Title of Study:

TRY
Trastuzumab and AUY922 in HER2-aberrant NSCLC

A phase II study to evaluate safety and efficacy of combined trastuzumab and AUY922 in advanced non-small cell lung cancer with HER2-overexpression or –amplification or -mutation

Short Title / Acronym: TRY

Eudra-CT Number: 2011-005655-13

ID of Trial Protocol: TRY

Start of Study – Completion of Study

First patient enrolled on April 22, 2013, last study visit of last patient June 9, 2016.

Final Study Report

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Signatures

By their signatures the authors approve of the content of the present final report. The clinical trial described herein was conducted in compliance with the principles of the Helsinki Declaration, Good Clinical Practice (GCP), and pursuant to all applicable legislation

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Principal Investigator	Professor Dr. med. Jürgen Wolf	9.4.19 Cologne,
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2 Synopsis

Full Study Title	A phase II trial to evaluate safety and efficacy of combined trastuzumab and AUY922 in advanced non-small cell lung cancer (NSCLC) with HER2-overexpression or -amplification or - mutation.
Short title	TRY: Trastuzumab and AUY922 in HER2-aberrant NSCLC
Study Sponsor	University of Cologne, Germany, represented by the Principal Investigator (PI)
Principal Investigator	Prof. Dr. Jürgen Wolf, University Hospital Cologne
Study Centers	LCGC, Dep. I of Internal Medicine, Center for Integrated Oncology, University Hospital Cologne, Germany Clinic for Internal Medicine (Tumor research), West German Cancer Center, University Hospital Essen, Essen, Germany
Biostatistics	Institute for Statistics, University Hospital Cologne
Study Drugs	Trastuzumab (Herceptin®, Roche) and AUY922 (Novartis)
Study Design	Phase II, oligocentric, single-arm
Primary Objective	To evaluate efficacy of combined trastuzumab and AUY922 in HER2-overexpressed or -amplified or -mutated NSCLC
Primary Endpoint	Response rate (RR) of the combination therapy with trastuzumab and AUY922
Secondary Objectives and Endpoints	• To evaluate the response rate in patients treated with trastuzumab monotherapy • To evaluate the tolerability of trastuzumab and AUY922 in combination (endpoints: assessment of adverse events (AEs) according to CTC-AE V4.0) • To evaluate the clinical efficacy of trastuzumab monotherapy and the combination descriptively (endpoints: progression-free survival (PFS), overall survival (OS)) • To assess correlation of outcome parameters with the type of genetic aberration of HER2 (amplification, mutation) descriptively • To assess pharmacokinetics of AUY922 and trastuzumab • To establish a pharmacokinetic / pharmacodynamic model with regard to response rate and adverse events
Treatment Design	Patients started with 4mg/kg loading dose of trastuzumab monotherapy i.v. on day 1 and continued with 2mg/kg trastuzumab i.v. weekly. CT (or MRI) restaging was performed after every 6 weeks of treatment. Patients with clinical benefit (complete remission, partial remission, stable disease according to RECIST 1.1 criteria) continued on trastuzumab monotherapy until disease progression. In case of disease progression, 70 mg/m^2 AUY922 was administrated i.v. weekly in combination with trastuzumab. AUY922 was administrated directly after the trastuzumab infusion. Patients

who responded to the combination treatment (complete response, partial response, stable disease) continued on combination therapy until disease progression. Patients with progress on combination therapy discontinued treatment and were assessed during follow-up visits up to 6 months after the last dose of trastuzumab (and AUY922).

Pharmacokinetic (PK) assessment of trastuzumab and of AUY922 and trastuzumab were performed at the following time points: before the first trastuzumab infusion and before the first combined AUY922 + trastuzumab infusion, in each case directly after the end of the infusion(s) and 1h, 24h, 96h and 168h after the end of the infusion(s). If logistically necessary, the time points for 24h and 96h PK sampling were adapted, but the 24h sample were withdrawn between 18-40h and the 96h sample between 72-120h. Thereafter, PK was assessed every other week before start and at the end of infusion(s). For combination therapy, "end of infusion" related to the end of the infusion of the second substance, and an additional sample was taken just after the end of the infusion of the first substance.

For safety reasons, patients remained at hospital site for at least 4 hours after first dosing of trastuzumab and AUY922. Before discharging of patient, final clinical findings and anamnesis including questions about adverse events were done by investigator. It was not necessary to hospitalize patients for this reason. However, if there were other reasons (i.e. patients arriving to study center from long distance, patient general condition and others) not allowing patient to stay in the outpatient clinic for 4 hours after first dosing, the hospitalization was organized.

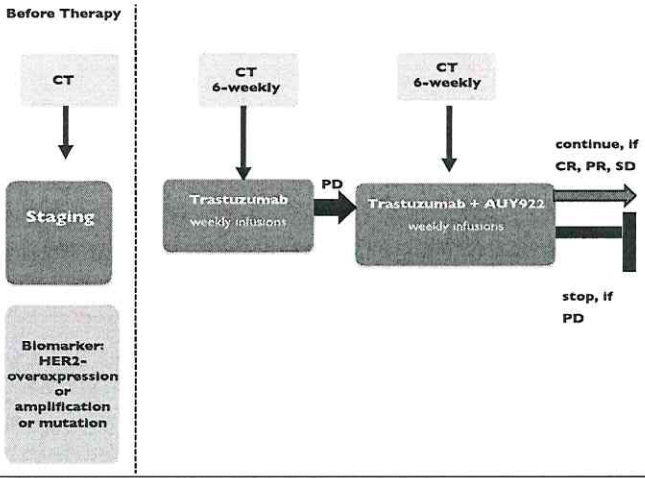


Figure 1: Flow-sheet of the trial

Patient number was calculated with respect to response on combination treatment. All patients on combination treatment received trastuzumab monotherapy initially and progressed on this treatment.

The statistical analysis for the primary endpoint with patient number calculation considered only patients treated on the combination. The analysis of patients on trastuzumab monotherapy was a secondary endpoint of the study.

Background for the statistical analysis:

The null hypothesis (ineffective treatment) that the response rate was at most 5% was tested against the alternative hypothesis (effective treatment) that the response rate was at least 20%.

	Patient sample size was based on Simon two-stage design (Simon 1989) with error rates alpha=0.05 and beta=0.20. Stage 1: Ten patients should have been treated with the combination in the first stage. If less than 1 response according to RECIST would have been observed in this first trial phase, the trial would have been terminated for inactivity of combination therapy. Stage 2: If at least 1 partial response was observed with the combination therapy in the first trial phase, another 19 patients would have been recruited and treated as described above. There was an 89% probability of continuing to the second stage if the true response rate is at least 20%, while if the true response rate is 5% or less there is a 60% probability of stopping. If at least 4/29 of patients responded, it would have been concluded that the combination treatment shows sufficient promise of effectiveness for further investigation.
Molecular Analyses	Tumor samples were prescreened locally for HER2 expression using standard immunohistochemistry. Only patients with positive HER2 prescreening result (immunohistochemical score 2+ or 3+) were considered for the study and tested further during the central screening as described below. Immunohistochemistry was carried out using the DAKO HercepTest. Cases with a strong membranous staining of > 30% of the tumor cells were given a score 3+. Cases with a weak to moderate membranous staining of > 10% of the tumor cells or a strong membranous staining of < 30% of the tumor cells were given a score of 2+. No membranous or weak membranous staining in <10% of tumor cells is defined score 0 or 1+, respectively. Protein overexpression is defined as an immunohistochemical score of 3+. HER2 amplification status was analyzed by FISH using a HER2 FISH probe set provided by ZytoVision, Bremerhaven, Germany. The probe set includes a green labelled HER2 probe as well as an orange-labelled chromosome 17 centromeric enumeration probe. The ratio of HER2/neu signals to centromere signals was determined in 20 nuclei. If the resulting ratio amounts between 1.8 and 2.2, further 40 nuclei were counted. If the ratio is < 2.0 no amplification was reported. If the ratio was ≥ 2.0, HER2 amplification (FISH-positive) was reported. Tumors with an average HER2 gene copy number of ≥6 per tumor cell were considered HER2 amplified (FISH-positive). In NSCLC, HER2 mutations are found in exon 20 encoding apart of the tyrosine kinase domain. Mutation analysis of exon 20 was done as central screening on fresh or formalin-fixed, paraffin-embedded tissue by PCR amplification of genomic DNA followed by Sanger sequencing and capillary electrophoresis.
Pharmacokinetic / Pharmacodynamic Analyses	A population pharmacokinetic evaluation (NONMEM VI or higher) was applied to the data. Individual exposure estimates was used as independent variables for a PK/PD analysis, dependent variables are adverse event and response metrics.
Number of Patients	In the first stage, recruitment was planned to proceed until 10 patients are evaluable for the combination treatment. The recruitment in the second stage was planned until additional 19 patients were evaluable on the com-

	bination. All patients on the combination treatment have received trastuzumab monotherapy initially and progressed on this treatment.
Inclusion Criteria	• Stage IV NSCLC patients after failure of at least one standard therapy with HER2 protein overexpression (HER2 score 3+) or gene amplification (FISH positive) or mutation • Age ≥ 18 years • ECOG performance status 0 to 2 • Life expectancy of at least 12 weeks • Evaluable disease or disease measurable per Response Evaluation Criteria in Solid Tumors (RECIST) • Adequate bone marrow, liver and renal function and adequate electrolyte balance as assessed by following laboratory requirements conducted 14 days prior to treatment: o Hemoglobin ≥ 9.0 g/dL o Absolute neutrophil count (ANC) ≥ 1500 /mm3 o Platelet count ≥ 100,000/µL o Total bilirubin ≤ 2 x ULN o ALT, AST and alkaline phosphatase (AP) ≤ 2.5 x ULN or ≤ 5.0 x ULN, if liver metastasis are present o PT-INR/PTT < 1.5 x ULN o Creatinine clearence (CrCl) ≥ 60ml/min calculated by either MDRD-formel or by 24 hours urine collection o Total calcium (corrected for serum albumin) within normal limits or correctable with supplements o Magnesium within lower normal limits or correctable with supplements o Potassium within normal limits or correctable with supplements • Written informed consent (after adequate explanation of the trial) to participate in the trial and to adhere to trial procedures, as well as consenting to data protection procedures • In case of females with childbearing potential (definition of menopause is no bleeding at least 12 months after the last menstruation): - negative serum pregnancy test in women with childbearing potential - effective method of contraception (Pearl-Index not greater than 1%)
Exclusion Criteria	• Known hypersensitivity to any study medication • Other history of ongoing malignancy that would potentially interfere with the interpretation of efficacy • Previous treatment with Hsp90 inhibitors (e.g.17-AAG)

	• Treatment with therapeutic doses of coumarin derivatives. Low doses of coumarin derivatives (e.g. < 2mg/day) are permitted • Patients with concurrent severe and/or uncontrolled medical conditions (e.g. uncontrolled diabetes mellitus, active untreated or uncontrolled infection, chronic obstructive or chronic restrictive pulmonary disease including dyspnea at rest from any cause or requiring supplementary oxygen therapy) that could cause unacceptable safety risks or compromise compliance with the protocol • Impaired cardiac function including any of the following: o History (or family history) of long QT syndrome o Mean QTcF ≥ 450 msec on screening ECG o History of clinically manifest ischemic heart disease including myocardial infarction, stable or unstable angina pectoris, coronary arteriography or cardiac stress testing/imaging with findings consistent with infarction or clinically significant occlusion ≤ 6 months prior to start of the study o History of heart failure or left ventricular (LV) dysfunction (LVEF ≤ 45%) by transthoracic echocardiography o Clinically significant ECG abnormalities including one or more of the following: left bundle brunch block (LBBB), right bundle brunch block (RBBB) with left anterior hemiblock (LAHB), ST segment elevations or depressions > 1 mm or 2nd (Mobitz II) or 3rd degree AV block o History or presence of atrial fibrillation, atrial flutter or ventricular arrhythmias including ventricular tachycardia or torsades de pointes o Other clinically significant heart disease (e.g. congestive heart failure, uncontrolled hypertension or history of unstable hypertension) o Clinically significant resting bradycardia (< 50 beats per minute) o Patients who are currently receiving treatment with any medication which has a relative risk of prolonging the QTc interval or inducing torsades de pointes and cannot be switched or discontinued to an alternative drug prior to commencing AUY922 o Obligate use of a cardiac pacemarker o Angina pectoris requiring a medicinal product o Evidence of transmural infarction on ECG o Clinically significant valvular disease • Known diagnosis of HIV, active hepatitis B and/or C (testing is not

	mandatory) • Clinically symptomatic leptomeningeal or brain metastases (patients with clinically stable brain metastases may be enrolled) • Any person being in an institution on assignment of the respective authority • Any medical, mental or psychological condition which in the opinion of the investigator would not permit the patient to complete the study or understand the patient information • Any serious medical condition with organ impairment • Parallel participation in another clinical trial • Experimental or other therapy within the last 30 days or 5 half-lives, whatever is of longer duration (with exception of trastuzumab, if patient is recruited directly for combination treatment) • Pregnancy, breast feeding
Study Duration / Timelines	Inclusion first patient (FPFV): 05/2013 Inclusion last patient: 05/2015 Last patient last visit (LPLV): 05/2016 Closure of database: 07/2016
Monitoring / QM	Clinical Trials Center Cologne
Study results	In the study, 8 patients were treated with trastuzumab. Of these 8 patients, only 3 patients received combination on trastuzumab and AUY922. No response was seen in patients on the study medication (no response on trastuzumab and no response on combination trastuzumab with AUY922). No new significant safety findings were observed from the study. The study was terminated prematurely due to slow recruitment.

3 Table of Contents

4 Acronyms/ Abbreviations and Definitions

AE	Adverse event (*cf.* SAE below)
CRF	Case Report Form
CRO	Clinical Research Organization
CV	Curriculum vitae
D	Day of treatment
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
HER2	Human epidermal growth factor receptor 2
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent To Treat
i.v.	Intravenously
LCGC	Lung Cancer Group Cologne
NSCLC	Non-small cell lung cancer
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per Protocol
Pts	Patients
RECIST	Response Criteria in Solid Tumors
SAE	Serious Adverse Event
ULN	Upper Limit of Normal

5 Ethics

5.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The study and all required documents (study protocol, ICF, amendments) were submitted to the responsible Ethics Committees of Cologne and Essen and were approved by the Committee.

5.2 Conduction of the study under ethical aspects

This study was conducted in compliance with the Declaration of Helsinki (in its version of 1996) and the AMG (German Medicinal Products Act), esp. its §§ 40-42, in its current versions, as well as the principles of the proper conduction of clinical trials (ICH-GCP).

As provided in the AMG, the study sponsor took out the insurance for every patient who agreed to participate in this clinical trial.

5.3 Patient informed consent

Prior to their inclusion in the study, a registered investigator discussed the study with every patient informing them in detail about the objectives, risks, and the study procedures. The Patient Informed Consent form was provided to the patients, and if, after a minimum time of 24hs for consideration and another discussion of potentially open questions, they agreed to participate, patient and investigator signed the Informed Consent form. In the TRY study, patients and investigators signed two informed consent forms – one for molecular screening and one for the clinical part of the study.

6 Investigators and Administrative Structure

The trial was carried out as a multicenter study at the University of Cologne (Lung Cancer Group Cologne - LCGC) and at University of Essen (West German Tumor Center) as the study sites. Medical treatment and management of the patients were in the hands of the investigators and study nurses at both sites. Imaging procedures to be applied during the study included computer-tomography at both sites. The pharmacokinetic data was obtained by the Institute of Pharmacology of the Cologne University Hospital. The following persons contributed to the planning, implementation and evaluation of the study:

- Prof. Dr. med. Jürgen Wolf, University Cologne (PI)
- PD Dr. med. Lucia Nogova, MSc, University Cologne (coordinating physician, Sub-PI)
- Prof. Dr. med. Martin Schuler, University Essen (PI at University Essen)
- Dr. Wilfried Eberhardt, University Essen (Sub-PI)
- Investigators at the University Cologne and at the University Essen
- Study nurses at the University Cologne and at the University Essen
- Data Safety Monitoring Committee (Dr. Kirsten Kliem, Dr. Nicole Skoetz, Dr. Corinne Brillant)
- Dr. rer. medic. Jeremy Franklin, University Cologne (biometry analysis)
- Prof. Dr. rer. medic. Martin Hellmich, University Cologne (biometry planning)
- Prof. Dr. med. Uwe Fuhr (pharmacokinetics) Institute of Pharmacology Cologne
- Center for Clinical Trials Cologne (monitoring, setting up the database, data management, safety management)

- Lung Cancer Group Cologne (Project Management)

7 Introduction

Amplifications and mutations in the HER2 gene have been identified in various solid tumors such as breast cancer, gastric cancer, glioblastoma, endometrial cancer and lung cancer and have been shown to be critically involved in tumor development due to activation of the RAS-MAPK signaling pathway (Slamon, Godolphin et al. 1989) .

7.1 Treatment with trastuzumab

Therapeutic activity of trastuzumab, a humanized monoclonal antibody against the extracellular domain of HER2, is well established in HER2-positive early and metastatic breast cancer. HER2 positivity evaluation is based on results on immunhistochemical analysis (IHC 3+ status or IHC 2+ status confirmed by FISH; appr. 20% of stage IV pts). Trastuzumab is effective and approved in metastatic breast cancer in combination with paclitaxel (benefit in mTTP: 3.9 m, p<0.001, mOS-benefit 3.7m, p:0.17) (Slamon, Leyland-Jones et al. 2001), anthracycline + cyclophosphamide (Slamon, Leyland-Jones et al. 2001) (benefit in mTTP 1.7m, p<0.001, mOS-benefit 5.4m, p=0.16) and docetaxel (benefit in TTP 5.6m, p=0.0001, OS-benefit 8.5m, p=0.0325) (Marty, Cognetti et al. 2005). Providing significant clinical benefit in the adjuvant setting, trastuzumab in combination with chemotherapy is also the foundation of care for all patients with HER2-positive early breast cancer (Slamon et al. 2011; Gianni et al. 2011; Perez et al. 2011, Piccart-Gebhart et al. 2005). A phase II study investigating trastuzumab monotherapy in previously untreated metastatic breast cancer patients with HER2-positive tumors showed a clinical benefit rate (complete and partial responses plus stable disease for at least 6 months) in 36% and a median time to progression of 3.4 months (Baselga, Carbonell et al. 2005).

In gastric cancer, the ToGA trial recruited patients with advanced gastric cancer positive for HER2 expression (appr. 20% of stage IV patients). The trial compared fluoropyrimidine/cisplatin based chemotherapy with or without trastuzumab. In the final analysis, median OS improved from 11.1 months with chemotherapy alone, to 13.8 months with the addition of trastuzumab (p=0.0046) (Bang, Van Cutsem et al. 2010). A post-hoc exploratory analysis revealed an improvement in OS from 11.8 to 16 months through the addition of trastuzumab in patients with IHC 3+ or IHC 2+/FISH-positive tumors.

HER2 mutations are found in up to 4% of NSCLC patients (Stephens, Hunter et al. 2004) and preclinical and clinical data suggest that HER2-targeted antibodies are active in such tumors (Wang, Narasanna et al. 2006).

However, phase II studies combining chemotherapy and trastuzumab in HER2 positive advanced NSCLC patients did not show a particular efficacy increase in favor of trastuzumab (Langer, Stephenson et al. 2004),(Zinner, Glisson et al. 2004), (Krug, Miller et al. 2005) probably due to inclusion of all HER2 IHC positive patients.

In a randomized phase II trial recruiting chemotherapy-naive advanced NSCLC patients after stratification based on their HER2 IHC status for treatment with trastuzumab and either paclitaxel or docetaxel response rates and median OS were not significantly different between both arms (32% vs. 23%; 14 vs. 16 months) (Krug, Miller et al. 2005).

Another randomized trial testing trastuzumab in advanced HER2 positive NSCLC patients recruited 103 chemotherapy-naive patients randomized between gemcitabine/cisplatin with and without trastuzumab. Efficacy was similar in the trastuzumab and control arm with response rates of 36 vs. 41% and a median PFS of 6.1 vs. 7 months. However, in the six trastuzumab treated patients with a HER2 IHC3+ status or FISH positivity, the

response rate of 83% and the median PFS of 8.5 months appeared remarkably good (Gatzemeier, Groth et al. 2004). These data indicate that in NSCLC patients as well as in gastric cancer patients, the response rates are linked with HER2 IHC3+ status or HER2-amplification as determined by FISH.

Taken these results together, only NSCLC cases with mutations and/or high amplification of HER2 appear to be candidates for future evaluation of anti HER2 therapies.

7.2 Treatment with Hsp90 inhibitors

The heat shock protein Hsp90 is a molecular chaperone that modulates the stability and/or transport of intracellular proteins. Many Hsp90 clients are oncogenes including HER2, EGFR, c-RAF, AKT, BCR-ABL, mutant p53, and hTERT. Preclinical data in KRAS transgenic mice showed shrinkage of tumors dependent on the activity of Hsp90-client oncogenes (Sos, Michel et al. 2009), (Biamonte, Van de Water et al. 2010). Phase-I trials with hsp90 inhibitors in patients with advanced solid tumors showed clinical tolerability (Solit and Chiosis 2008), (Solit, Osman et al. 2008). However, only marginal clinical efficacy was observed in these trials recruiting patients without selection based on preclinical data (Banerji, O'Donnell et al. 2005), (Solit, Ivy et al. 2007).
AUY922 is a novel Hsp90 inhibitor. Two studies investigated the maximum tolerated dose (MTD) of AUY922 in combination with bortezomib in patients with relapsed multiple myeloma and in solid tumors as well as efficacy in breast cancer (NCT00526045).

7.3 Combination of trastuzumab and Hsp90 inhibitors

Since HER2 is one of the Hsp90 client proteins, combination of an anti HER2 agent and an Hsp90 inhibitor is an attractive treatment option in HER2 dependent cancer. The feasibility and efficacy of the combination of trastuzumab with the Hsp90 inhibitor tanespimycin (17-AAG, KOS-953) in a trial recruiting trastuzumab-refractory and HER2-amplified breast cancer patients showed responses in 5/25 pts (2 PRs, 3 SDs) (Modi, Stopeck et al. 2007). Another phase I trial investigating the combination of the Hsp90 inhibitor alvespimycin and trastuzumab in pretreated breast and ovarian cancer patients showed clinical activity in 5/21 patients (2 PR, 3 SD) (Miller, Rosen et al. 2007).

7.4 Rationale for the study objectives

Based on findings in a genetically and phenotypically validated cell-line panel and in vivo data, there is strong evidence for an efficacy of the combination therapy with trastuzumab and AUY992 in patients with HER2-overexpressed or amplified (as measured by FISH) or mutated lung, gastric and breast cancer patients. In order to increase the efficacy of HER2-targeted therapy in these patients, a genetically guided selection of HER2–driven cancers and the combination of an HER2 antibody with an Hsp90 inhibitor was expected to be effective in these patients. In our trial, the HER2-overexpressed or amplified or mutated NSCLC patients were treated with trastuzumab monotherapy first to select patients who may benefit from trastuzumab alone. In case of disease progression, patients started with AUY922 in combination with trastuzumab. They continued with the combination as long as they benefited from the treatment. The primary objective of the trial was to investigate whether patients benefited from the combination therapy. The comparison of trastuzumab monotherapy versus combination of trastuzumab and AUY922 was performed descriptively only.

8 Study Objectives

The combination of trastuzumab and AUY922 is a promising treatment with a strong biological background in patients with HER2-overexpressed or -ampflied or -mutated NSCLC. The trial aimed at improvement of RR in this patients group with disastrous median overall survival of about 12 months (at the time point of the study) in unselected metastatic NSCLC (Sandler, Gray et al. 2006).

8.1 Primary objectives

- To evaluate the efficacy of combined trastuzumab and AUY922 treatment in HER2-overexpressed or –amplified or -mutated NSCLC patients (endpoint: response rate) progressing after treatment with trastuzumab monotherapy

8.2 Secondary objectives

- To evaluate response rate in patients treated with trastuzumab monotherapy descriptively
- To evaluate tolerability of trastuzumab and AUY922 in combination (endpoints: assessment of adverse events (AEs) according to CTC-AE V4.0)
- To evaluate progression-free survival (PFS) and overall survival (OS) of trastuzumab monotherapy and combination descriptively
- To assess correlation of outcome parameters with the type of genetic aberration of HER2 (amplification, mutation) descriptively
- To access pharmacokinetics of AUY922 and trastuzumab
- To establish a pharmacokinetic / pharmacodynamic model with regard to response rate and adverse events

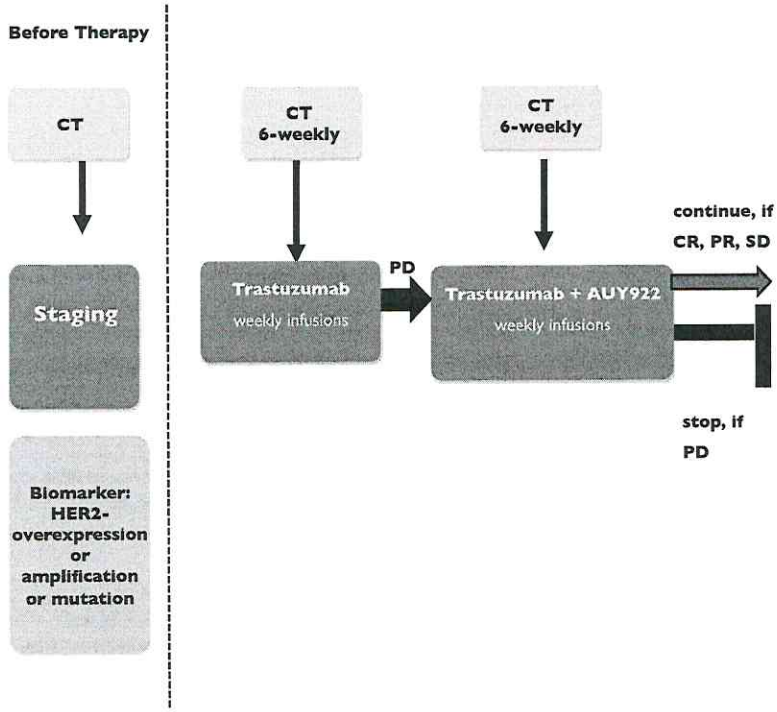
9 Study Protocol

9.1 General study protocol / study design

TRY was a phase II, genetically preselected, multicentric, single arm study.

Only patients with a confirmed HER2 overexpression or amplification or mutation were enrolled into the trial. Patients were identified within the Network Genomic Medicine for Molecular Screening of Lung Cancer and the Lung Cancer Center of the West German Cancer Center in Essen. The SOP for patient identification (prescreening) was described in the Network Protocol. The tissue processing is described in section 4.9.1.1 (scientific procedures) of study protocol. HER2 prescreening testing as well as all molecular tests in this study were performed in samples obtained for routine clinical diagnostics (in the rule paraffin sections). No additional diagnostic biopsy was necessary.

Figure 1: Flow-sheet of the trial



NSCLC patients were prescreened for HER2 protein expression at local sites. If the immunohistochemical score of the local HER2 pre-screening was 2+ or 3+, tumors were re-tested under conditions of this study. If re-tests within this study revealed HER2 overexpression (immunohistochemistry: score 3+) or gene amplification (FISH: HER2/CEN17 ratio ≥2.2 or average HER2 gene copy number ≥6 per tumor cell) or mutation (Sanger sequencing), patients were enrolled for the treatment in the study.

Patients consented to central molecular screening and study treatment (2 separate consents). Since Amendment 2 (June 8, 2014) also patients without central screening were allowed to entry study. The decision was based on the slow recruitment by the low frequency of HER2 genetic alterations.

After patient enrolment, baseline procedures were performed within 21 days before first treatment administration. The baseline procedures include clinical examination; ECGs, echocardiography, CT (or MRI) scans of involved areas and laboratory assessments.

All recruited patients started with trastuzumab monotherapy. The treatment started on day 1 with 4mg/kg loading dose of trastuzumab i.v. and – if well tolerated, continued with 2mg/kg trastuzumab i.v. weekly. Restaging CTs (or MRI) were performed every 6 weeks. In case of disease progression (defined according to RECIST 1.1), patients started with the combination of AUY922 70mg/m^2 i.v. and trastuzumab 2mg/kg i.v. weekly. The combination was given as long as no further progression occurred. In case of disease progression, patients discontinued study treatment and were further assessed by monthly follow-up visits until 6 months after the last administration of study treatment.

Pharmacokinetic (PK) assessment of trastuzumab and of AUY922 and trastuzumab was performed at the following time points: before the first trastuzumab infusion and before the first combined AUY922 + trastuzumab infusion, in each case directly after the end of the infusion(s) and 1 h, 24 h, 96 h and 168 h after the end of the infusion(s). If logistically necessary, the time points for 24 h and 96 h PK sampling were adapted, but the 24 h sample should have been withdrawn between 18-40 h and the 96 h sample between

72-120 h. Thereafter, PK was assessed every other week before start and at the end of infusion(s). For combination therapy, "end of infusion" related to the end of the infusion of the second substance, and an additional sample was taken just after the end of the infusion of the first substance.

9.2 Discussion of the study design and of control groups

At time point of the study conduction, the only drugable genetic alteration was EGFR mutation with significantly increased overall survival comparing to standard chemotherapy. Advanced NSCLC patients without a drugable EGFR mutation had at time point of the study a poor prognosis of about 12 months. The challenge of this genetically preselected clinical trial was to find an effective treatment for advanced NSCLC patients with HER2 alterations. The preclinical data as well as Phase-I studies in breast cancer patients had indicated, that targeting HER2 with HER2-antibody trastuzumab caused tumor shrinkage in about 20-30% of advanced patients.

Furthermore, preclinical and early clinical studies in HER2 patients had shown increased responses, if an inhibitor of heat shock protein Hsp90 was added to trastuzumab.

Thus, the primary objective of the trial was to increase response rate of patients with HER2 genetic alterations treated with trastuzumab in combination with AUY922 (an inhibitor of heat shock protein Hsp90) comparing to standard chemotherapy.

However, the challenging aspect of the study was the low frequency of HER2 genetic alterations in advanced NSCLC patients. According to available published data, we assumed the frequency of HER2 genetic alterations of about 4% in advanced NSCLC. Due to this fact we proposed a Simon 2-stage designs for the study in order to early indicate low/high clinical benefit and avoid including of necessary high patient numbers. This decision was in line with a "proof of concept" study: to stop the recruitment early, if no clinical benefit is seen.

The above reasons led to decision not investigate the study treatment in a control arm. In case of positive trial according to Simon 2-stage designs we would have started a phase-II trial with classical design and statistical power to confirm the clinical benefit. In case of positivity of such a Phase-II trial we would have decided to start a control trial.

9.3 Selection of study population

9.3.1 Inclusion criteria

- Stage IV NSCLC patients after failure of at least one standard therapy with HER2 protein overexpression or gene amplification (FISH-positive) or mutation
- Age ≥ 18 years
- ECOG performance status 0 to 2
- Life expectancy of at least 12 weeks
- Evaluable disease or disease measurable per Response Evaluation Criteria in Solid Tumors (RECIST)
- Adequate bone marrow, liver and renal function and adequate electrolyte balance as assessed by following laboratory requirements conducted 14 days prior to treatment:
    - Hemoglobin ≥ 9.0 g/dL
    - Absolute neutrophil count (ANC) ≥ 1500 /mm3
    - Platelet count ≥ 100,000/µL
    - Total bilirubin ≤ 2 x ULN
    - ALT, AST and alkaline phosphatase (AP) ≤ 2.5 x ULN or ≤ 5.0 x ULN, if liver metastasis are present
    - PT-INR/PTT < 1.5 x ULN
    - Creatinine clearance (CrCl) ≥ 60ml/min calculated by either MDRD-formel or by 24 hours urine collection
    - Total calcium (corrected for serum albumin) within normal limits or correctable with supplements
    - Magnesium within lower normal limits or correctable with supplements
    - Potassium within normal limits or correctable with supplements
- Written informed consent (after adequate explanation of the trial) to participate in the trial and to adhere to trial procedures, as well as consenting to data protection procedures
- In case of females with childbearing potential (definition of menopause is no bleeding at least 12 months after last menstruation):
    - negative serum pregnancy test in women with childbearing potential
    - effective method of contraception (Pearl-Index not greater than 1%)

9.3.2 Exclusion criteria

- Known hypersensitivity to any study medication
- Other history of ongoing malignancy that would potentially interfere with the interpretation of efficacy
- Previous treatment with Hsp90 inhibitors (e.g.17-AAG)
- Treatment with therapeutic doses of coumarin derivatives. Low doses of coumarin derivatives (e.g. < 2mg/day) are permitted
- Patients with concurrent severe and/or uncontrolled medical conditions (e.g. uncontrolled diabetes mellitus, active untreated or uncontrolled infection, chronic obstructive or chronic restrictive pulmonary disease including dyspnea at rest from any cause or requiring supplementary oxygen therapy) that could cause unacceptable safety risks or compromise compliance with the protocol
- Impaired cardiac function including any of the following:
    - History (or family history) of long QT syndrome
    - Mean QTcF ≥ 450 msec on screening ECG
    - History of clinically manifest ischemic heart disease including myocardial infarction, stable or unstable angina pectoris, coronary arteriography or cardiac stress testing/imaging with findings consistent with infarction or clinically significant occlusion ≤ 6 months prior to start of the study

- History of heart failure or left ventricular (LV) dysfunction (LVEF ≤ 45%) by transthoracic echocardiography
- Clinically significant ECG abnormalities including one or more of the following: left bundle brunch block (LBBB), right bundle brunch block (RBBB) with left anterior hemiblock (LAHB), ST segment elevations or depressions > 1 mm or 2nd (Mobitz II) or 3rd degree AV block
- History or presence of atrial fibrillation, atrial flutter or ventricular arrhythmias including ventricular tachycardia or torsades de pointes
- Other clinically significant heart disease (e.g. congestive heart failure, uncontrolled hypertension or history of unstable hypertension)
- Clinically significant resting bradycardia (< 50 beats per minute)
- Patients who are currently receiving treatment with any medication which has a relative risk of prolonging the QTc interval or inducing torsades de pointes and cannot be switched or discontinued to an alternative drug prior to commencing AUY922
- Obligate use of a cardiac pacemaker
- Angina pectoris requiring a medicinal product
- Evidence of transmural infarction on ECG
- Clinically significant valvular disease

- Known diagnosis of HIV, active hepatitis B and/or C (testing is not mandatory)
- Clinically symptomatic leptomeningeal or brain metastases (patients with clinically stable brain metastases may be enrolled)
- Any person being in an institution on assignment of the respective authority
- Any medical, mental or psychological condition which in the opinion of the investigator would not permit the patient to complete the study or understand the patient information
- Any serious medical condition with organ impairment
- Parallel participation in another clinical trial
- Experimental or other therapy within the last 30 days or 5 half-life's, whatever is of longer duration (with exception of trastuzumab, if patient is recruited directly for combination treatment)
- Pregnancy, breast feeding

-

9.3.3 Excluding patients from treatment and/or analysis

Patients who didn't fulfill any of inclusion criteria or fulfill any of the exclusion criteria were not included in the study. Five patients were documented as screening failure: 01-001, 01-004, 01-007, 01-008 and 02-003. The particular reasons for screening failure are listed in the section 10.1.

No patient in the study was excluded from trastuzumab treatment for other reasons than screening failure. However, 5 of 8 patients on trastuzumab didn't receive AUY922. Four patients (02-001, 02-002, 02-004 and 02-005) experienced a deterioration of general conditions at time of progression on trastuzumab and were not recommended for the combination treatment of trastuzumab and AUY922 by treating physician. Patient 01-005 developed cardiac toxicity (arrhythmia) on trastuzumab, discontinued the treatment and thus didn't proceed in the combination treatment with AUY922.

The main analysis was performed on the full analysis set, defined in agreement with the intention-to-treat principle as all recruited patients, who began study treatment. The full analysis set was defined separately for the analysis of response rate under monotherapy and for the analysis of response rate under combination therapy.

As a result, out of the total of 13 patients who were screened for study participation, 8 were included in the endpoint-related analyses.

9.4 Investigational products

9.4.1 Investigational products

The investigational products assessed in the study were trastuzumab (Herceptin®, Roche) and AUY922 (Novartis). These were administered as described under 9.1 using the 2mg/kg dose of trastuzumab (with singular loading dose of 4mg/kg) and 70mg/m^2 AUY922 weekly. Patients started with trastuzumab monotherapy and continued with the combination of trastuzumab and AUY922 after progression on trastuzumab. The combination treatment of trastuzumab and AUY922 continued until progression or unacceptable toxicity occurred.

9.4.2 Description of investigational products

The two investigational products were provided as follows:

Trastuzumab	i.v. infusion, provided by Roche
AUY922	i.v. infusion, provided by Novartis

The investigational products were stored at Pharmacy Departments of University Hospital Cologne and University Hospital Essen.

9.4.3 Method of assigning patients to investigational products

Since the trial was a single arm study, no randomization was required. The study medication consisted of i.v. monotherapy of trastuzumab and the combined administration of trastuzumab and AUY922 after progression on trastuzumab. Patients were assigned chronologically in each center in order of their signatures on informed consent form. Patients kept their identification numbers through the study. The

identification number consisted of study center and the number of patient enrolled (i.e. 01-001, 01-002, 02-001, etc).

Because of the heterogeneity of the patient population (pretreatments, molecular pathological findings, etc), a comparison with a historical or other control group had not been intended from the outset.

9.4.4 Dose-ranging

After consenting, signing the patient informed consent, receiving the identification number and finalizing the screening, patients started with a loading dose of trastuzumab of 4mg/kg i.v (infusion time 90 min) at day 1.

If patients tolerated the loading dose of trastuzumab, further therapy proceeded with 2 mg/kg trastuzumab over 30 minutes. In case of disease progression as assessed in the 6-weekly CT (or MRI) -restaging, patients started with the combination of AUY922 70 mg/m^2 (infusion time 60 min) and 2 mg/kg trastuzumab i.v. (infusion time 30 min). Trastuzumab infusion started directly after AUY922 infusion.

Recommendations for study drug reductions or withdrawal by adverse events were as follows:

9.4.4.1 TRASTUZUMAB

It remained investigator responsibility to withdraw trastuzumab in cases of adverse reactions. The protocol recommended the dose reduction or even discontinuation in cases of cardiotoxicity, especially in cases of decreased cardiac function. Furthermore, the dose reduction, discontinuation or/and supportive therapy was recommended in cases of infusion reaction, allergic-like reactions and hypersensitivity. The particular drug changes were described in protocol section 7.4.

9.4.4.2 AUY922

It remained investigator responsibility to reduce/withdrawn AUY922 in cases of adverse reactions. The protocol recommended to reduce/withdrawn AUY922 especially in cases of bleeding, diarrhea, by changes in blood count and in liver and renal metabolism and in case of eye disorders. Cardiac changes were measured clinically and by ECG (especially assessing of QTcF interval). The particular changes were described in protocol section 7.4.

9.4.4.3 Discontinuation of one drug

If patients could not continue treatment of one or both drugs even after study drug reduction, the administration of one or both drugs was discontinued. If trastuzumab was discontinued, patients could be treated on AUY922 as long as they do not meet criteria for discontinuation.
In case of interruption of both drugs, depending on the individual course and performance state of the patient and the declared intention of the patient, standard therapy, which comprised continuous mono-chemotherapy, small molecule in another study or best supportive care were offered as well as participation in another clinical trial after discontinuation of the trial. Further treatment was offered at the study site. The patient might nevertheless change the treating institution, if wished. The follow-up visits on day 14 and 28 and once a month for up to 6 months were performed at the study site. In case of patient worsening conditions, these study visits were performed on phone between the patient and the site investigator.

9.4.4.4 Dose ranges

In the monotherapy, trastuzumab ranged from 94.0 to 330.8 mg in treated patients. In the combination therapy, the dose of trastuzumab ranged from 111.6 to 160.0 mg and the dose of AUY922 from 109.1 to 136.9 mg. Please refer also to the statistics analysis, section 5.

9.4.5 Selecting dosage and timing administration for each patient

All patients started with 4mg/kg trastuzumab i.v. over 90 minutes (loading dose) on day 1 and – if well tolerated - continued with 2mg/kg trastuzumab i.v. over 30 minutes weekly. Patients with response to the treatment (i.e. complete response, partial response, stable disease) continued the monotherapy treatment until disease progression. If patients progressed on trastuzumab monotherapy, the combination treatment of AUY922 and trastuzumab was commenced. Thus, all patients on combination treatment had received trastuzumab monotherapy previously.

Patients on combination therapy received both therapies (AUY and trastuzumab) on the same day. Trastuzumab was always administered before AUY922 in order to monitor potential allergic drug reactions.

On the treatment day, patients started with trastuzumab 2mg/kg i.v over 30 minutes. AUY922 70mg/m2 i.v was given over 60 minutes directly after trastuzumab. AUY922 was administered as close as possible but not sooner than 10 minutes from the end of the trastuzumab infusion. The AUY922 infusion started not later than 24 hours from the end of the trastuzumab infusion.

If well tolerated, patients proceeded to receive the combination therapy weekly.

For safety reasons, patients remained at hospital site for at least 4 hours after first dosing of trastuzumab and AUY922. Before discharging of patients, final clinical findings and anamnesis including questions about adverse events was done by investigator.

Administration of both drugs was performed by LCGC and by West German Cancer Center Essen. Drug accountability per patient was performed by qualified staff of Medical Drug Dispensary of University Hospital Cologne, Division of Central Cytotoxics Agents (Zentrale Zytostatika Zubereitung – ZZZ) and of Central Pharmacy of University Hospital Essen, corresponding to GCP guidelines and contractual agreements with Roche and Novartis.

9.4.6 Blinding

As this was a single-arm open-label study, blinding did not apply.

9.4.7 Previous treatment and concomitant treatment

As required in the inclusion criteria under 9.3.1, all patients participating in the study had already received treatment, and it was mandatory for participation that all patients received at least one standard treatment line. Due to the heterogeneity of the patient population no detailed information can be provided on the individual pretreatments.

In line with the inclusion and exclusion criteria described, no other systemic antineoplastic therapy was allowed in addition to the study regimen. Limitations did not concern the concomitant treatment of pre-existing or newly acquired diseases that were independent of the tumor disease or were necessary for symptom control. All

concomitant medication and its potential alterations were documented at baseline and during all study visits in the patient medical records and in the case report forms (CRFs).

9.4.8 Compliance

Patients received the study medication intravenously at study site at the same day. Medical supervision was provided during the whole infusion time and afterward as needed. The dose infused, as well as remaining medications were documented. The infusion time and the time between both infusions (trastuzumab and AUY922) were documented as well.

Compliance with study visits was ensured due to a printed study plan, which patients received during the screening. In case, the patient didn't appear to the planned study he/she was contacted via phone. If medically indicated, the unscheduled visits were scheduled as well.

9.5 Effect variables for safety and efficacy

9.5.1 Measuring the effect variables for safety and efficacy, plus flow chart

Effect variables were measured as described under 8. Specifically, the parameters surveyed for safety and efficacy were as follows:

Effect variable	Time and method of evaluation	Parameter or variable incl. units	Staff / institute responsible for data acquisition	Staff responsible for interpretation and assessment
Adverse events	Structured questioning at each study visit; face-to-face or phone contact at any time between visits, data acquisition using written or electronic sources like e. g. medical reports, lab findings, imaging	Naming the AE (AE term) Degree of severity acc. to the CTCAE Criterion for "seriousness" (SAE) Relatedness with investigational medication	Study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) at University Hospital Cologne and at the West German Tumor Center at University Hospital Essen	Investigators at the Lung Cancer Group Cologne (LCGC), University Hospital Essen and at the West German Tumor Center at University Hospital Essen; Data Safety Monitoring Committee
Lab values	Blood collection as described in the study protocol	Parameters as in study protocol, normal values and units conforming to standards of the Institute of Clinical Chemistry of Cologne University and University Essen	Study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) and the University Hospital Essen Institute of Clinical Chemistry of Cologne University and University Essen	Investigators at the Lung Cancer Group Cologne (LCGC) and University Hospital Essen
Concentrations of substances investigated	Blood collection as in study protocol during screening and before first treatment, directly at the end of infusion and 1h, 24h, 96h and 168h after end of infusion. Thereafter	Measurements acc. to methodology of Institute of Pharmacology, Cologne University	Blood collection: study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) and the University Essen Measurements: Prof.	Prof. Dr. med. Uwe Fuhr, Institute of Pharmacology Cologne University Hospital

	every other week before start and at the end of infusion.		Dr. med. Uwe Fuhr, Institute of Pharmacology Cologne University Hospital	
Restaging by CT	CT of affected region (screening and every 6 weeks until progression or discontinuation of treatment. In case of disease progression, CT if clinically indicated)	Measurement and documentation of target and non-target lesions and of any new tumor manifestations acc. to RECIST 1.1	Institute of Diagnostic and Interventional Radiology, Cologne University Hospital and Institute of Diagnostic and Interventional Radiology and Neuroradiology, University Hospital Essen	Institute of Diagnostic and Interventional Radiology, Cologne University Hospital and Institute of Diagnostic and Interventional Radiology and Neuroradiology, University Hospital Essen
Descriptive coverage of treatment and survival data	Data coverage from inclusion into study, start of treatment, end of treatment, progression of disease, death	Calculations for "progression-free survival" und "overall survival"	Study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) and University Hospital Essen	Investigators at the Lung Cancer Group Cologne (LCGC) and University Hospital Essen
HER2 genetic alteration	Ascertained during screening from pre-existing findings from pathological examination, follow-up verification from available tissue samples if necessary	Presence of a HER2-genetic alteration	Pre-existing findings: Study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) and University Hospital Essen Central testing: Prof. Dr. med. Reinhard Büttner, Institute of Pathology, Cologne University Hospital	Pre-existing findings: Study nurses/ investigators at the Lung Cancer Group Cologne (LCGC) and University Hospital Essen Central Testing: Prof. Dr. med. Reinhard Büttner, Institute of Pathology, Cologne University Hospital

9.5.2 Suitability of effect variables

The primary effect variable for assessing treatment response was the CT scan and its evaluation according to the RECIST 1.1 criteria. Restaging based on CT scans was in line with the current standard of care for patients with tumor disease receiving treatment.

Further effect variables were investigated for a descriptive analysis that would answer the secondary research questions as presented in the above table and in the sections below.

9.5.3 Primary effect variables/ endpoints

The primary endpoint of the study was the response rate (RR) of the combination therapy with trastuzumab and AUY922.

9.5.4 Determining serum concentrations of the investigational products

Pharmacokinetic (PK) assessment of trastuzumab and the combination of AUY922 and trastuzumab was performed at the following time points: before the first trastuzumab infusion and before the first combined AUY922 + trastuzumab infusion, in each case directly after the end of the infusion(s) and 1 h, 24 h, 96 h and 168 h after the end of the infusion(s). If logistically necessary, the time points for 24 h and 96 h PK sampling was adapted, but the 24 h sample was withdrawn between 18-40 h and the 96 h sample between 72-120 h. Thereafter, PK was assessed every other week before start and at the end of infusion(s). For combination therapy, "end of infusion" was related to the end of the infusion of the second substance, and an additional sample was taken just after the end of the infusion of the first substance.

It was not intended to analyze individual patient samples during the study in order to assess individual patient safety profile. It was intended to establish a pharmacokinetic / pharmacodynamic model with regard to response rate and adverse events. However, due to small number of patients (n=8) who received therapy, an informative pharmacokinetics analysis/model was not feasible.

9.6 Data quality assurance

As required by the GCP / ICH guidelines, the study physicians of the LCGC and the University Hospital Essen had been trained and registered as investigators and were familiar with the content of the study protocol. Formalized training on study procedures and protocol was carried out during the study initiation. There were regular meetings discussing the study content and procedures and briefing on the current situation of the patients receiving the treatment under investigation during the regular weekly meetings of the LCGC Cologne.

External quality assurance was based on the regular monitoring by the Cologne Center for Clinical Studies (ZKS). These monitoring visits primarily concentrated on reviewing the Patient Information and Consent for correctness, assessed the measures carried out for conformance with the protocol, the handling of adverse events, completeness of Case Report Forms incl. reconciliation of source data, management of the study medication and overall progress of the study. A detailed list of the individual aspects of the monitoring and the frequency of monitoring visits is included in the separate Monitoring Manual (Annex).

Using the source data (paper or electronic patient records), the study nurses of the LCGC and the University Hospital Essen documented the data material obtained in the course of the study in standardized electronic Case Report Forms (eCRFs) which were signed off by an investigator. ZKS staff then entered the CRF documented data in the database that the ZKS had set up on the basis of the CRFs and which also was being managed by the ZKS.

Lab parameters were evaluated at the Institutes of Clinical Chemistry of Cologne University and University Essen following their institutional standards. Measurements were documented in the above eCRFs.

Same-day evaluation of the CT-based restagings under clinical aspects during the course of the study was carried out by the radiologists of the Institute for Diagnostic and Interventional Radiology, Cologne University Hospital and of the Institute for Diagnostic and Interventional Radiology and Neuroradiology, University Essen.

9.7 Statistical methods under the study protocol and planning of sample size

9.7.1 Planned analyses

All variables were planned using appropriate descriptive summary tables according to the following:

- continuous variables: number of observations, mean, standard deviation, minimum, median, maximum;
- categorical variables: number of observations, absolute and relative frequency;
- time to event variables: number of observations, number and percentage of censored observations, median (with 95% confidence interval), Kaplan-Meier survival curves and estimate of survival at appropriate time points (with standard error).

9.7.2 Study populations

The main analysis was performed on the full analysis set, defined in agreement with the intention-to-treat principle as all recruited patients qualified according to the inclusion and exclusion criteria that began study treatment. The full analysis set was defined separately for the analysis of response rate under monotherapy and for the analysis of response rate under combination therapy.

9.7.3 Primary objectives

The response rate (RR) of the combination therapy with trastuzumab and AUY922 was calculated as the proportion of the full analysis set for the combination that attain a partial or complete response under study treatment.

9.7.4 Secondary objectives

- The response rate in patients treated with trastuzumab monotherapy was planned to be calculated as the proportion of the full analysis set for the monotherapy who attain a partial or complete response under this treatment.
- The tolerability of trastuzumab and AUY922 in combination was evaluated by listing all adverse events (AEs) according to CTC-AE V4.0) reported during or after combination treatment. Frequencies of classes of AEs, defined according to the MedDRA system if appropriate, was displayed.
- The progression-free survival (PFS), overall survival (OS) of trastuzumab monotherapy and the combination was analyzed using the Kaplan-Meier method.
    - OS of monotherapy (with subsequent use of the combination treatment at progression) was defined as the time from initial staging until death from any cause or last documentation of vital status.
    - PFS of monotherapy was defined as the time from initial staging until progression, death from any cause or last clinical examination.
    - OS of the combination therapy was defined as the time from progression under monotherapy until death from any cause or last documentation of vital status.
    - PFS of the combination therapy was defined as the time from progression under monotherapy until progression under combination therapy, death from any cause or last clinical examination.

9.7.5 Subgroup analysis

Analyses were performed descriptively for subgroups according to gender and age (cut-point: median age of the analysis set), respectively.

9.7.6 Interim analysis

An interim analysis was planned to be performed when 10 patients were evaluable for response to the combination treatment. The response rate to the combination and the tolerability (adverse events) of this therapy was planned to be analyzed.

9.8 Sample size calculation

Patient sample size was based on the Simon two-stage design (Simon 1989) with error rates alpha=0.05 and beta=0.20.

The null hypothesis (ineffective treatment) that the response rate is at most 5% was tested against the alternative hypothesis (effective treatment) that the response rate is at least 20%.

Stage 1: Ten patients on the combination were planned to be treated in the first stage. If less than 1 response on the combination according to RECIST was observed in the first trial phase, recruitment was planned to be stopped and it would have been concluded that the treatment is ineffective.

Stage 2: If at least 1 partial response on the combination had been observed in the first trial phase, another 19 patients would have been recruited and treated as described above. There was an 89% probability of continuing to the second stage if the true response rate was at least 20%, while if the true response rate was 5% or less there was a 60% probability of stopping.

If at least 4/29 of patients treated with combination therapy would have responded, it would have been concluded that the treatment showed sufficient promise of effectiveness for further investigation.

9.9 Pharmacokinetic / pharmacodynamic analyses

A population pharmacokinetic evaluation (NONMEM VI or higher) was applied to the data. Individual exposure estimates were planned be used as independent variables for a PK/PD analysis, dependent variables are adverse event and response metrics.

However, as mentioned above, due to small number of patients (n=8) who received therapy, an informative pharmacokinetics analysis/model was not feasible.

9.8 Modifications in the conduction of the study or the proposed methods of analysis

Four amendments to the protocol became necessary during the course of the study. The following describes the content-related changes, the reasons for them and the submission days. The details on amendments are given in Annex.

***Amendment 1* (19-Nov-2012)**

Amendment 1 arose from initiation of the trial and contained corrections in in- and exclusion criteria as the wording was not consistent on two different places in protocol (in the synopsis at the beginning of the protocol and in the protocol under the section "Inclusion and exclusion criteria").

Amendment 2 (08-Jun-2014)

Amendment 2 comprised several aspects related to implementation of the study, administration of the study medication and patient's safety.

1. New timelines. During the course of the study, it became clear that the initial timelines have to be extended due to low frequency of HER2 genetic alterations in NSCLC patient population.
2. The delivery of AUY922 was changed from 10ml ampoules of Solution for Infusion (each containing 50mg AUY922) to Concentrate for Infusion in 20ml Vials (each containing 50mg AUY922).
3. Clear formulation that AUY922 infusion can be started directly after trastuzumab. This was not clearly expressed in the version before.
4. In the previous version, the central testing was mandatory. Due to Amendment 2, also patients tested as HER2 positive (as per inclusion criteria) by local pathologist could be included in the trial. This change arose from the low frequency of HER2 positive patients. With this amendment we also wanted to avoid new biopsy (if necessary for central testing) for patients tested locally.
5. Due to recommendation of DMSC, management of bleeding was added to the management of adverse events. The necessity became obvious after an adverse event (bleeding) of one study patient. Bleeding was a known adverse event related to AUY922 according to IB.

Amendment 3 (24-Feb-2015)

Amendment 3 arose due to changes in trastuzumab label. The label changes, secondary packagers and changed module 1 were accepted by the German Federal Institute for Drugs and Medical Devices and by both Ethics Committees (University Cologne and University Essen).

Amendment 4 (25-Jun-2015)

Amendment 4 concerned changes in the quality documents of AUY922 and was submitted and accepted by the German Federal Institute for Drugs and Medical Devices.

10 Study Population

10.1 Patients participating in the study

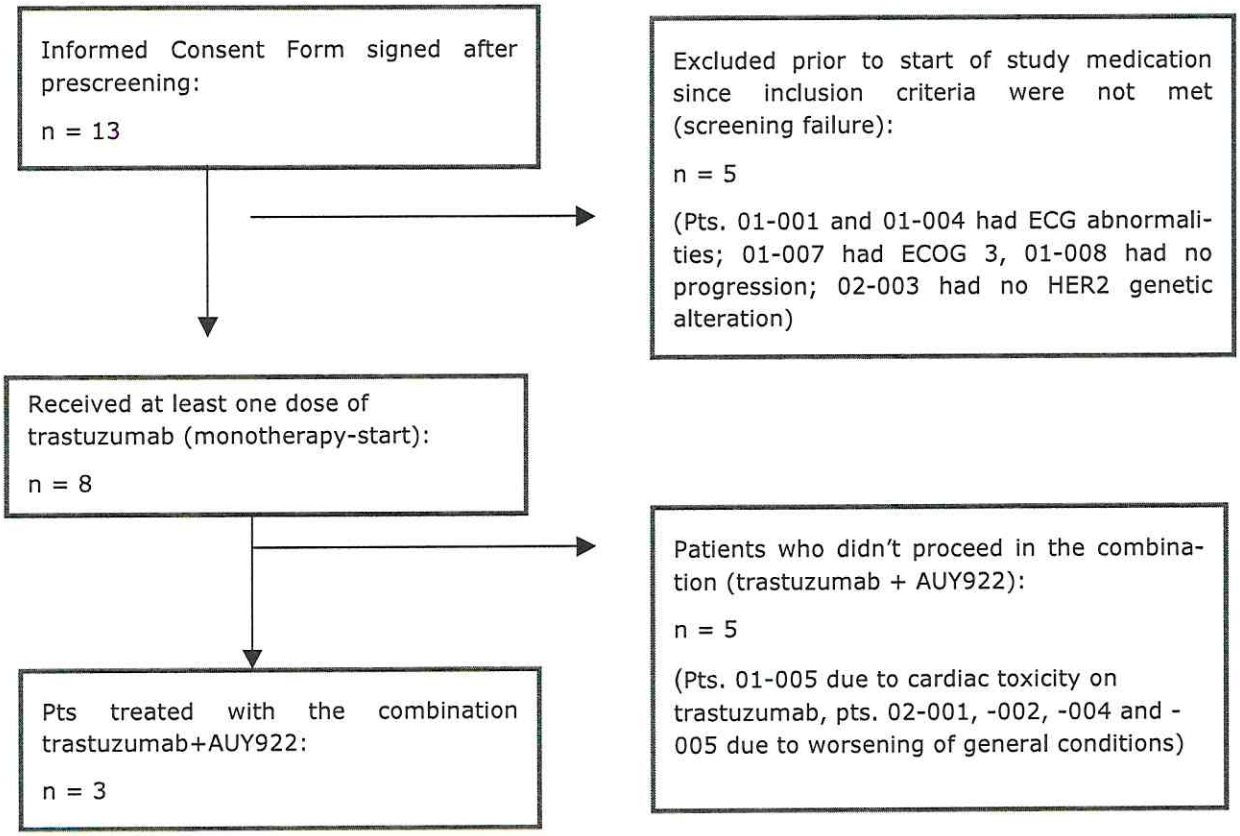
Investigators of the Lung Cancer Group Cologne and Department of Internal Medicine University Cologne as well as investigators at West German Tumor Center were in charge of the prescreening of potentially suitable study candidates, which was carried out on the basis of the outpatient or inpatient care in Department I of Internal Medicine of the Cologne University Hospital and Department of Internal Medicine University Essen. Potentially suitable candidates were patients of local oncological practices or of other departments of Cologne and Essen University Hospitals who were evaluated within the scope of the Second Opinion Services.

Patients were prescreened for HER2 genetic alterations locally. Centrally confirmed HER2-alterations were not needed after Amendment 2.

Patients found suitable according to the findings available at prescreening received detailed information about the study and if they consented to participate were subjected to the screening examinations as provided in the study protocol. If they met all inclusion and no exclusion criteria, treatment was started with trastuzumab loading dose of 4mg/kg. If well tolerated, the treatment was continued with trastuzumab monotherapy of 2mg/kg. In case of disease progression, AUY922 of 70mg/m^2 was added to trastuzumab. In this single-arm study patients were not randomized, but suitable candidates were included in the order of their screening in a sequential manner.

If an exclusion criterion was identified within the screening phase, i.e. after patients had signed the Informed Consent Form and thus had been formally included in the study, these cases were considered "screening failures", and the patients received no treatment with the study medication(s).

Flow charts for included patients



10.2 Deviations from protocol

As described under 10.1, the study regimen could be started in compliance with the protocol in 8 out of the 13 patients who had been enrolled. Five patients had to be considered "screening failures" since exclusion criteria were identified in the screening process, i.e. after they had signed the Informed Consent Form and thus had been formally admitted to the study. This concerned the following patients:

1-001	1-007	2-003
1-04	1-008	

Patient 1-001 had mean QTcF ≥ 450 msec as exclusion criterion:

1-004 had total calcium not within normal limits (not fulfilled inclusion criterion) and had history/presence of atrial fibrillation (exclusion).

1-007 had ECOG 3 (not fulfilled inclusion criterion);

1-008 had no progression (not fulfilled inclusion criterion)

2-003 had no HER2 genetic alteration (exclusion).

From 8 patients who started trastuzumab monotherapy, 3 proceeded to combination therapy. The reasons for 5 patients, who did not receive the combination therapy, were as follows:

Patient 1-005 developed cardiac arrhythmia, so we suspected cardiotoxicity due to trastuzumab.

Patient 2-001, -002, -004 and -005 didn't receive the combination due to worsening of general conditions.

11 Efficacy Assessment

11.1 Patient groups analyzed

The efficacy of the combination of trastuzumab and AUY922 was the primary endpoint of the study. Efficacy on trastuzumab monotherapy was the secondary endpoint. However, all 8 patients treated on trastuzumab monotherapy had stable disease as best response. Due to slow recruitment, we didn't recruit sufficient patient number to answer the primary endpoint. The slow recruitment was caused mainly due to very low frequency (about 4%) of NSCLC patients with HER2 alterations. Secondly, the weekly infusion treatment represented another logistical problem for patient with advanced cancer.

Summarized, we recruited 13 patients, 5 were screening failures, 8 received trastuzumab monotherapy and 3 got the combination of trastuzumab and AUY922. So far, we haven't had sufficient amount of patients to answer the primary endpoint.

The descriptive analysis of efficacy (overall survival, progression free survival, response acc. to RECIST 1.1) considered all qualified patients who had received "per protocol" treatment and for which the corresponding data was available.

The details on patient analysis are presented in Statistics Annex (statistical data).

11.2 Demographics and other baseline characteristics

The demographic characteristics of the entire patient population included in the study are given in the following table:

Pat-ID	patid_d	Date of signed Informed Consent Form	Her 2 status	Year of birth	Age (years)	Gender	Smoking status	Ethnic group	Other ethnic group
01-002	01-002	06/05/2013	amplification	1961	52	female	never smoker	caucasian / white	
01-003	01-003	04/07/2013	amplification	1957	56	female	ex-smoker	caucasian / white	
01-005	01-005	10/12/2013	amplification	1961	52	male	smoker	caucasian / white	
01-006	01-006	20/01/2014	overexpression	1955	59	female	ex-smoker	caucasian / white	
02-001	02-001	04/12/2013	overexpression	1956	57	female	ex-smoker	caucasian / white	
02-002	02-002	17/02/2015	mutation	1953	61	female	.	caucasian / white	
02-004	02-004	27/02/2015	amplification	1954	61	female	never smoker	caucasian / white	
02-005	02-005	21/08/2015	amplification	1963	52	female	ex-smoker	caucasian / white	

11.3 Assessment of compliance

There was no formal evaluation of patient compliance. The intravenous study medication was documented for each patient. The pharmacokinetics analysis was initially planned after collection of all samples for all patients.

Due to low patient number, serum levels of both medications were not determined.

11.4 Results of the analysis of efficacy and tabulated presentation of individual patient data

11.4.1 Analysis of efficacy

For the descriptive analysis of efficacy, please see the Statistics Annex under 7-8.

Since this trial was a single-arm Phase II study, a comparative evaluation of efficacy against standard therapies or against a similar historic patient population was not feasible or even useful.

11.4.2 Statistical methods

The statistical evaluation of the study was carried out as described under 9.7. The various subitems are given below in as much as these aspects apply to the study.

11.4.2.1 Control of covariables

The medical histories of the patients in the ITT group are listed under Point 3a of the Statistics Annex. The concomitant medication is listed in Statistics Annex as well.

11.4.2.2 Dealing with dropouts and non-availability of data

As mentioned, 13 patients consented for the study, 5 of them were screening failures. All 8 patients received at least one dose of trastuzumab. From 8 patients, 5 didn't receive the combination therapy of trastuzumab and AUY922 due to worsening of general conditions (4 patients) and due to cardiac toxicity (one patient).

11.4.2.3 Interim analysis and data monitoring

The study protocol required annual meetings of the Data Safety Monitoring Committee (DMC). The minutes of the meetings are presented in the Annex.

11.4.2.4 Multicenter studies

The study was conducted as a multicenter trial – at the Universities Cologne and Essen. University Cologne was the Sponsor of the study.

11.4.2.5 Multiple comparisons

With only one primary dependent variable and one treatment group, adjusting for alpha error was not necessary.

11.4.2.6 "Per Protocol" analysis

Statistical analysis concerning the primary endpoint could not be performed due to low patient number, who received the combination therapy of trastuzumab and AUY922.

We plotted Kaplan-Meier curve for all patients who started monotherapy with trastuzumab, including patients treated with the combination of trastuzumab and AUY922. We didn't plot the Kaplan-Meier curve for the combination treatment as we had only 3 patients treated on the combination.

11.4.2.7 Equivalence tests with active controls

The study did not include the testing of active controls.

11.4.2.8 Evaluation of subsets

The sample size of the study was too small for subset analysis.

11.4.3 Tabulated presentation of individual data

Please see Statistics Annex. All sections included individual data.

11.4.4 Relation between dose/ concentration and effect

Study results do not support a correlation between dose/ concentration and effect.

11.4.5 Intervention-intervention interaction and intervention-disease interaction

Study results revealed no apparent interaction between the effects of the investigational product and any concomitant treatment or comorbidity.

11.4.6 Patient profiles

See listing of the Statistics Annex sections 1-2.

11.4.7 Upshot of efficacy analyses

Response according RECIST

Statistics Annex, Sections 7-8 and the following tables give the assessment based on the CT results according to the RECIST 1.1.

Pat. ID	Best CT response trastuzumab	Best CT response trastuzumab+AUY922
01-002	SD	PD
01-003	PD	PD
01-005	SD	Not applicable
01-006	PD	SD
02-001	PD	Not applicable
02-002	SD	Not applicable

02-004	SD	Not applicable
02-005	PD	Not applicable

Mortality, overall survival (OS)

Mortality details are listed in Statistics Annex, Section 12. One patient (01-005) survived 21.22 months after starting the study medication (death was not reported before closure of database). For all other patients overall survival ranged from 0.82 to 15.38 months.

In all cases death was caused by progression of the primary disease. Median survival using the Kaplan-Meier estimate was 9.6 months (confidence interval 95% (1.9; not reached)). The Kaplan-Meier curve is pictured in Statistics Annex, Section 12.

Progression-free survival (PFS)

The PFS details have been listed in Statistics Annex, Section 12. All patients suffered progression between 1.48 and 9.33 months after starting the study medication. Median PFS after Kaplan-Meier was 3.22 months (confidence interval 95% (1.5; 9.3)). The Kaplan-Meier curve for PFS is in Statistics Annex, Section 12).

12 Safety Analysis

12.1 Range of patient exposure

In the course of the study, a total of 8 patients were treated with at least one dose of the study medication. Eight patients received at least one dose of trastuzumab. Of them, 3 patients got the combination treatment. All patients were included in the analyses for safety and efficacy.

Treatment exposure for trastuzumab is detailed in the section 5 of the Statistics Annex and for the combination of trastuzumab and AUY922 in the section 6 of the Statistics Annex.

12.2 Adverse events

12.2.1 Brief overview of adverse events

Already at entry in the study, many of the patients had relevant tumor-associated signs and in many cases their physical condition was noticeably reduced.

Consistent with this background, from 8 patients that received treatment, all developed at least one adverse event. We registered 7 SAEs in 5 patients. From 7 SAEs, one was related to AUY922, all remaining had no relation to study medication and were mainly caused due to progressive disease. The SAE "bowel bleeding" related to AUY922 occurred in patient 01-002 and led to Protocol Amendment 2 (as described in section 9.8) with detailed instruction to AUY922 dose reduction in case of bleeding events.

12.2.2 Detailed compilation of adverse events

For a detailed list of adverse events please refer to the Statistics Annex, Sections 9, 10 and 11.

12.2.3 Analysis of adverse events

The incidence and nature of the adverse events documented in the study corresponded to the known side-effect profiles of the two study medications trastuzumab and AUY922. In addition to these undesirable effects, the adverse events that frequently developed were directly or indirectly related to the metastatic disease. Adverse events that were associated with concomitant diseases of the patients were rare. As described above, the SAE "bowel bleeding" related to AUY922 occurred in patient 01-002 and led to Protocol Amendment 2 (as described in section 9.8) with detailed instruction to AUY922 dose reduction in case of bleeding events.

Furthermore, patient 01-005 developed cardiac side effect (arrhythmia) that is known by Herceptin. This patient discontinued the Herceptin treatment on the basis of these side effects and didn't proceed to the combination treatment.

12.2.4 Listing adverse events by patient

Please see Statistics Annex, Section 9.

12.3 Deaths, other Serious Adverse Events, and further significant undesirable incidents

12.3.1 Listing deaths, other Serious Adverse Events, and further significant undesirable incidents

Serious Adverse Events are listed in the Statistics Annex under 10 and deaths are given under 1a 'Course of disease' und under 12a 'Survival'.

12.3.1.1 Deaths

From eight patients who received study medication, 7 died due to progressive underlying disease, one patient who discontinued trastuzumab treatment due to cardiac toxicity was still alive at time of data close. At the time of data base closure, 4 patients died. Remaining 3 patients died after data base closure.

12.3.1.2 Other SAEs

All SAEs are listed in Statistics Annex in the Section 10.

12.3.1.3 Other significant AE

All have been mentioned.

12.3.2 Qualitative description of fatalities, other SAE and specific other significant AE

See 12.3.1.1 and sections 9 to 12 in the Statistics Annex.

12.3.3 Analysis and discussion of fatalities, other SAE and other significant AE

The majority of the deaths that occurred were related to progression of the metastatic disease. Considering the inclusion criterion "patients with solid tumors after standard therapies", this was to be expected. There were two adverse events that should be mentioned as they influenced the clinical course of both patients.

Patient 01-005 developed sinusarrythmia and supraventricular extrasystoles that were indicated as related to trastuzumab. The patient discontinued treatment from this reason and didn't continue on the combination with trastuzumab and AUY922. Cardiotoxicity is a known side effect of trastuzumab.

Patient 01-002 developed melaena which we indicated as related to AUY922. The patient was hospitalized, so we graded the AE as SAE. Bleeding is a known side effect of AUY922. The patient developed simultaneously progressive disease and discontinued the

treatment. The event melaena led to Amendment 2 of protocol with recommendations to AUY922 dose reduction by bleeding.

12.4 Evaluation of clinical laboratory values

12.4.1 List of laboratory values by patient and abnormal single parameters

Please see Statistics Annex under section "Laboratory data".

12.4.2 Evaluation of individual laboratory parameters

Please see Statistics Annex under section "Laboratory data". There were no new aspects on laboratory data and given therapy.

12.3.1.4 Changes of laboratory values over time

Please see Statistics Annex under section "Laboratory data".

12.3.1.5 Patient-related presentation of changes

Please see Statistics Annex under section "Laboratory data".

12.3.1.6 Individual, clinically significant deviations

There were no clinical significant changes in patient individual laboratory results.

12.4 Vital signs, physical findings, and other safety-critical observations

Please see section "Vital signs" of the Statistics Annex. Concerning the ophthalmologic examinations, no significant observations related to both medications were observed.

12.5 Upshot of the safety analysis

All related AEs were in line with the known safety profile of the two investigational products. AEs that were indicated as not related were in most cases in line with symptoms caused due to underlying disease.

13 Discussion and Overall Conclusion

This Phase II study evaluated the efficacy of trastuzumab and AUY922 in advanced NSCLC patients harboring genetic alterations in HER2 gene. Based on preclinical and early clinical data we calculated the response rate at least 20% in patients in advanced NSCLC with HER2 genetic alteration. Regarding the Simon two-stage design we would have needed to recruit 10 patients in the first stage. If 1 of 10 patients had responded, we would have needed to recruit additional 19 patients. If 4/29 patients responded, we would have included that the combination treatment showed sufficient promise of effectiveness for further investigation.

However, the recruitment in the study was extremely slow. This was mainly due to very low frequency (about 4%) of NSCLC patients with HER2 alterations. Secondly, the weekly infusion treatment represented another logistical problem for patient with advanced cancer. Due to impaired general conditions in the majority case, patients saw the weekly treatment with trastuzumab and combination as physically demanding and decided not to participate in the study.

As a result, we could screen 13 patients. Five patients failed the screening and 8 received treatment with trastuzumab. From 8 trastuzumab patients, only 3 patients received combination of tratuzumab and AUY922. The reason not to proceed to the combination was in the majority of patients the deteriorated general conditions due to malignant disease.

Regarding the safety, we observed the known adverse drug reactions, which were already mentioned in the prescribing information and investigational brochure, respectively. There were 2 adverse drug reactions, which were in fact described, but which meant treatment changes for both patients. One patient developed cardiotoxicity in terms of cardiac arrhythmia, so we decided to discontinue the trastuzumab treatment and not proceed for the combination. In another case, patient developed bleeding as an adverse drug reaction of AUY922 and was hospitalized. We recommended continuing with reduced dose of AUY922. However, patient developed progressive disease and discontinued the combination treatment. We amended the protocol with special AUY922 dose recommendations in case of bleeding for further patients.

As regards efficacy, no outcome in the form of a partial response (PR) was attained. From 8 patients treated with trastuzumab, 4 patients had stable and 4 progressive disease as best response. From 3 patients treated with the combination trastuzumab and AUY922, one patient reached stable disease as best response and 2 patients were progressive at their first CT scan.

Median progression free survival (PFS) was 3.2 months and overall survival (OS) 9.6 months for all included patients.

In summary, the study failed to recruit sufficient number of patients to answer the clinical question if NSCLC patients with HER2 genetic alterations may benefit from the combination of HER2 antibody trastuzumab and the heat shock protein inhibitor AUY922. However, the rather high number of progressive diseases at first CT scans in monotherapy and the combination therapy indicates that the treatment with trastuzumab and the combination of trastuzumab and AUY922 respectively might be insufficient to induce responses in these patients.

We suggest that further preclinical studies with potential development of new targeted compounds are needed to identify effective treatment for NSCLC patients with HER2 genetic alterations.

14 Literature

Please see List of References in the study protocol.

15 Annexes

1. Statistics Annex
2. Protocol and Amendments
3. CRF
4. Patient Informed Consent with Amendments
5. DMSC
6. Monitoring manual