5. Synopsis

Title	An open-label, multicenter, phase I dose-escalation trial of EGF816 and trametinib in patients with non-small cell lung cancer and acquired EGFR p.T790M positive resistance to 1st or 2nd generation EGFR TKI therapy
Coordinating Investigator	Prof. Dr. Jürgen Wolf, University Hospital of Cologne, Kerpener Str. 62, 50937 Cologne, Germany
Study sponsor	University of Cologne, Albertus-Magnus-Platz, 50923 Cologne, Germany Represented by: Prof. Dr. Jürgen Wolf, Department I of Internal Medicine, Center for Integrated Oncology, University Hospital Cologne, Kerpener Str. 62, 50937 Cologne, Germany
Study drugs	EGF816 tablets or capsules (25 mg, 50 mg), trametinib filmcoated tablets (0.5 mg, 2 mg)
Primary indication	Patients with advanced non-small cell lung cancer harbouring sensitizing *EGFR* mutations who have not received any EGFR-targeted treatment or who have progressed while on continuous treatment with a first-, second-, or third-generation EGFR TKI
Trial design	Phase I, dose escalation, genetically pre-selected, international, multicentre, open-label
Trial rational	Resistance to EGFR TKI treatment inevitably develops upon therapy with first- or second-generation EGFR TKIs (i.e. erlotinib, gerfitinib, afatinib) and third-generation EGFR TKIs (i.e. osimertinib) in first- or second-line. Mechanisms described so far in preclinical models and biopsies involve secondary *EGFR* mutations, *HER2* amplification, *MET* amplification and others. Multiple mechanisms of activation of the RAS/RAF/MEK pathway, among them, acquired activating mutations in *NRAS* and *KRAS* as well as amplifications and gain of copy number of *KRAS*, *MAPK1* and *NRAS* have been described to contribute to acquired resistance [Eberlein et al., 2015; Ercan et al., 2012; Sharifnia et al., 2014; Thress et al., 2015]. Preclinical models have also shown that activation of the RAS/MEK pathway results in reduced EGFR dependency, which can be overcome by inhibition of MEK [Tricker at al., 2015]. We thus hypothesise that combined inhibition of EGFR and MEK may restore sensitivity to EGFR inhibition in patients with acquired RAS/MEK activation and may as well prolong the acquisition of RAS/MEK-mediated resistance to third-generation EGFR TKI treatment in first- or second-line.

Summary of the study strategy and aims	The population of interest for this trial is defined as patients with NSCLC harbouring sensitizing *EGFR* mutations, who have not received any EGFR TKI treatment or who developed *EGFR* p.T790M-positive or -negative resistance to treatment with EGFR TKIs including osimertinib. A high-level amplification of *MET* as well other EGFR mutations than *EGFR* del19, p.L858R or p.T790M may not be detected. *EGFR* mutation status is assessed locally by DNA sequencing (e.g. Sanger sequencing, massively parallel sequencing). *MET* status will be assessed locally by FISH or sequencing methods. The aim of the trial is to identify the maximum tolerated dose (MTD)/recommended phase II dose (RP2D) for a continuous treatment with EGF816 and trametinib. The recommendations for dose level escalations will be based on an “up and down” design proposed by Storer, 1989. The dose limiting toxicity (DLT) period comprises the first 28 days of treatment with EGF816 and trametinib at the designated dose level (Cycle 1). Preliminary efficacy data of EGF816 and trametinib in the trial population will be generated according to RECIST v1.1. Throughout the study blood samples will be collected to monitor cell free plasma DNA (cfDNA). Patients who develop resistance upon treatment with the study drugs may undergo an optional rebiopsy to identify potential mechanisms of resistance.
Primary objective	1. To assess the maximum tolerated dose (MTD)/recommended phase II dose (RP2D) of a combination treatment of EGF816 and trametinib
Primary endpoint	1. Incidence of dose limiting toxicities (DLTs)
Secondary objectives	1. To characterize the safety of EGF816 in combination with trametinib 2. To characterize the tolerability of EGF816 in combination with trametinib 3. To assess the preliminary clinical efficacy of EGF816 in combination with trametinib 4. To define pharmacokinetic (PK) variables of the combination treatment and to explore the relationship between PK and pharmacodynamics (PD) concerning therapeutic and/or adverse effects (PK/PD analysis)
Secondary endpoints	1. Incidence, severity and grading of AEs and SAEs 2. Dose interruptions, reductions and dose intensity 3. Objective response rate (ORR), progression free survival (PFS), duration of response (DOR) and disease control rate (DCR), overall survival (OS) according to investigators assessed RECIST v1.1 4. Plasma concentration vs time profiles - plasma PK parameters of EGF816 and trametinib - and explorative compartmental population PK/PD models

Exploratory objectives	1. To analyse pre-treatment samples for multiple cancer related genes in order to assess potential predictive markers for response and resistance 2. To determine mechanisms of primary and acquired resistance to a combination treatment of EGF816 and trametinib in post-treatment samples 3. To assess the value of cell-free plasma DNA (cfDNA) for assessment of predictive molecular markers of response and resistance and for monitoring those under therapy 4. To evaluate the value of conditionally reprogrammed tumour cells (CRCs) established from tumour biopsies (baseline or upon progression) of fresh tissue for the analysis of molecular resistance mechanisms and drug sensitivity assessment in selected centres
Exploratory endpoints	1+2. Massively parallel sequencing (MPS), FISH, phospho-immunoblots of pre-treatment tumour samples and progression tumour samples, and whole exome or genome sequencing if possible 3. MPS of cfDNA at baseline, during treatment and at progression 4. CRCs will be made at the Department of Translational Genomics and the Institute of Pathology of the University Hospital of Cologne according to the established protocols. Long-term cultivation of CRCs and functional analyses including drug treatments, DNA and RNA sequencing, protein analyses, immunohistochemical analyses will be performed.
Patient number calculations and statistics	Dose level escalation will be based on a modified traditional cumulative 3+3 dose (C33D) design, i.e. the “up and down” “Design D” proposed by [Storer, 1989]: Starting with the first dose level (dose level 1: 100 mg EGF816 QD + 1 mg trametinib QD) groups of 3 patients will be treated. Escalation occurs if no DLTs or other toxicities ≥ Grade 2, that to the discretion of the sponsor fulfil the criteria of a DLT, are seen (for DLT definition see *Section 10.5.4*). De-escalation will be necessary if more than one patient exhibits such an event. If only a single patient has toxicity as described above, then the next group of three patients is treated at the same dose level. At a first stage, 18 (6×3) patients will be treated and evaluated. Based on these data, the “virtual MTD” (product of daily doses of EGF816 and trametinib in mg) is estimated by inverse prediction at 1/3 from exact logistic regression (with 95% confidence interval). At a second stage, 6 further patients (2×3) will be treated on the highest (already investigated) dose level (i.e. the actual MTD) equal or below the virtual MTD (extension cohort). No formal statistical sample size calculation was performed for this trial. A total number of 24 patients will be treated.

<table>
<tr><td>Treatment regimen and dose levels</td><td>Patients will receive continuous doses of EGF816 and trametinib at the designated dose levels.

The starting dose of EGF816 will be 100 mg QD. The dose will be escalated by 50.0% in dose level 3.

The starting dose of trametinib will be set at 1.0 mg daily. Dose levels will be increased from the previous dose by 50.0% (dose level 2) and 33.3% (dose level 4).

Dose levels and treatment regimens

<table>
<tr><th>Dose level</th><th>EGF816 daily dose (mg, QD)</th><th>trametinib daily dose (mg, QD)</th></tr>
<tr><td>-1</td><td>100</td><td>0.5</td></tr>
<tr><td>1</td><td>100</td><td>1.0</td></tr>
<tr><td>2</td><td>100</td><td>1.5</td></tr>
<tr><td>3</td><td>150</td><td>1.5</td></tr>
<tr><td>4</td><td>150</td><td>2.0</td></tr>
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</td></tr>
<tr><td>Molecular analyses</td><td>Tumour samples of all patients eligible for trial participation will be molecularly characterised.

For the purpose of screening and inclusion into the trial EGFR status will be determined by single gene sequencing (e.g. Sanger sequencing) or massively parallel sequencing (MPS). MET status will be determined by fluorescence in-situ hybridisation (FISH) or sequencing methods. High-level MET amplification is defined as a tumour fulfilling the following criteria:

a MET/CEN7 ratio ≥2.0 and/or b) an average MET gene copy number per cell of ≥6.0 [modified Schildhaus et al., 2015].

These analyses will be performed locally.

After inclusion into the trial, pre-treatment biopsy tumour samples of all patients will be sent to NGM for massively parallel sequencing, FISH and phospho-protein analysis by immunohistochemistry to determine cancer related aberrations that may predict response or resistance to the combination treatment of EGF816 and trametinib.

At baseline, during the course of treatment and at progression, blood samples will be collected and sent to NGM for analysis of circulating cfDNA by MPS.

At progression according to RECIST, an optional rebiopsy will be scheduled to determine mechanisms of acquired resistance to the combination treatment of EGF816 and trametinib. Tumour specimens will be analysed centrally by MPS and FISH (NGM).

In selected centres fresh-frozen and vital cell biopsies will be collected at baseline and progression for phospho-protein analyses, WES or WGS as well as for the establishment of functional CRC models.</td></tr>
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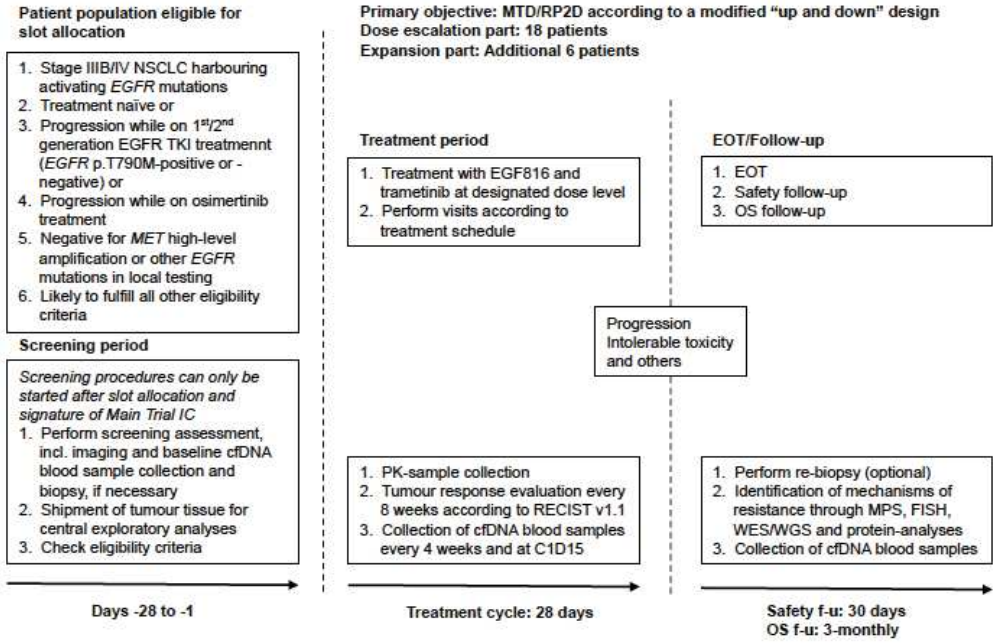
Summary of trial procedures	*Flow chart of trial procedures.*



Before signing the Main Trial Informed Consent a slot for participation in the trial should have been allocated for the individual patient. A patient for whom a slot for participation has been requested should be able to start treatment within the next 28 days and presumably fulfil the eligibility criteria. In patients who are undergoing rebiopsy after signature of the Main Trial IC fresh frozen tissue will preferentially be collected. Patients whose tumour harbour an *EGFR* p.T790M mutation and no high level *MET* amplification at local testing will be eligible for screening for the main trial.

The screening period (d -28 to -1) will only start, once a slot has been allocated to the patient by the sponsor and after the signing of the Main Trial Informed Consent. After the screening period and if the patient meets eligibility criteria, treatment will start at the designated dose level and drug administration schedule.

Patients will be treated on a continuous schedule of EGF816 and trametinib.

Treatment cycles are defined as 28 days (4 weeks) for the purpose of scheduling procedures and evaluation.

Tumour response evaluation will be performed by CT and/or MRI scans every 8 weeks and assessed according to RECIST v1.1.

Treatment will be conducted until disease progression, occurrence of intolerable toxicity, withdrawal of IC or treatment discontinuation at the discretion of the investigator.

At progression an optional biopsy may be collected to determine potential mechanisms of acquired resistance (*Section 11.3*).

At baseline, throughout the trial treatment and at progression blood samples will be collected for analysis of circulating cfDNA by MPS.

Treatment beyond progression will be allowed after approval by the PI, as long as the patient clinically derives benefit from the treatment.

Inclusion criteria	1. Written informed consent must have been obtained prior to any screening procedures. 2. Patients (male or female) ≥ 18 years of age. 3. Histologically documented, locally advanced or recurrent (stage IIIB who are not eligible for combined modality treatment) or metastatic (stage IV) non-small cell lung cancer. 4. Presence of at least one measurable lesion according to RECIST v.1.1. 5. ECOG performance status ≤ 2 6. Patients must have NSCLC harbouring *EGFR* p.L858R or *EGFR* del19 as assessed by local testing. 7. Patients must be EGFR TKI treatment naïve (prior chemotherapy treatment is allowed) or must have progressed while on continuous treatment with a first- or second-generation EGFR TKI (*EGFR* p.T790M-negative or -positive) or must have progressed while on continuous treatment with osimertinib or other experimental third-generation EGFR inhibitors (*EGFR* p.T790M-negative or -positive) 8. In patients who have received no prior EGFR TKI treatment, an archival biopsy sample, defined as a sample being obtained prior to any anti-cancer treatment is mandatory. If an archival biopsy fulfilling this criterion is not available, patients must be suitable and willing to undergo baseline biopsy according to the local institution's guidelines (newly obtained biopsy). 9. In patients who have received prior EGFR TKI treatment, an archival biopsy sample, defined as a sample being obtained after or during progression upon the last anti-cancer treatment is mandatory. No consecutive line of treatment must have been given after collection of the rebiopsy and inclusion into this trial. If an archival rebiopsy fulfilling these criteria is not available, patients must be suitable and willing to undergo baseline biopsy according to the local institution's guidelines (newly obtained biopsy). 10. In patients who have received prior EGFR TKI treatment, *EGFR* p.T790M mutation status must have been assessed by local testing in the tumour sample fulfilling the requirements of inclusion criterion 9. 11. Patients who have received prior osimertinib treatment, may only be eligible if no standard treatment approach outside this trial is available or feasible (e.g. chemotherapy) 12. Patients who have progressed while on continuous treatment with a first- or second-generation EGFR inhibitor and whose tumour has been tested EGFR p.T790M-negative may only be eligible if no standard treatment approach outside this trial is available or feasible (e.g. chemotherapy). 13. In patients who have received prior EGFR TKI treatment, progression of disease according to RECIST v1.1 while on continuous treatment with an EGFR TKI (e.g. erlotinib, gefitinib, afatinib or osimertinib) must be documented.
Exclusion criteria	1. History of allergic reactions or hypersensitivity to one of the study drugs or to any component of the study drugs 2. Prior treatment with any investigational agent known to inhibit EGFR (mutant or wild-type), except experimental third-generation EGFR inhibitors or EGFR-targeting antibodies 3. Prior treatment with any agent known to inhibit MEK/ERK or other mediators of RAS pathway. 4. Patients with high level *MET* amplification in the archival or newly obtained biopsy sample as determined by local testing. High-level MET amplification is defined as:

	a) a MET/CEN7 ratio ≥2.0 and/or b) an average MET gene copy number per cell of ≥6.0 [modified Schildhaus et al., 2015]. 5. Patients with EGFR mutations other than *EGFR* del19, p.L858R or p.T790M. 6. Patients with brain metastases. However, if radiation therapy and/or surgery has been completed at least 4 weeks prior to screening for the trial and evaluation by CT (with contrast enhancement) or MRI at study baseline demonstrates the disease to be stable and if the patient remains asymptomatic and off steroids, then patients with brain metastases may be enrolled. 7. Patients with presence or history of carcinomatous meningitis. 8. Any acute or chronic medical, mental or psychological condition, which in the opinion of the investigator would not permit the patient to participate or complete the study or understand the patient information 9. History of hepatitis B (HBV) or hepatitis C (HCV) or positive result in mandatory testing for acute or chronic hepatitis B or hepatitis C 10. Known HIV infection or history of HIV infection independent from the cellular immune status 11. Patients who receive any continuous, long term immunosuppressive treatment, including long term treatment with steroids at immunosuppressive doses at the time of study entry 12. Patients who underwent bone marrow or solid organ transplantation, including patients who do not receive any immunosuppressive treatment. 13. Presence or history of any other primary malignancy other than NSCLC within 5 years prior to enrolment into the trial. Except from this: Adequately treated basal or squamous cell carcinoma of the skin or any adequately treated in situ carcinoma 14. Any of the following within 6 months prior to first trial drug administration: Myocardial infarction (NSTEMI or STEMI), severe/unstable angina pectoris, symptomatic congestive heart failure (> NYHA II), uncontrolled hypertension, coronary/peripheral artery bypass graft, cerebrovascular accident or transient ischemic attack, atrial fibrillation of CTCAE Grade ≥ 2, ongoing cardiac dysrhythmias of CTCAE Grade ≥ 2, including corrected QTcF prolongation of > 480 ms, 15. Aortic valve stenosis with mean gradient ≥ 25 mmHg and aortic valve area of ≤ 1.5 cm^2 16. Any other cardiac valve abnormality of more than mild degree/stage 17. Left ventricular ejection fraction (LVEF) of < 50 % 18. History of congenital long QT-syndrome or Torsades de Pointes 19. History of retinal vein occlusion (RVO) or retinal pigment epithelial detachment (RPED) 20. Unable or unwilling to swallow tablets or capsules 21. Patients with impaired gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of EGF816 (e.g., ulcerative diseases, uncontrolled nausea, vomiting diarrhoea, or malabsorption syndromes 22. Patients have received anticancer treatment within the following time frames prior to the first dose of study treatment: a. Conventional cytotoxic chemotherapy: ≤ 4 weeks (≤ 6 weeks for nitrosoureas, mitomycin-C and suramin) b. Biological therapy (e.g., antibodies, excluding PD-1 or PD-L1 antibodies): ≤ 4 weeks c. PD-1/PD-L1 antibodies (e.g., nivolumab, pembrolizumab): ≤ 5 half-times d. Non-cytotoxic anti-cancer therapeutic (e.g., tyrosine kinase inhibitors): ≤ 5 half-times or ≤ 1 weeks (whichever is longer)

	e. Other investigational agent: ≤ 4 weeks f. Radiation therapy (excluding palliative radiation, e.g., of bone metastases): ≤ 4 weeks g. Major surgery (excluding minor surgical interventions, e.g., vascular device implantation): ≤ 2 weeks 23. Laboratory values as listed below, that cannot be corrected to normal limits within screening : a. Absolute Neutrophil Count (ANC) < 1.5 x 10^9/L b. Haemoglobin (Hb) < 9 g/dL c. Platelets (PLT) < 100 x 10^9/L d. Total bilirubin > 1.5 x upper limit of normal (ULN). For patients with confirmed Gilbert's disease total bilirubin > 2.5 x ULN e. AST and/or ALT > 3 x ULN f. AST and/or ALT > 5 x ULN in patients with liver involvement g. Serum creatinine > 1.5 x ULN h. Measured or calculated creatinine clearance ≤ 45 mL/min i. Serum amylase and/or lipase CTCAE Grade > 2 j. Potassium, magnesium, phosphorus, total calcium (corrected from serum albumin) > ULN 24. Patients receiving treatment with any medication that are known to be a. Strong inhibitors or inducers of CYP3A4/5 b. Substrates of CYP2D6 with narrow therapeutic index c. and that cannot be discontinued at least 7 days prior to the first dose of the study drugs. *d. For further information please refer to Section 11.7* and the *Concomitant Medication Manual.* 25. Patients with a history of or presence of interstitial lung disease or interstitial pneumonitis, including clinically significant radiation pneumonitis 26. Pregnancy or breastfeeding/nursing women 27. Women of child-bearing potential (for definition see *Section 8.3.3*) unless they use highly effective methods of contraception during treatment and for four months after withdrawal of study treatment (for methods of contraception see *Section 8.3.4*) 28. Sexually active males unless they use a condom during intercourse for the time of study treatment and for four months after the withdrawal of study treatment.
Trial duration / timelines	Inclusion first patient (FPFV): 02/2018 Inclusion last patient: 12/2022 Last patient last visit (LPLV): 05/2023