EUDRACT-NO.: 2013-001269-18	**Protocol No.**: D-IV

Sponsor: Universitätsklinikum Ulm (AöR), Albert-Einstein-Allee 29, D-89081 Ulm
Responsible person: Prof. Dr. Wolfgang Janni, University Hospital Ulm

Coordinating Investigator („Leiterin der klinischen Prüfung" acc. to German Drug Law):
Prof. Dr. Tanja Fehm, University Hospital Düsseldorf

Title: DETECT IV – A prospective, multicenter, open-label, phase II study in patients with HER2-negative metastatic breast cancer and persisting HER2-negative circulating tumor cells (CTCs).

Investigational medicinal products (IMP): DIVa: Everolimus tablets 5 mg/Ribociclib capsules 3 x 200 mg /d d 1-21 q28d or **DIVb:** Eribulin mesylate 1.23 mg/m² d1+8 q3w

Indication/Clinical Trial population

Everolimus/Ribociclib cohort:
Postmenopausal female patients with hormone-receptor positive, HER2-negative metastatic breast cancer with only HER2-negative circulating tumor cells (CTCs) and indication for standard endocrine therapy.

Eribulin cohort:
Patients with hormone-receptor positive, HER2-negative metastatic breast cancer and indication to chemotherapy or patients with triple-negative metastatic breast cancer, both with only HER2-negative circulating tumor cells (CTCs).

Clinical Trial Design
A prospective, multicenter, open-label, phase II study.

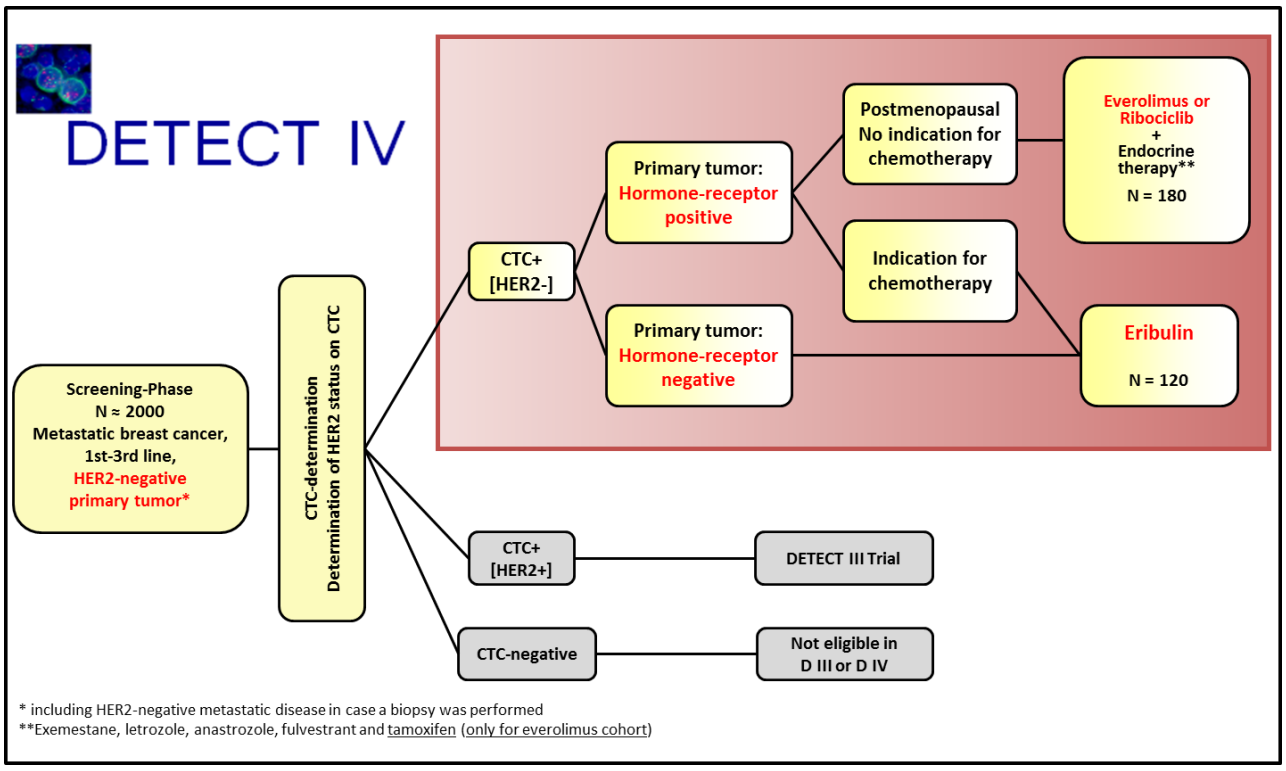

Figure 1: Screening and Design of DETECT IV

Planned duration of the entire study
The study starts when the necessary approval has been given by the competent authority (BfArM). Furthermore the competent ethics committee must have given a favorable opinion on study conduct before the first patient is included.
The trial is terminated after the last patient has either died or completed the 24-months follow-up period.
The maximum study duration is 72 months and 3 weeks.

Planned duration of individual study participation
The individual study participation begins with the screening visit and ends when the patient has either died or completed the 24-months follow-up period.

- Maximum duration of pre-treatment evaluation period (from screening to recruitment): 3 weeks
- Maximum duration of treatment period: 12 months

- Maximum duration of follow-up period: 24 months

Thus, the maximum duration of individual study participation is 36 months and 3 weeks.

Number of Clinical Trial Centers Planned to be Involved

A maximum of 100 clinical trial centers is planned to participate in the study.

Inclusion Criteria

In General for both study cohorts

- Metastatic breast cancer, which cannot be cured by surgery or radiotherapy. The primary tumor and/or biopsies must have be confirmed as cancer by histolopathology.
- HER2 status (as investigated on all primary tumor tissue and/or biopsies from metastatic sites or loco regional recurrences) must be negative. HER2-negativity is defined as (i.e.: immunohistochemistry (IHC) score 0-1+ or 2+ and fluorescent in situ hybridization (FISH) negative or just FISH negative, whichever was performed) in all tissue samples
- Evidence of CTCs. At least one CTC has been detected in 7.5 ml patient blood by means of the CellSearch® Circulating Tumor Cell Kit (Veridex LLC, Raritan, USA).
- HER2 negativity of all detected CTCs.
- Adequate organ function within 7 days before date of recruitment, evidenced by the following laboratory results:
  - absolute neutrophil count ≥ 1500/µL
  - platelet count ≥ 100000/µL
  - hemoglobin ≥ 9 g/dL
  - ALT (SGPT) ≤ 3.0 × ULN
  - AST (SGOT) ≤ 3.0 × ULN
  - bilirubin ≤ 2.0 × ULN
  - creatinine ≤ 2.0 × ULN.
- Written informed consent in study participation.
- Undergoing a re-biopsy prior to inclusion if tissue is accessible, which can be safely biopsied, is otional but desirable.
- Tumor evaluation has been performed within 6 weeks before date of recruitment and results are available.
- Patients must have at least one not previously irradiated lesion that can be evaluated according to RECIST version 1.1 (Eisenhauer 2009). Patients with measurable and non-measurable disease are eligible. Presence of clinically and/or radiologically documented disease.
- Age ≥ 18 years.
- ECOG Performance Status ≤ 2.

Everolimus/Ribociclib cohort (DIVa)	Eribulin cohort (DIVb)
Both cohorts: • Indication for an endocrine therapy (Histological confirmation of estrogen receptor positive (ER+) and/or progesterone receptor positive (PgR+) breast cancer). • Up to two lines of previous cytostatic treatment for MBC. • Any endocrine therapy in the history is allowed. • Disease progression following prior treatment with endocrine therapy (endocrine therapy does not have to be the last therapy before inclusion in the trial). • Postmenopausal women. The investigator must confirm postmenopausal status Postmenopausal status is defined either by - Age ⩾ 55 years and one year or more of amenorrhea • - Age < 55 years and one year or more of amenorrhea and postmenopausal levels of FSH and LH • - Prior hysterectomy and has postmenopausal levels of FSH and LH • - Surgical menopause with bilateral oophorectomy • Everolimus cohort: • Cholesterol ≤ 2.0 × ULN • Ribociclib cohort: • Standard 12-lead ECG values assessed by the local laboratory: • - QTcF interval at screening < 450 msec (using Fridericia's correction) • - Resting heart rate 50-90 bpm • INR ≤ 1,5 (ribocilclib cohort)	• Either hormone-receptor negative MBC or hormone-receptor positive MBC with indication for chemotherapy • Up to three previous chemotherapy treatment lines for metastatic disease • In case of patients of child bearing potential: - Negative pregnancy test (minimum sensitivity 25IU/L or equivalent units of HCG) within 7 days prior to recruitment - Contraception by means of a reliable method (i.e. non-hormonal contraception, IUD, a double barrier method, vasectomy of the sexual partner, complete sexual abstinence). Patient must consent in maintaining such contraception until 3 months after completion of study treatment

• Patients must have the following laboratory values within normal limits or corrected to within normal limits with supplemets before the first dose of study medication: • -Sodium • -Potassium • -Total calcium	

Exclusion Criteria

In General for both study cohorts

1. Treatment with other investigational agents of any type or anticancer therapy during the trial, within 2 weeks prior to the start of treatment.
2. Adverse events due to prior anticancer therapy which are > Grade 1 (NCI CTCAE) and therapeutically relevant at time of treatment start.
3. Known HIV infection.
4. Current active hepatitis B or C, cliniclally relevant known liver dysfunction, e.g. according to Child Pugh Classification class B and C, or biliary disease (with exception of patients with Gilbert's syndrome, asymptomatic gall-stones, liver metastases or stable chronic non-viral liver disease per investigator assessment).
5. Concurrent disease or condition that might interfere with adequate assessment or evaluation of study data, or any medical disorder that would make the patient's participation unreasonably hazardous.
6. Other malignant diseases within the last 3 years (apart from carcinoma in situ of the cervix or non-melanoma skin cancer)
7. Dementia, altered mental status, or any psychiatric or social condition which would prohibit the understanding or rendering of informed consent or which might interfere with the patient's adherence to the protocol.
8. Life expectancy < 3 months.
9. Male gender.

Everolimus/Ribociclib cohort (DIVa)	Eribulin cohort (DIVb)
• Known hypersensitivity to any of the excipients of ribociclib, everolimus or any of the other given drugs. • Known hypersensitivity to lecithin (soya) and peanuts (ribocilib-cohort) • Disease or condition, which might restrain the ability to take or resorb oral medication. This includes malabsorption syndrome, requirement for intravenous (IV) alimentation, prior surgical procedures affecting absorption (for example resection of small bowel or stomach), uncontrolled inflammatory GI disease (e.g., Crohn's disease, ulcerative colitis) and any other diseases significantly affecting gastrointestinal function as well as inability to swallow and retain oral medication for any other reason.	• History of hypersensitivity reactions attributed to eribulin. • Pre-existing neuropathy grade 3 or higher. • Severe Congenital long QT syndrome. • Pregnancy or nursing.

Treatment Plan

Treatment with everolimus in combination with standard endocrine therapy:

The investigational drug used in the course of this trial is everolimus in combination with standard endocrine therapy as defined below (see TABLE 1).

On the first day of each cycle, patients will receive an adequate drug supply (everolimus not combined with exemestane) or a prescription (everolimus combined with exemestane) for self-administration at home. The investigator must emphasize compliance and will instruct the patient to take everolimus and standard endocrine therapy exactly as prescribed.

All patients will receive everolimus + standard endocrine therapy. All patients will take everolimus tablets orally per day and will also take standard endocrine therapy once daily (dosage according to the label).

Endocrine treatment		**+Everolimus***
Exemestane (Baselga et al. 2012 – BOLERO 2-trial) Prior treatment with Letrozol/ Anastrozol	25 mg/d	10 mg/d*
Tamoxifen (Bachelot et al. 2011 – TAMRAD-trial) Prior treatment with AI	20 mg/d	10mg/d*
Letrozole (Baselga et al. 2009 [neo-adjuvant, Phase II); (Awada et al. 2008 [MBC, Phase I])	2,5mg/d	10mg/d*
Anastrozole	1mg/d	10mg/d*

***Everolimus will be prescribed according to the approved label. The prescribing physician can decide about dose modifications according to the individual medical need of the patient, and a starting dose of 5mg daily is allowed if medically indicated. The decision for treatment of the patient with everolimus will be made independently of the study.**

Table 1: Recommended treatment regimen for combination with everolimus

Everolimus will be dosed starting on treatment Day 1 (Inclusion). Patients will be instructed to take the everolimus tablet(s) orally with a large glass of water once daily at the same time each day with or without food.

Exemestane / letrozole / anastrozole / tamoxifen will be dosed starting on treatment Day 1 according to the recommended daily dosage (see Table 1). Package insert instructions should be followed.

Everolimus and endocrine therapy will be taken daily from treatment Day 1 up to 12 months or until disease progression, unacceptable toxicity, death, or withdrawal from the study for any other reason. If medically indicated everolimus treatment may be continued during follow up.

Treatment with ribociclib in combination with standard endocrine therapy:

The investigational drug used in the course of this trial is ribociclib in combination with standard endocrine therapy as defined below (see TABLE 2)

On the first day of each cycle, patients will receive an adequate drug supply for self-administration at home. The investigator must emphasize compliance and will instruct the patient to take ribociclib and standard endocrine therapy exactly as prescribed.

All patients will receive ribociclib + standard endocrine therapy. All patients will take ribociclib capsules orally per day (3-weeks-on/1-week-off schedule) and will also take standard endocrine therapy once daily (dosage according to the label).

Endocrine treatment		**+Ribociclib**
Exemestane	25 mg/d	600 mg/d*
Letrozole (Hortobagyi et. al 2016 - MonaLEEsa-2 trial)	2,5mg/d	600mg/d*
Anastrozole	1 mg/d	600mg/d*
Fulvestrant	500 mg/q4w	600mg/d*

* 1-21 d q28d

Table 2: Recommended treatment regimen for combination with ribociclib

Ribociclib will be dosed for the first 21 days out of the 28 day cycle. Patients should be instructed to take the ribociclib capsules orally with a large glass of water at the same time; however dietary habits around the time of dosing should be as consistent as possible thoughout the study.
Exemestane / letrozole / anastrozole / fulvestrant will be dosed starting on treatment Day 1 according to the recommended dosage (see TABLE 2). Package insert instructions should be followed.
Ribociclib and endocrine therapy will be taken from treatment Day 1 up to 12 months or until disease progression, unacceptable toxicity, death, or withdrawal from the study for any other reason. If medically indicated ribociclib treatment may be continued during follow up.

Treatment with eribulin:
Eribulin as the ready to use solution will be dosed 1.23 mg/m^2 which should be administered intravenously over 2 to 5 minutes on Days 1 and 8 of every 21-day cycle. Eribulin should be given 12 months or until disease progression, unacceptable toxicity, death, or withdrawal from the study for any other reason. If medically indicated eribulin treatment may be continued during follow up.

End of treatment:
The end of treatment (EOT) is defined as the last date that the patient has taken the study drug, excluding interruption for less than 4 weeks. Maximum Treatment Period in both cohorts is 12 months. A Follow-up visit 4 weeks after the last date that the patient has taken the study drug will be conducted to report any adverse events during this period.

Treatment in Follow-Up Period:
Therapy after treatment period (i.e. treatment in the follow-up period) is at the discretion of the investigator.

Clinical Trial Visits

Screening Visit

- Informed consent in blood sampling for CTC count and assessment of HER2 status on CTC is obtained (patient information and consent form - part 1)*
- Allocation of a patient identification number via eCRF *

Data being obtained:

- Year of birth*
- General condition (ECOG, menopause status)**
- Information on primary tumor: date of primary tumor diagnosis*, stage of primary breast cancer*, localization of primary breast cancer**, surgical therapy**
- Information on metastases: date of metastases diagnosis*, localization*, bone/visceral/other*, multiple/single*, surgical therapy**
- HER2 status on primary tumor tissue and/or biopsies from metastatic sites or locoregional recurrences*
- Hormone receptor status found in solid tumor tissue (estrogen and progesterone status each graded positive or negative)*
- Information on adjuvant/neoadjuvant therapy**
- Information on palliative therapy: number of prior chemotherapy lines for metastatic disease*, type(s) of palliative therapy lines**, therapy with bisphosphonates**
- Blood sampling for CTC count and assessment of HER2 status on CTC with ***Screening Kit****
  - If CTC count is negative or HER2 status on CTC is positive study participation is terminated
  - If HER2 status on CTC is negative the patient is invited to the Inclusion / Recruitment Visit.
- If applicable: additional blood sampling in patients who consent to participate in translational research (part of patient information and consent form – part 1). If a patient objects to blood sampling for this purpose, she may nevertheless participate in the study.

*Data must be obtained within the Screening Visit

**Patients may be screened without obtaining this additional information. Documentation of these data will be reimbursed additionally.

Inclusion Visit

- If HER2 status on CTC is negative, informed consent in study participation is obtained (patient information and consent form – part 2). Before this no study procedure must be carried out except procedures planned on the Screening Visit.
- In addition, informed consent in blood sampling for translational investigations is obtained (patient information and consent form – part 3). If a patient objects to blood sampling for this purpose, she may nevertheless participate in the study.
- Medical/surgical history, concomitant diseases and ongoing toxicities attributed to prior anticancer therapies (denomination, date of onset, end date / specification if ongoing; in case of toxicities additionally: severity according to NCI CTCAE 4.03, treatment the toxicity is attributed to).
- Prior anti-cancer medication, other relevant prior medication (e.g. treatment of toxicities), concomitant medication (denomination, start date, dosage, route, end date/specification if ongoing, indication).
- Physical examination.
- Vital signs (heart rate, blood pressure, body temperature), height and weight.
- Standard 12-lead ECG
- Blood sampling for hematology and biochemistry (see also TABLE 12 below for parameters and allowed time intervals).
- Blood sampling for translational medical investigations with ***Analysis Kit*** only in patients who have given informed consent – part 3.
- Tumor evaluation (see section 10.2.1 for examinations to be done and TABLE 12 below for allowed time intervals).
- Cardiac investigations (see TABLE 12 below examinations to be done and allowed time intervals)**.**
- Quality of life assessment (see TABLE 12 below questionnaires and allowed time intervals)**.**
- Pregnancy test (eribulin cohort only; see below for allowed time intervals)**.**
- Review of the inclusion or exclusion criteria.
  - If during pre-treatment evaluation period a patient is found not to be eligible due to one of the inclusion or exclusion criteria, she is excluded from the study. Beyond the respective finding and the respective inclusion or exclusion criterion no further data need to be documented in the eCRF.
    Patients who are excluded from the study and who consent in additional assessment will be monitored during their course of disease as part of their routine treatment in order to gain further information about the impact of CTC

detection on disease progression.
    - If the patient meets all inclusion criteria and none of the exclusion criteria, she is eligible according to section 7.0 of the protocol and for treatment according to section 8 of the protocol.
- Start of treatment according to the protocol / Dispense everolimus/ribociclib to patients according section 8.2.

Control Visits

Every 2 to 4 weeks (Everolimus/Ribociclib cohort) and every 6 weeks (eribulin cohort):

- Vital signs
- Physical examination
- Standard 12-lead ECG (ribociclib cohort)
- Adverse events
- Documentation of protocol treatment
- Concomitant medication
- Blood sampling for hematology and biochemistry (APPENDIX II)
- Blood sampling with Analysis Kit in week 3-4 and 9-12 only in patients who have given informed consent - part 3 for translational medical investigations (TraFo-Project)
- Blood sampling with Analysis Kit afterwards every 3 months in everolimus/ribociclib cohort only nse
- Tumor evaluation every 12 weeks (in the everolimus/ribociclib cohort together with blood sampling for CTC count)
- Quality of life assessment: EORTC QLQ-C30 and EORTC QLQ-BR23
- Tablet count, additional dispense of everolimus/ribociclib if necessary
- Pregnancy test (eribulin cohort only)
- Survival

Conclusion Visit of the Treatment Period

As soon as possible after disease progression, completion or premature discontinuation of protocol treatment or respectively 12 months after inclusion:

- Vital signs
- Physical examination
- Standard 12-lead ECG
- Adverse events
- Concomitant medication
- Documentation of end of protocol treatment and planned therapy after end of protocol treatment
- Blood sampling for hematology and biochemistry
- Blood sampling with Analysis Kit
- Quality of life assessment: EORTC QLQ-C30 and EORTC QLQ-BR23
- Tablet count, collection of unused everolimus/ribociclib
- Pregnancy test (eribulin cohort only)
- Survival
- Reminding patient of the follow-up procedures planned

Follow up assessments

Between 2 and 4 weeks after Conclusion Visit of the Randomized Treatment Period and then every 3 months:

- Adverse events which are ≥ grade 3 NCI CTCAE and/or serious
- Concomitant medication
- Survival

Objectives and Endpoints of Clinical Trial

Primary objective:

Everolimus/Ribociclib cohort (DIVa)

The primary objective is to investigate the clinical efficacy of everolimus/ribociclib (as assessed by the CTC clearance rate) in combination with endocrine therapy in postmenopausal patients with hormone-receptor positive, HER2-negative metastatic breast cancer and persisting only HER2-negative circulating tumor cells (CTCs).

Eribulin cohort (DIVb)

The primary objective is to investigate the clinical efficacy of eribulin (as assessed by progression-free survival, PFS) both in patients with HER2-negative, hormone-receptor positive metastatic breast cancer and indication to chemotherapy and triple-negative metastatic breast cancer both with persisting only HER2-negative CTCs.

Secondary objectives:

In General

- Evaluation of efficacy of the study treatment as assessed by overall response rate (ORR), disease control rate (DCR), and overall survival (OS)
- Assessment of toxicity, safety and tolerability of the study treatments (everolimus/ribociclib or eribulin)
- Assessment of the dynamic of CTCs by longitudinal comparisons of CTC counts before during and after treatment and evaluation of the value of different measures of CTC dynamics for prognosis and assessing therapy efficacy
- Assessment of Quality of Life (QoL) as evaluated based on the EORTC QLQ-C30 and EORTC QLQ-BR23 questionnaires
- Assessment of quality-adjusted survival (as calculated by the Q-TWiST method)
- Evaluation of compliance to study procedures

Everolimus/Ribociclib cohort (DIVa)	**Eribulin cohort (DIVb)**
• *Evaluation of efficacy of the study treatment as assessed by progression-free survival (PFS)* • *Establishing of immune histochemistry and assessment of the response of phosphorylated ribosomal protein S6 (pS6) analysed in CTCs to treatment* • *Evaluation of the correlation of pS6 levels analysed in CTCs with clinical outcome (PFS)* • *Assessment of the activation of the PI3K/Akt/mTOR-pathway in CTCs (SNaPshot methodology for PI3KCA mutations)* • *Establishing and assessment of immune histochemistry for pAKT and PTEN in CTCs* • *Establishing the analysis of estrogen-receptor 1 (ESR-1) mutations via SNaPshot methodology in CTCs* • *Expression of Epithelial Mesenchymal Transition inducing transcription factors in CTCs* • *Expression of stem cell markers in CTCs* • *Resistance to anoikis in CTCs* • *Expression of LKB1 in CTC* • *Molecular profiling of CTCs in breast cancer* • *Quantification of circulating microRNAs miR-125a, miR-125b, miR-18a und miR18b in the serum of breast cancer patients*	• *Evaluation of efficacy of the study treatment as assessed by new metastasis-free survival (nMFS)* • *To determine the androgen receptor (AR) expression on CTCs using AR specific monoclonal antibody* • *To determine mutation status of AR by PCR amplification of AR exons followed by sequencing analysis* • *To determine PIK3CA mutations on CTC based on SNaPshot technology* • *To isolate Eribulin resistant CTCs from TNBC patients and to characterize them on the molecular level with a special focus on the correlation between EMT and their capacity to overcome anoikis. The cells will be also interrogated to assess the expression of stem cell markers.* • *To determine the predictive values of the detection of aberrant 53BP1 signals on CTCs and genetic alterations in BRCA1 in peripheral blood samples as potential biomarkers for responsiveness to therapy with Eribulin in patients with TNBC and persisting HER2-negative CTCs.* • *To assess the dynamic of 53BP1 signals on CTCs by longitudinal comparisons before, during, and after treatment, to evaluate the suitability of the different measures for assessing therapy efficacy*

Primary endpoint:

Everolimus/Ribociclib cohort (DIVa)

CTC clearance rate: Proportion of patients with at least one CTC detected in 7.5 ml of peripheral blood drawn before treatment that show no evidence of CTCs in the blood after treatment (CTC prevalence as assessed using the Cell-Search® System; Veridex LLC, Raritan, USA)

Eribulin cohort (DIVb)

The primary endpoint is progression-free survival (PFS), defined as time interval from date of recruitment until progressive disease (PD) or death from any cause, whichever comes first (as defined by RECIST guideline version 1.1). If a patient has not had an event, progression-free survival is censored at the date of last adequate tumor assessment.

Secondary endpoints:

In General

Overall response rate (ORR): rate of complete (CR) and partial responses (PR) in patients in whom target lesions were defined

Disease control rate (DCR): rate of patients who were assessed as having a PR or a CR or who had stable disease (SD) for at least 6 months

Overall survival (OS), defined as the time interval from start of treatment until death due to any cause. If a patient is not known to have died, survival is censored at the date of last contact

Dynamic of CTC: Descriptive statistics of regular CTC counts

Level of compliance to the study protocol

The assessment of safety will be based mainly on the frequency of adverse events. Other safety data (e.g. laboratory values, vital signs, and special tests) will be considered as appropriate.

Quality of life (QoL) as assessed by evaluation of the EORTC QLQ-C30 and EORTC QLQ-BR23 questionnaires

Quality-adjusted survival (as assessed by the Q-TWiST method), with the utility scores for the different health states being prospectively determined in the clinical trial subjects based on the EORTC QOL C30 questionnaire.

(CR, PR, and SD are defined according to the RECIST Version 1.1 criteria) (Eisenhauer 2009)

Everolimus/Ribociclib cohort (DIVa)	**Eribulin cohort (DIVb)**
• Progression-free survival (PFS), defined as time interval from date of recruitment until progressive disease (PD) or death from any cause, whichever comes first (as defined by RECIST guideline version 1.1). • Dynamic of CTCs: changes in CTC counts from baseline numbers (start of treatment) to the time of first radiological tumor evaluation after about 12 weeks, and every 12 weeks thereafter until progression • Levels of pS6 at baseline, at first radiological tumor assessment after about 12 weeks, and at the time of progression • Change in the activation of the PI3K/Akt/mTOR-pathway in CTCs as assessed by longitudinal comparisons (at baseline, after 12 weeks, at time of progression) • Estrogen-receptor 1 (ESR-1) mutations in CTCs at baseline, after 12 weeks and at time of progression.	• Dynamic of CTCs: changes in CTC counts from baseline numbers (start of treatment) to first (6 weeks) and second (12 weeks) control visit as well as the time of progression or end of treatment. • New metastasis-free survival (nMFS), defined as time from recruitment to death or progression due to appearance of a new metastasis, whichever comes first. If a patient has not had an event, nMFS is censored at the date of last adequate tumor assessment.

Sample size estimation and principles of analysis

Study Populations

The following study population sets will be examined:

Intention to Treat (ITT) Set: All recruited patients who received at least one dose of the study treatment (endocrine therapy plus everolimus/ribociclib or eribulin).

Safety Set: All recruited patients who received at least one dose of the study treatment and had at least one post-baseline safety assessment (the statement that a patient had no adverse events on the Adverse Event CRF is also regarded as a safety assessment). Patients who have received at least one dose of study treatment but who have no post-treatment safety data of any kind will be excluded from the safety set.

Per Protocol (PP) Set: All patients of the ITT set who did not violate any inclusion or exclusion criteria and who were treated according to protocol.

If necessary, further data sets required for additional analyses may be specified in separate analysis plans.

Statistical Methods

Statistical analysis of experimental data will be performed at the end of the studies, and there is no pre-planned interim

analysis. The primary objective of the DETECT IV trial is to estimate treatment efficacy in patients with HER2-negative metastatic breast cancer and persisting exclusively HER2-negative circulating tumor cells; treatment efficacy will be assessed by the CTC clearance rate in patients treated with everolimus/ribociclib combined with endocrine therapy (everolimus/ribociclib cohort) or by progression-free survival (PFS) in patients treated with eribulin (eribulin cohort). There is no statistical hypothesis underlying the primary analysis. CTC clearance rate will be evaluated using non-parametric statistical methods appropriate for the analysis of frequencies and rates. PFS will be estimated using the Kaplan-Meier method, and median, 95% confidence limits and additional descriptive statistics as well as the Kaplan-Meier survival function will be presented. The effect of covariates on PFS will be evaluated using Cox regression models.

All analyses regarding the secondary objectives will have exploratory character only. A safety analysis will be performed by the coordinating investigator and the DSMB after recruitment of 15 patients.

Unless otherwise noted, all statistical calculations will be implemented using the SPSS system (IBM Cooperation, New York, USA).

Sample Size Assumptions

The primary objective of the DETECT IV trial is to estimate treatment efficacy in patients with HER2-negative metastatic breast cancer and persisting exclusively HER2-negative circulating tumor cells treated either with everolimus/ribociclib combined with endocrine therapy (everolimus/ribociclib cohort) or with eribulin (eribulin cohort). The everolimus/ribociclib cohort comprises postmenopausal patients with HER2-negative, hormone-receptor positive metastatic breast cancer with no indication for chemotherapy, and the eribulin cohort comprises both patients with HER2-negative, hormone-receptor positive metastatic breast cancer with indication for chemotherapy and patients with triple-negative metastatic breast cancer. There is no statistical hypothesis underlying the primary analysis.

The following assumptions were made regarding the sample size of patients available for the DETECT IV trial:

- anticipated number of about 2000 patients with HER2-negative metastatic breast cancer that are to be screened for CTCs in DETECT IV (and the related DETECT III trial (which focuses on patients with HER2-negative primary tumor and HER2-positive circulating tumor cells)
- evidence of CTCs (≥ 1) in 65% of patients with metastatic breast cancer (conservative estimate based on Botteri et al. 2010, Fehm et al. 2010, Pierga et al. 2012)
- evidence of exclusively HER2-negative CTCs in 70% of CTC-positive patients (conservative estimate based on experience from the related DETECT III trial)

Based on these assumptions, the screening will result in about 910 patients with HER2-negative metastatic breast cancer and exclusively HER2-negative CTCs.

Everolimus/ribociclib cohort (DIVa)

Assuming that 70% of breast cancer patients have a hormone-receptor positive primary tumor and 75% of these patients have postmenopausal status, there will be about 480 postmenopausal patients with hormone-receptor positive, HER2-negative metastatic breast cancer and persisting exclusively HER2-negative CTCs. Based on experience from the related DETECT III trial, a considerable proportion of these patients is expected to fulfill not all of the inclusion criteria, to meet some of the exclusion criteria, or to have an indication for chemotherapy, thus prohibiting the inclusion in the everolimus/ribociclib cohort. We estimate that only about 180 of the 480 patients with hormone-receptor positive, HER2-negative metastatic breast cancer and persisting exclusively HER2-negative CTCs can indeed be included in the everolimus/ribociclib cohort of the DETECT IV trial.

It is assumed that the CTC clearance rate in the everolimus/ribociclib group of our study is in a range similar to that reported in the clinical trial NCT00898014 (“Blood Levels of Tumor Cells in Predicting Response in Patients Receiving First-Line Chemotherapy for Stage IV Breast Cancer”), which showed a CTC clearance rate of 54% after treatment with standard chemo- or endocrine therapy. The anticipated sample size of 160 patients for this study (total of 180 patients enrolled, 90 patients treated with everolimus, 90 patients treated with ribociclib, about 10% loss to follow-up assumed), will then produce a 95% confidence interval for CTC clearance rate with a width of about 23% both for the 80 patients treated with everolimus and the 80 patients treated with ribociclib. Thus, the expected sample size of 160 patients in the everolimus/ribociclib cohort of this study will allow to estimate CTC clearance rate with a reasonable level of precision both for the first 90 patients recruited to the DIVa study that are treated with everolimus and the subsequent 90 patients recruited to the DIVa study that are treated with ribociclib.

Eribulin cohort (DIVb)

Assuming that 30% of breast cancer patients have a hormone-receptor negative primary tumor, there will be about 270 patients with triple-negative metastatic breast cancer and persisting exclusively HER2-negative CTCs. In addition, it is estimated that about 20% of all hormone-receptor positive patients have an indication for chemotherapy, resulting in a total of about 390 patients eligible for the eribulin cohort of the DETECT IV trial. Given that this patient cohort (triple-negative or hormone-receptor positive with indication for chemotherapy) is likely to have a worse general health status as compared to the everolimus/ribociclib cohort, a considerable proportion of these patients is expected to fulfill not all of the inclusion criteria, to meet some of the exclusion criteria, or to have other additional comorbidities that prevent the inclusion in the eribulin cohort. If we very conservatively estimate that only about a third of the patients can indeed be included, there will be about 120 patients available that can be recruited in the eribulin cohort of the DETECT IV trial.

It is assumed that the PFS obtained in the eribulin group of our study is in a range similar to that reported in the EMBRACE trial for the Eribulin group (n = 508, median PFS = 3.7 months, 95% confidence interval 3.3 – 3.9 months). With a sample size of 108 patients for this study (total of about 120 patients enrolled, 10% loss to follow-up assumed), a two-sided 95% confidence interval for median PFS with a width of about 1.3 – 1.5 months will be obtained, thus providing an estimate for median PFS with a reasonable precision.