
Adenocarcinoma	34 (92%)	59 (82%)	93 (85%)
Adenosquamous carcinoma	1 (3%)	1 (1%)	2 (2%)
Not otherwise specified	1 (3%)	2 (3%)	3 (3%)
Unknown	1 (3%)	10 (14%)	11 (10%)
ECOG performance status			
0	17 (46%)	36 (50%)	53 (49%)
1	18 (49%)	32 (44%)	50 (46%)
2	2 (5%)	4 (6%)	6 (6%)
Brain metastasis			
Yes	7 (19%)	14 (19%)	21 (19%)
No	30 (81%)	58 (81%)	88 (81%)
Type of *EGFR* mutation			
Deletion of exon 19	23 (62%)	47 (65%)	70 (64%)
L858R mutation in exon 21	14 (38%)	25 (35%)	39 (36%)
BRCA1 mRNA expression			
Low (<9·2)	10 (27%)	13 (18%)	23 (21%)
High (≥9·2)	10 (27%)	13 (18%)	23 (21%)
No material or no value	17 (46%)	46 (64%)	63 (58%)
AEG1 mRNA expression			
Low (<1)	11 (30%)	20 (28%)	31 (28%)
High (≥1)	12 (32%)	18 (25%)	30 (28%)
No material or no value	14 (38%)	34 (47%)	48 (44%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. *Age p=0·023; all other p values comparing characteristics between the T790M cohorts were non-significant.

Table 1: **Baseline characteristics**

Statistical analysis

The trial was designed to detect an improvement in the median progression-free survival in patients with advanced NSCLC and *EGFR* mutations after erlotinib and bevacizumab treatment. Sample size calculations were based on published results on the median progression-free survival of patients with advanced NSCLC with *EGFR* mutations who were given erlotinib treatment (9 months for T790M positive and 18 months for T790M negative), and the percentage of patients with *EGFR* mutations who were T790M positive (35% using the TaqMan assay).[8]

The main focus of our study was on patients who were T790M positive (substudy one). We chose an all-comers design to assess response to bevacizumab and erlotinib treatment in parallel in the patients who were T790M negative. In substudy one, we adopted a Simon's two-stage design to investigate the 12 month progression-free survival in the T790M-positive subgroup. The target was a 12 month progression-free survival of 63% (P_1), corresponding to a median progression-free survival of 18 months. A 12 month progression-free survival of 40% (P_0) was deemed inadequate (median of 9 months).[8] For one-sided $\alpha=0\cdot05$ and $\beta=0\cdot20$, a total of 35 patients who were *EGFR* T790M positive needed to enter the trial, with eight patients in the first stage of the trial. In the formal interim efficacy analysis, four or more patients needed to reach 12 months without a progression-defining event to proceed to the second stage (where 19 or more of the 35 patients had to be progression-free at 12 months to reject the null hypothesis of $P_0 \leq 40\%$). In substudy two, based on Fleming's single-stage design (one-sided $\alpha=0\cdot05$), the required sample size to target the 12 month progression-free survival of 65% (P_1) with 80% power (*vs* P_0 of 50%) was 67 patients who were T790M negative. This target corresponds to a median progression-free survival of 19 months, which

is slightly better than previously reported with erlotinib.[8] We planned to enrol 102 patients overall, thereby satisfying the required sample size in both substudies.

The intention-to-treat efficacy analysis included all enrolled patients irrespective of whether they received any treatment. The primary efficacy analysis was designed to be done separately for each substudy. Additionally, a secondary objective was to compare the efficacy between the pretreatment T790M-positive and T790M-negative cohorts. Interim safety analyses for the full cohort were done every 6 months, and reviewed by the European Thoracic Oncology Platform Independent Data Monitoring Committee. The final safety analysis included all patients that received at least one dose of trial treatment.

We estimated progression-free survival and other secondary time-to-event endpoints, along with their medians and 12 month rates, with the product-limit Kaplan-Meier method. We calculated the 95% CIs for the median values using the complementary log–log transformation. We used the log-rank test for the secondary objective comparisons between groups defined by T790M mutation status and other baseline characteristics. We used Cox proportional-hazard models, including each baseline characteristic or biomarker separately, along with the T790M status and their interaction, to assess the possible differential effect of T790M status for different levels of the variable of interest (depicted graphically in a forest plot). A multivariable Cox model was also chosen by the backward elimination procedure (p>0·10) to assess the effect of T790M mutation status adjusted for the variables of clinical interest. Adjusted hazard ratios (HRs) and corresponding 95% CIs were estimated from the Cox model. We assessed departures from the proportional hazards assumption for all Cox models on the basis of the Schoenfeld residuals. We used SAS version 9.3 for the statistical analysis. The final statistical analysis was implemented when the last enrolled patient completed 1 year of follow-up, and was done by substudy and for the full study cohort. Additional statistical methods are provided in the appendix (p 4).

This trial was registered with ClinicalTrials.gov, number NCT01562028.

Role of the funding source

The funder provided the study drug and financed the trial. As the sponsor, The European Thoracic Oncology Platform designed the trial, collected and analysed the data, and interpreted the results fully and independently from the funder. The authors vouch for the accuracy and completeness of the data. RR, RAS, and UD prepared the manuscript outline, but all authors contributed to subsequent drafts and made the decision to submit the report for publication.

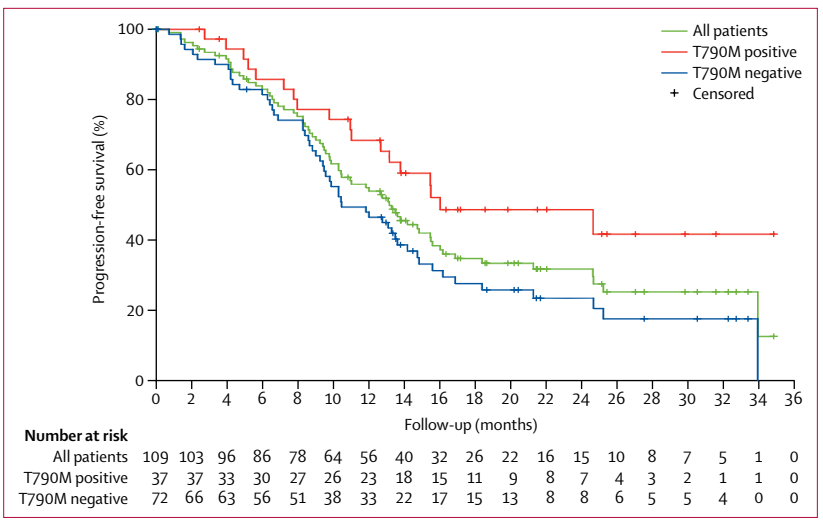

Figure 2: **Progression-free survival by pretreatment T790M mutation status in the intention-to-treat population**

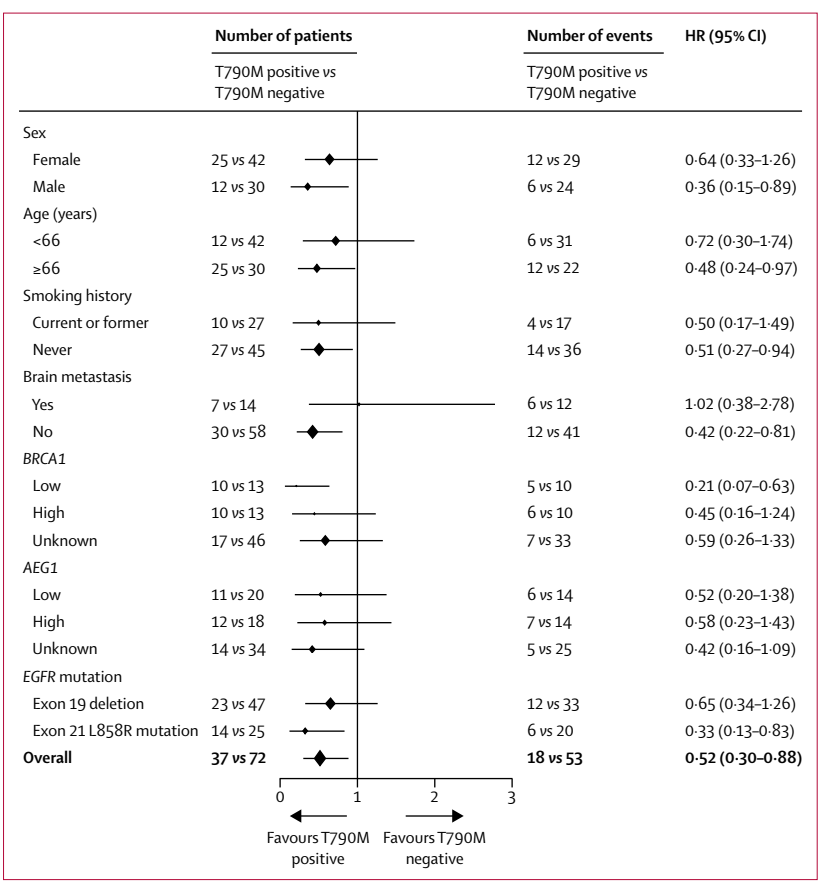

Figure 3: **Forest plot of hazard ratios for progression-free survival by baseline characteristics and biomarkers**
All interaction p values of each variable (baseline characteristic or biomarker) with T790M from separate Cox models are not significant at α=10%.

Results

Between June 11, 2012, and Oct 28, 2014, 109 eligible patients were enrolled and all were included in the efficacy analysis. Three patients never started

	Hazard ratio (95% CI)	p value
T790M mutation positive *vs* T790M mutation negative	0·37 (0·20–0·67)	0·0011
Female *vs* male	0·66 (0·40–1·08)	0·099
High *BRCA1 vs* low *BRCA1*	0·73 (0·36–1·50)	0·39
Unknown* *BRCA1 vs* low *BRCA1*	0·40 (0·21–0·78)	0·0074
No brain metastasis *vs* brain metastasis	0·45 (0·26–0·78)	0·0047

Best model according to backward elimination (p>0·10) with initially included explanatory variables: pretreatment T790M mutational status, type of *EGFR* mutation, sex, smoking history, *BRCA1* and *AEG1* mRNA expression, age, and brain metastases *Unknown is either no value or no material.

Table 2: **Multivariable Cox model for progression-free survival**

	T790M positive (n=37)	T790M negative (n=72)	All patients (N=109)
Complete response	3 (8%)	3 (4%)	6 (6%)
Partial response	24 (65%)	54 (75%)	78 (72%)
Stable disease	8 (22%)	9 (13%)	17 (16%)
Progressive disease	1* (3%)	3 (4%)	4 (4%)
Non-assessable*	1 (3%)	3 (4%)	4 (4%)

*Patients with only one tumour assessment are classified as non-assessable because their objective response cannot be assessed. A total of five patients were non-assessable; among them one was classified as having progressive disease.

Table 3: **Overall best objective responses by pretreatment T790M mutation**

treatment (two were lost to follow-up and one withdrew; figure 1). The cutoff date for the final analysis was Dec 17, 2015. With the PNA probe-based TaqMan assay, 37 (34%, 95% CI 26–43) patients were T790M positive and the remaining 72 (66%, 57–74) were T790M negative (figure 1). In a post-hoc exploratory analysis, T790M status was assessed with orthogonal methods (appendix pp 4–5).

Baseline patient and tumour characteristics for the full cohort and by T790M mutation status were recorded (table 1). In the full cohort, median age was 66·1 years (IQR 57·1–72·4) and most were female and never smokers. Age was the only characteristic found to significantly differ between the two T790M groups (p=0·023).

At a median follow-up of 21·4 months (IQR 15·9–30·7), a progression event or death had occurred in 71 (65%) of 109 patients with a median progression-free survival of 13·2 months (95% CI 10·3–15·5). The 12 month progression-free survival was 55% (95% CI 45–64) for all patients, 68% (50–81) for the T790M-positive patients, and 48% (36–59) for the T790M-negative patients (log-rank p=0·014; figure 2). In the substudy of patients who were T790M positive, the first stage (interim analysis for futility) of Simon's two-stage trial was successful and the study continued accruing. Among the first 35 patients who were T790M positive, 23 reached 12 months without a progression-free survival event and thus, according to Simon's two-stage design, the T790M-positive substudy showed that erlotinib plus bevacizumab is a promising treatment in this subgroup.

Median progression-free survival was 16·0 months (95% CI 12·7 to not estimable) in the T790M-positive group and 10·5 months (9·4–14·2) in the T790M-negative group (unadjusted HR 0·52, 95% CI 0·30–0·88; p=0·016; figure 2). Median progression-free survival was 14·7 months (12·0–18·4) for patients without brain metastases and 8·8 months (6·0–10·5) for patients with brain metastases (adjusted for T790M mutation HR 0·48, 95% CI 0·27–0·82; p=0·0078). Figure 3 compares the risk of progression between the T790M mutation groups for different levels of baseline characteristics and biomarkers. No significant interaction was found between any variable and T790M mutation (all interaction p>0·10, from separate Cox models of progression-free survival). In the T790M-negative group, patients with high *BRCA1* mRNA expression had a median progression-free survival of 9·4 months (95% CI 4·1–14·7), which was non-significantly longer than the 6·5 months (95% CI 1·6–11·8) of patients with low *BRCA1* mRNA expression (p=0·33; appendix p 13), a result based on small numbers of events. In the group of patients with deletion 19, median progression-free survival was 15·5 months for those who were T790M positive versus 13·3 months for those who were T790M negative (p=0·22). In the group of patients with L858R, median progression-free survival was 24·6 months for those who were T790M positive versus 9·7 months for those who were T790M negative (p=0·022; appendix pp 16–17).

A multivariable Cox proportional hazards model for progression-free survival was fitted with the following clinically interesting variables: T790M mutational status, type of *EGFR* mutation, sex, smoking history, age, brain metastases, and *BRCA1* and *AEG1* mRNA expression. The presence of the T790M mutation and absence of brain metastases contributed significantly to progression-free survival. Patients with the T790M mutation were less likely to progress than patients without the mutation, and patients without brain metastases were less likely to progress than patients with brain metastases (table 2). Proportionality assumption has been tested and it holds in all Cox models used.

Median overall survival was 28·2 months (95% CI 21·4–41·8) with an estimated 84% (75–90) 12 month overall survival; for overall survival by T790M mutation status see the appendix (p 18). No further analysis on overall survival is presented in this report due to immature data. The median time-to-treatment failure was 9·2 months (6·6–11·2), with treatment failures in 87 (80%) of 109 patients overall. For the

T790M-positive group, time-to-treatment failure was 13·4 months (5·6–19·6), with treatment failures in 26 (70%) of 37 patients, whereas for the T790M-negative group, time-to-treatment failure was 8·3 months (6·3–9·8), with treatment failures in 61 (85%) of 72 patients (p=0·073). Overall, 84 (77%) of 109 patients achieved an objective response. Six (6%) patients achieved a complete response and 78 (72%) a partial response. Overall, 101 (93%) of 109 patients achieved disease control. The proportion of patients achieving an objective response was similar in the two T790M groups (table 3, figure 4). Median duration of response was 14·7 months (10·6–32·5) overall, not yet reached (14·7 to not estimable) for the T790M-positive group, and 12·0 months (8·2–20·2) for the T790M-negative group.

The relative dose intensity was 94% (range 39–100) for erlotinib and 98% (4–117%) for bevacizumab. The relative dose intensity for erlotinib was similar in the two T790M groups (89% in the T790M-positive group and 95% in the T790M-negative group). Similarly, the relative dose intensity for bevacizumab did not differ between the two groups (99% *vs* 97%). Overall, 19 (17%) of 109 patients discontinued bevacizumab because of toxicity and continued with erlotinib alone. Four patients discontinued bevacizumab due to rectal bleeding, two due to proteinuria, and two due to pancreatitis, whereas three discontinued erlotinib due to rash. The median time to bevacizumab discontinuation was 9·2 months (95% CI 6·6–11·3), and the median time to erlotinib discontinuation was 11·1 months (9·2–13·6).

All but one patient had at least one adverse event and 31 (29%) of 106 patients also had a serious adverse event. Hypertension and rash were the most frequent grade 3 adverse events (table 4). There were five grade 4 (acute coronary syndrome, biliary tract infection, colonic perforation, and other neoplasms) and one grade 5 (death due to sepsis) events. The worst adverse event for 74 (70%) of 106 patients was of grade 3, and for 23 (22%) was of grade 2.

Due to the potential clinical relevance of T790M mutations, a post-hoc analysis with the COBAS EGFR test and a highly sensitive droplet digital PCR approach was done. As expected, the COBAS test showed high concordance with the PNA probe-based TaqMan assay for activating *EGFR* mutations, but did not detect any T790M. This is consistent with the findings in the EURTAC study.[9] With respect to the comparison between the PNA probe-based Taqman assay and the droplet digital PCR assay, fair agreement was obtained, which was better in samples with higher abundance of the T790M mutation (appendix p 10). Notably, the distribution of the T790M mutation within positive tumours is heterogeneous, and different tumour areas were selected for the PNA probe-based TaqMan assay and the droplet digital PCR analyses.

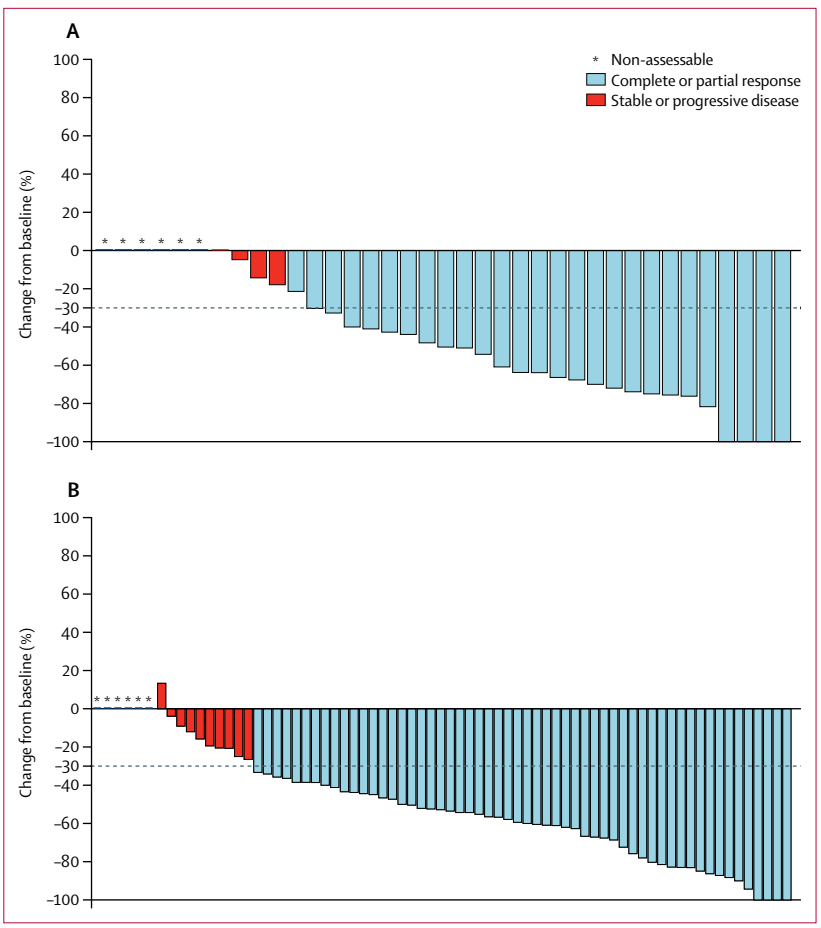

Figure 4: **Waterfall plot of best percentage change from baseline in the sum of longest tumour diameters**
(A) T790M-positive patients. (B) T790M-negative patients. Responders were confirmed according to Response Evaluation Criteria in Solid Tumors 1.1. Of 109 patients, seven had only non-target lesions and five had only one available tumour assessment; as such, no comparison with the baseline could be implemented for these 12 patients, who are shown as non-assessable.

Discussion

This is the first study assessing the efficacy of the combination of erlotinib and bevacizumab in patients with NSCLC with both activating *EGFR* and T790M mutations. The patients carrying the *EGFR* activating mutation and T790M had a longer median progression-free survival than the patients without T790M (p=0·016).

The effect of bevacizumab in patients with *EGFR*-mutant NSCLC was first noted in the phase 3 BeTa study[21] of second-line treatment of patients with NSCLC, in which the median progression-free survival of patients with *EGFR* mutation given erlotinib plus bevacizumab (17·1 months) was higher than that of patients given erlotinib alone (9·7 months). While the BELIEF study was ongoing, another phase 2 randomised study[22] in patients with NSCLC with activating *EGFR* mutation, excluding patients with a T790M mutation or brain metastases, was published. The median progression-free survival was 16·0 months

	All (N=106)	Grade 1–2	Grade 3	Grade 4	Grade 5
Hypertension	95 (90%)	56 (53%)	39 (37%)	0	0
Diarrhoea	87 (82%)	77 (73%)	10 (9%)	0	0
Rash maculopapular	84 (79%)	63 (59%)	21 (20%)	0	0
Proteinuria	61 (58%)	52 (49%)	9 (8%)	0	0
Fatigue	57 (54%)	52 (49%)	5 (5%)	0	0
Cough	54 (51%)	54 (51%)	0	0	0
Epistaxis	38 (36%)	37 (35%)	1 (1%)	0	0
Dry skin	37 (35%)	37 (35%)	0	0	0
Nausea	33 (31%)	33 (31%)	0	0	0
Dyspnoea	30 (28%)	29 (27%)	1 (1%)	0	0
Mucositis oral	30 (28%)	29 (27%)	1 (1%)	0	0
Alanine aminotransferase increase	29 (27%)	24 (23%)	5 (5%)	0	0
Anorexia	28 (26%)	27 (25%)	1 (1%)	0	0
Aspartate aminotransferase increase	28 (26%)	25 (24%)	3 (3%)	0	0
Rash acneiform	23 (22%)	20 (19%)	3 (3%)	0	0
Pain	22 (21%)	20 (19%)	2 (2%)	0	0
Skin and subcutaneous tissue disorders	22 (21%)	22 (21%)	0	0	0
Abdominal pain	21 (20%)	18 (17%)	3 (3%)	0	0
Bone pain	21 (20%)	20 (19%)	1 (1%)	0	0
Headache	21 (20%)	19 (18%)	2 (2%)	0	0
Back pain	20 (19%)	20 (19%)	0	0	0
Constipation	20 (19%)	19 (18%)	1 (1%)	0	0
Alopecia	19 (18%)	19 (18%)	0	0	0
Conjunctivitis	18 (17%)	18 (17%)	0	0	0
Dysgeusia	18 (17%)	18 (17%)	0	0	0
Vomiting	18 (17%)	17 (16%)	1 (1%)	0	0
Dizziness	17 (16%)	16 (15%)	1 (1%)	0	0
Pruritus	16 (15%)	16 (15%)	0	0	0
Urinary tract infection	15 (14%)	12 (11%)	3 (3%)	0	0
Thromboembolic event	8 (8%)	4 (4%)	4 (4%)	0	0
Lung infection	6 (6%)	2 (2%)	4 (4%)	0	0
Aphonia	4 (4%)	0	4 (4%)	0	0
Acute coronary syndrome	3 (3%)	2 (2%)	0	1 (1%)	0
Pleural effusion	3 (3%)	0	3 (3%)	0	0
Colonic perforation	2 (2%)	0	0	2 (2%)	0
Sepsis	2 (2%)	0	1 (1%)	0	1 (1%)
Biliary tract infection	1 (1%)	0	0	1 (1%)	0
Neoplasms benign, malignant, and unspecified (including cysts and polyps)	1 (1%)	0	0	1 (1%)	0

Adverse events reported by 15% or more patients for grades 1–2, 2% or more patients for grade 3, and all adverse events for grades 4 and 5 (safety population).

Table 4: **Adverse events**

with erlotinib plus bevacizumab and 9·7 months with erlotinib alone (HR 0·54, 95% CI 0·36–0·79; p=0·0015).[22] The most common grade 3 or worse adverse events were rash, hypertension, and proteinuria[22]—at a similar frequency as those reported in the present study. The Swiss Group for Clinical Cancer Research noted that therapy with erlotinib and bevacizumab was well tolerated in patients with advanced *EGFR*-mutant NSCLC, who had a median progression-free survival of 14 months.[23] A second phase 2 study[24] combining gefitinib plus bevacizumab in patients with *EGFR*-mutant NSCLC reported similar outcomes, with a median progression-free survival of 14·4 months.

Several questions arise from these findings. First, why is overall survival longer in patients with acquired T790M? Patients with *EGFR* T790M have longer median post-progression survival than those with *EGFR* T790M-negative tumours (1·9 *vs* 1·6 years, p=0·015).[25] Acquired resistance occurs either by emergence of the pre-existing resistant clone, T790M, or by evolution of initially *EGFR* T790M-negative drug-tolerant cells that acquire T790M during the course of EGFR tyrosine kinase inhibitor therapy.[26]

Second, several studies have detected low frequency of *EGFR* T790M-positive clones in pre-therapy patient specimens. Maheswaran and colleagues[27] reported a progression-free survival of 8 months with erlotinib for patients with pretreatment *EGFR* T790M-positive tumours, compared with 17 months for patients with *EGFR*-mutant tumours without T790M (p<0·001). Additionally, in the EURTAC study,[3] in patients with the *EGFR* mutation treated with erlotinib, the median progression-free survival was 9·7 months for those with T790M, and 15·6 months for those without T790M (p=0·018)[9]. Su and colleagues[28] also showed shorter progression-free survival for patients with T790M than without (6·7 months *vs* 10·2 months; p=0·030).[28]

Finally, why does the combination of erlotinib with bevacizumab prolong progression-free survival only in the T790M-positive subgroup? The T790M mutation, when combined with the activating mutations L858R or deletion 19, results in a substantial enhancement of EGFR activity.[5] EGFR-mediated signalling upregulates neuropilin 1, VEGFR, and VEGF expression, promoting angiogenesis, which results in synergistic crosstalk between EGFR and VEGFR (appendix pp 19–20).[29] Erlotinib resistance is associated with an increase in both tumour cell and host stromal VEGF, and the combination of bevacizumab with erlotinib abrogates primary resistance in the H1975 tumour model.[12] However, bevacizumab was not active in *EGFR*-mutant cell lines without T790M (the H3255 cell line with L858R mutation and the HCC827 cell line with deletion 19).[12] Additionally, the combination of afatinib with bevacizumab suppressed tumours harbouring the T790M mutation.[29] In the current study, a substantial benefit was noted from the combination of erlotinib plus bevacizumab in the subgroup of patients with pretreatment T790M. The BELIEF results mirror the naive-treated H1975 (L858R plus T790M) cell line, in which the combination of gefitinib or afatinib with bevacizumab suppressed tumour growth.[12,13,30]

When we examined the prespecified molecular markers *BRCA1* and *AEG1*, the results were not conclusive, probably because the combination of erlotinib and bevacizumab can partly obscure their biological significance. We are performing additional gene expression profiling of formalin-fixed, paraffin-embedded tumour tissue from the BELIEF trial to identify the potential relation between the interleukin 6–STAT3–VEGF pathway and double *EGFR* mutations.

In conclusion, the BELIEF trial further validates previous studies—supporting the compelling evidence for combinatorial therapy in patients with *EGFR*-mutant NSCLC. On June 8, 2016, the European Medicines Agency approved the use of bevacizumab in combination with erlotinib as first-line treatment for patients with advanced metastatic or recurrent NSCLC with activating *EGFR* mutations on the basis of the results of a randomised phase 2 trial and supporting evidence from other trials,[22,24] including the BELIEF trial presented here. A phase 2 clinical trial assessing the safety and efficacy of osimertinib as a first-line treatment for patients with pretreatment T790M is ongoing (AZENT, NCT02841579). Additionally, the European Thoracic Oncology Platform is assessing the combination of osimertinib with bevacizumab in an investigator-initiated randomised phase 2 trial as second-line therapy in patients with advanced NSCLC with confirmed activating *EGFR* and T790M mutation (BOOSTER, EudraCT number 2016-002029-12).

Contributors

RR and RAS designed the trial. UD was responsible for statistical planning and data analysis. RR, RAS, SPe, and UD were involved in data interpretation and preparation of the report. All other authors were involved in data collection, and preparation and finalisation of the report.

Declaration of interests

SPo is consultant to Ariad, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Merck Sharp and Dohme, Novartis, Pfizer, and Eli Lilly, has received honoraria from Boehringer Ingelheim, Pfizer, and Eli Lilly, travel expenses from Boehringer Ingelheim, Bristol-Myers Squibb, Merck Sharp and Dohme, and Pfizer, and research funding from Boehringer Ingelheim and Pierre Fabre. MP and RS have received honoraria as consultants on a Roche advisory board. RT and DSS are affiliated with Genentech and own stock in Roche Holdings. RAS has received honoraria as a consultant on advisory boards from Abbvie, AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Eli Lilly, Merck Sharp and Dohme, Pfizer, and Roche, and as a speaker from Astellas, AstraZeneca, Lilly, Merck Sharp and Dohme, Novartis, and Roche. All other authors declare no competing interests.

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