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Rationale and Study Design of the IRENE-Trial (NVALT-16): A Phase II Trial to Evaluate Iressa Rechallenge in Advanced NSCLC Patients With an Activating *EGFR* Mutation Who Responded to an EGFR-TKI Used As First-Line or Previous Treatment

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Abstract

Background: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have shown improved progression-free survival (PFS) and overall survival (OS) over chemotherapy in a molecularly defined subgroup of advanced non–small-cell lung cancer (NSCLC) patients (ie, patients with an activating mutation in the *EGFR* gene). Nevertheless, all *EGFR*-mutated NSCLC patients develop TKI resistance eventually and there is no registered treatment or therapeutic strategy available for these patients. Several retrospective or small cohort studies have described patients who re-responded to EGFR-TKI treatment after a TKI-free interval ('drug holiday'). To date, no large prospective evaluation of the clinical effects of EGFR-TKI rechallenge in *EGFR*-mutated NSCLC patients has been performed. **Patients and Methods:** The IRENE (Iressa RE-challenge in advanced, *EGFR*-mutated NSCLC patients who responded to an EGFR-TKI used as first-line or previous treatment) (Dutch association for pulmonologists [NVALT]-16) trial is a multicenter, open-label, single-arm, single-stage, phase II study to evaluate gefitinib rechallenge in *EGFR*-mutated NSCLC patients who were previously treated with a TKI followed by a subsequent line of treatment (excluding EGFR-TKIs). The primary objective is disease control rate according to Response Evaluation Criteria in Solid Tumors criteria. Secondary objectives are objective response rate, PFS, OS, mutation characterization of sequential biopsies, VeriStrat correlation to PFS and OS, analysis of tumor-derived RNA in blood platelets and analysis of cell-free DNA in blood plasma. **Results:** The IRENE (NVALT-16) trial will evaluate the safety, efficacy, and feasibility of readministration of gefitinib after an EGFR–TKI-free interval in *EGFR*-mutated NSCLC patients. **Conclusion:** The study will evaluate gefitinib re-challenge in *EGFR*-mutated NSCLC patients. The study will also provide more insight into the dynamic development of molecular characteristics of *EGFR*-mutated NSCLC along the course of the disease.

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide.[1] Most lung cancer concerns non–small-cell lung cancer (NSCLC) and prognosis after diagnosis of stage IV NSCLC is poor, with median overall survival (OS) of 9 to 12 months.[2] In recent years, the introduction of targeted therapies has improved survival for subgroups of molecularly defined NSCLC patients. NSCLC-patients with an activating mutation in the epidermal growth factor receptor (*EGFR*$^+$) are the most prevalent 'druggable' subgroup. Activating *EGFR* mutations occur in 9.4% of all NSCLC patients, with higher prevalence in adenocarcinoma, Asian individuals, women, and nonsmokers.[3-5] Treatment with EGFR tyrosine kinase inhibitors (TKIs) is the cornerstone of treatment for these patients and 70% to 80% of these patients respond to EGFR-TKIs in first-line treatment.[6-11] Erlotinib (Tarceva), gefitinib (Iressa), and afatinib (Gilotrif) are registered TKIs for first-line and maintenance treatment of *EGFR*$^+$ NSCLC patients. Despite the high response rate, virtually every patient will develop EGFR-TKI resistance after a median progression-free survival (PFS) of 8.4 to 13.1 months.[6-11]

Rebiopsy studies have elucidated several mechanisms of resistance. At the time of acquired resistance, a secondary mutation in exon 20 of *EGFR*, the T790M mutation, is detected in more than half of the patients.[12] Other, less frequently detected mechanisms of resistance are: transformation to small-cell lung cancer (1%-14%), HER2 amplification (13%), and c-MET amplification (5%-21%).[12-14] However, in a substantial number of patients the resistance mechanism remains unknown.

There is currently no registered treatment or therapeutic strategy available for *EGFR*-mutated NSCLC patients with acquired EGFR-TKI resistance. Shortly after the introduction of gefitinib and erlotinib as first-line treatment for *EGFR*-mutated NSCLC patients, successful reinitiation of gefitinib in an *EGFR*-mutated NSCLC patient after an EGFR–TKI-free period was described.[15] Later, other groups[16-25] reported the beneficial effect of a so-called 'drug-holiday' as well (Table 1).[16-25] Moreover, EGFR-TKI rechallenge has been significantly correlated with improved OS in *EGFR*-mutated NSCLC patients.[20]

The phenomenon might be explained biologically according to the selection model.[26] *EGFR*-mutated NSCLC cells are thought to have a higher proliferation rate than *EGFR* wild type cells[27] and are therefore the most abundant population of cancer cells at baseline. These cells are highly sensitive to EGFR-targeted treatment and the initiation of EGFR-TKI treatment usually causes rapid elimination of these cells, leading to evident responses in most patients. However, meanwhile some (preexistent) resistant populations of tumor cells are provided the opportunity to proliferate, eventually leading to progressive disease. A substantial portion of patients are subsequently treated with chemotherapy after having acquired EGFR-TKI resistance, which is directed against all dividing cells instead of a particular molecularly defined subset. If progression occurs during this subsequent line of treatment, most progressing cancer cells might again be the sensitive *EGFR*-mutated cancer cells, because of the more rapid growth rate of this cell population compared with the resistant tumor cells with an inherent slower proliferation rate. This theory of 'dynamic adaptation' of predominant cancer cell populations in tumor lesions might explain the phenomenon of re-responses.

Reintroduction of EGFR-TKI treatment after a TKI-free period has been described several times, but all studies were retrospective and/or conducted in small cohorts of patients.[15-25] We therefore initiated the IRENE (Iressa RE-challenge in advanced NSCLC *EGFR*-mutated patients who responded to an EGFR-TKI used as first-line or previous treatment) trial (NVALT [Dutch association for pulmonologists]-16); a prospective, multicenter phase II trial to evaluate the reintroduction of gefitinib in *EGFR*-mutated NSCLC

Table 1 Studies Describing EGFR-TKI Rechallenge in *EGFR*-Mutated NSCLC Patients Who Responded to First-Line EGFR-TKI Treatment After a TKI-Free Period[a]

First Author, Year	Patient (*N*)	Prospective or Retrospective	EGFR Mutation Status	PR Rate	SD Rate	PFS2, Months	OS, Months
Watanabe, 2011[22]	8	Retrospective	Positive: 2 Unknown: 6	12.5%	50%	Median: 3.4	Median: NA Range: 2.1-24.6
Becker, 2011[17]	14	Retrospective	Positive: 12 Unknown: 2	36%	50%	Median: 6.5	NA
Oh, 2012[21]	23	Prospective	Positive: 14 Wild type: 1 Unknown: 8	21.7%	65.2%	Median: 3.4	Median: 11.3
Nishino, 2013[20]	65	Retrospective	NA	NA	NA	—	Median: 41.8
Yano, 2005[23]	3	Retrospective	NA	NA	NA	12, 7, and 12 months	NA
Asahina, 2010[16]	16	Prospective	Positive: 3 Negative: 3 Unknown: 10	0%	44%	Median: 2.5	Median: 14.7
Yokouchi, 2007[24]	5	Retrospective	Unknown: 5	20%	60%	Median: NA Range: 0.6-7.8	NA
Koizumi, 2012[19]	20	Prospective	Positive: 9 Negative: 1 Unknown: 10	15%	45%	Median: 2.0	Median: 12.0

Abbreviations: EGFR = epidermal growth factor receptor; NSCLC = non–small-cell lung cancer; PFS2 = progression-free survival with second line of EGFR-TKI treatment, after TKI-free period; TKI = tyrosine kinase inhibitor; NA = not available.
[a]Case reports with < 3 patients were not included in this table.[15,18,25]

patients who previously had been treated with an EGFR-TKI followed by another (non-TKI) line of treatment.

Patients and Methods

Study Design and Duration

The IRENE trial is a multicenter, open-label, single-arm, single-stage phase II study to characterize the efficacy of gefitinib rechallenge in standard dose in *EGFR*$^{+}$ NSCLC patients who responded to previous treatment with an EGFR-TKI followed by a subsequent anticancer therapy (excluding EGFR-TKIs) as their last treatment. The study is investigator-initiated by 'stichting NVALT studies.' The Dutch Cancer Society ("KWF kankerbestrijding") provided funding for data management. The study will be open in at least 35 academic and nonacademic centers with expertise in treating NSCLC throughout the Netherlands. Study duration is estimated at 2.5 years.

Inclusion Criteria

To be eligible for inclusion in the IRENE trial, patients must have histologically or cytologically confirmed NSCLC with an activating sensitizing EGFR mutation (including, but not limited to, EGFR exon 19 deletions or EGFR exon 21 insertions) using a validated methodology.[28] Stage of disease should be locally advanced (stage IIIB), not suitable for therapy of curative intent, or metastatic (stage IV). Patients should previously have been treated with an EGFR-TKI (erlotinib, gefitinib, or afatinib) with documented stable disease (SD) for at least 12 weeks or CR or PR followed by a subsequent line of non-TKI treatment (eg, chemotherapy) during which their disease must have progressed. Patients must be 18 years or older, have World Health Organization performance status of 0 to 2, must have a life expectancy of $\geq$ 12 weeks, and must have measurable disease defined as at least 1 lesion, not previously irradiated, that can be accurately measured at baseline as $\geq$ 10 mm in the longest diameter (except lymph nodes, which must have a short axis $\geq$ 15 mm) with spiral computed tomography (CT) scan and is suitable for accurate repeated measurements. Finally, there must be a possibility of obtaining tumor tissue before the start of gefitinib rechallenge for molecular characterization.

Exclusion Criteria

All patients who do not meet the inclusion criteria will be excluded. Other criteria for which patients will be excluded are known severe hypersensitivity to gefitinib, consideration to require radiotherapy to the lung at the time of the study entry, a past medical history of interstitial lung disease or radiation pneumonitis, known or suspected brain metastases unless locally treated with surgery and/or stereotactic radiotherapy, any unresolved chronic toxicity higher than Grade 2 (according to Common Terminology Criteria for Adverse Events[29]) from previous anticancer therapy, concomitant use of known CYP 3A4 inducers, pregnancy or breastfeeding, severe or uncontrolled systemic disease (eg, unstable or uncompensated respiratory, cardiac, hepatic, or renal disease as judged by the investigator), other coexisting malignancies or malignancies diagnosed within the past 2 years (basal cell carcinoma or cervical cancer in situ excluded), and treatment with a nonapproved or investigational drug within 30 days before the first day of study treatment. Finally, patients who are involved in the planning or conduct of the study and patients who were previously enrolled in this study will be excluded from participation.

Objectives

The primary objective is the disease control rate (confirmed complete response [CR] or PR, or SD) using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[30] The secondary objectives include objective response rate according to RECIST, PFS according to RECIST, OS, molecular characterization of sequential biopsies, the association between the VeriStrat assay[31] and PFS and OS, analysis of blood platelets for detection of (mutant) tumor cell RNA,[32] and analysis of blood plasma for detection of cell-free tumor DNA (cfDNA).[33]

Treatment and Safety

The treatment scheme and interventional procedures are depicted in Figure 1. Patients will be treated with gefitinib in standard dose (250 mg once daily). Physical examination and routine blood test (for liver function control) will be performed at follow-up visits in the outpatient clinic. Treatment will be continued until disease progression, withdrawal of informed consent, or unacceptable risks to the patient as judged by the investigator and/or principal investigator. Adverse events and serious adverse events will be evaluated according to the Common Terminology Criteria for Adverse Events version 4.0.[29] At disease progression, gefitinib may be continued at the discretion of the investigator outside the trial.

Assessment and Follow-Up

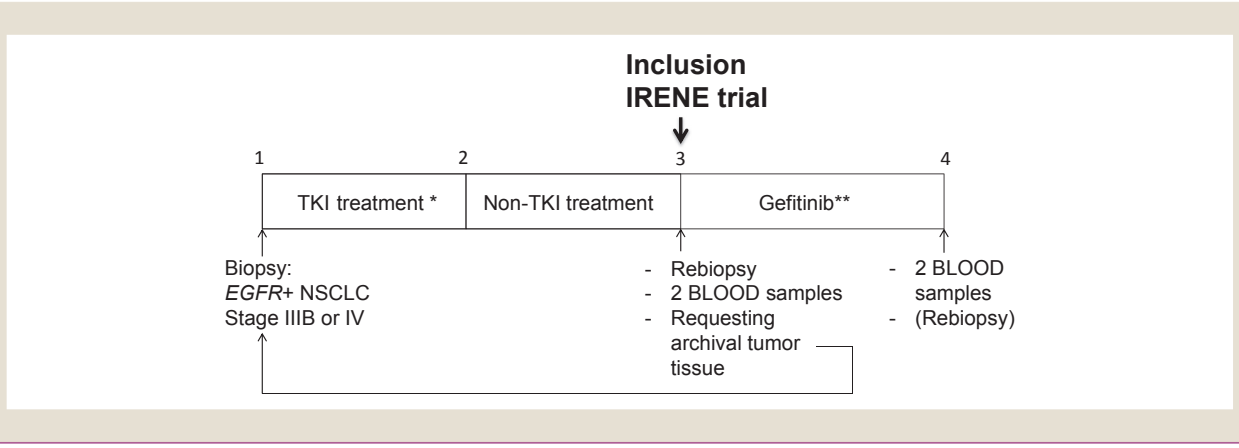
The study plan is depicted in Table 2. During the screening period, the local investigator will assess eligibility for inclusion in the study. If the patient fulfills all inclusion criteria and is eligible for the study, informed consent must be obtained before inclusion in the study. The first day of treatment is considered to be day 1. From then on, during the first 12 weeks of the study, patients will visit the outpatient clinic every 3 weeks. After 12 weeks, outpatient visits will be every 6 weeks. Tumor response will be evaluated every 6 weeks (within a window of approximately 7 days of the scheduled date), on CT scan of thorax and abdomen (including adrenal gland) according to RECIST 1.1.[30] Any other areas of disease involvement or the time at which new disease is suspected should be additionally investigated based on signs and symptoms of individual patients. Baseline assessments should be performed no more than 28 days before the start of treatment, and ideally should be performed as close as possible to the start of study treatment.

Biomarker and Pathology Analysis

Before the start of the study, a rebiopsy (preferably from a growing lesion) will be obtained. The original pretreatment biopsy (the 'archival biopsy') will as well be requested for analysis. Both biopsies will be analyzed for the presence of somatic gene mutations in a panel of genes, including *EGFR*, Kirsten rat sarcoma viral oncogene homolog (*KRAS*), phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*), and *MET*.[34]

Before the start and at the end of study, 2 blood samples will be obtained. One of these paired samples will be used for VeriStrat analysis. VeriStrat is a clinically validated proteomic blood test that can identify patients who are likely to have a good or poor outcome

Figure 1 Treatment Scheme and Interventional Procedures. (1) Time of First Diagnosis; *EGFR*$^+$ NSCLC Stage IV. Start of TKI Treatment. (2) Progression of Disease During TKI Treatment. Start of Subsequent, Non-TKI Treatment (Eg, Chemotherapy). (3) Inclusion in the IRENE Trial; Start of Treatment With Gefitinib Rechallenge. (4) (Potential) Progression of Disease During Gefitinib Rechallenge. * Registered TKIs for First-Line TKI Treatment: Erlotinib, Gefitinib, or Afatinib. ** Follow-Up During Gefitinib Rechallenge: First 12 Weeks: Every 3 Weeks. After 12 Weeks: Every 6 Weeks



Abbreviations: NSCLC = Non–Small-Cell Lung Cancer; TKI = Tyrosine Kinase Inhibitor.

with treatment with an EGFR-TKI.[31] In this study, the predictive value of VeriStrat in pretreated *EGFR*-mutated NSCLC patients will be evaluated. The other paired blood sample will be used for analysis of blood platelets to detect (mutant) tumor RNA[32] and analysis of blood plasma to detect (mutant) tumor cfDNA.[33] Tumor RNA from platelets and tumor cfDNA from plasma will be evaluated for *EGFR* exon 18 to 21 mutations. Depending on the results obtained with molecular analysis of tumor tissue (ie, mutations in genes other than *EGFR*), additional genes can be evaluated in the analysis of tumor RNA and cfDNA.

Statistical Design

In this study we plan to recruit a total of 92 patients. It is expected that the screening of approximately 108 patients will be sufficient to obtain the 92 evaluable patients with *EGFR*$^+$ NSCLC (15% screening failure). Screening and recruitment will stop when 92 patients have started study treatment. A one-arm binomial sample size method[35] was used to calculate the number of subjects required. An exact binomial test with a nominal 0.050 2-sided significance level will have 90% power to detect the difference between the null hypothesis proportion of 0.20 and the alternative proportion of 0.35 when the sample size is 92. The experimental treatment will be considered sufficiently active if 26 or more patients with CR, PR, or SD are observed out of the 92 subjects. All subjects who are enrolled and receive study treatment will be considered the intention-to-treat (ITT) population. The analysis population for all efficacy outcome variables will be the ITT population. Response rates and disease control rates will be summarized according to proportions together with a 95% confidence interval. Durations of PFS and OS will be summarized using Kaplan–Meier methods. Tolerability will be summarized according to the appropriate standard summary statistics.

Data Collection

Patient data will be collected with electronic case record forms by the Dutch Comprehensive Cancer Centers (IKNL). Consistency checks will be performed and queries will be issued in case of inconsistencies. Data monitoring will be performed. The trial master file (containing all essential documents for the trial) will be archived for at least 15 years. Every participating center will keep an investigator site file on site, also archived for at least 15 years.

Ethical and Legal Aspects

The protocol will be conducted according to the guidelines of Good Clinical Practice and the ethical principles described in the Declaration of Helsinki. Informed consent will be obtained from every patient before inclusion in the study. The study protocol was centrally approved by the VU University medical ethical assessment committee (METc). Subsequently, every center that participates in the study also obtained approval from their local METc. At last, according to federal law, the protocol was approved by the Dutch national competent authority (CCMO). The study was assigned the EudraCT number 2012-005272-34 and is registered at ClinicalTrials.gov (NCT02025218).

Results

Results of the study are expected to be published when data are available for publication.

Discussion

The discovery of the *EGFR* mutation as a 'druggable' target in the treatment of NSCLC has had major clinical implications. Testing for *EGFR* mutations in advanced stage NSCLC is now common practice and every *EGFR*-mutated NSCLC patient should be treated with at least 1 line of EGFR–TKI-based treatment. The development of resistance to EGFR-TKIs is however inevitable and up until today there is no consensus on the best treatment strategy after a patient has acquired EGFR-TKI resistance. Moreover, development of resistance along the course of the disease is not well understood. The treatment strategy of reinitiating EGFR-TKI treatment after a TKI-free period has been described various times[15-25] and there is a biological rationale available to explain this phenomenon.

Table 2 Study Plan of the IRENE Trial (NVALT-16)

	Screening	Enrollment	Start of Study Treatment	Treatment Visits[a]					Discontinuation From Treatment[b]	Post-Progression Survival Follow-Up[c]
Visit Number	S	1	2	3	4	5	6	≥7	–	–
Day	−28 to −1	−7 to −1	1	22 ± 3	43 ± 3	64 ± 3	85 ± 3	127 ± 3	–	–
Week	−4 to −1	1	0	3	6	9	12	18	–	–
Informed Consent	X	–	–	–	–	–	–	–	–	–
Inclusion/Exclusion Criteria	X	–	–	–	–	–	–	–	–	–
Physical Examination and Routine Laboratory Testing	–	X[d]	–	X[e]	X[e]	X[e]	X[e]	–	X[e]	–
AEs (CTC 4.0)	–	X	X	X	X	X	X	–	X	–
SAEs	–	X[f]	X[f]	X	X	X	X	–	X[g]	X[g]
Pregnancy Test	–	X	–	As clinically indicated						
Blood Sample for VeriStrat Analysis	–	X	–	–	–		–	–	X	–
Blood Sample for EGFR Mutation and Blood Platelet Analysis	–	X	–	–	–	–	–	–	X	–
Archival Biopsy	–	X	–	–	–	–	–	–	–	–
Repeat Biopsy	–	X[h]	–	–	–	–	–	–	–	–
Tumor Assessment (RECIST 1.1)	–	X	–	–	X	–	X	–	–	–
Survival Data	–	–	–	–	–	–	–	–	–	X

Abbreviations: AE = adverse event; CTC = Common Terminology Criteria; EGFR = epidermal growth factor receptor; IRENE = Iressa RE-Challenge in Advanced NSCLC *EGFR*-Mutated Patients Who Responded to an EGFR-TKI Used As First-Line or Previous Treatment; NSCLC = non–small-cell lung cancer; NVALT = Dutch association for pulmonologists; RECIST = Response Evaluation Criteria in Solid Tumors; S = screening; SAE = serious adverse event.

[a]Patients will have treatment visits every 3 weeks from start of study treatment (visit 2) until visit 6 (week 12), and every 6 weeks after visit 6.

[b]Discontinuation from treatment, in case of: progressive disease, withdrawal of consent, unmanageable toxicity.

[c]Survival information should be collected after objective disease progression at every 8 weeks until death, withdrawal of consent, loss to follow-up or closure of the study.

[d]Clinical chemistry/hematology at baseline will be performed at a local laboratory at the center; basic tests: hemoglobin, thrombocytes, leukocytes, absolute neutrophil count, Na, K, Ca, albumin, creatinine, urea, bilirubin, alkaline phosphatase, gamma-glutamyl transferase, aspartate transaminase, alanine transaminase, and lactate dehydrogenase.

[e]Clinical chemistry/hematology in the follow-up phase will be limited to liver function tests only unless the clinical situation of the patient indicates that other tests are needed.

[f]Data on occurrence of SAEs will be collected from the time of signing the screening informed consent.

[g]New-onset SAEs will be reported until 30 days after discontinuation of study treatment. All SAEs ongoing at 30 days after discontinuation of study treatment must be followed until resolution, unless, in the investigator's opinion, the condition is unlikely to resolve because of the patient's underlying disease. SAEs occurring > 30 days after discontinuation of study treatment should be reported if they are considered by the investigator to be related to the study treatment.

[h]Repeat biopsy sample (including needle biopsy, surgical biopsy, trucut biopsy, fine-needle aspiration biopsy, cytology sample, or pleural or pericardic effusion sample or any other tumor biopsy sample obtained using the center's standard core biopsy techniques) for EGFR mutation analysis at time of progression from previous anticancer treatment, before the start of study treatment.

Nevertheless, the strategy has never been evaluated prospectively. For this reason, the IRENE (NVALT-16) trial was designed. If this treatment strategy proves to be effective, it will provide a feasible treatment option in the treatment of *EGFR*-mutated NSCLC patients with acquired EGFR-TKI resistance.

This trial will, by means of the longitudinal follow-up (re)biopsies, also provide more insight into the development of resistance along the course of the disease in a homogeneous population in terms of previous treatment. Several rebiopsy studies have evaluated resistance mechanisms in *EGFR*-mutated NSCLC patients at the time of acquired resistance to EGFR-TKI treatment.[12,14,36] Although it is known that chemotherapy might influence *EGFR* mutation status,[37] the effect of chemotherapy as a subsequent line of treatment to resensitize *EGFR*-mutated NSCLC patients for EGFR-TKI treatment has never been investigated.

Some substudies will be performed in this trial. Blood platelets from blood samples will be evaluated for mutant tumor RNA[32] and mutant tumor cfDNA.[33] 'Liquid biopsies' have already been described to be able to provide relevant information on tumor characteristics,[38] even in the setting of developing resistance to targeted treatment.[39] These techniques are promising methods of obtaining information about tumor characteristics in a less invasive manner to the patient compared with tumor biopsy.

Subsequently, the predictive value of VeriStrat will be evaluated as side study in this trial. Although this test has successfully been evaluated in various trials,[31] its value for this pretreated population of *EGFR*-mutated NSCLC patients will be investigated in this study.

Altogether, the IRENE trial is expected to provide more insight into effective treatment strategy at time of acquired EGFR-TKI resistance in *EGFR*-mutated NSCLC patients. With the analysis follow-up repeat biopsies of a homogeneous population in terms of pretreatment, the study will increase our understanding of development of resistance to targeted treatment along the course of the disease.

Conclusion

To our knowledge, the IRENE (NVALT-16) trial is the largest prospective trial to investigate EGFR-TKI rechallenge in previously treated *EGFR*-mutated NSCLC patients.

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Disclosure

Tom Würdinger is cofounder and shareholder of ThromboDX BV. The remaining authors have stated that they have no conflicts of interest.

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