Version 5.0
12.03.2018

DETECT IV

A multicenter phase II study in patients with HER2-negative metastatic breast cancer and persisting HER2-negative circulating tumor cells (CTCs).

In cooperation with

This study has been designed according to the 'International Conference on Harmonization Good Clinical Practice Guideline 1998'(1)

EudraCT Number 2013-001269-18

ClinicalTrials.gov Identifier: NCT02035813

Sponsor:	**Universitätsklinikum Ulm (AöR)** **Albert-Einstein-Allee 29** **D-89081 Ulm**
Sponsor's Responsible Person:	**Prof. Dr. Wolfgang Janni** **Department of Gynecology** **University Hospital Ulm** **Prittwitzstr. 43** **D-89075 Ulm**
Coordinating Investigator: („Leiterin der klinischen Prüfung" according to §4 (25) German Drug Law)	**Prof. Dr. Tanja Fehm** **Department of Gynecology** **University Hospital Düsseldorf** **Moorenstraße 5** **D-40225 Düsseldorf**
Biomaterial Coordinating Lead Labaratory	**Frauenklinik der LMU - Campus Innenstadt** **Tumorimmunologisches Labor, Zi. 123** **c/o PD Dr. B. Rack/ L. Majunke** **Maistraße 11** **D-80337 München**

RESPONSIBILITIES

Sponsor

The Universitätsklinikum Ulm (AöR) is sponsor in terms of §4 (24) AMG (German Drug Law).

Responsible person for the observance of the sponsor's trial-related duties and functions is:

Prof. Dr. Wolfgang Janni
Director of Dpt. Obstetrics and Gynecology
University Hospital Ulm
Prittwitzstr. 43
D-89075 Ulm
Tel.: +49 (0) 731 500 58501
Fax: +49 (0) 731 500 58502
Email: wolfgang.janni@uniklinik-ulm.de

Coordinating Investigator

(„Leiterin der klinischen Prüfung“ according to §4 (25) AMG (German Drug Law))

Prof. Dr. Tanja Fehm
Director of Dpt. of Gynecology and Obstetrics
University Hospital Düsseldorf
Moorenstraße 5
D-40225 Düsseldorf
Tel.: +49 (0) 211 81 17500
Fax: +49 (0) 211 81 18483
Email: tanja.fehm@med.uni-duesseldorf.de

Monitoring, Randomization, Data Management

Alcedis GmbH
Winchesterstraße 3
D-35394 Gießen
Tel.: +49 (0) 641 94436 0
Fax: +49 (0) 641 94436 70
Email: info@alcedis.de

Medical Monitor

Prof. Dr. Wolfgang Janni
Dpt. Obstetrics and Gynecology
University Hospital Ulm
Prittwitzstr. 43
D-89075 Ulm
Tel.: +49 (0) 731 500 58501
Fax: +49 (0) 731 500 58502
Email: wolfgang.janni@uniklinik-ulm.de

Pharmakovigilance
Dpt. Obstetrics and Gynecology
Study Office
University Hospital Ulm
Prittwitzstr. 43
D-89075 Ulm
Tel.: +49 (0) 731 500 58520
Fax: +49 (0) 731 500 58526
Email: studienzentrale.ufk@uniklinik-ulm.de

Statistics

PD Dr. rer. nat. Thomas Friedl
Dpt. Obstetrics and Gynecology
University Hospital Ulm
Prittwitzstr. 43
D-89075 Ulm
Tel.: +49 (0) 731 500 58501
Fax: +49 (0) 731 500 58526
Email: thomas.friedl@uniklinik-ulm.de

SAE-MANAGMENT

Dpt. Obstetrics and Gynecology
Study Office
University Hospital Ulm
Prittwitzstr. 43
D-89075 Ulm
Tel.: +49 (0) 731 500 58520
Fax: +49 (0) 731 500 58526
Email: studienzentrale.ufk@uniklinik-ulm.de

Central Laboratories for Circulating Tumor Cell Count and Assessment of their HER2 Status

Biomaterial Coordinating Lead Laboratory:

Frauenklinik der LMU - Campus Innenstadt
Tumorimmunologisches Labor, Zi. 123
z. Hd. PD Dr. B. Rack/
Dr. rer. nat. M. Alumni-Fabbroni/ L. Majunke
Maistraße 11
D-80337 München
Tel.: +49 (0) 89 4400 54239
Fax: +49 (0) 89 4400 54715
Email: leonie.majunke@med.uni-muenchen.de

Endokrinologisch-onkologisches Labor (EOL)
Frauensteige 14, Haus 19
z. Hd. J. Kaufmann
D-89075 Ulm
Tel.: +49 (0) 731 500 58599
Fax: +49 (0) 731 500 58804
Email: julia.kaufmann@uniklinik-ulm.de

Universitätsklinikum Hamburg-Eppendorf
Institut für Tumorbiologie
Campus Forschung N27
z. Hd. Dr. Riethdorf/ C. Coith
Martinistraße 52
D-20246 Hamburg
Tel.: +49 (0) 407410 57497
Fax: +49 (0) 407410 55379
Email: tumorbiologie@uke.de

Forschungslabore der Frauenklinik
Life Science Center Düsseldorf
Merowingerplatz 1A
z. Hd. Dr. rer. nat. Dieter Niederacher
D-40225 Düsseldorf
Tel.: +49 (0) 211 385428 120
Fax: +49 (0) 211 385428 160
Email: Niederac@med.uni-duesseldorf.de

The study is performed on behalf of the German Senology and the DETECT- Study Group

Protocol Board:
Prof. Dr. Tanja Fehm, Düsseldorf
Prof. Dr. Wolfgang Janni, Ulm
Prof. Dr. Andreas Schneeweiss, Heidelberg
Prof. Dr. Klaus Pantel, Hamburg
Prof. Dr. Erich-Franz Solomayer, Homburg
Prof. Dr. Volkmar Müller, Hamburg
Prof. Dr. Jens Huober, Ulm
PD Dr. Brigitte Rack, München
PD Dr. Thomas Friedl, Ulm
Dr. A. De Gregorio
Dr. B. Jäger, Düsseldorf
Dr. C. Melcher, Düsseldorf
Dr. C. Hagenbeck, Düsseldorf
A. Polasik, Ulm
T. Romashova, Ulm
Dr. F. Schochter, Ulm

Steering Committee:
Prof. Dr. Tanja Fehm, Düsseldorf
Prof. Dr. Wolfgang Janni, Ulm
Prof. Dr. Andreas Schneeweiss, Heidelberg
Prof. Dr. Klaus Pantel, Hamburg
Prof. Dr. Erich-Franz Solomayer, Homburg
Prof. Dr. Volkmar Müller, Hamburg
PD Dr. Brigitte Rack, München
PD Dr. Bahriye Aktas, Essen
Dr. Friedrich Overkamp, Essen
Prof. Dr. Hans Tesch, Frankfurt
Dr. Georg Heinrich, Fürstenwalde, BNGO
Dr. Hans-Joachim Hindenburg, Berlin, BNGO
Prof. Dr. Nadia Harbeck, München
Prof. Dr. Christian Jackisch, Offenbach
Prof. Dr. Michael Untch, Berlin
Prof. Dr. Gunter von Minckwitz, Neu-Isenburg, GBG
Prof. Dr. Christoph Thomssen, Halle
Prof. Dr. Volker Möbus, Frankfurt
Prof. Dr. Peter Fasching, Erlangen
Prof. Dr. Elmar Stickeler, Freiburg
Prof. Dr. Jens Huober, Ulm
Prof. Dr. Hans-Joachim Lück, Hannover
Prof. Dr. Diethelm Wallwiener, Tübingen
Prof. Dr. Jens-Uwe Blohmer, Berlin

Advisory Board:
Prof. Dr. Susanna Hegewisch-Becker, Hamburg
Dr. Norbert Marschner, Freiburg
Prof. Dr. Ute-Susann Albert, Marburg
Prof. Dr. Ulrich Kleeberg, Hamburg-Altona
PD Dr. Diana Lüftner, Berlin
PD Dr. Stephan Schmitz, Köln
Prof. Dr. Rainer Souchon, Tübingen
Prof. Dr. Dirk Elling, Berlin-Lichtenberg
Prof. Dr. Hans-Heinrich Kreipe, Hannover
PD Dr. Marcus Schmidt, Mainz
Prof. Dr. Achim Rody, Lübeck
Prof. Dr. Matthias W. Beckmann, Erlangen

Prof. Dr. Klaus Friese, München
Dr. Jana Barinoff, Essen
Prof. Dr. Olaf Ortmann, Regensburg
Prof. Dr. Anton Scharl, Amberg
Prof. Dr. Rolf Kreienberg, Landshut
Prof. Dr. Walter Jonat, Kiel
Prof. Dr. Bernd Gerber, Rostock
Prof. Dr. Jalid Sehouli, Berlin
Prof. Dr. Rüdiger Schulz-Wendtland, Erlangen
Prof. Dr. Katharina Pachmann, Jena
PD Dr. Sibylle Loibl, Neu-Isenburg, GBG

Self Help Support Groups:
Fr. Ursula Goldmann-Posch, Mammazone e.V
Fr. Renate Haidinger, Brustkrebs Detschland e.V.
Fr. Doris C. Schmitt, Brustkrebs Deutschland e.V.
Fr. Karin Meißler, Selbsthilfe nach Brustkrebs e.V.

Data Safety Monitoring Board (DSMB):

- Prof. Dr. Harald Sommer, Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Klinikum der Universität München, Maistr. 11, 80337 München
- Dr. Lothar Häberle Universitätsstraße 21-23, 91054 Erlangen
- Dr. Dan Hayes, Breast Care Center, University of Michigan, Michigan
- Prof. Dr. Matthias Schwab, Robert-Bosch-Krankenhaus, Institut für Klinische Pharmakologie, Stuttgart

SPONSOR'S STUDY OFFICE:

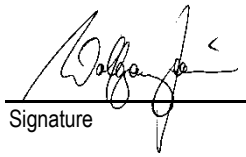
Universitätsfrauenklinik Ulm
Studienzentrale
Prittwitzstr. 43
D-89075 Ulm
Germany

Physician: Dr. F. Schochter; Dr. S. Albrecht, Dr. A. De Gregorio, A. Polasik, T. Romashova, Prof. J. Huober, Prof. W. Janni
Studycoordinators: Evelyn Ziel, Jessica D'Andrea, Heike Karl

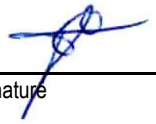
Tel.: +49 (0) 731 500 58520
Fax: +49 (0) 731 500 58526
Email: studienzentrale.ufk@uniklinik-ulm.de

SIGNATURES

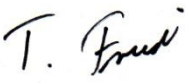
Sponsor:
The Universitätsklinikum Ulm (AöR) is sponsor in terms of §4 (24) AMG (German Drug Law). Responsible person for the observance of the sponsor's trial-related duties and functions is Prof. Dr. Wolfgang Janni, Director of Gynecological Clinic, University Hospital Ulm, Prittwitzstr. 43, D-89075 Ulm, Tel.: +49 (0) 731 500 58501, Fax +49 (0) 731 500 58502, Mobile: +49 (0) 170 3101034:

12.03.2018	Prof. Dr. Wolfgang Janni	
Date	Name	Signature

Coordinating investigator:
"Leiterin der klinischen Prüfung" according to §4 (25) AMG (German Drug Law).

12.03.2018	Prof. Dr. Tanja Fehm	
Date	Name	Signature

Statistician:

12.03.2018	PD Dr. Thomas Friedl	
Date	Name	Signature

Principal Investigator at the Study Site:

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein, and according to Good Clinical Practice and any applicable local regulations. I will make a reasonable effort to complete the study within the time designated. I confirm that I and study personnel participating under my supervision have adequate resources to fulfill their responsibilities as outlined in this protocol. I will maintain documentation of any investigator responsibilities assigned to participating study personnel. I confirm that all data will be submitted in a timely manner and will be accurate, complete and supported by source documents.

I will provide copies of the protocol and access to all further available information on the trial to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the investigational product and the study.

The contents may not be used in any other clinical trial and may not be disclosed to any person except study personnel who will be committed to secrecy. Any supplemental information that may be added to this document is also confidential and must be kept in confidence in the same manner as the contents of this protocol.

I am informed that I may terminate or suspend enrollment of the study at any time if it becomes necessary to protect the best interests of the study subjects. However, I will give prompt notice to the coordinating investigator. The sponsor and the coordinating investigator may terminate the study at any time with or without stating a cause.

I understand that the coordinating investigator cares for the request of study approval by the competent authority and of a favorable opinion from the competent ethics committee. Before these have been obtained no patient will be included in the trial. The CRO will notify the local authority as required by §67 AMG (German Drug Law) in conjunction with §12 GCP-V. I will inform the CRO about any change in study personal, so that the notifications can be done timely.

Principal Investigator	Representative

Site Address

Telephone No.	Fax No.	Email Address

Date	Principal Investigator's Signature
Date	Representative Signature

TABLE OF CONTENT

ABBREVIATIONS

ABC	Advanced Breast Cancer
AE	Adverse Event
AGO	“Arbeitsgruppe gynäkologischer Onkologen” (Working Group for Gynecological Oncology)
AI	Aromatase Inhibitor
ALT (SGPT)	Alaninaminotransferase
AMG	Arzneimittelgesetz (German Drug Law)
ANC	Absolute neutrophil count
AR	Androgen receptor
ASCO	American Society of Clinical Oncology
AST (SGOT)	Aspartataminotransferase, (Glutamic Oxalacetic Transaminase)
AUC	Area under the (Concentration - Time) – Curve
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German competent authority)
BP	Bisphosphonates
BUN	Blood Urea Nitrogen
CIN	Cervical Intraepithelial Neoplasia
CPK	Creatine Phosphokinase
CR	Complete Response
CRF	Case Report Form
CT	Computerized Tomograph
CTC	Circulating Tumor Cells
CTCAE	Common Toxicity Criteria for Adverse Events
CYP	Cytochrome P
DCR	Disease Control Rate
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EGFR	Epidermal Growth Factor Receptor
EORTC	European Organization for Research and Treatment of Cancer
ER	Estrogen Receptor
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration (USA)
FISH	Fluorescent In Situ Hybridization
GCP	Good Clinical Practice
GCP-V	Verordnung über die Anwendung der Guten Klinischen Praxis bei der Durchführung von klinischen Prüfungen mit Arzneimitteln zur Anwendung am Menschen (German regulation on GCP)
G-CSF	Granulocyte Colony-Stimulating Factor
GI	Gastrointestinal
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HER2	Human Epidermal Growth factor Receptor 2

HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IF	Immunofluorescence
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ITT	Intention to Treat
IU	International Units
IV	Intravenous
L	Liter
LEE011	Ribociclib
LD	Lesion Diameter
MBC	Metastatic Breast Cancer
mL	Milliliter
MRI	Magnetic Resonance Imaging
MRPs	multidrug resistant proteins
mTOR	Mammalian Target of Rapamycin
NCI	National Cancer Institute
NRS	Numeric Rating Scale
NSAI	Non-steroidal Aromatase Inhibitor
OATPs	organic anion-transporting proteins
OD	Once Daily
ORR	Overall Response Rate
OS	Overall Survival
pCR	Pathological Complete Response
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
Pgp	P-glycoprotein
PgR	Progesterone Receptor
PI3K	Phosphatidylinositol 3-Kinase (upstream effector of the mTOR signaling pathway)
PP	Per Protocol
PR	Partial Response
QOL	Quality of Life
RAD001	Everolimus
RANKL	Receptor activator of nuclear factor kappa B ligand
RECIST	Response Evaluation Criteria In Solid Tumors
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SD	Stable Disease
SGPT	Serum Glutamic-Pyruvic Transaminase

SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TK	Tyrosine Kinase
TNBC	Triple negative breast cancer
TTP	Time To Progression
YOB	Year of Birth

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.

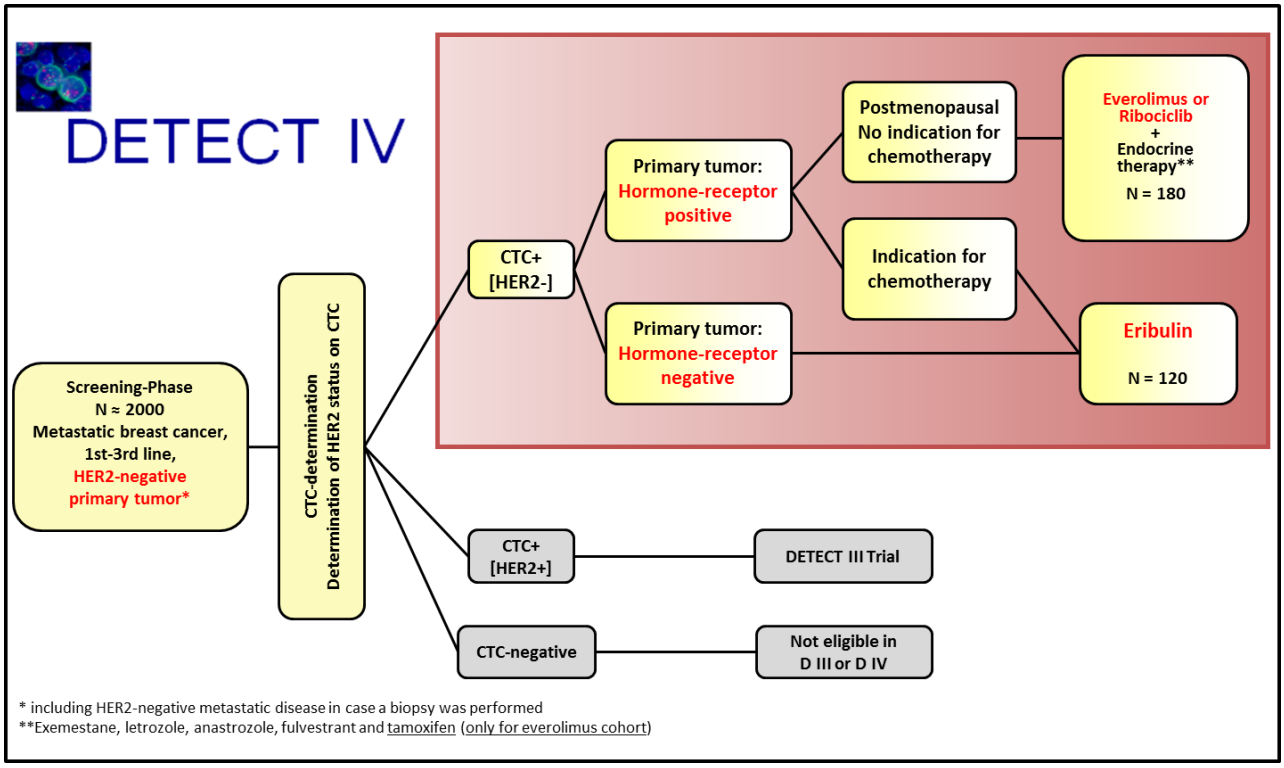


Figure 1: Screening and Design of DETECT IV

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:	• 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

• - QTcF interval at screening < 450 msec (using Fridericia's correction) • - Resting heart rate 50-90 bpm • INR ≤ 1,5 (ribocilclib cohort) • 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.

1 OBJECTIVES AND ENDPOINTS

1.1 Objectives

1.1.1 Primary Objective:

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

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

1.1.2 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 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)
- Assessment of toxicity, safety and tolerability of the study treatments (everolimus/ribociclib or eribulin)
- 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 • 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. • 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

1.2 Endpoints

1.2.1 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.

1.2.2 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.

2 BACKGROUND INFORMATION AND RATIONALE

2.1 Metastatic Breast Cancer

With an estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers), breast cancer is by far the most frequent cancer among women. Incidence rates vary from 19.3 per 100,000 women to 89.7 per 100,000 women, and are considerably higher (greater than 80 per 100,000) in developed regions of the world (except Japan) as compared to most of the developing regions (less than 40 per 100,000). Mortality rates range from approximately 6-19 per 100,000, and breast cancer ranks as the fifth cause of death from cancer overall (458 000 deaths), still being the most frequent cause of cancer death in women. Despite significant improvements in early diagnosis and treatment of breast cancer, about 40% of women with breast cancer will relapse and ultimately die of metastatic disease. Recurrent or metastatic breast cancer (MBC) is an incurable malignancy with a median survival of 24 – 30 months or less. Treatment for MBC is palliative, and the goals are to reduce tumor size, to slow down progression and metastasis, to reduce complications such as fatigue, bone fracture and hypercalcemia, and to increase quality of life. Although initial treatments did not achieve these goals, in the last decade a number of studies using new contemporary agents have shown promising results with increased response rates and benefits in terms of prolonged progression-free and overall survival.

2.2 Hormon-Receptor positive Breast Cancer

The presence of estrogen receptor (ER) and/or progesterone receptor (PgR) is one of the most important predictive and prognostic markers in human breast cancers. Approximately 70% of all invasive breast cancers are positive for ER and/or PgR expressions at the time of diagnosis. Consequently, anti-estrogen therapies that antagonize ER functions (such as tamoxifen) or inhibit estrogen production (e.g. aromatase inhibitors [AIs]) have been extensively developed in oncology (Jensen and Jordan 2003, Smith and Dowsett 2003). Deprivation of estrogenic signaling with the anti-estrogen tamoxifen has been the main form of hormonal treatment for over 30 years. Tamoxifen is indicated for the treatment across the whole continuum of breast cancer, ranging from risk reduction for women with increased risk of breast cancer, as an adjuvant treatment and also for metastatic disease. While therapies interfering with ER functions such as tamoxifen have significantly contributed to mortality reduction in advanced breast cancer (ABC) patients, at best 50-60% of ER positive patients respond to anti-estrogen therapy. Consequently, a number of aromatase inhibitors (AI) that reduce peripheral estrogen synthesis have been developed for the treatment of ABC. The AIs block the conversion of androgens to estrogens, which is the primary way estrogens are produced in post-menopausal women. At present, third generation aromatase inhibitors have been approved for use in postmenopausal women with hormone receptor positive BC after tamoxifen. The third generation AIs can be broadly classified into two groups: non-steroidal aromatase inhibitors (NSAI), mainly letrozole (Femara®) and anastrozole (Arimidex®) and steroidal aromatase inactivators, represented by exemestane (Aromasin®).

2.3 Circulating tumor cells

Hematogenous tumor cell dissemination is a crucial step in tumor progression, and blood-derived metastases account for the majority of breast cancer-related deaths. Cells that are able to disseminate into the circulation are of biologic relevance as potential founder cells for new metastases, and CTC detection and characterization have already improved our understanding of the complex process underlying tumor cell dissemination and metastatic progression in breast cancer.

Previous research has shown that CTCs (≥ 1 / 7.5 ml blood) are present in 65-85% of patients with MBC (Fehm et al. 2010, Botteri et al. 2010, Müller et al. 2012, Pierga et al. 2012), and five or more CTCs are detected in about 40-50% of MBC patients (Cristofanilli et al. 2004, Budd et al. 2006, Fehm et al. 2010, Müller et al. 2012, Pierga et al. 2012). There is good evidence that CTCs detected in the peripheral blood of patients with MBC can both provide prognostic information (Budd et al. 2006, Cristofanilli et al. 2005, Hayes et al. 2006, Giuliano et al. 2011, Giordano et al. 2012, Wallwiener et al. 2013) and indicate therapy success (Liu et al. 2009, Pierga et al. 2012).

The FDA approved standardized and semi-automatic CellSearch™ system (Veridex, LLC, USA) will be used for capture, isolation and enumeration of CTCs. The method has been described in detail in a validation study by Riethdorf et al. 2007, and is now routinely being used in the laboratories responsible for conducting the translational research program in the DETECT IV study. Briefly, blood samples are collected into special CellSave tubes, centrifuged to separate solid blood components from plasma, and then placed in the CellTracks® AutoPrep® System. Using ferrofluid nanoparticles with antibodies that target epithelial cell adhesion molecule (anti-EpCAM), CTCs are magnetically separated from the other cells in the blood. CTCs are then stained with the fluorescent nucleic acid dye 4',6-diamidino-2-phenylindole (DAPI), cytokeratin monoclonal antibodies (specific to epithelial cells) and a

monoclonal antibody to identify CD45 (a marker specific to leukocytes) to be able to distinguish epithelial cells from leukocytes. Cells are put in a magnet cartridge that applies a magnetic force pulling the cells to a single focal depth. The cartridge containing stained CTCs is placed into the CellTracks Analyzer II® for scanning, and the system presents tumor cell candidates (nucleated cells lacking CD45 and expressing cytokeratin) to an operator for final review. HER2 expression of CTCs is characterized within the CellSearch™ system by the addition of a fluorescein-labeled anti-HER2 antibody (CellSearch™ tumor phenotyping reagent HER2; Veridex, LLC), as described previously (Riethdorf et al. 2010).

2.4 Overview of Everolimus (RAD001)

Everolimus (RAD001) has been in clinical development as immunosuppressant in solid organ transplantation since 1996. Since 2003, everolimus is approved in Europe (trade name: Certican®) via the Mutual Recognition Procedure (MRP) for the prevention of organ rejection in patients with renal and cardiac transplantation. Certican® is also approved in other global markets. In 2010, everolimus was approved in the United States under the trade name Zortress® for the prevention of organ rejection in adults receiving kidney transplants who are at a low to moderate immunologic risk.

Everolimus was first approved under the trade name Afinitor® for patients with advanced renal cell carcinoma. It is now approved in the EU, US, and many other countries worldwide.

Afinitor® received accelerated approval in the US for the treatment of patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TS) who require therapeutic intervention but are not candidates for curative surgical resection. In the EU conditional approval was received under the trade name Votubia® for the treatment of patients aged 3 years and older with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC) who require therapeutic intervention but are not amenable to surgery. Afinitor® was also approved in advanced pancreatic neuroendocrine tumors in the EU, US, and several other countries.

Everolimus is a derivative of Rapamycin that acts as a signal transduction inhibitor. The target of this class of agents is mTOR, a multi-functional signal transduction protein, which obtains signals from many upstream inputs, and propagates the information via regulation of multiple downstream pathways. The serine-threonine kinase, mTOR acts as a nutrient sensor and monitor of the cellular metabolic state, regulating protein synthesis and ultimately cell growth and cell proliferation, angiogenesis and survival. mTOR serves a key role in normal mammalian cell physiology, and is centrally involved in tumor-cell physiology, (for example, facilitating cell-cycle progression from G1-S phase) and consequently inhibition of this target has received considerable attention as an anti-cancer approach, as reviewed by Bjonrnsti et al. 2004 and Abraham et al. 2007.

mTOR regulates global mRNA translation. (Beuvink et al. 2005) Indeed, downstream from mTOR is the serine / threonine kinase p70S6 kinase (S6K), of which there are two forms (S6K1 and S6K2). S6K phosphorylates key residues on the ribosomal protein S6, permitting its activation and full function as a protein involved in ribosomal biogenesis. The mTOR kinase also modulates phosphorylation of 4E-BP1, releasing its inhibition of eIF-4E and consequently permitting efficient cap-dependent translation. (Beuvink et al. 2005) Specifically, the Rapamycin-sensitive part of mTOR occurs through the mTOR-RAPTOR complex (mTORC1), while a Rapamycin-insensitive pathway occurs when mTOR is complexed with RICTOR (mTORC2). (Sarbassov et al. 2004). Rapamycins inhibit the activity of mTOR by directly adhering to FKBP-12 which binds to mTORC1 (i.e. the mTOR-raptor complex), and also indirectly inhibit the mTOR-RICTOR complex (mTORC2) by sequestering free mTOR and thus also preventing its assembly into mTORC2 complexes. (Sarbassov et al. 2004) Downstream of PI3/Akt, mTOR can be considered as a component in the PI3K/Akt/mTOR pathway, which is known to be dysregulated in numerous human cancers.

Molecular epidemiological studies show that in addition to a high frequency of activation in specific cancers, activation of the PI3K/Akt/mTOR pathway is frequently a characteristic of worsening prognosis through increased aggressiveness, resistance to treatment and progression. (Luo et al. 2003) A variety of preclinical studies have confirmed the role of this pathway in tumor development. Gain of function models have demonstrated that constitutive activation of kinases such as Akt can lead to the inexorable development of cancers resembling those in patients which are characterized by frequent activation of the same kinase. This is complemented by the demonstration of anti-tumor activity of kinase inhibitors acting in vitro and in vivo. Experiments carried out in Novartis laboratories, as well as elsewhere, show that everolimus is capable of inhibiting the proliferation and growth of a wide spectrum of tumor cell lines and tumors, respectively. The anti-proliferative effects of everolimus are achieved at nanomolar concentrations, which can be reached in patients at the doses used in clinical trials. (Everolimus

Investigator Brochure: Report RD-2000-02546; Report RD-2002-03223; Report RD-2006-02213).

An important aspect of the anti-tumor effect of everolimus is its potential to act both on tumor cells directly (to inhibit growth) and indirectly (by inhibiting angiogenesis and displaying anti-vascular properties). The observation of in vivo sensitivity of xenografts comprised of tumor cells showing insensitivity to everolimus in vitro is attributed to the drug's potential to act on the vascular component of the supporting peritumoral stroma. The anti-angiogenic property of everolimus has been confirmed through experiments demonstrating the effect of everolimus in countering vascular endothelial growth factor (VEGF) -induced proliferation of human umbilical endothelial cells (HUVECs) in vitro, VEGF-driven angiogenesis in a chamber implant murine model, and neovascularisation in murine orthotopic melanoma and xenograft models. (Shinohara et al. 2005, Mabuchi et al. 2007a & 2007b, Manegold et al. 2008).

2.5 Overview of Ribociclib

In the mammalian cell cycle, entry into S phase is achieved by cyclin-dependet kinases 4 and 6 (CDK4/6). Ribociclib (formely LEE011) ia an orally bioavailable, highly selective small molecule inhibitor of CDK4/6 that induces G1 arrest at sub-micromolar concentrations in a variety of retinoblastoma protein (pRB)-positive cancer cells *in vitro*. Ribociclib has proven efficacious when combined with other targeted therapies *in vitro* and *in vivo* in cancers driven by a variety of oncogenic signaling pathways. Ribociclib may therefore be an effective anti-cancer agent in a variety of pRb-positive human neoplasms, especially in those that contain an activated CDK4/6-pRb pathway.

MonaLEEsa-2 (CLEE011A2301) is controlled phase III study of ribociclib in combination with letrozole in postmenopausal woman with HR+ and HER2- advanced breast cancer who received no prior therapy for advanced disease. The combined treatment has shown that the duration of progression-free survival was significantly longer in the ribociclib group than in the placebo group (hazard ratio, 0,56; 95% CI, 0,43 to 0,72; P=3,29 x 10-6 for superiority). The median duration of follow-up was 15.3 months. After 18 months, the progression-free survival rate was 63% (95% confidence interval [CI], 54,6 to 70,3) in the ribociclib group and 42,2% (95% CI, 34,8 to 49,5) in the placebo group. In patients with measurable disease at baseline, the overall response rate was 52,7% and 37,1%, respectively (P<0,001).

2.6 Overview of Eribulin

Eribulin (E7389) is a structurally simplified synthetic analog of halichondrin B, a natural product isolated from the marine sponge Halichondria okadai (Towle et al. 2001), which shows potent anticancer effects in both cell-based and animal models of cancer (Bai et al. 1991, Towle et al. 2001. Eribulin encompasses the biologically active portion of the polyether macrolide halichondrin B, and shows similar or identical anticancer properties in preclinical models.
The antimitotic mechanism of eribulin is different from other microtubule-targeted agents such as taxanes. Eribulin exerts its antiproliferative action by inhibiting microtubule dynamics and tubulin polymerization into microtubules without having an effect on microtubule shortening. Inhibition is exerted by induction of complete mitotic block at G2/M (GAP 2/mitosis stages of cell cycle), disruption of mitotic spindles formation and initiation of apoptosis following prolonged mitotic blockage. The mitotic blocks induced by eribulin are sustained after drug washout and correlate with lack of cell viability in cultures five days later (Towle et al. 2001), suggesting that they are functionally irreversible.

In vitro studies showed that eribulin inhibits cell growth in several established human cancer lines including the breast cancer cell line MDA-MB-435 (mean IC_{50} 1.8 Nm; range 0.09-9.5 nM) and in vivo anticancer efficacy of eribulin was demonstrated in several human tumor xenograft studies in mice (Rusnak et al. 2001). Interestingly, eribulin shows full in vitro activity in taxane-resistant human ovarian cancer cells with mutations in the beta-tubulin I isotype gene (Giannakakou et al. 1997, Kuznetsov et al. 2004) and also in taxane-resistant tumor cells over-expressing beta-tubulin 3 isotype (Mozzetti et al. 2005), indicating that eribulin might be an effective anticancer agent for taxane-resistant patients.

2.7 Rationale for Current Study

2.7.1 Everolimus cohort

The multi-functional signal transduction protein mTOR plays a key role in normal tumor cell physiology, i.e. progression from G1 to S-Phase (reviewed by Bjornsti et al. 2004 and Abraham et al. 2007). During oncogenesis mTOR is deregulated and thereby disrupts a cascade that regulates basic cellular functions, such as proliferation, apoptosis and angiogenesis (Chan et al. 2004). Thus the inhibition of this target is a promising approach in anti-cancer treatment.

RAD001 (everolimus) is a novel macrolide which interacts with and inhibits mTOR. The anti-neoplastic effect of everolimus is mainly based on two mechanisms: first everolimus can act directly on tumor cells (by growth inhibition) and second it can act indirectly by countering VEGF-induced proliferation of cells (Shinohara et al. 2005).

Activation of the mTOR pathway is a key adaptive change driving endocrine resistance. Research into the mechanisms of resistance has shown that various signal transduction pathways are activated to escape the effect of endocrine therapy. For example, the PI3 kinase/Akt/mTOR pathway is constitutively activated in aromatase inhibitor resistant and long-term estrogen deprivation BC cells (Campbell et al. 2001, Santen et al. 2005, Tokunaga et al. 2006). Selective inhibitors of mTOR, e.g. Rapamycin, demonstrated a significant growth inhibition particularly in long-term estrogen deprivation BC cells (Yue et al. 2007).

Currently RAD001 is being evaluated in different types of tumors. The BOLERO-2 study was conducted on postmenopausal patients with hormone-receptor positive MBC refractory to non-steroidal aromatase inhibitors (letrozole and anastrozole). 724 patients were randomized in a 2:1 ratio to receive either 10 mg daily everolimus (N=485) or matching placebo (N=239) in addition to open-label 25 mg daily exemestane. The primary endpoint for the trial was PFS evaluated by RECIST, based on the investigators (local radiology) assessment. Supportive PFS analyses were based on an independent central radiology review. The study demonstrated a statistically significant clinical benefit of the everolimus + exemestane treatment as compared to the placebo + exemestane treatment. Adding everolimus improved median progression-free survival from 3.2 up to 7.8 months and so lowered the risk of cancer progression by 55% for these women. These findings were confirmed by an independent assessment (4.1 vs. 11.0 months, risk reduction: 62%).

The main purpose of this multicenter, open-label, single-arm study is to collect efficacy and safety data for the combination of everolimus with various endocrine therapy regimens (exemestane, tamoxifen, letrozole, anastrozole) in a broader patient population as compared to the BOLERO-2 trial, and to provide access to everolimus to patients who are without satisfactory treatment alternatives until everolimus is approved for this indication. Since the addition of everolimus was shown to significantly improve PFS in the previous BOLERO-2 trial, for ethical reasons no treatment with endocrine therapy only will be investigated in the present study, due to the low efficacy of exemestane monotherapy (PFS 3.2 months). If the study succeeds in proving efficacy of the combined treatment with everolimus and endocrine therapies, new options for the treatment of hormone-receptor positive MBC can be established.

2.7.2 Ribociclib cohort

In international guidelines for advanced breast cancer, endocrine treatment is the preferred treatment option for premenopausal and postmenopausal women and men with HR-positive, HER2-negative advanced breast cancer, unless there is concern or proof of endocrine resistance or rapidly progressive disease requiring a fast response (Cardoso 2012).

Multiple pathways are known in which alterations can facilitate endocrine resistance. Two related emerging mechanisms of endocrine resistance include the decoupling of cell cycle control from ER-signaling and the utilization of alternate growth signaling pathways such as the PI3K pathway (Miller et al 2011; Lange and Yee 2011). The currently available standard option for overcoming endocrine resistance targets the PI3K/AKT/mTOR pathway as well as the estrogen receptor pathway by combining the mTOR inhibitor Afinitor (everolimus) with the steroidal aromatase inhibitor exemestane. This combination demonstrated superior efficacy with a manageable safety profile compared to exemestane monotherapy. The median progression free survival in the pivotal phase III trial BOLERO-2 was 7,8 months for the combination of everolimus plus exemestane versus 3.2 months for exemestane and placebo (hazard ratio = 0.45; 95% confidence interval 0.38–0.54); log-rank P<0.0001) (Yardley 2013).

Besides the PI3K/AKT/mTOR pathway, one of the most frequently altered pathways in breast cancer is the cell cycle regulation by cyclin D1 and cyclin dependent kinases 4 and 6. By targeting two distinct pathways (aromatase inhibition by letrozole, CDK4/6 pathway inhibition by ribociclib), it is hypothesized that this combination will result in prolonged progression free survival. First clinical results demonstrated promising efficacy and safety data in a Phase Ib trial (CLEE011X2107). In this study, ribociclib in combination with letrozole demonstrated encouraging clinical activity, in both the treatment-naïve group (46% objective response rate, 88% disease control rate, 79% clinical benefit rate in pts with measurable disease) as well as pretreated patients (1 pt (5%) had a confirmed PR, 7 pts (37%) had NCRNPD (non-measurable disease), 7 pts (37%) had SD (measurable disease), and 4 pts (21%) had PD).The most common adverse events suspected to be study drug related were Neutropenia (70% all grades, 43% grade 3/4), Nausea (43% all grades, no grade 3/4), Fatigue (26% all grades, no grade 3/4), Alopecia, Anemia

and Diarrhea (23% all grades, no grade 3/4, respectively).

MonaLEEsa-2 (CLEE011A2301) is placebo controlled phase III study of ribociclib in combination with letrozole in postmenopausal women which has shown to prolong PFS in patients with HR+ and HER2- advanced breast cancer who received no prior therapy for advanced disease. Updated PFS analyses continued treatment benefit for patients receiving ribociclib + letrozole vs. placebo + letrozole. Median PFS was prolonged by 9.3 months, from 16.0 months, 24-months PFS rates were 54,7 % vs 35,9%. In US patients (n=213), meidan PFS was 27,6 months in the ribociclib + letrozole arm (n=100) vs 15.0 months in the placebo + letrozole arm (n=113), with a hazard ratio = 0,527 (95 % CI: 0,351 – 0,793). A numerical benefit in OS was observed in thr ribociclib + letrozole arm vs the placebo + letrozole arm, the study remains immature for OS analysis. The safety profile of ribociclib + letrozole was comparable to that senn at the first interim analysis and remains manageable, with minimal impact on duration of combination therapy and no evidence of cumulative toxicity.

The combined treatment has shown that the duration of progression-free survival was significantly longer in the ribociclib group than in the placebo group (hazard ratio, 0,56; 95% CI, 0,43 to 0,72; P=3,29 x 10^{-6} for superiority). The median duration of follow-up was 15.3 months. After 18 months, the progression-free survival rate was 63% (95% confidence interval [CI], 54,6 to 70,3) in the ribociclib group and 42,2% (95% CI, 34,8 to 49,5) in the placebo group. In patients with measurable disease at baseline, the overall response rate was 52,7% and 37,1%, respectively (P<0,001).

Ribociclib in combination with aromatase inhibitor therapy and goserelin is currently investigated in premenopausal patients in the phase III trial MonaLEEsa-7 (CLEE011F2301). Paloma-3, a phase III trial with a different CDK4/6 inhibitor in combination with fulvestrant, demonstrated equal efficacy in premenopausal patients and postmenopausal patients.

Preclinical and early clinical data suggests that CDK4/6 inhibition may play a key role in the treatment of subsets of breast cancer by abrogating endocrine-resistant cell proliferation. Therefore, the addition of a CDK4/6 inhibitor such as ribociclib to standard endocrine therapy is a promising therapeutic approach that will be explored in this study.

The main purpose of this multicenter, open-label, single-arm study is to collect efficacy and safety data for the combination of ribociclib with various endocrine therapy regimens (exemestane, letrozole, anastrozole, fulvestrant) in a broader patient population as compared to the MonaLEEsa-2 trial, and to provide access to ribociclib to patients who are without satisfactory treatment alternatives until ribociclib is approved for this indication. If the study succeeds in proving efficacy of the combined treatment with ribociclib and endocrine therapies, new options for the treatment of hormone-receptor positive MBC can be established.

These data will complement the data of using everolimus in combination with endocrine treatment in the same indication, which were acquired in a first cohort within the Detect IV study. Both primary, as well as secondary endpoints will be compared in terms of a cohort analysis between the two cohorts.

2.7.3 Eribulin cohort

In this treatment cohort both patients with triple-negative metastatic breast cancer and patients with hormone-receptor positive metastatic breast cancer and indication for chemotherapy will be included.

The clinical efficacy of eribulin in the treatment of metastatic breast cancer was established in the pivotal phase III open-label randomized trial EMBRACE (NCT00388726). In this study, 762 patients with locally advanced or metastatic breast cancer who received between two and five prior chemotherapies (at least two in the advanced disease) including anthracyclines and taxanes were enrolled. The patients were randomly allocated in a 2:1 ratio to receive either eribulin monotherapy or treatment of physician's choice, and the primary endpoint was overall survival in the intention-to-treat population. Patients in the eribulin group had a significantly improved overall survival (median 13.1 months) as compared to the group receiving treatment of physician's choice (median 10.6 months; hazard ratio 0.81, 95% confidence interval 0.66 – 0.99, p = 0.041). Based on these results, eribulin was approved by the FDA and the European Medicines Agency for the treatment of patients with metastatic breast cancer that have progressed after at least two chemotherapeutic regimes for advanced disease.

In another recent phase III open-label randomized clinical trial, eribulin was compared to capecitabine in patients with locally advanced or metastatic breast cancer that had received prior anthracycline- and taxane-based therapy, but were on first, second or third line of therapy for advanced disease (E7389-G000-301). The study did not meet the primary objective, as overall survival was not statistically improved for patients receiving eribulin (median 15.9

months) as compared to patients receiving capecitabine (median 14.5 months; hazard ratio 0.879, 95% confidence interval 0.770-1.003, p = 0.056). However, the study demonstrated that eribulin is active in earlier lines of treatment of metastatic breast cancer, with numerically better overall survival as compared to capecitabine.

Explorative subgroup analyses of the data gained in the 301-study showed a numerically better overall survival for hormone-receptor positive patients in the eribulin group (median 18.3 months) as compared to patients receiving capecitabine (median 16.4 months; hazard ratio 0.869, 95% confidence interval 0.720-1.049).

Explorative data analyses of the 301-study also indicated that eribulin might be particularly effective in triple-negative patients. Specifically, median overall survival in triple negative subjects was 14.4 months and 9.4 months for eribulin and capecitabine, respectively, corresponding to an improvement of 5.1 months (hazard ratio 0.702, 95% confidence interval 0.545 – 0.906, nominal p of log rank test = 0.0062; data provided by Eisai). The difference in progression-free survival between eribulin-treated and capecitabine-treated triple-negative patients was considerably smaller and not significant, but PFS was still numerically higher for the eribulin group (median PFS eribulin group 2.9 months, median PFS capecitabine group 2.3 months, hazard ratio 0.802, 95% confidence interval 0.613 – 1.049; data provided by Eisai). Interestingly, new metastasis-free survival (nMFS; defined as time from recruitment to death or progression due to appearance of a new metastasis, whichever comes first) was significantly higher in the eribulin group (median nMFS 4.2 months) as compared to the capecitabine group (median nMFS 3.1 months; hazard ratio 0.749, 95% confidence interval 0.564 – 0.995, nominal p of log rank test = 0.0466; data provided by Eisai).
There is compelling evidence that CTCs detected in the peripheral blood of patients with MBC can be used as surrogate marker for overall survival (Budd et al. 2006, Cristofanilli et al. 2005, Hayes et al. 2006, Giuliano et al. 2011, Giordano et al. 2012, Wallwiener et al. 2013) and indicate therapy success (Liu et al. 2009, Pierga et al. 2012). A detailed assessment of the dynamic of CTCs by longitudinal comparisons of CTC counts before treatment, during treatment, and at the time of progression could be especially useful to elucidate the factors responsible for the observed discordance between PFS benefit and OS benefit of eribulin observed in triple-negative patients.

2.8 Risk/Benefit Analysis

Risks patients may be exposed due to study participation
If a patient is treated with everolimus/ribociclib (in DIVa) or eribulin (in DIVb), the side effects may cause risk according to the well-known spectrum of adverse effects of these investigational drugs. The frequency of adverse effects is well published. There is a very small risk of life threatening side effects.
Except for blood sampling for CTC assessments and quality of life evaluations there are no additional impairments due to study participation.

Benefits accruing to patients by study participation
If patients are treated with eribulin, there is a considerable chance that they are treated more effectively and profit from prolonged progression free and/or overall survival compared to other chemotherapy.

Scientific benefit
If the application of eribulin is shown to be efficacious, this study will lead to improved treatment of triple-negative or hormone-receptor positive metastasizing breast cancer with indication to chemotherapy. In addition, the study will increase the knowledge on the role of the dynamic of CTCs for prognosis and assessing therapy efficacy in metastasizing breast cancer.

Risk/benefit assessment
Anticancer therapy generally is associated with considerable side effects. The potential benefit of increased efficacy of treatment with eribulin would be of high relevance given the unfavorable prognosis of MBC. Taking into account possible benefits resulting from the study, the risks of the study appear acceptable.

2.9 Quality of Life

Quality of life (QoL) is relevant to metastatic cancer patients. From the patient perspective it measures possible drawbacks and – beyond prolongation of survival – possible benefits from an intervention. It provides additional information compared to measuring adverse events alone. (Huschka et al. 2007, Paul et al. 1991)

As measuring tool the EORTC QLQ-C30 core questionnaire and the supplementary breast cancer module QLQ-BR23 are used. They are designed to capture the multidimensionality of QoL in metastatic breast cancer. The

EORTC QLQ-C30 and QLQ-BR23 are widely used, cancer specific health-related QoL questionnaires which are well accepted by patients (Aaronson et al. 1993, Conroy et al. 2004). The EORTC QLQ-C30 core questionaire comprises 30 questions assessing five functional subscales (physical, role, cognitive, emotional, social), three multi-item symptom subscales (fatigue, nausea and vomiting, and pain), six single items (dyspnoea, insomnia, appetite loss, constipation, diarrhoea, financial impact) and a global health measure (physical condition and global QoL). The QLQ-BR23 breast-cancer module comprises 23 questions and incorporates five multi-item scales (systemic therapy side effects, arm symptoms, breast symptoms, body image, sexual functioning) and three single-item questions (sexual enjoyment, hair loss, future perspective). The questionnaires use 4 and 7-point scales. For evaluation each scale is linearly transformed into a scale ranging from 0 to 100. Convergent and criterion validity has been demonstrated for this questionnaire in metastatic breast cancer (Bottomlay et al. 2004, McLachlan et al. 1998) and reliability is adequate (Aaronsonet al. 1993, Hjermstad et al. 1995). The EORTC QLQ-C30 + BR23 has been shown to be responsive to change associated with chemotherapy and with disease progression (Osoba et al. 1998, Lemieux et al. 2007). The questionnaire is available in German (see APPENDIX V – QUALITY OF LIFE ASSESSMENTS).

2.10 Translational Medical Investigations

2.10.1 In General

Translational medical investigations will be performed in patients who consent in blood sampling for this purpose (patient information and consent form - part 3). At predefined time points during study participation, peripheral blood will be drawn from the patients and collected in tubes provided by the sponsor:

Provided Kit	Blood volume
Screening Kit	Approx. 40 ml (10 ml for CTC detection, 30 ml for additional research)
Analysis Kit	Approx. 50 ml (10 ml for CTC detection, 40 ml for additional research)

Table 3: Bloodkits for translational research

2.10.2 Everolimus/Ribociclib cohort

The translational research program in the DETECT IVa study mainly investigates the association of CTC-presence and their molecular characteristics with treatment response and prognosis.

Within the DETECT IV trial, prevalence and number of CTCs in the peripheral blood will be determined parallel to tumor evaluation at baseline (i.e. before the start of treatment; only in patients with informed consent for translational research projects), at week 4 and week 12 (only in patients with informed consent for translational research projects), and every 12 weeks thereafter until progression or the regular end of treatment after 12 months (always in correlation with Re-Staging, e.g. CT-Scan).

The objectives of the DETECT IV trial in the everolimus/ribociclib cohort regarding the translational research program are as follows:

- 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
- Establishing of immune histochemistry and assessment of the response of phosphorylated ribosomal protein S6 (pS6) to treatment
- Evaluation of the correlation of pS6 levels 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 CTCs
- Quantification of circulating microRNAs miR-125a, miR-125b, miR-18a und miR18b in the serum of breast cancerpatient

	Baseline	Week 12*	Progression
CTC count (within study)	+	+*	Every 3 months and at progression
pS6 (IHC)	+	+*	+
pAKT (IHC)	+	+*	+
PTEN (IHC)	+	+*	+
PIK3CA mut (Snapshot)	+	+*	+ (only at progression)
ESR-1 mut (SNaPshot)	+	+*	+ (only at progression)

* biomarkers will only be assessed in selected subgroups

Table 4: Overview biomarker program DETECT IV (Everolimus/ribociclib cohort)

The molecular characteristics of CTCs will be analyzed using SNaPshot technology or immunohistochemistry.

2.10.2.1 Analyses based on SNaPshot technology

The Department of Obstetrics and Gynecology of the University Hospital Tübingen (Prof. Tanja Fehm) established an approach based on the SNaPshot technology published by Hurst et al. 2009 to analyze single nucleotide polymorphisms (SNPs) within the exon 9 and 20 of PIK3CA. The protocol was modified and adapted for single cell analysis.
This assay combines multiplex PCR amplification with a multiplex primer extension approach to allow targeted detection of several mutations in one reaction. The method was conducted by Hurst et al. 2009 using samples that had previously been analyzed for mutations by high-resolution melting analysis and sequencing. All mutations detected for PIK3CA were concordant and no false-positive results were obtained. Sensitivity tests showed that the SNaPshot assay could detect mutant DNA when it represents 5–10% of the total DNA present. The application of the method to the analysis of DNA extracted from formalin-fixed paraffin-embedded samples was also demonstrated.

The following figure indicates the single steps:

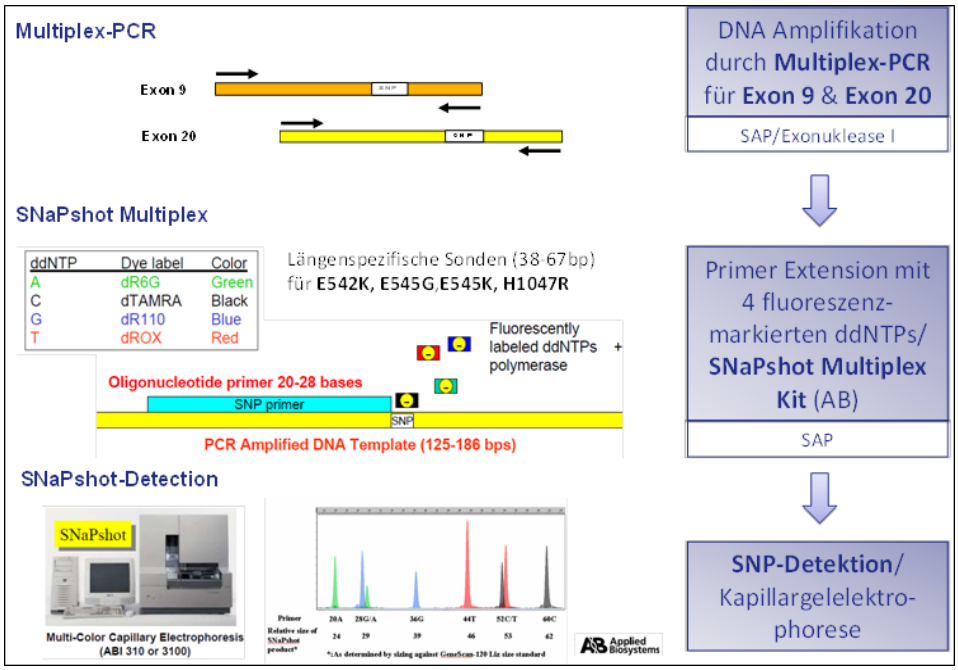


Figure 1: SNaPshot-Technology

Analogous to the SNP analysis for PI3K, the SNaPshot assay will be established for analysing SNPs in estrogen receptor (ESR-1).
To date, the number of naturally occurring mutations within the ESR-1 identified in breast cancers is relatively low: the Y537N (Tyr537Asn) mutation was discovered in a metastatic breast tumor. This mutation eliminates a carboxy-terminal tyrosine residue that is considered to be an important c-Src phosphorylation site with potential roles in regulating ligand binding, homo-dimerization, and transactivation of ERα. Y537N may allow ERα to escape phosphorylation-mediated controls, providing cells with a potential selective advantage (Zhang et al. 1997, Fuqua et al. 2000).
Mutation A908G has been identified in about a third of premalignant breast hyperplasias and one half of invasive breast tumors from untreated patients. The A908G mutation introduces a lysine-to-arginine transition at residue 303 (termed K303R) within the hinge domain. K303R mutation allows ERα to be more highly phosphorylated by PKA and Akt kinase signaling, and alters the dynamic recruitment of co-activators and co-repressors, such as BRCA-1 or calmodulin (Herynk et al. 2007, Barone et al. 2010, Ma et al. 2010).
Overexpression of the K303R ERα mutation in ERα-positive MCF-7 breast cancer cells confers estrogen hypersensitivity, and decreased sensitivity to tamoxifen treatment when engaged in cross-talk with growth factor receptor signaling pathway (Giordano et al. 2010). Enhanced growth factor receptor cross-talk with ERα is a known mechanism of hormone resistance in breast cancer. Expression of the K303R ERα mutation also conferred resistance to the non-steroidal aromatase inhibitor anastrozole in ERα-positive cells, via a dynamic interaction between the K303R ERα mutation, S305 phosphorylation, and the IGF-1R signaling pathway (Barone et al. 2009). K303R ERα mutation was associated with poor outcomes in univariate analyses of tumors from untreated breast cancer patients, and its presence was correlated with older age, larger tumor size, and lymph node-positive disease, all clinical factors associated with worse outcomes (Conway et al. 2005, Herynk et al. 2007).

2.10.2.2 Analyses based on immunohistochemistry

The ribosomal protein pS6 is a downstream target of the PI3K/Akt/mTOR pathway. Protein expression status of pS6 as well as for activated AKT (pAKT) and PTEN will be analysed in CTCs. In our laboratories several protocols in order to establish immunohistochemical detection of proteins (e.g. for antigen retrieval etc.) are available.
Normally, several antibodies are tested on cell lines which have been immobilised on glass slides. Antibodies with corresponding blocking peptides are preferred to verify specificity and will be evaluated in breast cancer cell lines by Western blots.

Current Status:
SNaPshot analysis for hot spot mutations of PI3K3CA from Veridex cartridge is established and functional. SNaPshot analysis for ESR1 has to be established and will take approx. 2-3 months. Immunohistochemistry has to be established and will take approx. 2 months.

2.10.2.3 Expression of Epithelial Mesenchymal Transition inducing transcription factors in CTCs

In order to undergo metastasis, epithelial tumour cells need to start a number of processes such as loss of cell-cell contact and cell polarity, down regulation of epithelial associated genes, up regulation of mesenchymal gene expression and cytoskeleton modification. These processes are as a whole known as Epithelial-Mesenchymal Transition (EMT). EMT plays a key role in tumour progression and potentially represents an important limitation to CTC analysis via standard methods. These are in fact based mainly on the detection of epithelial markers (such as EpCam) which disappear during EMT, making mesenchymal CTCs undetectable.
Several studies have been conducted to unravel the presence of CTCs which underwent the EMT in different tumour types and in particular in primary breast cancer (PBC) or metastatic breast cancer (MBC) patients (Aktas et al. 2009, Kasimir-Bauer et al. 2012, Mego et al. 2012a, Mego et al. 2012b, Yu et al. 2013).
Goal of this project will be to characterize CTCs isolated from HER2 negative MBC patients, assessing the expression of EMT transcription factors as well as stem cell markers and correlating the results with clinical findings.

Short experimental design:
1. EpCam positive CTC detection, enumeration and analysis:
   Blood samples withdrawn from patients who received or not the everolimus/ribociclib treatment in combination with the endocrine therapy, staining with anti-EpCam antibodies according to standard protocols (Cell Search System), CTC enumeration, analysis of EMT inducing Transcription Factors (TWIST1, AKT2, PI3K, SNAIL1, SLUG, Zeb1, Vimentin, N-Cadherin) and stem cell markers (CD24, CD 44) by qPCR.

2. EpCam negative CTC analysis:
   Blood samples withdrawn from patients who received or not everolimus/ribociclib treatment in combination with the endocrine therapy, EpCam positive cells depletion, PBMC enrichment, CD45 depletion, analysis of EMT inducing TFs (TWIST1, AKT2, PI3K, SNAIL1, SLUG, Zeb1, Vimentin, N-Cadherin) and stem cell markers (CD24, CD44) by qPCR.
3. Immunostaining
   EpCam positive and EpCam negative CTCs will be immunostained with antibodies recognizing EMT inducing TFs.

Current Status:
The enumeration of CTCs by the Cell Search System is a well-established method in the laboratory and there is no need for further development. The protocol to isolate EpCam negative CTCs must be established (3-6 months). The qPCR protocol to quantitatively analyse the EMT inducing TFs needs to be established (3-6 months). Immunostaining protocol needs to be established (2-4 months).

2.10.2.4 Resistance to anoikis in CTCs

Epithelial cells are programmed to stay in close contact to their substrate. When they lose contact with the matrix, anoikis (a form of apoptosis induced by the lack of cellular adhesion) is triggered and the cells finally die (Frisch and Rouslhati 1997, Liotta et al. 2004).
Cancer cells are able to overcome anoikis, since they have activated specific molecular pathways that keep them alive and proliferative even when they lose matrix contact. This process is considered a key step in the early phases of metastasis.
A potent and specific inhibitor of anoikis is TrkB, a tyrosine kinase receptor which activates the PI3K and AKT pathways, both of them responsible for the disruption of the caspase pathway and prevention of anoikis (Douma et al. 2004).
Interestingly, EMT can also induce resistance to anoikis (Tiwari et al. 2012, Jia et al. 2012). This observation would be clinically relevant if applicable to CTCs. If EMT and anoikis resistance are indeed both active in CTCs, the existence of CTCs with mesenchymal characteristics and insensible to caspase activity might explain why patients, only apparently CTCs negative, finally develop metastasis.
Up to now, the signalling pathways inducing survival of tumour cells once they enter in the blood stream are still not fully understood. It would be certainly interesting to expand the data since defining better the CTCs surviving mechanisms could lead to a more effective therapeutic approach.
Goal of this project will be to further characterize CTCs isolated from HER2 negative MBC patients, assessing the expression of anti-anoikis transcription factors and correlating the results with clinical findings.

Short experimental design:
1. Epcam positive CTC detection, enumeration and analysis:
   Blood samples withdrawn from patients who received or not the everolimus/ribociclib treatment, staining with anti-Epcam antibodies according to standard protocols (Cell Search System), CTC enumeration, analysis of anoikis markers (TrkB, list to be expanded) by qPCR.
2. Epcam negative CTC analysis:
   Blood samples withdrawn from patients who received or not everolimus/ribociclib treatment, EpCam positive cells depletion, PBMC enrichment, CD45 depletion, analysis of anoikis markers (TrkB, list to be expanded) by qPCR

Current Status:
The enumeration of CTCs by the Cell Search System is a well established method in the laboratory and there is no need for further development.
The method to isolate EpCam negative CTCs must be established (3-6 months).
The qPCR protocols to quantitatively analyse the anoikis marker(s) must be established (3-6 months).

2.10.2.5 Expression of LKB1 in CTC

LKB1 (Liver Kinase B1) is a tumor suppressor gene which has been shown to suppress, in breast cancer, cell migration, invasion, tumor growth, microvessel density and metastasis (Shen et al, 2002; Zhuang et al., 2006; Tagliaferro-Smith et al., 2009). LKB1 is also involved in cell cycle regulation and apoptosis. Overexpression of LKB1 in cells induces cell cycle arrest, while knocking down LKB1 triggers a cell cycle progression from G1 to S

(Tiainen et al., 2002; Liang et al., 2010).
LKB1 is an upstream inhibitor of mTOR (mammalian Target of Rapamycin) via the activation of the AMPK pathway (Shackelford et al., 2009). AMPK itself is a major p53 dependent tumor suppressor kinase which controls cell growth, proliferation and autophagy through a negative regulation of mTOR (Chapuis et al., 2010). On the contrary, mTOR is a PI3K related kinase which regulates cell growth, proliferation and survival and its pathway is often aberrantly activated in cancer. Therefore LKB1 can module cell proliferation via an AMPK mediated- mTOR inhibition, counteracting in this way the positive effect of the PI3K cascade on mTOR activity.
Some preclinical and clinical studies are currently monitoring the efficacy of Rapamycin and its analogues in anticancer therapy. Among others, everolimus has been tested in combination with tamoxifen in frame of the TAMRAD randomized Phase II study on ER+HER2- breast cancer patients. Interestingly, the researchers observed a greater benefit from the therapy in patients with a low LKB1 expression profile in the primary tumor with respect to those with a high PI3K expression profile, suggesting a stronger effect of everolimus in patients showing a PI3K independent activation of mTOR (Treilleux et al., data presented at ASCO 2013). These results might have potentially a great impact on the treatment choice, since only a restricted group of patients might be responsive to everolimus.
Goal of this project will be to further characterize CTCs isolated from HER2 negative MBC patients, assessing the expression of LKB1 in primary tumors as well as in CTCs and correlating the results with clinical findings.

Short experimental design:

1. Epcam positive CTC detection, enumeration and analysis: blood samples withdrawn from patients who received or not the everolimus treatment, staining with anti-Epcam antibodies according to standard protocols (Cell Search System, AdnaTest), CTC enumeration, analysis of LKB1 by qPCR and immunofluorescence.
2. Epcam negative CTC analysis: blood samples withdrawn from patients who received or not everolimus treatment, EpCam positive cells depletion, PBMC enrichment, CD45 depletion, analysis of LKB1 by qPCR and immunofluorescence.
3. Immunostaining
4. EpCam positive and EpCam negative CTCs will be immunostained with antibodies recognizing LKB1.

Current Status:
The enumeration of CTCs by the Cell Search System is a well-established method in the laboratory and there is no need for further development.
The method to isolate EpCam positive CTCs must be established (3-6 months).
The method to isolate EpCam negative CTCs must be established (3-6 months).The qPCR protocols to quantitatively analyse the expression level of LKB1 must be established (3-6 months).
The immunostaining protocol to identify LKB1 in CTC must be established (2-4 months)

2.10.2.6 Molecular profiling of CTCs in breast cancer

Background: Great hope rests in the development of therapies that target specific molecular structures on cancer cells. In most instances, therapy targets are genetically activated molecules or pathways, because genetic activation renders the cancer cells addicted to the pathway or molecule. The selection of a drug in an “individualized” therapy setting then depends on the molecular characteristics of the primary tumour, which for example is tested for point mutations or gene amplifications. This approach rests on the assumption that genomic alterations in the target cells of systemic therapies, which can be isolated as circulating tumour cells (CTCs), are identical to those in matched primary tumours.
The DETECT study questions the hypothesis of identical mutational profiles between primary tumours and CTCs by searching for the HER2 gene amplification in CTCs whose primary was initially defined as HER2-negative. However, the general concept may be addressed by asking whether the molecular profiles of CTCs are even more informative than the molecular patterns of primary tumour cells. Therefore, we would like to assess the prevalence of important genomic alterations in breast cancer CTCs. In this project we will test the seven most frequent genomic alterations, which are thought to drive cancer progression, comprising the gene amplifications of ERBB2, CCND1, MYC, FGFR1 and the point mutations in TP53, GATA3 and PI3KCA in individually isolated CTCs. All seven alterations are currently used as therapy targets or are investigated as potential markers to predict therapy response. A systematic analysis of these alterations in CTCs within the DETECT study should help to identify molecular profiles that are associated with survival data or with response to selected therapies.
Approach and specific questions: We have developed several methods to analyse single CTCs. CTCs will be isolated from the CellSearch cartridges by the help of the DEPArray technology and amplified by using the Ampli1 whole genome amplification kit. We are currently setting up the downstream assays, which have been established for five of the seven alterations (TP53, GATA3, PIK3CA, HER2, CCND1). We will use all available cartridges from Ulm, Munich and Düsseldorf to isolate as many CTCs as possible. Following questions will be addressed:

- What is the prevalence of the individual mutations in CTCs?
- Which combinations of alterations are found in individual cells?

- What is the heterogeneity among single CTCs?
- What are the links between primary tumour data and CTC mutational profiles?
- Are specific mutational profiles linked to disease courses?
- Are specific mutational profiles linked to therapy response?

2.10.2.7 Quantification of circulating microRNAs miR-125a, miR-125b, miR-18a und miR18b in the serum of breast cancer patients

The aim is to quantify the expression of circulating microRNAs miR-125a, miR-125b, miR-18a and miR18b in the serum of patients with breast cancer. To compare the serum levels of the microRNAs with clinical outcome of the patients, the impact of the therapy on the expression levels in the patients will be investigated using paired samples (before and after therapy).

The identification of microRNAs and their targets has provided new insight in the complexity of their functions in biological processes. MicroRNAs are evolutionary conserved, small non-coding RNA molecules consisting of approximately 22 nucleotides. They inhibit the gene expression post-transcriptionally by binding specifically to the 3' untranslated-region (UTR) of their target mRNA. This gene silencing can occur through translational inhibition of the mRNAs or their cleavage. Computational analyses indicate that a sole microRNA has binding affinity to hundreds of different mRNAs and hence, they are involved in the regulation of various cellular processes, such as development, cell differentiation and proliferation. Since microRNAs frequently map to fragile chromosomal regions harboring DNA amplifications, deletions or translocations, their expression is often deregulated during tumorigenesis. Thus, the developmental lineage and differentiation state of breast cancer may be reflected by the microRNA signature. In blood microRNAs circulate in a remarkably stable form, because most of them are included in apoptotic bodies, microvesicles or exosomes and can withstand known mRNA degradation factors.

MicroRNAs are quantified by real-time PCR. RNA is extracted from 1 ml serum by the NucleoSpin RNA Plasma kits. Following conversion of RNA into cDNA, cDNA is amplified by TaqMan universal PCR master mix and TaqMan microRNA assays containing the different primer sets specific for microRNAs. The experimental data are normalized by the amplified miR-16. The method is established in the laboratory. For this project, it is planned to examine paired samples (pre- and post-surgery) of app. 100 patients.
The results of this project could help to evaluate the potential role of the kinetics of serum changes of miR-125a, miR-125b, miR-18a und miR18b.

2.10.3 Eribulin cohort

The main focus of the translational research program in the DETECT IVb study is on investigating the association of CTC prevalence and numbers with treatment response and prognosis. As eribulin seems to be even more effective in preventing the formation of new metastases than in preventing increase in size of preexisting lesions, the high efficacy of eribulin in prolonging OS in this subgroup might be based on mechanisms of action that particularly affect CTCs as the potential founder cells for blood-derived metastases.

2.10.3.1 Expression and mutation status of androgen receptor

Triple negative breast cancer (TNBC) is the most lethal form of breast cancer. Treatment options for advanced disease are limited, with a median survival from the time of developing metastases rarely exceeding 1 year. TNBC is heterogeneous, and harbors several molecular alterations. Up to now, clinical trials combining targeted agents and chemotherapy have failed to show substantial survival improvement; therefore, chemotherapy remains the backbone of treatment.
Greater than 70% of human breast cancers express the androgen receptor (AR). Emerging preclinical and clinical data suggest that AR may play a role in breast cancer pathogenesis and may serve as a therapeutic target in certain more difficult-to-treat breast cancer subtypes, such as TNBC. In the last several years advances have been achieved regarding the underlying biology of AR signaling in breast cancer development and the available clinical data for the use of androgen inhibition in the treatment of AR(+) TNBC. Recent data suggest that loss of AR in TNBC is associated with a worse prognosis (Sutton et al. 2012; Thike et al. 2013).
Not only the expression of AR but also its activation status may have prognostic relevance in TNBC. Regarding AR mutations only few data are published in breast cancer: e.g. in the MDA-MB-453 cells a AR-Q865H variant has been identified (Moore et al. 2012). Furthermore, in breast cancer increased AR levels seem to be associated with PIK3CA mutation in the kinase domain (Gonzalez-Angulo et al. 2009).

Regarding the analysis of AR mutations in circulating tumor cells in patients with advanced prostate cancer coding mutations in the AR have been detected in CTC-enriched peripheral blood samples from 20 out of 35 castration-resistant prostate cancer patients (Jiang et al. 2010). The relative abundance of the mutants in the amplified products ranged from 5% to 50% (Lilja and Scher 2010). In addition, activation of AR signal transduction of the AR has been determined in CTCs as a marker of hormonally responsive prostate cancer (Miyamoto et al. 2012).

Based on these data the aims of this translational research project are (1) to determine the AR expression on CTCs using AR specific monoclonal antibody SP61 combined with fluorescence labeled secondary antibody, (2) to determine mutation status of AR by PCR amplification of AR exons followed by sequencing analysis in analogy to Lilja and Scher (2010), and (3) to determine PIK3CA mutations on CTC based on SNaPshot technology (Schneck et al. 2013).

2.10.3.2 Resistance to anoikis in EMT tumor cells isolated from TNBC patients treated with Eribulin

In physiological conditions, when epithelial cells lose contact with their substrate, anoikis, a form of apoptosis induced by the lack of cellular adhesion, is activated (Frisch and Rouslhati 1997; Liotta et al. 2004). Prevention of anoikis can be considered a key step in the early phases of metastasis. Cancer cells can overcome anoikis, since they are able to activate specific molecular pathways which keep them alive and able to proliferate even when they lose matrix contact. A central role in this process is played by the PI3K/AKT pathway activated by the tyrosine kinase receptor TrkB, with the consequent disruption of the caspase cascade and block of cellular death (Douma et al., 2004).
Another key step in tumor progression is the so called Epithelial-Mesenchymal Transition (EMT). In physiological conditions EMT is manly involved in development and tissue regeneration and defines a number of cellular processes such as lose of cell-cell contact and cell polarity, down regulation of epithelial associated genes, up regulation of mesenchymal gene expression and cytoskeleton modification. EMT has been linked to tumor progression, since epithelial cells acquiring mesenchymal features become able to metastasize. Also CTCs can lose epithelial markers developing mesenchymal characteristics (Aktas et al., 2009; Kasimir-Bauer et al., 2012; Mego et al., 2012a; Mego et al., 2012b; Yu et al., 2013). EMT in CTC has relevant consequences on their detection and enumeration. Standard identification methods are in fact based on epithelial markers such as EpCam. Since these markers disappear during EMT, mesenchymal CTC become in this way undetectable.
In recent years, it has been shown that EMT and anoikis are linked together, EMT having a role in the resistance to anoikis (Oredr et al., 2008; Tiwari et al., 2012; Jia et al., 2013). This observation is clinically quite relevant when applicable to CTCs. If EMT and anoikis resistance are indeed both active in CTCs, the existence of CTCs with mesenchymal characteristics and insensible to caspase activity might explain why patients, only apparently CTCs negative, might be resistant to the therapy and finally develop metastasis.
Understanding how these processes impact each other might lead to the development of new therapeutic protocols with a more targeted effect on mesenchymal anoikis-resistant CTC.
The eribulin cohort of the Detect IV study will involve TNBC patients who in most cases present tumor cells with a basal like gene expression (Foulkes et al, 2010) and with a clear up-regulation of EMT markers (Sarrió et al, 2008). Goal of this project will be 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. CTC will be discriminated in EpCam positive and EpCam negative cells according to an already established depletion-based method, gene expression variations will be analysed by qPCR and protein distribution will be investigated by immunofluorescence. The signalling pathways inducing anoikis resistance and EMT will be interrogated in parallel and the results will be correlated to the clinical findings.

2.10.3.3 Evaluation of DNA damage and repair marker to predict therapeutic responsiveness to eribulin treatment

Most known breast cancer susceptibility genes such as BRCA1 directly or indirectly promote accurate DNA double-strand break (DSB) repair mechanism by homologous regulation and concomitantly suppress alternative, error-prone, i.e. mutagenic DSB repair mechanisms (Stratton and Rahman 2008; Walsh and King 2010). A subgroup of triple-negative breast cancers (TNBCs), i.e. tumors devoid of HER2, ER and progesterone receptor (PgR) expression, resemble hereditary tumors due to defectiveness in the BRCA1 homologous recombination repair pathway, however, mostly in the absence of BRCA1 mutations (Linn and van't Veer 2009; Joosse et al. 2011). Additionally, BRCA1 inhibits centrosome overduplication in response to DNA damage, which explains why BRCA1 deficiency causes lower responsiveness to microtubule-modulatory drugs such as Paclitaxel (Sung and Giannakakou 2013; Brodie et al. 2012; Brodie and Henderson 2012). Resistance mechanisms are not limited to BRCA1 mutations but also include expression changes of BRCA1, BRCA1-interaction partners such as BARD1, and nuclear exclusion of BRCA1.

To monitor BRCA1 pathway dysfunction in patient samples as a potential predictive biomarker of therapeutic responsiveness, several groups analysed discrete focal nuclear accumulations (foci) specific for the DNA damage marker γH2AX or RAD51, the essential component of the homologous recombination machinery (Redon et al. 2010; Mukhopadhyay et al. 2010; Oplustilova et al. 2012). Like γH2AX, focal accumulations (foci) of the nuclear protein 53BP1 represent another surrogate marker for DSBs (see e.g. Asaithamby and Chen 2009). 53BP1 foci analysis post genotoxic treatment enables detection of DSB accumulation and subsequent DSB removal, i.e. DSB repair activity. More recently, it has become clear that 53BP1 foci may represent a more specific DSB (repair) marker than γH2AX foci (see e.g. Gagou et al. 2010). In the context of the DETECT IV study, it is of major interest that reduced overall expression of 53BP1 (immunoblotting, immunohistochemistry) was found in subsets of TNBC and BRCA-associated cancers, was associated with poor prognosis, partially restored the homologous recombination defect in BRCA1-mutated cells, and modulates therapeutic responsiveness (Bouwman et al. 2010; Bunting et al. 2012).

Based on these data the aims of this translational research project are 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 triple negative, metastatic breast cancer and persisting HER2-negative CTCs. In addition, the dynamic of 53BP1 signals on CTCs will be assessed by longitudinal comparisons before, during, and after treatment, and the suitability of the different measures for assessing therapy efficacy will be evaluated.

Qualitative and quantitative immunofluorescence microscopic detection of nuclear 53BP1 foci in immortalized and primary cells from various human cell types has been established in the Section of Gynecological Oncology, Department of Obstetrics and Gynecology, Ulm University (see e.g. Volcic et al. 2012). The protocol will be adapted for use in the Cell Search® System (Cell Search® Epithelial Cell, Cell Search® CXC vs. Profile Kit; choice of fluorophore; cut-offs regarding signal intensity vs. foci numbers; exposure/integration time etc.) using blood samples spiked with well-described BRCA-mutated and/or TNBC cell lines (HCC1937, MDA-MB-436, MDA-MB-468) following guide lines established with γH2AX detection on CTCs (Wang et al. 2010).

The optimized protocol will be applied to blood samples (10 ml CellSave Preservative Tubes, Veridex Inc.) collected from TNBC patients during the Randomization visit, if CTCs (≥ 1 / 7.5 ml blood) with HER2-negative status were detected in blood samples retrieved during the Screening visit. Additional blood samples will be collected 0, 6, and 12 weeks after treatment start with Eribulin. CTC enrichment and enumeration from samples in CellSave tubes will be performed following the instructions by Veridex LLC. CTCs, captured from peripheral blood by EpCAM-antibody and identified by cytokeratin8/18/19-positivity and CD45-negativity, will be analysed for 53BP1 signals within the DAPI-stained nucleus by use of corresponding antibody dilutions (according to optimized protocol). Homogeneous, diffuse 53BP1 staining will be categorized into negative (0), weak (1+), moderate (2+), and strong (3+) staining analogous to HER2-specific immunofluorescence. Discrete, focal 53BP1 signals will be scored per nucleus and positivity defined by use of the cut-off level established in the optimized protocol.

Genetic analysis of BRCA1 in genomic DNA isolated from EDTA blood samples by Sanger sequencing and MLPA analyses will follow established protocols (Keimling et al. 2008; Herman et al. 2012).

3 BACKGROUND THERAPEUTIC INFORMATION

3.1 Investigational Medicinal Product (IMP)

3.1.1 Everolimus

Everolimus tablets 5 mg in combination with endocrine treatment (either exemestane 25mg, tamoxifen 20mg, letrozole 2,5mg or anastrozole 1mg).

Standard endocrine therapy is performed in accordance with the Working Group for Gynecological Oncology (AGO) guidelines. However, only agents and daily doses are administered whose combination with everolimus is either approved (see SPC of Afinitor® 10 mg tablets) or has been investigated in prior clinical trials. Patients can only participate in this clinical trial after decision for a standard endocrine therapy fulfilling these inclusion criteria.

3.1.2 Ribociclib

Ribociclib capsules 200 mg in combination with endocrine treatment (either exemestane 25mg, letrozole 2,5mg,

anastrozole 1mg or fulvestrant 500mg)

Standard endocrine therapy is performed in accordance with the Working Group for Gynecological Oncology (AGO) guidelines. However, only agents and daily doses are administered whose combination with ribociclib has been investigated in prior clinical trials. Patients can only participate in this clinical trial after decision for a standard endocrine therapy fulfilling these inclusion criteria.

3.1.3 Eribulin

The recommended dose of eribulin as the ready to use solution is 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.

3.2 Everolimus

Everolimus tablets 5 mg (for further information please refer to the investigator's brochure (IB)).

3.2.1 Name, Chemical and Physical Information

Everolimus as an inhibitor of mammalian target of rapamycin (mTOR) works similarly to sirolimus of which it is the 40-O-(2-hydroxyethyl) derivative.

3.2.2 Chemical Structure

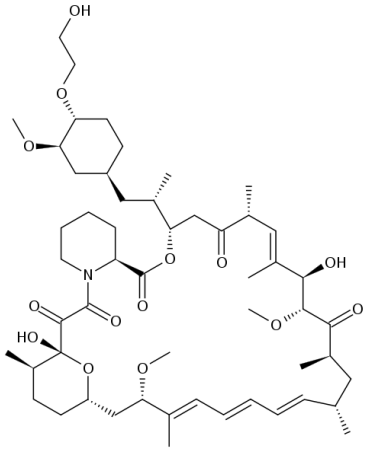


Figure 3: chemical structure of everolimus

3.2.3 Mechanism of Action

Everolimus is a derivative of Rapamycin that acts as a signal transduction inhibitor specifically targeting mTOR, a multi-functional signal transduction protein, which obtains signals from many upstream inputs, and regulates multiple downstream pathways. Everolimus inhibits cell signaling through the PI3K/Akt/mTOR pathway and thus reduces cell proliferation and angiogenesis (please refer to section 2.4 for more details).

3.2.4 Experimental Non-clinical Data

Everolimus inhibits the proliferation of a range of human tumor cell lines *in vitro* including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS) *in vitro*, with particular potency against VEGF-induced proliferation suggesting that everolimus may also act as an anti-angiogenic agent. The anti-angiogenic activity of everolimus was confirmed *in vivo*: Everolimus selectively inhibited VEGF-dependent angiogenic response at well-tolerated doses. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density when compared to controls.

Everolimus administered daily p.o. was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and "relatively resistant" *in vitro*. In general, everolimus was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity

in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of everolimus-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

All significant adverse events observed in toxicology studies with everolimus in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant and at least in part reversible after a 2 or 4 week recovery period with the exception of the changes in male reproductive organs, most notably testes.

3.2.5 Everolimus Pharmacokinetics

Everolimus is rapidly absorbed after oral administration, with a median time to peak blood levels (t_{max}) of 1-2 hours post dose. The extent of absorption is estimated at above 11%. The area under the blood concentration-time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration (C_{max}) appears to plateau at dose levels higher than 20 mg. The elimination half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation (CV) of approximately 50%. In healthy subjects, high fat meals reduced systemic exposure to Afinitor 10 mg (as measured by AUC) by 22% and the peak plasma concentration C_{max} by 54%. Light fat meals reduced AUC by 32% and C_{max} by 42%. Food, however, had no apparent effect on the post absorption phase concentration-time profile. Everolimus is eliminated by metabolism, mainly by hydroxylation, then excreted into the feces >80%. (Everolimus Investigator Brochure, Clinical Study Report RAD001C2120).

Steady-state trough levels are highly predictive of AUC, with a coefficient of determination of 0.96, as has been reported in renal transplantation patients (Kovarik_et al. 2001).

Everolimus is mainly metabolized by the cytochrome P450 isoenzyme 3A4 (CYP3A4) in the liver and to some extent in the intestinal wall. Everolimus is also a substrate of P-glycoprotein (P-gp). Therefore, medicinal products that interact with CYP3A4 and/or P-gp may influence absorption and subsequent elimination of systematically absorbed everolimus. Strong CYP3A inhibitors (such as Ketoconazole, Itraconazole, Ritonavor) and inducers (such as Rifampicin, Rifabutin) should be avoided.

3.2.6 Everolimus Pharmacodynamics

Pharmacokinetic/pharmacodynamic modeling based on inhibition in a peripheral biomarker (S6 kinase inhibition in peripheral blood mononuclear cells) suggests that 5-10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition. Furthermore, molecular pharmacodynamic (MPD) studies using IHC in biopsied tumor tissue assessed the degree of inhibition and its duration (for p-S6, p-4E-BP1 and p-Akt expression) with the daily and weekly dosing. The pathologist was blinded for the biopsy sequence. There was almost complete inhibition of p-S6 at all doses and schedules studied (p=0.001). Preliminary results suggest a dose-related decrease in p-4E-BP1 and increase in p-Akt expression with maximal effect at 10 mg daily and ≥ 50 mg weekly.

In [study C2107], molecular changes in tumor were subsequently investigated through serial biopsying of patients before and while on treatment (Tabereno et al. 2008). Biopsying of patients on treatment took place in week 4 (pharmacokinetic steady-state). All patients underwent a 24-hr post-dose biopsy. Patients following the weekly regimen had a further biopsy on Day 4-5 during the same week.

Molecular activity was measured by immunohistochemistry. In the absence of a reliable technique for measuring mTOR phosphorylation itself, the phosphorylation status of downstream markers S6 and eIF4G, for which reliable antibodies exist, was selected as reflecting the immediate pharmacodynamic effect of everolimus. Also measured were changes in the phosphorylation status of upstream AKT and the proliferation index Ki67. The daily regimen was associated with a high inhibition of p-S6 and p-eIF4G at 5mg/d which was complete at 10mg/d. In patients on the weekly schedule, p-S6 inhibition was complete and sustained at all dose levels while that of p-eIF4G was complete and sustained at 50mg/d but not at 20mg/wk. On both regimens numerous patients demonstrated apparent up-regulation of AKT which tended, however, not to persevere in the patients at 50mg/wk. The proliferation index was reduced in most patients, recovering in some of those on the 50mg/wk regimen.

3.2.7 Safety

Data are available from phase I-III clinical studies of everolimus given as a single agent or in combination with other agents to cancer. Such studies included various doses and schedules (weekly dosing, range 5-70 mg and

daily dosing 5-10 mg). Approximately, 46% of patients reported rash or erythema and 40% of the patients presented with stomatitis/mucositis. The most frequent adverse events suspected to be drug-related observed in three studies using everolimus as a single agent are listed in the TABLE 5 below.

	Weekly			Daily		
	5-30 mg	50 mg	70 mg	5mg	10 mg	Total
	n=30	n=18	n=38	n=16	n=45	n=147
No. Pts with AEs						
Any event	23 (1)	17 (2)	38 (10)	14 (1)	43 (14)	135 (28)
By event						
Rash	5	8	18	10	27 (1)	68 (1)
Stomatitis/mucositis	6	8 (2)	16 (2)	6 (1)	23 (3)	59 (8)
Fatigue	8	7 (1)	14 (1)	1	17 (1)	47 (3)
Nausea	5	4	8	2	18 (1)	37 (1)
Anorexia	1	6	10	3	15	35
Diarrhea	1	7	7	-	9	24
Vomiting	4	5	5	-	10	24
Headache	7	4	6	6	4	20
Pruritus	2	1	6	3	4	16
Infections1	1	3	3 (1)	1	6 (2)	14 (3)
Constipation	-	1	2	2	9	14

The numbers of patients (by dose level and dose schedule) who have reported grade ≥ 3[1] toxicities is given in brackets.

[1] events included in brackets reached no more than grade 3 severity

[2] Infections noted as drug-related included:

Herpes simplex:	5 pts (1 at 50 mg/wk; 1 at 5 mg/d; 3 at 10 mg/d)
Oral candidiasis:	5 pts (1 at 50 mg/wk; 3 at 70 mg/wk, 1 at 10 mg/d)
Pneumonia (gr3)	1 pt (10 mg/d)
Pustular rash	1 pt (20 mg/wk)
Rhinitis	2 pts (50 mg/wk)
URT Infection	1 pt (50 mg/wk)
Urinary Tract Infect	1 pt (50 mg/wk)

Table 5: Adverse events suspected to be drug-related in greater or equal to 10% of patients with advanced cancer reported in Phase I Everolimus monotherapy

Reduced blood cell counts at the initiation of treatment are frequent but remain mostly within the normal range or limited to grade 1 however a grade 3 neutropenia was a DLT in one patient. In addition a grade 3 thrombocytopenia was observed in a patient receiving everolimus with letrozole where pharmacodynamic interaction is unlikely. This suggest that some patients may be particularly sensitive to the myelosuppressive effect of everolimus making it necessary to monitor carefully blood cell counts at initiation of treatment.

Metabolic changes (hyperlipidemia and hyperglycemia) may be observed during treatment with everolimus. Both events may be medically managed. Hyperlipidemia has been reported as an adverse drug reaction (ADR) in 10% of patients although review of the laboratory values suggests that as many as a quarter of patients develop grade 1-2 hyperlipidemia on treatment, mostly hypercholesterolemia. Hyperglycemia has been reported as ADR in 7% of patients. Grade 3 hyperglycemia has been observed, especially in diabetics receiving everolimus treatment. Therefore, patients with diabetes should have their blood glucose monitored carefully and their medications adjusted, as needed, to maintain adequate control of their blood glucose levels.

Outside the particular context of hemorrhagic gastritis in advanced GIST patients treated with everolimus and Imatinib, serious, suspected drug-related hemorrhages have been exceptional. Nevertheless, everolimus should be considered as predisposing patients to hemorrhage, potentially fatal, should they develop severe drug-related thrombocytopenia. Patients with on-going thrombocytopenic or with a known bleeding diathesis should be subject to careful evaluation and more frequent monitoring. Platelet counts should be monitored. Imatinib (Glivec® / Gleevec™), a 3A4 and Pgp substrate, has been shown to increase the AUC of everolimus more than 3-fold, most probably the consequence of competitive inhibition.

Everolimus is an immunosuppressant and consequently patients taking the drug are at an increased risk of infection. This may be the case even if the patient has a normal white cell count. Physicians and patients/carers should be aware of this risk, and vigilant for signs and symptoms of infection. Prompt treatment with appropriate anti-infectives should be given as clinically appropriate.

Non-infectious pneumonitis is a known side effect of Rapamycin analogues including everolimus. Clinically significant pneumonitis is typically accompanied by non-specific symptoms including dyspnea, nonproductive cough, fatigue, and fever. Diagnosis is generally suspected in individuals receiving mTOR inhibitors who develop these symptoms or in asymptomatic individuals in whom a routine chest CT scan reveals a new ground glass or alveolar infiltrate. The frequency of symptomatic pulmonary toxicity (all grades) was approximately 13% in a phase III study of everolimus in patients with metastatic renal cell carcinoma (CRAD001C2240). Severe (CTC grade 3) pneumonitis occurred in 4% of patients, and an occasional fatality was reported. The lung toxicity was partly or completely reversible in the majority of cases with interventions including drug interruption, discontinuation and the use of corticosteroids.

A randomized phase II study comparing two schedules of everolimus (10 mg daily and 70 mg weekly) in patients with recurrent/metastatic breast cancer (Ellard et al. 2009), demonstrated that in general, adverse effects were those predicted from preclinical and early clinical studies (O'Donnell et al. 2008, Tabernero et al. 2008) including hyperglycemia and hyperlipidemia (mostly in patients with preexisting abnormalities), and were reversible. The most common grade 3 or 4 adverse events were fatigue, non-infectious pneumonitis and neutropenia. Pneumonitis occurred more frequently than anticipated, but was reversible in all affected patients and in general, manageable, although some patients required discontinuation. None of the 16 patients recruited to the weekly 70 mg arm responded, with four patients with a stable disease. Among the 33 patients treated in the daily 10 mg arm, response was evaluable in 30 patients; one patient had a complete response, three patients had a partial response and 15 patients had stable disease. Ellard et al. 2009 concluded that continuous daily dosing of everolimus, but not weekly dosing, has single agent activity in this disease setting (Ellard et al. 2009).

For the most up to date safety information, please refer to the most current edition of the Investigator's Brochure.

3.2.8 Marketing Authorization Status

Everolimus is approved for the combination with exemestane in the treatment of progressed or metastasizing breast cancer showing expression of HR receptors (see SPC of Afinitor® tablets). Combinations with other standard anticancer therapies are not approved.

3.2.9 Pharmaceutical Data

Supply:
The IMP everolimus is provided by Novartis Pharma GmbH. Novartis Pharma GmbH is responsible for shipment of the IMP everolimus to the clinical trial centers. The investigator will prescribe everolimus + exemestane according to the label. Everolimus in combination with any other endocrine regimen will be provided by Novartis to the participating sites in the study setting (see TABLE 1) and will be dispensed by the study center personnel on an outpatient basis. Investigator's request of everolimus will be done through the eCRF (initial order automatically with recruitment, follow up orders as required).

Packaging:
Everolimus is formulated as tablets of 5 mg strength for oral administration. Commercially available exemestane will be supplied as tablets of 25 mg strength. Anastrozole will be supplied as tablets of 1 mg strength, Letrozol as tablets of 2.5 mg strength and tamoxifen as tablets of 20 mg strength for oral administration. Complete guidelines for management and administration of exemestane, anastrozole, letrozole and tamoxifen can be found in the package insert. All study medication will be packaged into blister packs. The blisters should be opened only at the time of administration, as the drugs are both hygroscopic and light sensitive. All blisters will conform to all local regulatory requirements.

Labeling:
Everolimus for combination with anastrozole, letrozole or tamoxifen is supplied in blister packs with 30 Tablets of 5 mg. Packages will be signed with the warning "Zur klinischen Prüfung vorgesehen". Everolimus for combination with exemestane will be prescribed according approval for use with 10 mg dosis of everolimus.

Stability:
Unopened blisters with everolimus tablets are stable until the date indicated on the package label when stored under the storage conditions described below.

Storage:
Storage conditions for everolimus and standard endocrine therapy will be described on the local package insert.

The study drug should be stored in a secure, locked area while under the responsibility of the investigator. An authorized person at the investigator's site throughout the entire study must record receipt and dispensing of supplied study drugs. Persons allowed to handle the study medication are indicated in the site delegation log to be kept in every study center.

All drugs, which are supplied by Novartis, are to be stored below 30°C room temperature in dry and secured space. The storage temperature must be recorded weekly in the temperature log.

3.3 Ribociclib

Ribociclib capsules 200 mg (for further information please refer to the investigator's brochure (IB)).

3.3.1 Name, Chemical and Physical Information

Ribociclib (LEE011) is an orally bioavailable, highly selective small molecule inhibitor of CDK4/6 that potently induces G1 arrest with sub-micromolar IC50's in a variety of pRb-positive cancer cells.

3.3.2 Experimental Nonclinical Data of Ribociclib

A panel of human breast cancer cell lines was treated with increasing doses of ribociclib and dose-dependent inhibition of proliferation was observed across the panel with enhanced activity against ER+ breast cancer cell lines with IC50 < 1µM being observed for most ER+ breast cancer lines (Novartis internal data, ribociclib Investigator Brochure figure 4-3). Ribociclib as a single agent has been shown to have activity in preclinical models of ER+ breast cancer (Novartis internal data). In in vivo studies, combinations with the mTOR inhibitor RAD001 and PI3K inhibitor, BYL719, resulted in either improved or prolonged anti-tumor effects in tumor models derived from ER+ breast cancer.

In Jeko-1 MCL cells that overexpress cyclin D1 as a result of the t(11;14) chromosomal translocation, LEE011 inhibits the phosphorylation of pRb at CDK4/6-specific sites with an average IC50 of 60 nM. In nude rats bearing Jeko-1 subcutaneous xenografts, ribociclib demonstrates dose-dependent target inhibition in the tumors. LEE011 doses that induce >75% inhibition of pRb phosphorylation in this model are associated with complete tumor regression (see ribociclib Investigator Brochure Sections 4.1.1.2.1, 4.1.1.2.2). Ribociclib also inhibits the growth of many other tumor cell types in vitro and in vivo, including liposarcoma, melanoma, rhabdoid cancer, and carcinomas of the esophagus, breast, lung and pancreas. Regardless of the various genetic aberrations that may be present in the cancer cells, the anti- tumor activity of ribociclib requires the presence of functional pRb. Refer to ribociclib Investigators Brochure for more details.

Preclinical data generated using a primary model of ER+ breast cancer compared ribociclib at clinically relevant doses with the combination of ribociclib plus letrozole (ribociclib Investigator Brochure Figure 4-10). Animals treated with the combination showed complete inhibition of tumor growth. Body weight was monitored throughout the treatment showing comparable results in all groups supporting the predicted lack of overlapping toxicity between ribociclib and letrozole.

The pharmacokinetics (PK) of ribociclib was investigated in mouse, rat, dog and monkey. Ribociclib showed high clearance (CL) in the mouse, rat, dog and monkey. The volume of distribution was large across species and the terminal elimination half-life (T1/2) was moderate in rodents and monkey (~2 to 5 h) and longer in dog (18 h).

Bioavailability was low to moderate in rat (37%) and cynomolgus monkey (17%); moderate in mouse (65%) and dog (64%). Following oral administration, time to reach maximal plasma concentrations (Tmax) occurred between 2 to 4 h across species.

Gender dependent toxicokinetics were observed in rats with higher exposure to ribociclib in males as compared to females and higher exposure to the metabolite, LEQ803. Plasma protein binding was moderate in all species (unbound fraction (fu) in human: 30%).
In a rat ADME (absorption, distribution, metabolism and excretion) study, extensive distribution of [3H]ribociclib and its metabolites was observed. In pigmented rats, radioactivity was specifically found in melanin-containing structures; the highest exposure to total radiolabeled components was observed in eye ciliary body, eye choroid, meninges, tactile hair and hair follicles. Radioactivity was not detected in the brain. Tlast (last observation timepoint) was $\leqslant$ 48h for most tissues, but long (168 to 840h) for lymph nodes, preputial gland, testis, eye and meninges. At one week $\leqslant$ 0.04% of the dose was retained in the carcass. LEQ803 (N-demethylation) was a prominent metabolite found in mouse, rat, dog, monkey and human hepatocytes. This metabolite retains some pharmacologic activity and interacts with human Ether-a-go-go Related Gene (hERG) channels in vitro. In male rats, unchanged

ribociclib (24.7% of [3H]AUC0-24h) and its metabolite M11 (26.3% of [3H]AUC0-24h) were the major components in plasma.

In rats, ribociclib was eliminated mainly by metabolism with direct sulfation as the major pathway. Direct ribociclib secretion accounted for 18.2% of the total plasma clearance. In male dogs, metabolism was the major elimination route. The most prominent components in plasma were ribociclib (55.9% of [14C]AUC0-48h) and its metabolite LEQ803 (1.61% of [14C]AUC0-48h).

Results from the ADME (male rats) study showed that 3H-components were predominantly excreted with bile (61.4% of dose). Minor urinary excretion was observed (5.9% of dose after p.o.). The majority of the administered dose (87.3%) was excreted within 24 h via urine, feces (enteric secretion) and bile.

In vitro, ribociclib was a reversible inhibitor of cytochrome P450 (CYP) enzymes CYP1A2, CYP2E1 and CYP3A4 and a time-dependent inhibitor of CYP3A4. Ribociclib may inhibit CYP3A4 under therapeutic conditions. No induction of CYP1A2, CYP2B6 or CYP3A4 was observed. The in vitro inhibitory potency of ribociclib observed for the transporters OATP1B1 (organic anion transporting polypeptide 1B1), BCRP (breast cancer resistance protein), OCT1 (organic cation transporter 1), OCT2, MATE1 (multidrug and toxin extrusion protein 1), MATE2K and BSEP (bile salt export pump) may translate into clinically relevant inhibition at therapeutic doses.

Elimination of ribociclib is dominated by oxidative metabolism mainly via CYP3A4 with a minor contribution by flavin-containing monooxygenase 3 (FMO3). The elimination of ribociclib may be affected by co-administered drugs that inhibit or induce CYP3A4. Although ribociclib is a substrate of the P-glycoprotein (P-gp) efflux transporter, this process is likely not clinically relevant due to the high passive permeability of ribociclib.

Refer to ribociclib Investigators Brochure for more details.

3.3.3 Clinical Experience with Ribociclib

Ribociclib is currently being investigated in patients as a single agent in 3 phase I studies: (CLEE011X1101, CLEE011X2101, CLEE011X2102) in 2 phase II studies: (CLEE011X2201, CLEE011XUS03).

Ribociclib is being evaluated in several combination trials: letrozole (CLEE011A2201, CLEE011A2301, CLEE011A2115C), letrozole and alpelisib (CLEE011X2107), letrozole and buparlisib (CLEE011A2112C), goserelin, letrozole, anastrozole and tamoxifen (CLEE011E2301); fulvestrant (CLEE011F2301), fulvestrant and buparlisib (CLEE011X2108), everolimus and exemestane (CLEE011X2106), ceritinib (CLEE011X2110C) LDK378 (CLEE011X2110C), HDM201 (CHDM201X2103C), LGX818 (CLEE011X2105, CLGX818X2102), LGX818, MEK162 (binimetinib) buparlisib or LGX818, binimetinib and INC280 or LGX818, binimetinib and BGJ398 (CLGX818X2109) binimetinib (CMEK162X2114), or binimetinib and LGX818 (CMEK162X2110). These trials are ongoing with the exception of CLEE011A2201 and CLEE011A2112C where recruitment was prematurely terminated on 28-July-2014 and 20-Mar-2015 respectively. CLEE011A2301, CLEE011X2102 and CLEE011X2105 have also closed enrolment. The results of the phase I combination of letrozole and LEE011 (CLEE011X2107) are detailed in Section 1.2.6. The phase III trial CLEE011A2301, investigating the combination of letrozole and ribociclib, reached its primary endpoint prematurely at the preplanned interim analysis.
Ribociclib is also being investigated in 4 clinical pharmacology studies: CLEE011A2102, CLEE011A2103, CLEE011A2109 and CLEE0112116. Three clinical pharmacology studies in healthy subjects have been completed: CLEE011A2101, CLEE011A2106 and CLEE011A2111.
Refer to the ribociclib Investigator Brochure for additional details.

3.3.4 Clinical Safety of Ribociclib As Single Agent

As of 15-Jun-2015, 157 patients have been treated with single agent ribociclib in the first-in-human (FIH) phase I study; 85 patients have been treated in the initial dose escalation part for the 3 week on/1 week off regimen and 47 patients in the dose expansion part of the study; 18 patients were enrolled for the continuous dosing regimen with ribociclib and, 7 patients were enrolled in the liquid formulation cohort.

Patients with advanced solid tumors or lymphomas were treated with increasing doses of ribociclib orally, once daily (qd) for 21 days followed by a 1-week rest (28-day cycle). Doses ranging from 50 mg to 1200 mg were

evaluated on this schedule. Treatment has been discontinued in 120 (90%) patients; the primary reasons for treatment discontinuation were: disease progression (106 [80%] patients); adverse events (AEs) (7 [5%] patients); death (2 [1.5%] patients); withdrawal of consent (3 [2%] patient); and loss to follow up (1 [1%] patient).

The most frequently reported AEs (≥10%), regardless of grade, causality and ribociclib dose were: nausea (52.3%); fatigue (40.9%); diarrhea (37.1%); vomiting (35.6%); neutropenia (34.1%); anemia (32.6%); decreased appetite, thrombocytopenia (23.5% each); white blood cell count decrease (22.7%); leukopenia (22%); constipation (21.2%); dyspnea (20.5%); asthenia (19.7%); cough (18.2%); hyperglycemia (17.4%); headache, hypoalbuminemia (16.7% each); ECG QT prolonged (15.9%); abdominal pain, back pain, lymphocyte count decrease, pyrexia (15.2% each); AST increase, blood creatinine increased, dizziness, lymphopenia (14.4% each); peripheral edema (13.6%); neutrophil count decreased (12.9%); ALT increase, pain in extremity (12.1% each) and hypocalcemia (11.4%).

For either continuous or intermittent dosing, the onset of neutropenia (most frequently Grade 2) occurs by Day 15, reaching a nadir in the third or fourth week with recovery during the week of drug holiday for the three weeks on/one week off schedule. Some patients require additional time for recovery (7 to 14 days). QT changes become evident in the first cycle by Day 8 and later (once steady state is reached), are associated with the maximum drug levels between 1 to 8 h post-dose, and remain stable or improve in subsequent cycles.
As of 15-Jun-2015, asymptomatic Grade 2 QTcF prolongation was observed with increasing frequency when increasing the dose starting at 600 mg: ten patients (13.5%) in the 600 mg cohort, three patients (21%) in the 750 mg cohort, four patients (31%) in the 900 mg cohort, and two patients (67%) in the 1200 mg cohort. Four patients (5.4%) at 600 mg and two patients (15%) at 900 mg had asymptomatic QTcF prolongation that resulted in a QTcF interval of 500 ms or more. As compared to baseline value, QTcF prolongation was at least 30 msec in 2 patients (50%) at 250mg, 2 (40%) at 350 mg and 400 mg, 59 (79.7%) at 600 mg, 11 (78.6%) at 750 mg, 11 (85%) at 900 mg and 2 (67%) at 1200 mg; and at least 60 msec in 23%, 0%, 39% and 67% of patients at 600 mg, 750 mg, 900 mg and 1200 mg, respectively. One grade 1 atrioventricular block of first degree was reported as related to ribociclib given at the dose of 140 mg. One grade 1 atrioventricular block of first degree was reported as related to ribociclib given at the dose of 140 mg. No other cardiac abnormalities were observed as related adverse events in any patient.
There have been no deaths related to study drug reported on study [CLEE011X2101].The following serious adverse events shown in TABLE 1-1 have been reported with a suspected causal relationship in study [CLEE011X2101] as of 6-Aug-2015. For a complete list of AEs, all grades and Grade 3/4 that are suspected to be related to ribociclib refer to the ribociclib Investigators Brochure.

Serious suspected adverse events which have occurred with ribociclib (single agent)	
System Organ Class Preferred Term	Preferred Term
Blood and lymphatic system disorders	Anaemia, Febrile neutropenia, Neutropenia, Thrombocytopenia
Gastrointestinal disorders	Diarrhoea, Nausea,*Pancreatitis*
General disorders and administration site conditions	Generalized oedema
Infections and infestations	Herpes simplex
Investigations	***Blood creatinine increased,*** *Electrocardiogram QT prolonged*

Events in *italic font* indicate those events which are newly included since the previous edition of the reference safety information.

Refer to ribociclib Investigators Brochure for more details.

Table 6: Serious adverse events with a suspected causal relationhip with ribociclib single agent

3.3.5 Clinical Efficacy With Ribociclib As a Single Agent

Preliminary anti-tumor activity of ribociclib from trial [CLEE011X2101] was assessed across all dose levels (50 mg – 1200 mg). Out of 114 evaluable subjects as of 24-Apr-2014, 3 partial responses were observed at the 600 mg dose level; one each in BRAF/NRAS wild type with CCND1 amplified melanoma, and head and neck acinar carcinoma with CDKN2A loss (both on the 3 weeks on/1 week off regimen), and ER+/HER2-, PIK3CA mutant, CCND1 amplified breast cancer (on the continuous daily dosing regimen). Stable disease (SD) was the best overall response in 41 (37%) patients. Enrollment in this study is completed. Stable disease ≥4 cycles and ≥6 cycles was observed in 26 (24%) and 17 (15%) patients, respectively. Six patients with SD ≥4 cycles received treatment for >1 year, of these 2 patients were on study for >2 years (Jeffrey R Infante ASCO 2014 abstract 2528). Refer to ribociclib Investigators Brochure for more details.

3.3.6 Clinical Pharmacokinetics of Ribociclib

As of 15-Jun-2015, preliminary PK data were available from approximately 143 patients from the first-inhuman (FIH) study [CLEE011X2101] across the dose range of 50 to 1200 mg. Following oral dosing, ribociclib was rapidly absorbed with median Tmax ranging from 1 to 4 hours. Ribociclib plasma exposure (Cmax and AUC0-24h) exhibited slightly over-proportional increases in exposure across the dose range tested. Steady-state was generally reached by Day 8 and the mean effective T1/2 based on accumulation ratio (i.e., T1/2,acc) ranged from 12.3 to 42.9. The mean accumulation ratio (Racc) calculated from AUC0-24h at steady-state and AUC0-24h after a single dose across the studied doses ranged from 1.35 to 3.11.

At the recommended dose for future development (600 mg), steady-state plasma Cmax (n=56) ranges from 606-6170 ng/mL (geometric mean = 1790 ng/mL or 4.1 µM), median Tmax (n=72) is 2.1 h, and AUC0-24h (n=53) ranges from 6770-90600 ng*h/mL (geometric mean = 23600 h*ng/mL). At this dose, inter-patient variability in Cmax and AUC is 62% and 66%, respectively, as assessed by geometric coefficient of variation (CV%). At the 600 mg dose level, LEQ803, an active metabolite of ribociclib, accounted for approximately 8% (geometric mean) of ribociclib AUC0-24h after single and multiple doses. Refer to the ribociclib [Investigators Brochure] for more details.

In a food effect study [CLEE011A2111] in 24 healthy subjects, a single dose of ribociclib (600 mg) was administered as drug-in-capsule (DiC) with a high-fat, high-calorie meal and under fasted conditions. Compared to the fasted state, oral administration of a single 600 mg dose of ribociclib DiC with a high-fat, high-calorie meal decreased the rate of absorption of ribociclib resulting in a 23% decrease in Cmax (geometric mean ratio: 0.775; 90% confidence interval [CI]: 0.700, 0.858) and a median difference in Tmax of 2 hours. However, there was no effect on the extent of absorption of ribociclib as the overall exposure (AUCinf) was unaffected under fed conditions (geometric mean ratio: 0.994; 90% CI: 0.925, 1.070). A similar trend was observed for LEQ803, an active metabolite of ribociclib, with a decrease in Cmax (32%), a delay in median Tmax, and no substantial effect on overall exposure. Based on these data, ribociclib DiC can be taken without regard to meals.

In the human ADME study [CLEE011A2102], a single oral dose of 600 mg [14C]LEE011 was administered to 6 healthy male subjects. The majority of the administered dose was excreted in feces (69.1%), with a minor amount excreted in urine (22.6%). Absorption was estimated to be approximately 58.8%. Ribociclib accounted for approximately 23% of the total radioactivity in plasma, based on AUCinf. Metabolites M1 (glucuronidation of M15), M4 (LEQ803, N-demethylation) and M13 (CCI284, N-hydroxylation) were the most abundant metabolites in plasma, representing an estimated 7.78%, 8.60% and 9.39% of total [14C]AUC0-48h, and 17.9%, 19.8% and 21.6% of ribociclib AUC0-48h, based on metabolite profiles.

A DDI study with ritonavir (a strong CYP3A4 inhibitor) and rifampicin (a strong CYP3A4 inducer) was conducted in 48 healthy subjects [CLEE011A2101]. Compared to ribociclib alone, ritonavir (100 mg bid for 14 days) increased ribociclib Cmax and AUCinf by 1.7-fold and 3.2-fold, respectively, following a single oral dose of 400 mg ribociclib. Cmax and AUClast decreased by 96% and 98%, respectively. These results demonstrated that concurrent use of strong CYP3A4 inhibitors may markedly increase ribociclib exposure and are prohibited.

3.3.7 Clinical Pharmacodynamics of Ribociclib

Paired skin biopsies from 55 patients treated with LEE011 at doses ranging from 50 to 900 mg and paired tumor biopsies from 20 patients (16 patients at 600 mg, 2 patients at 900 mg, and 1 patient each at 70 and 750 mg) were assessed for changes in Ki67 and pRb levels. Preliminary results indicate the following: in skin biopsies, reductions in Ki67 from baseline were observed across all dose levels with a more consistent trend from 400 mg onwards; in tumor biopsies, reductions in Ki67 from baseline were observed in 18/20 patients; however, limited samples and varied tumor types prevent conclusions about any dose-response relationship from being drawn. Changes in pRb were not significant or consistent in either skin or tumor samples, possibly due to varied tumor types. For further details see Section 5.2.1 of the ribociclib (LEE011) investigator's brochure.

3.3.8 Pharmaceutical Data

Supply:
The IMP ribociclib is provided by Novartis Pharma GmbH. Novartis Pharma GmbH is responsible for shipment of the IMP ribociclib to the clinical trial centers. Ribociclib in combination with any endocrine regimen will be provided by Novartis to the participating sites in the study setting (see TABLE 2) and will be dispensed by the study center personnel on an outpatient basis. Investigator's request of ribociclib will be done through the eCRF (initial order

automatically with recruitment, follow up orders as required).

Packaging:
Ribociclib is formulated as film-coated tablets of 200 mg strength for oral administration. Anastrozole will be supplied as tablets of 1 mg strength, letrozol as tablets of 2.5 mg strength, exemestane as tablets of 25mg strength for oral administration and fulvestrant 250 mg for IM injections. Complete guidelines for management and administration of exemestane, anastrozole, letrozole and fulvestrant can be found in the package insert. All study medication will be packaged into bottles. The bottles should be opened only at the time of administration, as the drugs are both hygroscopic and light sensitive. All bottles will conform to all local regulatory requirements.

Labeling:
Ribociclib for combination with anastrozole, letrozole or fulvestrant is supplied in bottles with Capsules of 200 mg. Bottles will be signed with the warning "Zur klinischen Prüfung vorgesehen". Patients will be provided with an adequate supply of study drug for self-administration at home, including instructions for administration, until at least their next scheduled study visit. Patients will receive Ribociclib (LEE011) on an outpatient basis. The investigator shall provide the patient with instructions for Ribociclib (LEE011) administration according to the protocol.

Stability:
Unopened bottles with ribociclib capsules are stable until the date indicated on the package label when stored under the storage conditions described below.

Storage:
Storage conditions for ribociclib and standard endocrine therapy will be described on the local package insert. The study drug should be stored in a secure, locked area while under the responsibility of the investigator. An authorized person at the investigator's site throughout the entire study must record receipt and dispensing of supplied study drugs. Persons allowed to handle the study medication are indicated in the site delegation log to be kept in every study center.

All drugs, which are supplied by Novartis, are to be stored below 30°C room temperature in dry and secured space. The storage temperature must be recorded weekly in the temperature log.

3.3.9 Standard Endocrine Therapy

Deprivation of estrogenic signaling with the anti-estrogen Tamoxifen has been the main form of hormonal treatment for over 35 years. Tamoxifen is indicated for the treatment across the whole continuum of breast cancer, ranging from risk reduction for women with increased risk of breast cancer, as an adjuvant treatment and also for metastatic disease. Besides Tamoxifen, progesterone analogues or progestins have been used for the treatment of breast cancer for almost 50 years. Although the exact mechanism of action of progestins has not been established, their effect on estrogen levels is thought to play a role. While therapies interfering with ER functions such as tamoxifen have significantly contributed to mortality reduction in advanced breast cancer patients, at best 50-60% of ER-positive patients respond to anti-estrogen therapy. Consequently, a number of aromatase inhibitors (AI) that reduce peripheral estrogen synthesis have been developed for the treatment of ABC. The AIs block the conversion of androgens to estrogens, which is the primary way estrogens are produced in post-menopausal women. At present, third generation aromatase inhibitors have been approved for use in postmenopausal women with hormone receptor positive BC after tamoxifen. The third generation AIs can be broadly classified into two groups: non-steroidal aromatase inhibitors (NSAI), mainly Letrozole (Femara®) and Anastrozole (Arimidex®) and steroidal aromatase-inactivators, represented by Exemestane (Aromasin®).
Fulvestrant (Faslodex®) is the first-in-class unique ER down regulator with no known agonist effects. In fact, Fulvestrant mechanism of action is distinct from other endocrine agents; it binds, blocks and degrades the ER, completely inhibiting ER signaling. As result, there is less chance of the ER being activated by alternative pathways that are believed to cause resistance (e.g. growth factor-mediated mechanisms).

3.3.9.1 Exemestane

Exemestane is a third generation steroidal aromatase inactivator that irreversibly binds to the active site of the enzyme and has demonstrated efficacy in the treatment of postmenopausal patients with estrogen-receptor positive breast cancer. It is indicated for adjuvant treatment of postmenopausal women with estrogen receptor positive early BC who have received two to three years of tamoxifen and are switched to exemestane for completion of a total of five consecutive years of adjuvant hormonal therapy. It is also indicated for the treatment of advanced BC in postmenopausal women whose disease has progressed following tamoxifen therapy (in the USA) or following antiestrogen therapy (in Europe).

Combination Everolimus plus Exemestane

The combination of everolimus and exemestane was evaluated in the placebo controlled phase III study BOLERO-2 (CRAD001Y2301) study (Baselga et al. 2012, Piccart et al. 2012). The combined treatment has shown to prolong PFS in patients with ER+ and/or PgR+ and HER2- breast cancer refractory to initial non-steroidal aromatase inhibitors. 724 patients were randomized in a 2:1 ratio to receive either 10 mg daily everolimus (N=485) or matching placebo (N=239) in addition to open-label 25 mg daily exemestane. The primary endpoint for the trial was PFS evaluated by RECIST, based on the investigators (local radiology) assessment. Supportive PFS analyses were based on an independent central radiology review. The study demonstrated a statistically significant clinical benefit of the everolimus + exemestane treatment as compared to the placebo + exemestane treatment. Adding everolimus improved median progression-free survival from 3.2 up to 7.8 months and so lowered the risk of cancer progression by 55% for these women. These findings were confirmed by an independent assessment (4.1 vs. 11.0 months, risk reduction: 62%).

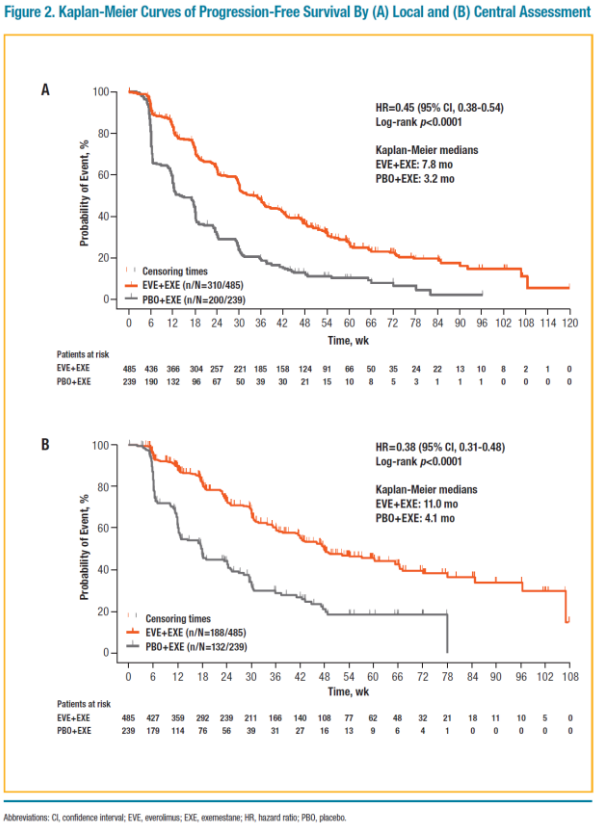


Figure 4: Kaplan-Meier Curves of Progression-Free Survival (Piccart et al. 2012)

Table 2. Adverse Events Irrespective of Relationship to Study Treatment (with at Least 10% Incidence in the Everolimus–Exemestane Group).

Adverse Event	Everolimus and Exemestane (N=482)			Placebo and Exemestane (N=238)		
	Any Event	Grade 3 Event	Grade 4 Event	Any Event	Grade 3 Event	Grade 4 Event
	percent					
Stomatitis	56	8	0	11	1	0
Rash	36	1	0	6	0	0
Fatigue	33	3	<1	26	1	0
Diarrhea	30	2	<1	16	1	0
Decreased appetite	29	1	0	10	0	0
Nausea	27	<1	<1	27	1	0
Cough	22	1	0	11	0	0
Dysgeusia	21	<1	0	5	0	0
Headache	19	<1	0	13	0	0
Decreased weight	19	1	0	5	0	0
Dyspnea	18	4	0	9	1	<1
Arthralgia	16	1	0	16	0	0
Anemia	16	5	1	4	<1	<1
Epistaxis	15	0	0	1	0	0
Vomiting	14	<1	<1	11	<1	0
Peripheral edema	14	1	0	6	<1	0
Pyrexia	14	<1	0	6	<1	0
Aspartate aminotransferase level increased	13	3	<1	6	1	0
Constipation	13	<1	0	11	<1	0
Hyperglycemia	13	4	<1	2	<1	0
Pneumonitis	12	3	0	0	0	0
Thrombocytopenia	12	2	1	<1	0	<1
Asthenia	12	2	0	3	0	0
Alanine aminotransferase level increased	11	3	<1	3	2	0
Pruritus	11	<1	0	3	0	0
Insomnia	11	<1	0	8	0	0
Back pain	11	0	0	8	1	0

Table 7:: Adverse Events Irrespective of Relationship to Study Treatment (Baselga 2012)

The quality of life data show a positive trend towards better results in the everolimus plus exemestane treatment arm. The adverse event profile of everolimus in this indication was similar to the well-known and established side effects seen in metastatic renal cell cancer and pNET indication. Stomatitis (8%), anemia, dyspnea, hyperglycemia, fatigue, AST increased and pneumonitis (3%) were the most common adverse events grade 3 / 4 (Baselga et al. 2012, Hortobagyi et al. 2011).

Based on these data, the FDA approved everolimus tablets (Afinitor®, Novartis Pharmaceuticals Corporation) for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer in combination with exemestane, after failure of treatment with letrozole or anastrozole (July 20, 2012). On July 23 2012 the combination of everolimus and exemestane was also approved by the European Medicines Agency EMA.

3.3.9.2 Tamoxifen

Tamoxifen is a non-steroidal agent with potent anti-estrogenic properties that competitively binds to estrogen receptors. It is approved for the treatment of both early and advanced estrogen-receptor positive breast cancer in pre- and post-menopausal women.

Combination Everolimus plus Tamoxifen

Efficacy and safety of everolimus in combination with tamoxifen were evaluated in a phase II trial for patients with hormone-receptor positive HER2-negative metastatic breast cancer resistant to aromatase inhibitors. The patients were randomized to receive either tamoxifen (20 mg/d) plus everolimus (10 mg/d) or tamoxifen alone (20 mg/d). Explorative analyses showed that both time to progression (TTP) and overall survival were significantly prolonged in the everolimus plus tamoxifen group as compared to the tamoxifen alone group.(Bachelot et al. 2012)

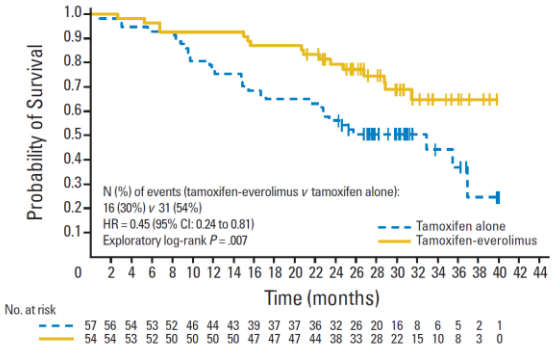


Figure 5: TTP Probability (Bachelot et al. 2012)

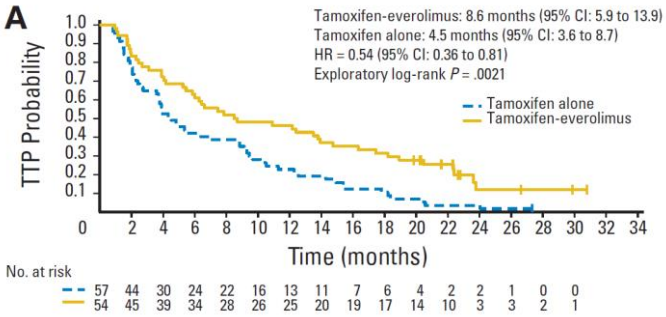


Figure 6: Probability of Survival (Bachelot et al. 2012)

Table 2. Adverse Events Experienced by ≥ 10% of Patients

	Tamoxifen Alone (n = 57)				Tamoxifen Plus Everolimus (n = 54)			
	Any Grade		Grade 3-4		Any Grade		Grade 3-4	
Adverse Event	No.	%	No.	%	No.	%	No.	%
Nonhematologic events								
Pain	49	86	10	18	44	82	5	9
Fatigue	30	53	6	11	39	72	3	6
Nausea	20	35	0	0	19	35	2	4
Stomatitis	4	7	0	0	30	56	6	11
Anorexia	10	18	2	4	23	43	4	7
Hot flashes	19	33	0	0	12	22	0	0
Infection	11	19	3	5	19	35	4	7
Rash	4	7	0	0	24	44	2	4
Diarrhea	6	11	0	0	21	39	1	2
Constipation	13	23	0	0	9	17	0	0
Vomiting	7	12	2	4	9	17	0	0
Pneumonitis	2	4	2	4	9	17	1	2
Hematologic events								
Decreased hemoglobin	20	35	2	4	37	69	1	2
Decreased leukocyte count	10	18	0	0	29	54	1	2
Decreased lymphocyte count	12	21	2	4	26	48	1	2
PNNs	11	19	3	5	26	48	1	2

NOTE. Adverse events listed regardless of relationship to study drug.
Abbreviation: PNN, polymorphonuclear neutrophil.

Table 8: Adverse Events experienced in >10% of Patients (Bachelot et al. 2012)

The AEs observed in the study were mostly grade 1 or 2 and consistent with those previously reported for tamoxifen and everolimus. Most common non-hematologic AEs besides pain were fatigue (72% tamoxifen plus everolimus vs. 53% tamoxifen alone), stomatitis (56% vs. 7%), rash (44% vs. 7%), anorexia (43% vs. 18%) and diarrhea (39% vs. 11%). Most common hematologic AEs were decreased hemoglobin (69% vs. 35%), decreased leukocyte counts (54% vs. 18%), and decreased lymphocyte counts (48% vs. 21%). Overall incidence of serious AEs was 32% in each group, and complete treatment discontinuation due to drug-related AEs was observed in 5 cases in the tamoxifen plus everolimus group and in 4 cases in the tamoxifen only group.

In summary, the combination of tamoxifen plus everolimus provides a suitable treatment option for patients with hormone-receptor positive metastatic breast cancer resistant to aromatase inhibitors, showing good efficacy combined with manageable toxicities.

3.3.9.3 Letrozole

Letrozole is a third generation non-steroidal aromatase inhibitor that competitively and reversibly binds to the active site of the enzyme. Letrozole is approved for the first-line treatment of postmenopausal women with hormone receptor positive locally advanced or metastatic breast cancer as it improves the time to progression compared to tamoxifen, (HR = 0.72, p < 0.0001, 907 patients), (Mouridsen 2003) and for the treatment of ABC in postmenopausal women with disease progression following antiestrogen therapy (Risk Ratio for TTP in favor of letrozole vs. megestrol acetate: 0.80; 95% CI 0.63 to 1.02; p = 0.07) (Dombernowsky 1998).

Combination Everolimus plus Letrozole

Safety and pharmacokinetics of the combined treatment with letrozole and everolimus was investigated in a phase I study in patients with metastatic breast cancer stable or progressing after 4 months or longer treatment with letrozole alone (Awada et al. 2008). Most common adverse events were stomatitis (50.0% of patients), fatigue (44.4%), anorexia and/or decreased appetite (44.4%), diarrhoea (38.9%), headache (33.3%) and rash (33.3%),

and the overall safety profile of the combination was consistent with that expected for everolimus monotherapy. Seven patients received the combination therapy for more than 6 months; one of these had a complete response, and one had a 28% reduction in liver metastases. The authors concluded that the daily therapy with everolimus plus letrozole is promising, with the results suggesting anti-tumour activity with no pharmacokinetic interactions, and recommended a daily dose of 10mg everolimus for further trials.

The combination of letrozole and everolimus (L-R) was also evaluated in a randomized double blind phase II trial against letrozole + placebo (L-P) as a 4-month neoadjuvant treatment for postmenopausal women with early BC (Baselga et al. 2009). 270 patients were enrolled in this trial (138 L-R vs. 132 L-P). Response rates on L-R and L-P were 68% vs. 59% (palpation, p = 0.062) and 58% vs. 47% (ultrasound, p= 0.021) respectively. Baselga et al. concluded that everolimus significantly increased the efficacy of letrozole in newly diagnosed hormone-receptor positive breast cancer in terms of clinical response rates.

Table 4. Calculated Overall Response in the Intent-to-Treat Population As Determined by Clinical Palpation, Ultrasound, and Mammography

Response by Evaluation Type	Treatment Arm: Everolimus + Letrozole (n = 138) No.	%	Placebo + Letrozole (n = 132) No.	%
Clinical palpation				
Complete response	18	13.0	12	9.1
Partial response	76	55.1	66	50.0
No change	34	24.6	39	29.5
Progressive disease	6	4.3	13	9.8
Not available/not assessable	4	2.9	2	1.5
Overall response*	94	68.1	78	59.1
95% CI		60.3 to 75.9		50.7 to 67.5
χ^2 test P		.0616		
Ultrasound				
Complete response	7	5.1	1	0.8
Partial response	73	52.9	61	46.2
No change	43	31.2	54	40.9
Progressive disease	4	2.9	9	6.8
Not available/not assessable	11	8.0	7	5.3
Overall response*	80	58.0	62	47.0
95% CI		49.7 to 66.2		38.5 to 55.5
χ^2 test P		.0352		
Mammography				
Complete response	13	9.4	8	6.1
Partial response	37	26.8	44	33.3
No change	57	41.3	54	40.9
Progressive disease	2	1.4	8	6.1
Not available/not assessable	29	21.0	18	13.6
Overall response*	50	36.2	52	39.4
95% CI		28.2 to 44.3		31.1 to 47.7
χ^2 test P		.7039		

NOTE. Tumor response was calculated by using measurements of tumor change from baseline, as measured by clinical palpation, ultrasound, and mammography. Responses were calculated from the 4-month assessment. If this assessment was missing, then the last nonmissing tumor assessment was used. Patients who had missing baseline or who had no postbaseline measurements were considered nonassessable. The 95% CIs were calculated by using the normal approximation to the binomial distribution. The χ^2 test without continuity correction (significance threshold: one-sided $P \leq .10$) was used.

*Overall response is complete plus partial responses.

Table 9: Calculated Overall Response in the Intent-to-Treat Population (Baselga 2009)

Table 5. Frequent Adverse Events by Preferred Safety Population

	Patients by Safety Population and Grade							
	Everolimus + Letrozole (n = 137)				Placebo + Letrozole (n = 132)			
	Overall		Grades 3 to 4		Overall		Grades 3 to 4	
Adverse Event	No.	%	No.	%	No.	%	No.	%
Any	123	89.8	31	22.6	84	63.6	5	3.8
Stomatitis	50	36.5	3	2.2	8	6.1	0	0
Rash	28	20.4	1	0.7	10	7.6	0	0
Asthenia	24	17.5	0	0	13	9.8	1	0.8
Hot flush	15	10.9	0	0	22	16.7	0	0
Hypercholesterolemia	22	16.1	1	0.7	8	6.1	0	0
Thrombocytopenia	25	18.2	2	1.5	1	0.8	0	0
Fatigue	17	12.4	2	1.5	6	4.5	0	0
Anorexia	17	12.4	0	0	5	3.8	0	0
Hyperglycemia	18	13.1	7	5.1	4	3.0	0	0
Headache	15	10.9	0	0	7	5.3	0	0
Increased ALT	16	11.7	2	1.5	5	3.8	0	0
Arthralgia	8	5.8	0	0	12	9.1	1	0.8
Pruritus	18	13.1	0	0	0	0	0	0
Anemia	16	11.7	0	0	1	0.8	0	0
Neutropenia	13	9.5	1	0.7	2	1.5	0	0
Dyspnea	10	7.3	1	0.7	2	1.5	0	0
Pneumonitis	4	2.9	3	2.2	0	0	0	0
Hypokalemia	4	2.9	2	1.5	1	0.8	0	0
Sleep disorder	3	2.2	1	0.7	2	1.5	0	0
Pneumonia	2	1.5	1	0.7	0	0	0	0
Mental disorder	2	1.5	1	0.7	0	0	0	0
Restlessness	2	1.5	1	0.7	0	0	0	0
Cellulitis	1	0.7	0	0	2	1.5	1	0.8

NOTE. Frequent adverse events were defined as those that occurred in at least 10% of patients, or in at least 1% of patients for grades 3 to 4 events.

Table 10: Frequent Adverse Events (Baselga 2009)

The most common grade 3/4 adverse events in the L-R arm were hyperglycemia (5%), stomatitis (2%), pneumonitis (2%), and infections (2%). All three cases of grade 3 pneumonitis completely resolved after discontinuation of everolimus. Daily therapy with everolimus plus letrozole (NSAI) showed no PK interaction. The safety profile was consistent with historical results of everolimus monotherapy; grades 3 to 4 adverse events occurred in 22.6% of patients who received everolimus and in 3.8% of patients who received placebo.

This trial demonstrated that everolimus significantly increased letrozole efficacy in patients with ER-positive breast cancer and has an acceptable level of tolerability in the neoadjuvant setting, justifying the application of this combined treatment. A daily dose of everolimus 10 mg was recommended for further trials (Baselga et al. 2009).

Combination Ribociclib plus Letrozole

The combination of ribociclib (600 mg) and letrozole (2,5 mg) was evaluated in the placebo controlled phase III MonaLEEsa-2 study (Hortobagyi et al. 2016). The combined treatment has shown that the duration of progression-free survival was significantly longer in the ribociclib group than in the placebo group (hazard ratio, 0,56; 95% CI, 0,43 to 0,72; P=3,29 x 10^{-6} for superiority). The median duration of follow-up was 15.3 months. After 18 months, the progression-free survival rate was 63% (95% confidence interval [CI], 54,6 to 70,3) in the ribociclib group and 42,2% (95% CI, 34,8 to 49,5) in the placebo group. In patients with measurable disease at baseline, the overall response rate was 52,7% and 37,1%, respectively (P<0,001).

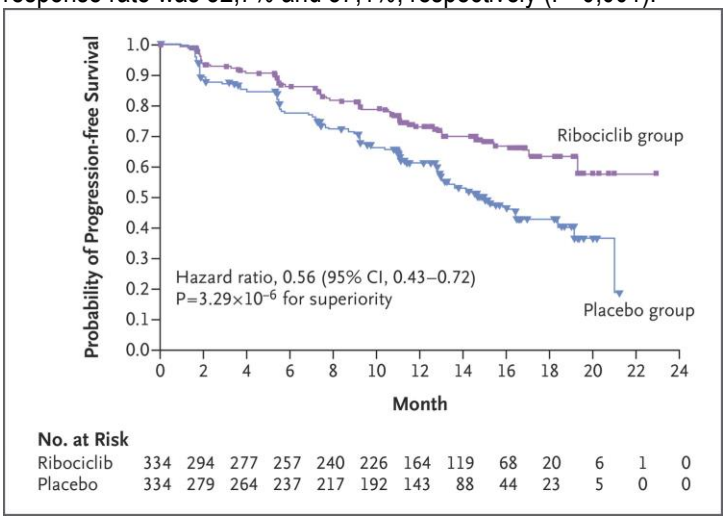


Figure 7: Kaplan-Meier Analysis of Progression-free Survival (Hortobagyi 2016)

In the safety population (334 patients in the ribociclib group and 330 in the placebo group), adverse events of any grade that occurred in at least 35% of the patients in either group were neutropenia (74,3% in the ribociclib group and 5,2% in the placebo group), nausea (51,5% and 28,5%, respectively), infections (50,3% and 42,4%), fatigue (36,5% and 30,0%, and diarrhea (35,0% and 22,1%) (TABLE 11). Nausea, infections, fatigue, and diarrhea were mostly grade 1 or 2. The most common grade 3 or 4 adverse events (≥5% of the patients in either group) were neutropenia (59,3% in the ribociclib group and 0,9% in the placebo group), leukopenia (21,0% and 0,6%, respectively), hypertension (9,9% and 10,9%), increased alanine aminotransferase level (9,3% and 1,2%), lymphopenia (6,9% and 0,9%), and increased aspartate aminotransferase level (5,7% and 1,2%). Febrile neutropenia occurred in 5 patients (1,5%) in the ribociclib group and in none in the placebo group.

Table 3. Adverse Events.*

Adverse Event	Ribociclib Group (N=334)			Placebo Group (N=330)†		
	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
	number of patients (percent)					
Any adverse event	329 (98.5)	221 (66.2)	50 (15.0)	320 (97.0)	105 (31.8)	3 (0.9)
Neutropenia‡	248 (74.3)	166 (49.7)	32 (9.6)	17 (5.2)	3 (0.9)	0
Nausea	172 (51.5)	8 (2.4)	0	94 (28.5)	2 (0.6)	0
Infections	168 (50.3)	12 (3.6)	2 (0.6)	140 (42.4)	7 (2.1)	1 (0.3)
Fatigue	122 (36.5)	7 (2.1)	1 (0.3)	99 (30.0)	3 (0.9)	0
Diarrhea	117 (35.0)	4 (1.2)	0	73 (22.1)	3 (0.9)	0
Alopecia	111 (33.2)	NA	NA	51 (15.5)	NA	NA
Leukopenia	110 (32.9)	66 (19.8)	4 (1.2)	13 (3.9)	2 (0.6)	0
Vomiting	98 (29.3)	12 (3.6)	0	51 (15.5)	3 (0.9)	0
Arthralgia	91 (27.2)	2 (0.6)	1 (0.3)	95 (28.8)	3 (0.9)	0
Constipation	83 (24.9)	4 (1.2)	0	63 (19.1)	0	0
Headache	74 (22.2)	1 (0.3)	0	63 (19.1)	1 (0.3)	0
Hot flush	70 (21.0)	1 (0.3)	0	78 (23.6)	0	0
Back pain	66 (19.8)	7 (2.1)	0	58 (17.6)	1 (0.3)	0
Cough	65 (19.5)	0	NA	59 (17.9)	0	NA
Anemia§	62 (18.6)	3 (0.9)	1 (0.3)	15 (4.5)	4 (1.2)	0
Decreased appetite	62 (18.6)	5 (1.5)	0	50 (15.2)	1 (0.3)	0
Rash	57 (17.1)	2 (0.6)	0	26 (7.9)	0	0
Increased alanine aminotransferase	52 (15.6)	25 (7.5)	6 (1.8)	13 (3.9)	4 (1.2)	0
Increased aspartate aminotransferase	50 (15.0)	16 (4.8)	3 (0.9)	12 (3.6)	4 (1.2)	0

* Listed are events that were reported in at least 15% of the patients in any group. One event of interest (hypertension) fell below the reporting threshold listed here. NA denotes not applicable, since grade 4 cough and grade 3 and 4 alopecia are not included in the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.
† Four patients who were randomly assigned to the placebo group did not receive either placebo or letrozole.
‡ Neutropenia includes a decreased neutrophil count and granulocytopenia.
§ This category includes both anemia and a decreased hemoglobin level.

Table 11: Adverse Events (Hortobagyi et al. 2016)

3.3.9.4 Anastrozole

Anastrozole is another third generation nonsteroidal aromatase inhibitor that – similar to letrozole - competitively and reversibly binds to the aromatase enzyme and is approved for treatment of primary and metastatic hormone-receptor positive breast cancer in both pre and post-menopausal women. Anastrozole has been shown to significantly prolong disease-free survival and time-to-recurrence as compared to tamoxifen in a large international double blind randomized phase III study (ATAC-study, Howell et al. 2005). In another large phase III study, anastrozole was compared to letrozole in postmenopausal patients with metastatic breast cancer that had progressed on tamoxifen. There were no significant differences between these two nonsteroidal aromatase inhibitors with regard to the primary endpoint time to progression or other efficacy measures, with the exception of overall response rate that was significantly higher for letrozole (Rose et al. 2003). In a randomized, open-label phase II trial anastrozole was compared to exemestane in patients with locally advanced or metastatic breast cancer. There were no statistically significant differences with regard to overall response rate, clinical benefit rate and time to progression, but the anastrozole group had numerically higher overall response and clinical benefit rates, as well as numerically longer median times to progression (Llombart-Cussac et al. 2012).

Combination Everolimus/Ribociclib plus Anastrozole

The combination of anastrozole plus everolimus/ribociclib has not been evaluated in a clinical study, but the similarity of the mechanism of aromatase inhibition between anastrozole and letrozole and the good clinical efficacy

data for anastrozole (equal to or even favourable when compared to exemestane or letrozole) justify the use of the combined treatment with everolimus and anastrozole as an alternative option in this study.

3.3.9.5 Fulvestrant

Fulvestrant is approved for the treatment of HR+ metastatic breast cancer in postmenopausal women with disease progression following anti-estrogen therapy (USA, Europe). However, also in postmenopausal women without symptomatic visceral disease also after reccurence or progression to an AI, current clinical practice and treatment guidelined include Fulvestrant as treatment option.
In postmenopausal patients who experienced progression after prior endocrine therapy (either AI or tamoxifen), fulvestrant 500 mg emerged as the optimal dose based on the results of the CONFIRM study, which showed that the higher dose (500 mg monthly) significantly prolonged PFS compared to the 250 mg dose. More specifically, subgroup analyses showed that PFS was prolonged in patients who had recurred during anti-estrogen therapy or during AI therapy although not reaching statistical significance for the latter. Median OS was 26,4 months for fulvestrant 500 mg and 22,3 months for 250 mg, irrespective of type of prior endocrine treatment.
Recently, data have been presented for the phase II randomized study (FIRST) of fulvestrant 500 mg compared to anastrazole as a first-line therapy in postmenopausal women with HR+ advanced breast cancer who received no prior endocrine therapy for advanced disease. The observed clinical benefit rate was 72,5 % for fulvestrant vs 67% for anastrozole. No significant difference was observed in ORR. The median Time to Progression (TTP) was significantly longer for fulvestrant than anastrozole.

Paloma-3 is a phase III trial that evaluated clinical efficacy and safety of the CDK4/6 inhibitor palbociclib in combination with fulvestrant. Fulvestrant plus palbociclib was associated with significant and consistent improvement in progression-free survival compared with fulvestrant plus placebo, irrespective of the degree of endocrine resistance, hormone-receptor expression level, and PIK3CA mutational status. The combination could be considered as a therapeutic option for patients with recurrent hormone-receptor-positive, HER2-negative metastatic breast cancer that has progressed on previous endocrine therapy.

3.4 Eribulin

3.4.1 Name, Chemical and Physical Information

HALAVEN 0.44 mg/ml solution for injection is a clear, colourless aqueous solution. One ml contains eribulin mesilate equivalent to 0.44 mg eribulin. Each 2 ml vial contains eribulin mesilate equivalent to 0.88 mg eribulin. This medicinal product contains small amounts of ethanol (alcohol), less than 100 mg per dose as well as water (for injections), hydrochloric acid (for pH-adjustment) and sodium hydroxide (for pH-adjustment).

3.4.2 Chemical Structure

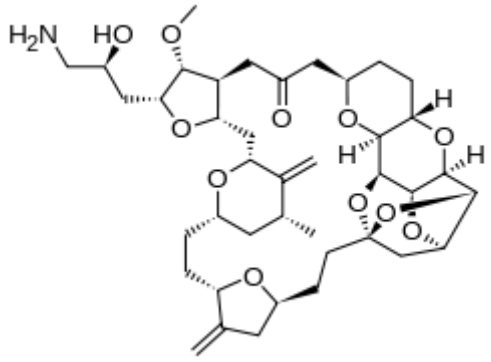


Figure 8: chemical structure of eribulin

3.4.3 Mechanism of Action

Eribulin is a non-taxane, microtubule dynamics inhibitor belonging to the halichondrin class of antineoplastic agents. It is a structurally simplified synthetic analogue of halichondrin B, a natural product isolated from the marine sponge *Halichondria okadai*.
Eribulin inhibits the growth phase of microtubules without affecting the shortening phase and sequesters tubulin into non-productive aggregates. Eribulin exerts its effects via a tubulin-based antimitotic mechanism leading to

G2/M cell-cycle block, disruption of mitotic spindles, and, ultimately, apoptotic cell death after prolonged mitotic blockage.

3.4.4 Experimental Non-clinical Data

Eribulin was not mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test). Eribulin was positive in the mouse lymphoma mutagenesis assay and was clastogenic in the *in vivo* rat micronucleus assay.
No carcinogenicity studies have been conducted with eribulin. An embryofoetal development study in rat confirmed the developmental toxicity and teratogenic potential of eribulin. Pregnant rats were treated with eribulin mesilate equivalent to 0.009, 0.027, 0.088 and 0.133 mg/kg eribulin at gestation days 8, 10 and 12. Dose related increased number of resorptions and decreased foetal weight were observed at doses ≥ 0.088 mg/kg and increased incidence of malformations (absence of lower jaw, tongue, stomach and spleen) was recorded at 0.133 mg/kg.

3.4.5 Eribulin Pharmacokinetics and Parmacodynamics

Distribution
The pharmacokinetics of eribulin are characterized by a rapid distribution phase followed by a prolonged elimination phase, with a mean terminal half-life of approximately 40 h. It has a large volume of distribution (range of means 43 to 114 l/m^2). Eribulin is weakly bound to plasma proteins. The plasma protein binding of eribulin (100-1000 ng/ml) ranged from 49% to 65% in human plasma.

Biotransformation
Unchanged eribulin was the major circulating species in plasma following administration of Ceribulin to patients. Metabolite concentrations represented <0.6% of parent compound, confirming that there are no major human metabolites of eribulin.
Elimination
Eribulin has a low clearance (range of means 1.16 to 2.42 l/h/m^2). No significant accumulation of eribulin is observed on weekly administration. The pharmacokinetic properties are not dose or time dependent in the range of eribulin doses of 0.22 to 3.53 mg/m^2.
Eribulin is eliminated primarily by biliary excretion. The transport protein involved in the excretion is presently unknown. Preclinical studies indicate that eribulin is transported by P-glycoprotein (Pgp). However, it is unknown whether Pgp is contributing to the biliary excretion of eribulin**.**

After administration of 14C-eribulin to patients, approximately 82% of the dose was eliminated in faeces and 9% in urine indicating that renal clearance is not a significant route of eribulin elimination. Unchanged eribulin represented most of the total radioactivity in faeces and urine.Eribulin is mainly (up to 70%) eliminated through biliary excretion. The transport protein involved in this process is unknown. Complete inhibition of the transport could in theory give rise to a more than 3-fold increase in plasma concentrations. It is not recommended to use substances which are inhibitors of hepatic transport proteins such as organic anion-transporting proteins (OATPs), Pgp, multidrug resistant proteins (MRPs) etc concomitantly with eribulin. Inhibitors of such transporters include but are not limited to: cyclosporine, ritonavir, saquinavir, lopinavir and certain other protease inhibitors, efavirenz, emtricitabine, verapamil, clarithromycin, quinine, quinidine, disopyramide etc. It cannot be excluded that concomitant treatment with inducing substances, such as carbamazepine, phenytoin, St John´s wort (Hypericum perforatum), could give rise to reduced plasma concentrations of eribulin, and co-administration with inducers should be carried out with caution considering a potential risk for reduced drug efficacy. No marked effects on eribulin exposure (AUC and Cmax) were observed during treatment with the CYP3A4 inducer rifampicin. However, rifampicin may due to its transporter inhibitory property counteract its possible inducing effect on eribulin elimination. Therefore, the effect of rifampicin may not presently be extrapolated to other inducers.
No drug-drug interactions are expected with CYP3A4 inhibitors. Eribulin exposure (AUC and Cmax) was unaffected by ketoconazole, a CYP3A4 inhibitor.

Effects of eribulin on the pharmacokinetics of other drugs
Eribulin may inhibit the important drug metabolising enzyme CYP3A4. This is indicated by in vitro data and no in vivo data is available. Concomitant use with substances that are mainly metabolised by CYP3A4 should be made with caution and it is recommended that the patient is closely monitored for adverse effects due to increased plasma concentrations of the concomitantly used substance. If the substance has a narrow therapeutic range, concomitant use should be avoided. Eribulin does not inhibit the CYP enzymes CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 2E1 at relevant clinical concentrations.

3.4.6 Safety

Haematology
Myelosuppression is dose dependent and primarily manifested as neutropenia. Monitoring of complete blood counts should be performed on all patients prior to each dose of eribulin. Treatment with eribulin should only be initiated in patients with ANC values ≥ 1500/µl and platelets > 100000/µl.
Febrile neutropenia occurred in < 5% of breast cancer patients treated with eribulin. Patients experiencing febrile neutropenia, severe neutropenia or thrombocytopenia, should be treated according to the recommendations in section 8.3.2.
Patients with ALT or AST >3 x ULN experienced a higher incidence of Grade 4 neutropenia and febrile neutropenia. Although data are limited, patients with bilirubin >1.5 x ULN also have a higher incidence of Grade 4 neutropenia and febrile neutropenia.Severe neutropenia may be managed by the use of G-CSF or equivalent at the physician's discretion in accordance with relevant guidelines.

Disseminated intravascular coagulation
Cases of disseminated intravascular coagulation have been reported, typically in association with neutropenia and/or sepsis.

Peripheral neuropathy
Patients should be closely monitored for signs of peripheral motor and sensory neuropathy. The development of severe peripheral neurotoxicity requires a delay or reduction of dose (see section 8.2.2)
In clinical trials, patients with pre-existing neuropathy greater than Grade 2 were excluded. However, patients with pre-existing neuropathy Grade 1 or 2 were no more likely to develop new or worsening symptoms than those who entered the study without the condition.
QT prolongation
In an uncontrolled open-label ECG study in 26 patients, QT prolongation was observed on Day 8, independent of eribulin concentration, with no QT prolongation observed on Day 1. ECG monitoring is recommended if therapy is initiated in patients with congestive heart failure, bradyarrhythmias, medicinal products known to prolong the QT interval, including Class Ia and III antiarrhythmics, and electrolyte abnormalities. Hypokalemia or hypomagnesemia should be corrected prior to initiating eribulin and these electrolytes should be monitored periodically during therapy. Eribulin should be avoided in patients with congenital long QT syndrome.

Hepatic impairment
A study evaluated the PK of eribulin in patients with mild (Child-Pugh A; n=7) and moderate (Child-Pugh B; n=4) hepatic impairment due to liver metastases. Compared to patients with normal hepatic function (n=6), eribulin exposure increased 1.8-fold and 3-fold in patients with mild and moderate hepatic impairment, respectively. Administration of eribulin at a dose of 0.97 mg/m^2 to patients with mild hepatic impairment and 0.62 mg/m^2 to patients with moderate hepatic impairment resulted in a somewhat higher exposure than after a dose of 1.23 mg/m^2 to patients with normal hepatic function. Eribulin was not studied in patients with severe hepatic impairment (Child-Pugh C). There is no study in patients with hepatic impairment due to cirrhosis.

Renal impairment
A study in patients with different degrees of impaired renal function showed that the exposure of eribulin in patients with moderate renal function (creatinine clearance ≥ 40 to 59 ml/min, n=6) was similar to patients with normal renal function while the exposure in patients with severe impairment was increased by 75% (creatinine clearance < 40 ml/min, n=4).

Elderly population
In studies of 1,222 patients treated with eribulin, 244 patients (20.0%) were > 65 - 75 years of age and 66 patients (5.4%) were > 75 years of age. Among the 827 of these patients who received the recommended dose of eribulin in the Phase 2/3 breast cancer studies, 121 patients (14.6%) were > 65 - 75 years of age and 17 patients (2.1%) were > 75 years of age. The safety profile of eribulin in elderly patients (> 65 years of age) was similar to that of patients ≤ 65 years of age. No dose adjustments are recommended for the elderly population.

Pregnancy
There are no data from the use of eribulin in pregnant women. Eribulin is embryotoxic, foetotoxic and teratogenic in rats. Eribulin should not be used during pregnancy and women of childbearing potential must be advised to avoid becoming pregnant whilst they are receiving eribulin by using effective contraception during and up to 3 months after treatment.

For the most up to date safety information, please refer to the most current edition of the Investigator's Brochure.

3.4.7 Marketing Authorization Status

Eribulin is indicated for the treatment of patients with locally advanced or metastatic breast cancer who have progressed after at least one chemotherapeutic regimen for advanced disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting unless patients were not suitable for these treatments.

3.4.8 Pharmaceutical Data

Supply:
The investigator will prescribe eribulin according to the label. Any change of label will be communicated. The IMP Eribulin for all other patients not covered by the label will be provided by Eisai to the participating sites in the study setting and will be administrated as i.v. injection at the study site. Eisai GmbH is responsible for shipment of the IMP eribulin to the clinical trial centers. Investigator's request of eribulin will be done through the eCRF (initial order automatically with recruitment, follow up orders as required).

Packaging:
5 ml type I glass vial, with teflon-coated, butyl rubber stopper and flip-off aluminium over seal, containing 2 ml of solution. The usual pack sizes are cartons of 6 vials, single vials will be available as well.

Labeling:
Packages will be signed with the warning "Zur klinischen Prüfung vorgesehen".

Stability:
Unopened vials are sable for 4 years when stored under the storage conditions described below.

Storage:
This medicinal product does not require any special storage conditions. From a microbiological point of view unless the method of opening precludes the risk of microbial contamination the product should be used immediately. If not used immediately eribulin as the undiluted solution in a syringe should not normally be stored longer than 4 hours at 25°C and ambient lighting, or 24 hours at 2°C - 8°C, in-use storage times and conditions are the responsibility of the user. Diluted solutions of eribulin (0.018 mg/ml to 0.18 mg/ml eribulin in sodium chloride 9 mg/ml (0.9%)) solution for injection should not be stored longer than 24 hours at 2°C - 8°C, unless dilution has taken place in controlled and validated aseptic conditions.
The storage temperature must be recorded weekly in the temperature log.

3.5 Concomitant treatment of neutropenia with lipegfilgrastim

3.5.1 *Indication, agent, dose regimens*

Lipegfilgrastim is used to reduce the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes). Lipegfilgrastim should be used in center´s standard practice according to general recommendations *[Smith 2006]* using G-CSF derivates, unless patients have known hypersensitivity to the active substance (lipegfilgrastim) or to any of the excipients or other G-CSF or derivates severe, untreated hypokalaemia (hypokalaemia must be corrected prior to initiating treatment with lipegfilgrastim), severe thrombozytopenia, myelogenous leukaemia or splenomegalia evidence of pulmonal infiltrates or pneumonia or ARDS (Adult Respiratory Distress Synome) sickle cell anaemia.

One 6 mg dose of lipegfilgrastim (a single pre-filled syringe of Lonquex) is recommended for each chemotherapy cycle, given approximately 24 hours after cytotoxic chemotherapy via the subcutaneous route. In clinical studies with a limited number of elderly patients, there was no relevant age-related difference with regard to the efficacy or safety profiles of lipegfilgrastim. Therefore, no adjustment of the dose is necessary for elderly patients. No recommendation on a posology can be made for patients with renal or hepatic impairment.

3.5.2 Warnings and Precautions

Haematopoietic system

Treatment with lipegfilgrastim does not preclude thrombocytopenia and anaemia caused by myelosuppressive chemotherapy. Lipegfilgrastim may also cause reversible thrombocytopenia. Regular monitoring of the platelet count and haematocrit is recommended. Special care should be taken when administering single or combination chemotherapeutic medicinal products that are known to cause severe thrombocytopenia. Leukocytosis may occur. No adverse events directly attributable to leukocytosis have been reported. Elevation in white blood cells (WBC) is consistent with the pharmacodynamic effects of lipegfilgrastim. A WBC count should be performed at regular intervals during therapy owing to the clinical effects of lipegfilgrastim and the potential for leukocytosis. If WBC counts exceed 50000 x 10/µl after the expected nadir, lipegfilgrastim should be discontinued immediately.

Splenic adverse reactions
Frequent but generally asymptomatic cases of splenomegaly and infrequent cases of splenic rupture, including fatal cases, have been reported following administration of G-CSF or derivatives. Spleen size should therefore be carefully monitored (e.g. clinical examination, ultrasound). A diagnosis of splenic rupture should be considered in patients reporting left upper abdominal pain or shoulder tip pain.

Pulmonary adverse reactions
Pulmonary adverse reactions, in particular interstitial pneumonia, have been reported after administration of lipegfilgrastim. Patients with a recent history of pulmonary infiltrates or pneumonia may be at higher risk.
The onset of pulmonary symptoms such as cough, fever and dyspnoea in association with radiological signs of pulmonary infiltrates and deterioration in pulmonary function together with an increased neutrophil count may be preliminary signs of Acute Respiratory Distress Syndrome (ARDS). In such circumstances lipefilgrastom should be discontinued at the discretion of the physician and appropriate treatment given.

Patients with sickle cell anaemia
Sickle cell crisis has been associated with the use of G-CSF or derivatives in patients with sickle cell anaemia. Physicians should therefore exercise caution when administering Lonquex in patients with sickle cell anaemia, monitor appropriate clinical parameters and laboratory results and be attentive to the possible association of lipegfilgrastim with splenic enlargement and vaso-occlusive crisis.

Hypokalaemia
Hypokalaemia may occur. For patients with increased risk on hypokalaemia due to underling disease or co-medications, it is recommended to monitor the serum potassium level carefully and to substitute potassium if necessary.
Excipients with known effect
This medicinal product contains sorbitol. Patients with rare hereditary problems of fructose intolerance should not use this medicinal product. This medicinal product contains less than 1mmol sodium (23mg) per pre-filled syringe, i.e. essentially 'sodium-free'.

Interaction with other medicinal products and other forms of interaction
Due to the potential sensitivity of rapidly dividing myeloid cells to cytotoxic chemotherapy, lipegfilgrastim should be administered approximately 24 hours after administration of cytotoxic chemotherapy. Concomitant use of lipegfilgrastim with any chemotherapeutic medicinal product has not been evaluated in patients. In animal models, concomitant administration of G-CSF and 5-fluorouracil (5-FU) or other antimetabolites has been shown to potentiate myelosuppression. The safety and efficacy of lipegfilgrastim have not been evaluated in patients receiving chemotherapy associated with delayed myelosuppression, e.g. nitrosoureas. The potential for interaction with lithium, which also promotes the release of neutrophils, has not been specifically investigated. There is no evidence that such an interaction would be harmful.

3.5.3 *Supply*

After randomization treatment with lipegfilgrastim can be started. Lipegfilgrastim may be prescribed according to its official approval.

3.5.4 *Marketing Authorization Status*

In July 2013 the EMA (European Medicines Agency) approved lipegfilgrastim for reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes).

3.5.5 *Safety*

Please refer to the current version of the SPC of lipegfilgrastim.
In addition, it is possible that unknown adverse reactions that are not yet listed in the current SPC of lipegfilgrastim

could occur. It is not possible to predict in advance if any adverse drug reactions will occur but, if they do, appropriate care will be provided to participants.

3.5.6 *Pharmaceutical Data*

Packaging and formulation:
Lonquex is a sterile, preservative free solution for injection, containing 6mg of lipegfilgrastim active pharmaceutical ingredient at a concentration of 10mg/mL. The concentration is declared based on protein content. Lonquex is presented in a 1mL type I glass pre-filled syringe to be stored in refrigerator. The solution is formulated with sodium acetate (formed by titrating acetic acid with sodium hydroxide), Sorbitol and Polysorbate 20. The pH is adjusted to 5.0.

Labeling and storage:
Store in a refrigerator (2 °C – 8 °C). Do not freeze. Keep the pre-filled syringe in the outer carton in order to protect from light. Lonquex may be removed from the refrigerator and stored below 25 °C for a maximum single period of up to 3 days. Once removed from the refrigerator, the medicinal product must be used within this period or disposed of.

Preparation and administration:
The solution should be visually inspected before use. Only clear, colourless solutions without particles should be used. The solution should be allowed to reach a comfortable temperature (15 °C - 25 °C) for injection. Vigorous shaking should be avoided. Excessive shaking may aggregate lipegfilgrastim, rendering it biologically inactive. Lonquex does not contain any preservative. In view of the possible risk of microbial contamination, Lonquex syringes are for single use only. Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

3.6 Drug Accountability

The investigator will keep an account of the trial medication and acknowledge the receipt of all shipments of the trial medication. The investigator will also keep accurate records of the quantities of trial medication dispensed, used, and returned by each subject. At the end of the trial, all unused trial medication and all medication containers will be destroyed at the investigator´s site. It will be assured that a final report of the drug accountability is prepared and maintained by the investigator. The site monitor will check this at the close out visit.

On receipt of study medication the following data are to be recorded: Confirmation of receipt including date, amount, batch number, information on potential damage and signature of the responsible person. With every shipment Novartis or Eisai will provide an appropriate form, which must be filled out, faxed back to Novartis/ Eisai and filed in the ISF.

Drug accountability is documented in the eCRF.

The following data are to be recorded:

- On dispensing study medication to the patient: patient identification number, date, batch number, number of tablets, signature of the responsible staff member.
- When study medication/packages are returned to the site: Date, number of tablets returned, signature of staff member, who received the medication/packages.
- On obliteration of the study medication: Amount, date and signature of the responsible staff member.

To assess treatment compliance (please refer also to section 8.2.1.6) patients are asked to present all tablets not yet used for tablet counts on every visit to the study site. At the end of the treatment period patients must return all unused tablets.

3.7 Safety

With respect to safety, it is possible that unknown adverse reactions that are not yet listed in the respective SPCs of the standard therapies could occur. It is not possible to predict in advance if any adverse drug reactions will occur but, if they do, appropriate care will be provided to participants.

4 TRIAL DESIGN

This is a prospective, multi-center, open-label, phase II study.

4.1 Number of Clinical Trail Centers planned to be involved

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

4.2 Number of Participating Patients

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

- anticipated number of at least 2000 patients with HER2-negative metastatic breast cancer that are to be screened for CTCs in the DETECT IV and related DETECT III trial (which focuses on patients with HER2-negative primary tumor and HER2-positive CTCs)
- 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 HER2-negative CTCs in 70% of CTC-positive patients (conservative estimate based on Fehm et al. 2010)

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:
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.

Eribulin cohort:
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 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.

4.3 Schedule

The individual study participation begins with the screening visit, consists of endocrine therapy plus everolimus/ribociclib or therapy with eribulin (depending on the treatment cohort) and ends with disease progression, the patient‘s death or with completion of the follow-up period. The estimated maximum duration of individual study participation is 36 months and 3 weeks both for the everolimus/ribociclib cohort and the eribulin cohort.

Patient participation is divided in 3 consecutive periods:

Pre-Treatment Evaluation Period:

Content:	Examinations and collection of data relevant to decide on patients‘ eligibility for further study participation. Informed consent 1
Period:	From screening to inclusion
Maximum duration:	3 weeks
Visits:	From Screening Visit to end of Inclusion Visit

Treatment Period:

Content:	Endocrine therapy combined with everolimus/ribociclib or chemotherapy with eribulin.

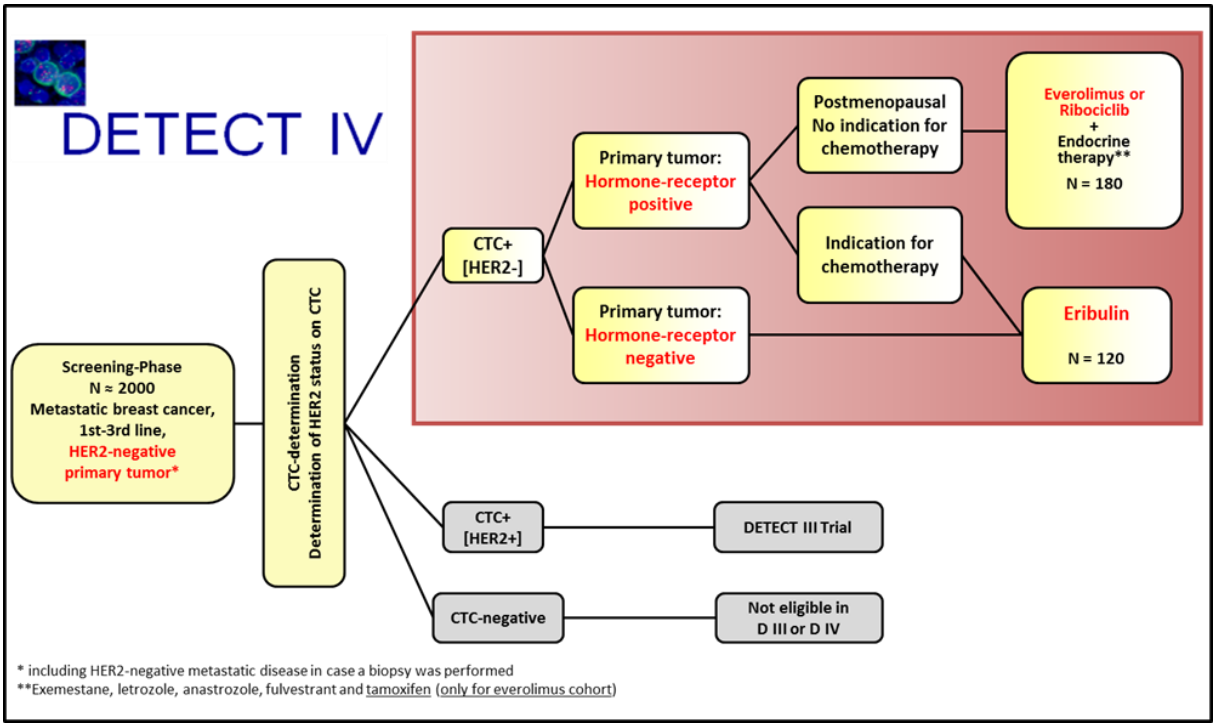
	Assessment of efficacy and safety data. Informed consent 2 and 3
Period:	From inclusion until disease progression or occurrence of other criteria for treatment discontinuation
Maximum duration:	12 months* (both for the everolimus/ribociclib cohort and the eribulin cohort) or until progress / death or discontinuation due to toxicities or other reasons
Visits:	From Inclusion Visit to Conclusion Visit (4 weeks after last application of everolimus/ eribulin)

*After treatment period the treatment with everolimus/ribociclib or eribulin can be extended if medically indicated. Patients will be followed up for toxicity and efficacy in such cases. The drug will be supplied by Novartis (everolimus/ribociclib), respectively Eisai (eribulin) on study terms for the duration of the individual study participation (including 2-year-period of follow-up assessments).

Follow-Up period:

Content:	Assessment of survival, adverse events and concomitant medication. Treatment of the metastatic disease at the investigator's discretion
Period:	From end of study treatment until the patient has either died or completed the 24-months follow-up period
Maximum duration:	24 months (estimation)
Visits:	From Conclusion Visit of the Treatment Period to the last Follow-Up Visit

Figure 9: Clinical Trial Design



Entire Study:

The study starts when the necessary approval has been given by the competent authority (BfArM). Furthermore the relevant ethics committee must have given a favorable opinion on study conduct before the first patient is included and consecutive all local ethical committees of the participating sites have to give their approval before the participation of one site.

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.

4.4 Inclusion

Patients with HER2-negative metastatic breast cancer who fulfill all inclusion criteria and do not meet any of the exclusion criteria will be recruited into this study.

5 STUDY POPULATION

Everolimus/ribociclib cohort
Postmenopausal women with hormone-receptor positive, HER2-negative metastatic breast cancer and evidence of ≥ 1 exclusively HER2-negative CTC in 7.5 ml blood according to Veridex technology.

Eribulin cohort
Women with hormone-receptor positive, HER2-negative metastatic breast cancer and indication to chemotherapy or with triple-negative metastatic breast cancer both with evidence of ≥ 1 exclusively HER2-negative CTC in 7.5 ml blood according to Veridex technology.

5.1 Eligibility Criteria

In General for both study cohorts

1. Metastatic breast cancer, which cannot be cured by surgery or radiotherapy. The primary tumor and/or biopsies must have been confirmed as cancer by histolopathology.
2. 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.: immunohisto-chemistry (IHC) score 0-1+ or 2+ and fluorescent in situ hybridization (FISH) negative or just FISH negative, whichever was performed) in all tissue samples.
3. 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).
4. HER2 negativity of all detected CTCs.
5. 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.
6. Written informed consent in study participation.
7. Undergoing a re-biopsy prior to inclusion if tissue is accessible, which can be safely biopsied, is optional but desirable.
8. Tumor evaluation has been performed within 6 weeks before date of recruitment and results are available.
9. 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.
10. Age ≥ 18 years.
11. ECOG Performance Status ≤ 2.

Everolimus/ribociclib cohort (DIVa)	Eribulin cohort (DIVb)
• 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.	• 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:

• 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) • 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	- 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

Ineligibility Criteria

Patients who fulfill any of the following criteria are not eligible for recruitment:

Exclusion Criteria

In General for both study cohorts

Treatment with other investigational agents of any type or anticancer therapy during the trial, within 2 weeks prior to the start of treatment.

1. Adverse events due to prior anticancer therapy which are > Grade 1 (NCI CTCAE) and therapeutically relevant at time of treatment start.
2. Known HIV infection.
3. Current active hepatitis B or C, clinically 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).
4. 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.
5. Other malignant diseases within the last 3 years (apart from carcinoma in situ of the cervix or non-melanoma skin cancer)
6. 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.
7. Life expectancy < 3 months.
8. 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.

There will be NO EXCEPTIONS to eligibility requirements at the time of recruitment into the study. Questions about eligibility criteria should be addressed PRIOR to requesting recruitment.
The eligibility criteria for this study have been carefully considered. Eligibility criteria are standards used to ensure that patients who enter this study are medically appropriate candidates for this therapy. For the safety of the patients, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions are made to these criteria for admission to the study.

6 PRE-TREATMENT EVALUATION

(cf. APPENDIX II – PATIENT EVALUATION FLOW SHEET)

6.1 In General

During the pre-treatment evaluation period there are **two visits**. The first visit is the Screening Visit; the second

one is the Inclusion Visit. Both are preceded by a specific informed consent.

The **Screening Visit** is only performed for CTC count and determination of the HER2 status of CTC. Thus, it is avoided to bother the patient with detailed information on the whole study before the CTC count and HER2 status is known. Blood sampling for CTC count should be scheduled at least one week after last application of investigational agents of any type or anticancer therapy.

The **Inclusion Visit** is only scheduled for a patient if CTCs are detectable and HER2 status on CTCs is negative. It serves to perform the required baseline examinations, to check the in- and exclusion criteria and to assign the patient to protocol treatment.

Corresponding to the arrangement of the visits, patient information and informed consent takes place in two steps (see section 16.4). The visits during the pre-treatment evaluation period are only performed if patients are left sufficient time to make their decision on study participation.
With the exception of quality of life assessments and the blood samplings for the examination of CTC and translational medical investigations all procedures planned in the pre-treatment period are part of the clinical routine usual in patients with metastasizing breast cancer.

6.2 Actions taken on Screening Visit

- Informed consent for blood sampling for CTC count and determination of HER2 status 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, menopausal status)**
- Hormone receptor status found in solid tumor tissue (estrogen and progesterone status each graded positive or negative)*
- HER2 status on primary tumor tissue and/or biopsies from metastatic sites or locoregional recurrences determined by ICH and/or FISH*
- Information on metastases: date of diagnosis of metastases*, localization*, bone/visceral/other*, multiple/single*, surgical therapy**
- Information on primary tumor: date of primary tumor diagnosis*, stage of primary breast cancer*, localization of primary breast cancer**, surgical therapy**
- Information on palliative therapy: number of prior chemotherapy lines for metastatic disease*, type(s) of palliative therapy lines**, endocrine therapy**, therapy with bisphosphonates** or Denosumab**.
- Information on adjuvant/neoadjuvant therapy**

- Blood sampling for CTC count and assessment of HER2 status on CTC (***Screening Kit****)
    - If CTC count is negative study participation is terminated.
    - If HER2 status on CTC is positive study participation within DETECT III trial may be possible (please check for eligibility an ineligibility criteria).
    - 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.

6.3 Actions taken on Inclusion/Recruitment Visit

- ***Note on Time schedule****:* Examinations and data collections for the so-called Inclusion/ Recruitment Visit are not necessarily to be performed on one single day, but can be done on several days over the following period of time: ***From obtaining informed consent - part 2 to day 21 after Screening Visit at the latest.*** Please

observe also the time intervals allowed for the results of several examinations (see TABLE 12 below). Results obtained before Inclusion/ Recruitment Visit may be employed if they meet the given time interval. However, the given flexibility during the Inclusion/ Recruitment Visit requires exact documentation of time and date of every result.

- If HER2 status on CTC is negative, informed consent in study participation is obtained (see section 17.4 and 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 (see section 17.4 and 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.
- 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 patient 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 (if applicable)
- 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 or eribulin to patients according to the treatment plan (section 8).
- Assessment of adverse events

Investigation		**Time interval before re-cruitment**
Blood Sampling with Screening Kit (Screening Visit) for CTC assessment	- Total number per 7,5 mL - HER2 status	3 weeks (Results must be available prior to IC part 2 and prior to start of Inclusion Visit)
Hematology	- Hemoglobin - Hematocrit - Red blood cell count - Differential white blood cell count - Platelet count	7 days
Biochemistry	- Total and direct bilirubin - ALT - AST - Serum creatinine - Albumin - BUN or urea	7 days

	- Fasting cholesterol and triglycerides (Everolimus/ribociclib cohort only) - Fasting glucose (Everolimus cohort only) - Sodium, potassium, calcium(ribociclib-cohort only) - Potassium and Magnesium (Eribulin-cohort)	
Pregnancy test	- (Eribulin cohort only)	7 days
Tumor evaluation (see section 10.2.1)	- Mandatory imaging	6 weeks
	- Other imaging as necessary to document all sites of disease	
	- Clinical lesion status	
	- Tumor markers (mandatory: CA15-3; optional: CEA, CA125)	
Cardiac investigations	- 12-lead ECG	3 weeks
Quality of life questionnaires	- EORTC QLQ-C30 - EORTC QLQ-BR23	1 week

Table 12: Information on special investigations during Pre-Treatment Period

7 RECRUITMENT PROCEDURE

If a patient meets all inclusion criteria and none of the exclusion criteria at Inclusion / Recruitment Visit she will be enrolled into the trial.

Online enrolment is possible 24 hours a day. The registration form of the eCRF has been saved, to complete the procedure. The enrolment lists will be kept in safe and confidential custody at Alcedis GmbH.

8 TREATMENT PLAN

8.1 General

Protocol treatment: During the treatment period all postmenopausal, hormone-receptor positive patients receive a standard endocrine therapy and the IMP everolimus/ribociclib (see section 8.2), all triple-negative and hormone-receptor positive patients with indication to chemotherapy receive the IMP eribulin (see section 8.3).

During the follow-up period treatment is at the discretion of the responsible investigator (see section 12.1.3 “Therapy after End of Protocol Treatment”).

8.2 Treatment with Everolimus

8.2.1 Drug Dispense, Administration and Treatment Regimens

8.2.1.1 Investigational drugs

The investigational drug used in the course of this trial is commercially available everolimus in combination with standard endocrine therapy.

The investigator will prescribe everolimus + exemestane according to the label. Everolimus in combination with any other endocrine regimen will be provided by Novartis to the participating sites in the study and will be dispensed by the study center personnel on an outpatient basis. Endocrine therapy in this case has to be prescribed according to study settings (see TABLE 13 below).

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.

8.2.1.2 Treatment arms

This is a non-randomized Phase II study.

8.2.1.3 Treatment assignment

All postmenopausal, hormone-receptor positive patients without indication to chemotherapy will be assigned to treatment with everolimus and standard endocrine therapy.

8.2.1.4 Dispensing the study drug

The investigator will prescribe everolimus + exemestane according to the label. In combination with any other endocrine regimen everolimus will be provided by Novartis to the participating sites in the study setting and will be dispensed by the study center personnel on an outpatient basis. All dosages prescribed to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

8.2.1.5 Study drug compliance

The investigator and/or study personnel will assess compliance at each patient visit. To accurately determine the patient's drug exposure throughout the study, the following information must be reported on the Drug Administration Record CRF pages for both everolimus and standard endocrine therapy and in the source document.

- Planned dose administration
- Actual total daily dose administered
- Start and end date of drug administration
- Dose change (no or yes)
- Reason for dose change (e.g. adverse event, dosing error, lab test abnormality etc.)

8.2.1.6 Patient Compliance

Compliance with daily everolimus is very important to the conclusions of this study. To assess treatment compliance patients are asked to present all tablets not yet used on every visit to the study site. At the end of the treatment period patients must return all unused tablets. Patients are instructed to notify the investigator of any missed doses. The investigator will make tablet counts at each patient visit during treatment. The tablet counts are to be documented in the eCRF. Dates of missed doses are also to be recorded on the eCRF.

8.2.1.7 Study drug accountability

The investigator or designee must maintain an accurate record of the shipment and dispensing of only for study drugs supplied by Novartis in a drug accountability log (please refer to section 3.4.). The field monitor will note drug accountability during site visits and at the completion of the study. Patients will be asked to return all unused Novartis-supplied study drugs and packaging on an ongoing basis or at the time of study drug discontinuation.

8.2.1.8 Disposal and destruction

The drug supply can be destroyed at the local site. Destruction must be recorded in the local Drug Accountability Log, and will be controlled by the field monitor.

8.2.1.9 Instructions for use of study drug

All patients will receive everolimus + standard endocrine therapy. All patients will take everolimus orally per day. All patients will also take standard endocrine therapy once daily (dosage according to the label, see * TABLE 13 below).

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 5 mg daily is allowed if medically indicated. The decision for treatment of the patient with everolimus will be made independently of the study.**

Table 13: Recommended treatment regimen for combination with everolimus

On treatment Day 1, patients will be provided or prescribed study drugs for self-administration at home. Enough tablets should be provided or prescribed to cover administration until next scheduled visit plus one week at minimum.

Everolimus will be dosed starting on treatment Day 1 (Inclusion / Recruitment Visit). 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 (refer to TABLE 13 above). Package insert instructions should be followed.

Everolimus should be swallowed whole with a glass of water and the tablets should not be chewed or crushed and grapefruit or citrus juices must be avoided for everolimus administration. If the tablets cannot be swallowed, the tablets should be disintegrated in approximately 30 ml of water. Immediately prior to administration, the contents should be stirred gently until the tablets have disintegrated into a suspension. The patient should then drink the contents. Afterwards, the glass should be rinsed with an additional 30 ml of liquid and drunk by the patient. If vomiting occurs no attempt should be made to replace the dose.

Everolimus and standard endocrine therapy will be taken daily from Inclusion / Recruitment Visit, Day 1 until disease progression, unacceptable toxicity, death, and withdrawal from the study for any other reason.

The End of treatment (EOT) is defined as the last date that the patient has taken the study drug everolimus, excluding an everolimus interruption for < 4 weeks. 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.

The investigator should promote compliance by instructing the patient to take the study drugs exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of the study. The patient should be instructed to contact the investigator if she is unable for any reason to take the study drug as prescribed.

All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

8.2.2 Dose Adjustments

In General

Doses may be held or reduced for hematologic (see section 8.2.3) and other adverse events (see section 8.2.4). Dose adjustments are to be made according to the system showing the greatest degree of toxicity. Once dose is reduced due to adverse events, patients should not be re-challenged to a higher dose level.

Dose reductions or delays will be considered for the standard endocrine treatment and everolimus and may occur independently.

Due to the wide spectrum of feasible combinations of standard endocrine treatment and everolimus dose reductions and delays of standard endocrine treatment should follow general GCP criteria for the application of antineoplastic drugs (Repetto et al. 2003, NCCN-Guidelines) and should be made according to the SPC and/or investigator's brochure. The latest versions of the SPCs ("Fachinformation" according to German Drug Law) of the standard therapy will be provided on the DETECT IV website or by contact the DETECT Study Office in Ulm (Univeristäts-frauenklinik Ulm, Studienzentrale, Prittwitzstr. 43, 89075 Ulm, Tel: 0731/500 58520, Fax: 0731/500 58526, Email: studienzentrale.ufk@uniklinik-ulm.de).

Subjects who require permanent discontinuation of one of the products due to unacceptable toxicities may switch to another treatment regime of the standard endocrine treatment if medically reasonable.

The antineoplastic treatment of a patient can be postponed for up to 4 weeks to allow resolution of toxicity. The investigator must consult the DETECT IV Study Office prior continuing therapy for any patient requiring a delay of study treatment of more than two weeks for any reason.

All patients who require permanent discontinuation of both everolimus and the endocrine agent in a given treatment combination will be withdrawn from the treatment intervention schedule (start of the 2-year follow-up period).

8.2.2.1 Permitted study drug dose adjustments and interruptions

For patients who are unable to tolerate the protocol-specified dosing scheme, dose adjustments and interruptions are permitted in order to keep the patient on the study drugs. The following guidelines should be followed.

These changes must be recorded on the Dosage Administration Record eCRF.

For patients with start dose of 5 mg daily and well tolerated treatment, increasing of everolimus dose to 10 mg daily is recommended according to the approved label.

Treatment with everolimus and standard endocrine therapy should continue as long as clinical benefit is observed or until unacceptable toxicity occurs. Management of severe and/or intolerable suspected adverse events may require temporary dose reduction and/or interruption.

For patients who do not tolerate the protocol-specified everolimus dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study. The following guidelines need to be applied:
Dose adjustments are permitted for any adverse event suspected to be related to everolimus in those patients unable to tolerate their individual once daily oral dose. If administration of everolimus must be interrupted because of unacceptable toxicity, everolimus dosing will be interrupted or modified according to the guidelines in TABLE 14, TABLE 15 and TABLE 16 below.
In addition, if any surgery is planned, everolimus dosing should be interrupted one week prior to surgery and should be re-started as soon as possible after wound healing.

If everolimus dosing is interrupted due to toxicity, everolimus should not be resumed unless recovery to grade ≤1 is achieved in less than 4 weeks. Then everolimus could be reintroduced at the initial dose or a lower dose level depending on the toxicity type and grade(see TABLE14 , TABLE 15 and TABLE 16 below).

All study drug interruptions or dose modifications must be recorded on the Dosage Administration Record page of the eCRF.

Dose level	Dose and schedule
0 = starting dose	Last administered daily dose
-1 dose level	5 mg daily (if starting dose 10 mg daily) 5 mg every other day (if starting dose 5 mg daily).

Minimal dose is 5 mg every other day.

Table 14: Everolimus dose reduction

If a patient has already decreased everolimus intake to 5 mg every other day, no further dose reduction is permitted. Patients requiring an additional everolimus dose reduction will be required to discontinue study treatment.

Patients who interrupt everolimus therapy for more than 4 weeks must be discontinued from the study.

TABLE 15 and TABLE 16 provide the procedure to be followed for everolimus dose modification in the event of toxicities suspected to be related to the everolimus treatment. Included are also instructions for re-initiation of everolimus dosing once sufficient recovery of toxicity is seen.

Toxicity	Actions
Metabolic events (e.g. Hyper-glycemia, Hyperlipidemia and/ or hypertriglyceridemia)	**Grade 1:** No dose adjustment required. Initiate an appropriate medical therapy an monitor. **Grade 2:** No dose adjustment required. Initiate an appropriate medical therapy an monitor. **Grade 3:** Interrupt everolimus until recovery to grade ≤1. Reintroduce everolimus at the next lower dose level. . Initiate an appropriate medical therapy an monitor **Grade 4:** Discontinue everolimus treatment
Stomatitis	**Grade 1:** No dose adjustment required. **Grade 2:** Interrupt everolimus until resolution to ≤ grade 1. Restart at the same dose. If stomatitis recurs at grade 2, interrupt dose until recovery to grade ≤ 1. Restart at a lower dose. **Grade 3:** Interrupt everolimus until recovery to grade ≤1. Reintroduce everolimus at the next lower dose level **Grade 4:** Discontinue everolimus treatment
Pneumonitis	**Grade 1:** No dose adjustment required. Initiale appropriate monitoring. **Grade 2:** Consider interruption of therapy, rule out infection and consider treatment with cortico-steroids until symptoms resolve to ≤ grade 1. Restart at a lower dose. Discontinue treatment if failure to recover within 4 weeks. **Grade 3:** Discontinue everolimus, rule out infection and consider treatment with corticosteroids until symptoms resolve to ≤ grade 1. Consider restart at a lower dose level. Discontinue treatment if failure to recover within 4 weeks. **Grade 4:** Discontinue everolimus treatment rule out infection and consider treatment with cortico-steroids.

Other toxicities	**Grade 1:** It toxicity is tolerable, no dose adjustment required. **Grade 2:** It toxicity is tolerable, no dose adjustment required. It toxicity becomes intolerable, interrupt everolimus until resolution to ≤ grade 1. Restart at the same dose. If toxicity recurs at grade 2, interrupt dose until recovery to grade ≤ 1. Restart at a lower dose. **Grade 3:** Interrupt everolimus until recovery to grade ≤1. Reintroduce everolimus at the next lower dose level **Grade 4** Discontinue everolimus treatment
Toxicity requiring interruption for >4 weeks	Permanently discontinue treatment.

No specific dose adjustments are recommended for Grade 1 toxicity. However, physicians should always manage patients according to their medical judgment based on the particular clinical circumstances.

Table 15: Everolimus dose modification guidelines for non-hematologic toxicities

Toxicity	Actions
Thrombocytopenia **Platelet count**	• **≥ 75000/mm³:** No change • **50000/ mm³ to 75000/ mm³** Hold everolimus treatment until recovery to ≥ 75000/mm³ Reintroduce everolimus at the same dose level • **< 50000/ mm³** Hold everolimus treatment until recovery to ≥ 75000/mm³ Reintroduce everolimus at the next lower dose level, if available.
Absolute Neutrophil count (ANC)	• **≥ 1000/ mm³** : No change • **500/ mm³ to 1000/ mm³** Hold everolimus treatment until recovery to ≥ 1000/ mm³ Reintroduce everolimus at the same dose level • **< 500/ mm³** Hold until recovery to ≥ 1000/ mm³. Reintroduce everolimus (at the next lowest dose level, if available.
Febrile neutropenia	Hold further dosing until ANC ≥ 1250/mm³ and no fever. Then resume dosing at the next lower dose level, if available.
Toxicity requiring interruption for > 4 weeks	Permanently discontinue everolimus treatment

Physicians should always manage patients according to their medical judgment based on the particular clinical circumstances.

Table 16: Everolimus dose modification guidelines for hematologic toxicities

8.2.3 Known undesirable effects of the study drugs

Adverse events most frequently observed with everolimus are rash, stomatitis / oral mucositis, non-infectious pneumonitis, fatigue, headache, anorexia, nausea, vomiting, diarrhea, and infections. Overall, the most frequently observed laboratory abnormalities include neutropenia, thrombocytopenia, hypercholesterolemia, and/or hypertriglyceridemia. The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2).

Recommendations for dose asjustments, should any of these treatment-related adverse events occur, are given in TABLE 14, TABLE 15 and TABLE 16.

8.2.3.1 Management of specific toxicities

Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to everolimus should be treated using local supportive care. Please note

that investigators in earlier trials have described the oral toxicities associated with everolimus as mouth ulcers, rather than mucositis or stomatitis. If examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. Please, follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers:

- For mild toxicity (grade 1), use conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.
- For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol) with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (Kenacort® A Orabase®).
- Agents containing hydrogen peroxide, iodine and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.
- Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (Ketoconazole, Fluconazole, Itraconazole, etc.) should be avoided in all patients due to their strong inhibition of everolimus metabolism, therefore leading to higher everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

Stomatitis/oral mucositis should be appropriately graded using the functional grading given on the NCI-CTC for Adverse Events, version 4.03.

Management of infections

Everolimus is an immunosuppressant. Patients taking everolimus are therefore at an increased risk of infection. In oncology patients, some infections have been severe and rarely have had a fatal outcome. Physicians should be aware of the increased risk of infection, and should warn patients and their caregivers to be vigilant for signs and symptoms of infection, and to seek medical attention immediately should such signs or symptoms occur. Should an infection occur, anti-infectives should be prescribed as clinically appropriate, and in the case of clinically significant infection, consideration should be given to withholding study medication until resolution of the infection.

Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Grade 2 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or Grade 2 or higher hypertriglyceridemia (>2.5 x ULN) should be treated with a statin (HMG-CoA reductase inhibitor), fibrate, or appropriate lipid-lowering medication in addition to diet. Patients should be monitored clinically and through serum biochemistry as required in the product label/data sheets for HMG-CoA reductase inhibitors.

Note: Concomitant therapy with fibrates and HMG-CoA reductase inhibitors are associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatine kinase (CPK) levels and myoglobinuria, acute renal failure and sometimes death. The risk versus benefit ratio should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia.

Hyperglycemia has been observed in patients receiving everolimus therapy. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Optimal glycemic control should be achieved before starting trial therapy.

Management of diarrhea

Appearance of diarrhea attributed to everolimus toxicity may be treated with Loperamide. Other medications for diarrhea may be used as needed.

Management of non-infectious pneumonitis

Clinically significant pneumonitis is typically accompanied by non-specific symptoms including dyspnea, nonproductive cough, fatigue and fever.

Worst Grade	Recommended Investigations	Management of Pneumonitis	Everolimus Dose Adjustment
Grade 1	CT scans with lung windows. Repeat chest x-ray/CT scan every 12 weeks until return to baseline.	No therapy required	Administer 100% of everolimus dose.
Grade 2	CT scan with lung windows. Repeat CT scan at least every 12 weeks until return to within normal limits. Consider bronchoscopy with biopsy and /or BAL.	Symptomatic only. Consider corticosteroids if cough is troublesome.	Consider interruption of everolimus until recovery to ≤ Grade 1. Study. Restart treatment at a reduced dose. Patients will discontinue everolimus if they fail to recover to <Grade 1 within 4 weeks
Grade 3	CT scan with lung windows. Repeat at least every 8 weeks until return to within normal limits. Bronchoscopy with biopsy and/or BAL is recommended.	Prescribe corticosteroids if infective origin ruled out. Taper as medically indicated.	Hold everolimus treatment until recovery to ≤Grade 1. May restart treatment within 4 weeks at a reduced dose (by one level**) if evidence of clinical benefit.
Grade 4	CT scan with lung windows and required pulmonary function testing including spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 8 weeks until return to within normal limits. Bronchoscopy with biopsy and/or BAL is recommended if possible.	Prescribe corticosteroids if infective origin ruled out. Taper as medically indicated.	Discontinue everolimus treatment.

Table 17: Management of non-infectious pneumonitis

Both asymptomatic radiological changes (Grade 1) and symptomatic non-infectious pneumonitis (Grade 2 = not interfering with activities of daily living and Grade 3 = interfering with activities of daily living and oxygen indicated) have been noted in patients receiving everolimus therapy. Non-infectious pneumonitis has been associated with everolimus and other mTOR inhibitors (Atkins et al. 2004). If non-infectious pneumonitis develops, a consultation with a pulmonologist should be considered. If the patient develops Grade 3 pneumonitis, treatment with everolimus must be interrupted and the patient treated as medically indicated (short course corticosteroids, oxygen, etc.). Management of non-infectious pneumonitis suspected to be associated with everolimus (TABLE 17) and dose modification instructions are provided in TABLE 14 , TABLE 15 and TABLE 16.

8.3 Treatment with Ribociclib

8.3.1 Drug Dispense, Administration and Treatment Regimens

8.3.1.1 Investigational drugs regimen will be provided by Novartis to the participating

The investigational drug used in the course of this trial is ribociclib in combination with standard endocrine therapy. Ribocilcib in combination with any endocrine regimen will be provided by Novartis to the participating sites in the study and will be dispensed by the study center personnel on an outpatient basis. Endocrine therapy in this case has to be prescribed according to study settings (see TABLE 18 below).

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.

8.3.1.2 Treatment arms

This is a non-randomized Phase II study.

8.3.1.3 Treatment assignment

All postmenopausal, hormone-receptor positive patients without indication to chemotherapy will be assigned to treatment with ribociclib and standard endocrine therapy.

8.3.1.4 Dispensing the study drug

In combination with any endocrine regimen ribocilib will be provided by Novartis to the participating sites in the study setting and will be dispensed by the study center personnel on an outpatient basis. All dosages prescribed to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

8.3.1.5 Study drug compliance

The investigator and/or study personnel will assess compliance at each patient visit. To accurately determine the patient's drug exposure throughout the study, the following information must be reported on the Drug Administration Record CRF pages for both everolimus and standard endocrine therapy and in the source document.

- Planned dose administration
- Actual total daily dose administered
- Start and end date of drug administration
- Dose change (no or yes)
- Reason for dose change (e.g. adverse event, dosing error, lab test abnormality etc.)

8.3.1.6 Patient Compliance

Compliance with daily ribociclib is very important to the conclusions of this study. To assess treatment compliance patients are asked to present all tablets not yet used on every visit to the study site. At the end of the treatment period patients must return all unused tablets. Patients are instructed to notify the investigator of any missed doses. The investigator will make tablet counts at each patient visit during treatment. The tablet counts are to be documented in the eCRF. Dates of missed doses are also to be recorded on the eCRF.

8.3.1.7 Study drug accountability

The investigator or designee must maintain an accurate record of the shipment and dispensing of only for study drugs supplied by Novartis in a drug accountability log. The field monitor will note drug accountability during site visits and at the completion of the study. Patients will be asked to return all unused Novartis-supplied study drugs and packaging on an ongoing basis or at the time of study drug discontinuation.

8.3.1.8 Disposal and destruction

The drug supply can be destroyed at the local site. Destruction must be recorded in the local Drug Accountability Log, and will be controlled by the field monitor.

8.3.1.9 Instructions for use of study drug

All patients will receive ribociclib + standard endocrine therapy. All patients will take ribociclib orally per day. All patients will also take standard endocrine therapy once daily (dosage according to the label, see TABLE 18)

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

Table 18: Recommended treatment regimen for combination with ribociclib

On treatment Day 1, patients will be provided or prescribed study drugs for self-administration at home. Enough tablets should be provided or prescribed to cover administration until next scheduled visit plus one week at minimum.

Ribociclib will be dosed starting on treatment Day 1 (Inclusion / Recruitment Visit). Patients will be instructed to take the ribociclib capsule(s) orally with a large glass of water once daily at the same time each day with or without food.

Exemestane / letrozole / anastrozole / fulvestrant will be dosed starting on treatment Day 1 according to the recommended daily dosage (refer to TABLE 18 above). Package insert instructions should be followed.

Patients should be instructed to take the study drug combination of ribociclib and standard endocrine therapy with a large glass of water (~250 mL) at the same time each day. Evening doses are strongly not recommended.

- Ribociclib can be admistered with or without food; however dietary habits aroud the time of dosing should be as consistent as possible throughout the study.

- Patients should be instructed to swallow the ribociclib whole and not to chew or crush them.

- If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next schedule dose. The occurrence and frequency of any vomiting during a treatment cycle must be noted in the adverse events section in the eCRF.

- Any doses that are missed (not taken within 6 hours of the intended time) should be skipped and should not be replaced or made up on subsequent day.

- Patients must avoid consumption of grapefruit, grapefruit hybrids, pummelos, starfruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medications, due to potential CYP3A4 interaction with the study medications.

Note: Orange juice is allowed.

1. No herbal or dietary supplements are permitted.

2. Multivitamis are permitted.

Ribociclib and standard endocrine therapy will be taken daily from Inclusion / Recruitment Visit, Day 1 until disease progression, unacceptable toxicity, death, and withdrawal from the study for any other reason.

The End of treatment (EOT) is defined as the last date that the patient has taken the study drug ribociclib, excluding an ribociclib interruption for < 4 weeks. 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.

The investigator should promote compliance by instructing the patient to take the study drugs exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of the study. The patient should be instructed to contact the investigator if she is unable for any reason to take the study drug as prescribed.

All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

8.3.2 Dose Adjustments

In General

Dose adjustments are to be made according to the system showing the greatest degree of toxicity. Once dose is reduced due to adverse events, patients should not be re-challenged to a higher dose level.

Dose reductions or delays will be considered for the standard endocrine treatment and ribociclib and may occur independently.

Due to the wide spectrum of feasible combinations of standard endocrine treatment and ribociclib dose reductions and delays of standard endocrine treatment should follow general GCP criteria for the application of antineoplastic drugs (Repetto et al. 2003, NCCN-Guidelines) and should be made according to the SPC and/or investigator's brochure. The latest versions of the SPCs ("Fachinformation" according to German Drug Law) of the standard therapy will be provided on the DETECT IV website or by contact the DETECT Study Office in Ulm (Univeristäts-frauenklinik Ulm, Studienzentrale, Prittwitzstr. 43, 89075 Ulm, Tel: 0731/500 58520, Fax: 0731/500 58526, Email: studienzentrale.ufk@uniklinik-ulm.de).

Subjects who require permanent discontinuation of one of the products due to unacceptable toxicities may switch to another treatment regime of the standard endocrine treatment if medically reasonable.

The antineoplastic treatment of a patient can be postponed for up to 4 weeks to allow resolution of toxicity. The investigator must consult the DETECT IV Study Office prior continuing therapy for any patient requiring a delay of study treatment of more than two weeks for any reason.

All patients who require permanent discontinuation of both ribociclib and the endocrine agent in a given treatment combination will be withdrawn from the treatment intervention schedule (start of the 2-year follow-up period).

8.3.2.1 Permitted study drug dose adjustments and interruptions

For patients who are unable to tolerate the protocol-specified dosing scheme, dose adjustments and interruptions are permitted in order to keep the patient on the study drugs. The following guidelines should be followed.

These changes must be recorded on the Dosage Administration Record eCRF.

Treatment with ribociclib and standard endocrine therapy should continue as long as clinical benefit is observed or until unacceptable toxicity occurs. Management of severe and/or intolerable suspected adverse events may require temporary dose reduction and/or interruption.
For patients who do not tolerate the protocol-specified ribociclib dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study. The following guidelines need to be applied:
All study drug interruptions or dose modifications must be recorded on the Dosage Administration Record page of the eCRF.

	Dose	**Number of tablets & strength**
Starting dose	600 mg/d	3 x 200 mg tablets
First Dose reduction	400 mg/d	2 x 200 mg tablets
Second dose reduction	200 mg/d	1 x 200 mg tablets

Table 19: Ribociclib Dose Modification guidelines

If a patient has already decreased ribociclib intake to 200 mg every other day, no further dose reduction is permitted. Patients requiring an additional ribociclib dose reduction will be required to discontinue study treatment.

Patients who interrupt ribociclib therapy for more than 4 weeks must be discontinued from the study.

TABLE 20, TABLE 21, TABLE 22 and TABLE 23 provide the procedure to be followed for ribociclib dose modification in the event of toxicities suspected to be related to the ribociclib treatment. Included are also instructions for re-initiation of ribociclib dosing once sufficient recovery of toxicity is seen.

Toxicity	Actions
HEPATOTOXICITY (BILIRUBIN; SGPT/ALT, SGOT/AST)	
TOTAL BILIRUBIN without ALT/AST increase above baseline value	**Grade 1 (> ULN-2.0 x ULN) :** Maintain dose level with LFTs monitored bi-weekly **Grade 2 (> 2.0 ULN - 3.0 x ULN):** Dose interruption of ribociclib If resolved to ⩽ grade 1 in ≤ 21 days, then maintain dose level If resolved to ≤ grade 1 in > 21 day or toxicity recurs, then reduce 1 dose level Repeat liver enzymes and bilirubin tests twice weekly for two weeks after dose resumption If toxicity recurs after two dose reductions, discontinue ribociclib **Grade 3 (> 3.0 ULN -10.0 x ULN)::** Dose interruption of ribociclib If resolved to ≤ grade 1 in ≤ 21 days, lower 1 dose level of ribociclib Repeat liver enzymes and bilirubin tests twice weekly for two weeks after dose resumption If resolved to ≤ grade 1 in > 21 days or toxicit recurs, discontinue ribociclib treatment **Grade 4 (> 10.0 ULN):** Discontinue ribociclib treatment
Confounding factors and/or alternative causes for increase of total bilitubin should be excluded before dose interruption/reduction. They include but are not limited to: evidence of obstruction, such as elevated ALP and GGT typical of gall bladder or bile duct disease, hyperbilirubinemia de to the indirect component (i.e. direct bilirubin component ≤ 1x ULN) due to hemolysis or Gilbert Syndrome, pharmocologic treatment, viral hepatitis, alcoholic or autoimmune hepatits, other hepatotoxic drugs. For patients with Gilbert Syndrome, these dose modifications apply to change in direct bilirubin only. Bilirubin will be fractionated if elevated.	
AST or ALT without bilirubin elevation > 2 x ULN	**Same grade as baseline or increase from baseline grade o to grade 1 (confirmed 48-72 h later) :** No dose adjustment required with LFTs monitored per protocol if same grade as baseline or bi-weekly in case of increase from baseline grade 0 to 1. **Increase from baseline 0 or 1 to grade 2 (> 3.0 – 5.0 x ULN):** Dose interruption of ribociclib If resolved to ≤ baseline grade in ≤ 21 days or toxicity recurs, then reduce 1 dose level Repeat liver enzyme and bilirubin tests twice weekly for 2 weeks after dose resumption If toxicity recurs after two dose reductions or recovery to ≤ baseline grade is > 28 days, discontinue ribociclib **Increase from baseline 0 or 1 to grade 3 (> 5.0 – 20.0 x ULN):** Dose interruption of ribociclib until resolved to ≤ baseline until resolved to ≤ basline grade, then lower 1 dose level of ribociclib Repeat liver enzyme ans bilitubin tests twice weekly for two weeks after dose resumption If recovery to ≤ baseline grade > 28 days, discontinue ribociclib If toxicity recurs, discontinue ribociclib **Increase from baseline from grade 2 to grade 3 (> 5.0 – 20.0 x ULN):** Dose interruption of ribociclib until resolved to ≤ baseline grde, than lower 1 dose lever of ribociclib Repeat liver enzyme ans bilirubin tests twice weekly for two wetoeks after dose resumption If toxicity recurs after two dose reductions or recovery to ≤ baseline grade is > 28 days, discontinue **Grade 4 (> 20.0 x ULN):** Discontinue ribociclib
AST or ALT and concurrent Bilirubin	**For patients with normal ALT and AST and total bilirubin at baseline: AST or ALT > 3.0 ULN combined with total bilirubin > 2 x ULN without evidence of cholestasis** **OR**

	For patients with elevated AST or ALT or total bilirubin at baseline: baseline: (AST or ALT > 2 x baseline AND > 3.0 x ULN) OR (AST or ALT 8.0 x ULN) – whichever is lower - -combined with (total bilirubin > 2 x baseline AND ≥ 2.0 ULN) Discontinue ribociclib treatment

Cinfounding factors and /or altenative causes for increased transaminases should be excluded before dose interruption/reduction. They include but are not limited to: concomitant medications, herbal preparations or dietary supplements, infection, hepato-biliary disorder or obstruction, new or progressive liver metastases, and alcohol intake.

Table 20: Ribociclib dose adjustment and management recommendation for hepatic toxicities

Toxicity	
Thrombocytopenia **Platelet count**	• **Grade 1 (≥ 75 x 10^9):** No change • **Grade 2 (50 x 10^9 – 75 x 10^9)** Dose interruption until recovery to grade ≤ 1 Reintroduce ribociclib at the same dose level • **Grade 3 (25 x 10^9 – 50 x 10^9)** Dose interruption until recovery to grade ≤ 1 Reintroduce ribociclib at the same dose level If toxicity recurs at grade 3: temporary dose interruption until recovery to grade ≤ 1 and reduce ribociclib to the next lowev dose level. • **Grade 4 (<25 x 10^9)** Dose interruption until recovery to grade ≤ 1 Reintroduce ribociclib at the next lower dose level. If toxicity recurs at grade 4: discontinue ribociclib.
Absolute Neutrophil count (ANC)	• **Grade 1 (≥ 1,5 x 10^9)**: No change • **Grade 2 (1,5 x 10^9 - 10^9)**: No change • **Grade 3 (0,5 x 10^9 – 1 x 10^9)**: Dose interruption until recovery to ⩾ 1.0 x 10^9/L Reindroduce ribociclib at the same dose level. If toxicity recurs at grade 3: temporary dose interruption until recovery to ≥ 1.0 x 10^9/L If resolved in ≤ 7 days, then maintain dose level. If resolved in > 7 days, then reduce ribociclib dose to the next lower dose level. • **Grade 4 (<0,5 x 10^9)**: Dose interruption until recovery to ≥ 1.0 x 10^9/L Reindroduce ribociclib at the next lower dose level. If toxicity recurs at grade 4: temporary dose interruption until recovery to ≥ 1.0 x 10^9/L And reduce ribociclib at the next lower dose level.
Febrile neutropenia	• **Grade 3 (ANC < 1.0 x 10^9/L with a single temperature of > 38.3˚ C or a sustained temperature of ≥ 38˚ for more than one hour)**: Dose interruption until improvement of ANC ≥ 1.0 x 10^9/L and no fever. Restart at the next lower dose level. If febrile neutropenia recurs, discontinue ribociclib. • **Grade 4 (Life-threatening consequences; urgent intervention indicated):** Discontinue ribociclib.
Anemia (Hemoglobin)	• **Grade 1 (⩾ 10.0 x LLN g/dl)**: No change • **Grade 2 (≥ 8.0 - < 10 x LLN g/dl)**: No change • **Grade 3 (< 8.0 x LLN g/dl)**:

Dose interruption until recovery to grade ≤ 2.
Reintroduce ribociclib at the same dose.

- **Grade 4 (Life-threatening consequences; urgent intervention indicated):**

Discontinue ribociclib

Physicians should always manage patients according to their medical judgment based on the particular clinical circumstances.

Table 21: Ribociclib dose asjustment and management recommendations for hematological adverse reactions

Additional follow up for QTc prolongation	
For All Grades	• Check the quality of the ECG and the QT value and repeat if it needed • Perform analysis of serum electrolytes (K+, Ca++, Phos, Mg++). If outside of the normal range, interrupt ribociclib administration, correct with supplements or appropriate therapy as soon as possible, and repeat electrolytes until documented as normal. • Review concomitant medication usage for the potential to inhibit CYP3A4 and/or to prolong the QT interval. • Check compliance with correct dose and administration of ribociclib. • Consider collecting a time matched PK sample; record date and time of last study drug intake
1* QTc 450-480 ms	No change
2* QTc 481-500 ms	Interrupt ribociclib. Perform a repeat ECG within one hour of the first QTcF of ⩾ 481 ms. If QTcF < 481 ms, restart ribociclib at the same dose. No dose adjustment required for first occurrence. If QTcF remains ⩾ 481 ms, repeat ECG as clinically indicated until the QTcF returns to < 481 ms. restart ribociclib at the same dose. No dose adjustment required for first occurrence. If QTcF ⩾ 481 ms recurs, ribociclib should be reduced by 1 dose level. Repeat ECGs 7 days and 14 days after dose resumption (then as clinically indicated) for any patients who had therapy interrupted due to QTcF ≥ 481 ms
3 QTc ⩾ 501 ms on at least two separate ECGs	Interrupt ribociclib. Transmit ECG immediately and confirm prolongation/abnormalities. Perform a repeat ECG within one hour of the first QTcF of ⩾ 501 ms. If QTcF remains ⩾ 501 ms, consult with a cardiologist (or qualified specialist) and repeat cardiac monitoring as indicated until the QTcF returns to < 481 ms. If QTcF returns to < 481 ms, ribociclib will be reduced by 1 dose level. Repeat ECGs 7 days and 14 days after dose resumption (then as clinically indicated) for any patients who had therapy interrupted due to QTcF ⩾ 501 ms If QTcF of ⩾ 501 ms recurs, discontinue ribociclib.
4* [QT/QTc ⩾ 501 or > 60 ms change from baseline] and [Torsades de pointes or polymorphic ventricular tachycardia, or signs/symptoms of serious arrhythmia]	Discontinue ribociclib. • Obtain local cardiologist (or qualified specialist) consultation and repeat cardiac monitoring as indicated until the QTcF returns to <481 ms.

*All values refer to the average of triplicate measurements

Table 22: Ribociclib dose adjustment and management recommendation for all other adverse reactions

8.3.3 Guidance for all other adverse reactions

Consider performing an analysis of serum potassium, calcium, phosphorous, and magnesium for all adverse reactions, if indicated. If electrolyte values are outside of the normal range, interrupt ribociclib administration, correct electrolytes with supplements or appropriate therapy as soon as possible, and repeat electrolyte testing until documented normalization of the electrolytes.

Patients who experience renal impairment (not due to other contributing factors) of grade 2 or higher during the treatment period should discontinue treatment and should be followed for safety assessments.

For all other adverse events please follow recommendations in Table 22.

Grade	Ribociclib dose adjustment and management recommendation
1	No dose adjustment recommended. Initiate appropriate medical therapy and monitor.
2	Dose interruption until recovery to grade ≤1. Initiate appropriate medical therapy and monitor. Re-initiate ribociclib at the same dose. If the same toxicity recurs at grade 2, interrupt ribociclib until recovery to grade ≤1. Re-initiate ribociclib at the next lower dose level.
3	Dose interruption until recovery to grade ≤1. Initiate appropriate medical therapy and monitor. Re-initiate ribociclib at the next lower dose level. If toxicity recurs at grade 2: temporary dose interruption until recovery to grade ≤1 and reduce ribociclib dose the next lower dose level. If toxicity recurs at grade 3, discontinue ribociclib.
4	Discontinue ribociclib and treat with appropriate medical therapy.

Table 23: Ribociclib dose adjustment and management recommendation for all other adverse reactions

8.3.3.1 Management of specific toxicities

Additional follow-up for hepatic toxocities
Increase in transaminases combined with total bilirubin (TBIL) increase may be indicative of drug-induced liver injury (DILI), and should be considered as clinically important events.

The threshold for potential DILI may depend on the patient's baseline AST/ALT and TBIL value; patients meeting any of the following criteria will require further follow-up as outlined below:
- For patients with normal ALT und AST and TBIL value at baseline: AST or ALT > 3.0 x ULN combined with TBIL > 2.0 x ULN
- For patients with elevated AST or ALT or TBIL value at baseline: (AST or ALT > 2 x baseline AND > 3.0 x ULN) OR (AST or ALT > 8.0 x ULN), whichever is lower combined with (TBIL > 2 x baseline AND > 2.0 x ULN)

Medical review needs to ensure that liver test elevations are not caused by cholestasis, defined as: ALP elevation > 2.0 x ULN with R value < 2 in patients without bone metastasis, or elevation of ALP liver fraction in patients with bone metastasis.

Note: (The R value is calculated by dividing the ALT by the ALP, using multiples of the ULN for both values. It denotes the relative pattern of ALT and/or ALP elevation is due to cholestatic or hepatocellular liver injury or mixed type injury)

In the absence of cholestasis, these patients should be immediately discontinued from study drug treatment, and repeat LFT testing as soon as possible, preferably within 48 hours from the awareness of the abnormal results. The evaluation should include laboratory tests, detailed history, physical assessment and the possibility of liver metastasis or new liver lesions, obstructions/compressions, etc.

Hepatic toxicity monitoring includes the following LFTs: albumin, ALT, AST, total bilirubin, direct and indirect bilirubin, alkaline phosphatase (fractionated if alkaline phosphatase is grade 2 or higher), creatine kinase, prothrombin time (PT)/INR and GGT. For patients with Gilbert Syndrome: total and direct bilirubin must be monitored, intensified monitoring applies to changes in direct bilirubin only.

Close observation is recommended in case of AST, ALT, and/or bilirubin increase requiring dose interruption, which involves:

1. Repeating liver enzyme and serum bilirubin tests **two or three times weekly**. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or return to normal values.
2. Obtaining a more detailed history of current symptoms.

3. Obtaining a more detailed history of prior and/or concurrent diseases, including history of any pre-existing liver conditions of risk factors.
4. Obtaining a history of concomitant drug use (including non-prescription medication, herbal and dietry supplemets), alcochol use, recreational drug use, and special diets.
5. Ruling out acute viral hepatitis types A, B, C, D, and E; hepatotropic virus infections (CMV, EBV or HSV); autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
6. Obtaining a history of exposure to environmental chemical agents.
7. Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
8. Considering gastroenterology or hepatology consultations.
9. Assessing cardiovascular dysfunction of impaired liver oxygenation, including hypotension or right heart failure as possible etiologies for liver dysfunction.
10. Liver biopsy as clinically indicated to assess pathological change and degree of pontetial liver injury.

All cases confirmed on repeat testing meeting the laboratory criteria defined above, with no other alternative cause for LFT abnormalities identified, should be considered as “medically significant”, thus met the definition of SAE (Section 11.2), and reported as SAE using the term “potential drug-induced liver injury”. All events should be followed up with the outcome clearly documented.

Exemestane
The most frequently reported adverse effects for exemestane are gastrointestinal disturbances, hot flushes, arthralgia, myalgia, sweating, fatigue and dizziness. Other reported effects include headache, insomnia, somnolence, depression, skin rashes, alopecia, asthenia and leg oedema. Thrombocytopenia and leucopenia have been reported occasionally. Reductions in bone mineral density can occur with long-term use of exemestane (Cheung et al. 2012). Refer to the package insert of the local supply of exemestane for more details.

Letrozole
The most frequently reported adverse effects for letrozole are hot flushes, night sweats, arthralgia, myalgia vaginal dryness, asthenia.. Other reported effects include depression, headache, dizziness, gastrointestinal disturbances, alopecia, skin rash osteoporosis, peripheral edema. Leucopenia, cataract, vaginal bleedings and insomnia have been occasionally reported.Refer to the package insert of the local supply of letrozole for more details.

Anastrozole
The most frequently reported adverse effects for anastrozole are hot flashes, night sweats. Other reported effects include arthralgia, myalgia vaginal dryness, asthenia, headache, dizziness, gastrointestinal disorders, alopecia, skin rash osteoporosis, peripheral edema .Vaginal bleedings, carpal tunne syndrome and insomnia, have been reported occasionally. Refer to the package insert of the local supply of anastrozole for more details.

Tamoxifen
The most frequently reported adverse effects for tamoxifen are hot flushes, night sweats, vaginal discharge. Other reported effects include Thromboembolic events, ischemic cerebrovascular events, head ache, dizziness, skin rash, peripheral edema, gastrointestinal disorders, cataract, retinopathy, occasionally endometrial cancer and ovarian cysts, neutropenia and thrombocytopenia. Refer to the package insert of the local supply of tamoxifen for more details.

Fulvestrant
The most common clinically significant adverse reactions occuring in ≥5% of patients receiving fulvestrant 5 mg were: injection site reactions, nausea, bone pane, arthralgia, hedache, back pain, fatigue, pain in extremity, hot flash, vomiting, anorexia, asthenia, musculoskeletal pain, cough, dyspnea and constipation. Pooled safety analysis (SmPC) identified Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) or alkaline phosphatase increases in approximately 15% of the treatment population with grade 3 increases seen in 1-2%. There was no difference in rates of AST, ALT and AP elevations between groups treated with 250 mg and 500 mg doses. Please refer to the SmPC for further pooled safety data for multiple fulvestrant 500 mg trials data.

8.4 Treatment with Eribulin

8.4.1 Drug Dispense, Administration and Treatment Regimens

8.4.1.1 Investigational drugs

The investigational drug used in the course of this trial is commercially available eribulin.

The investigator will prescribe eribulin according to the label. Any change of label will be communicated. The IMP Eribulin for all other patients not covered by the label will be provided by Eisai to the participating sites in the study setting and will be administrated as i.v. injection at the study site.

8.4.1.2 Treatment arms

This is a non-randomized Phase II study.

8.4.1.3 Treatment assignment

All hormone-receptor positive, HER2-negative patients with indication to chemotherapy and all triple-negative patients will be assigned to treatment with eribulin.

8.4.1.4 Dispensing the study drug

The investigator will prescribe eribulin according to the label. Any change of label will be communicated. The IMP Eribulin for all other patients not covered by the label will be provided by Eisai to the participating sites in the study setting and will be administrated as i.v. injection at the study site. All dosages given to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.
Eribulin is a cytotoxic anticancer medicinal product and, as with other toxic compounds, caution should be exercised in its handling. Eribulin should be administered in units specialised in the administration of cytotoxic chemotherapy and only under the supervision of a qualified physician experienced in the appropriate use of cytotoxic medicinal products.The use of gloves, goggles, and protective clothing is recommended. If the skin comes into contact with the solution it should be washed immediately and thoroughly with soap and water. If it contacts mucous membranes, the membranes should be flushed thoroughly with water. Eribulin should only be prepared and administered by personnel appropriately trained in handling of cytotoxic agents. Pregnant staff should not handle Eribulin. Using aseptic technique Eribulin can be diluted up to 100 ml with sodium chloride 9 mg/ml (0.9%) solution for injection. It must not be mixed with other medicinal products and should not be diluted in glucose 5% infusion solution. Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

8.4.1.5 Study drug compliance

The investigator and/or study personnel will assess compliance at each patient visit. To accurately determine the patient's drug exposure throughout the study, the following information must be reported on the Drug Administration Record CRF pages for eribulin and in the source document.

- Planned dose administration
- Actual total daily dose administered
- Start and end date of drug administration
- Dose change (no or yes)
- Reason for dose change (e.g. adverse event, dosing error, lab test abnormality etc.)

8.4.1.6 Patient Compliance

Not applicable.

8.4.1.7 Study drug accountability

The investigator or designee must maintain an accurate record of the shipment and dispensing of only for study drugs supplied by Eisai in a drug accountability log (please refer to section 3.4.). The field monitor will note drug accountability during site visits and at the completion of the study. Patients will be asked to return all unused Eisai-supplied study drugs and packaging on an ongoing basis or at the time of study drug discontinuation.

8.4.1.8 Disposal and destruction

The drug supply can be destroyed at the local site. Destruction must be recorded in the local Drug Accountability Log, and will be controlled by the field monitor.

8.4.1.9 Instructions for use of study drug

Patients will receive cycles of 1.23 mg/m^2 eribulin on days 1 and 8 of a 21-day cycle for 12 months or until disease progression, unacceptable toxic effects, patient or physician request for discontinuation, or serious protocol non-compliance. Eribulin as the ready to use solution is administered intravenously over 2 to 5 minutes. In the EU the recommended dose refers to the base of the active substance (eribulin). Calculation of the individual dose to be administered to a patient must be based on the strength of the ready to use solution that contains 0.44 mg/ml eribulin.

Monitoring of complete blood counts should be performed on all patients prior to each dose of eribulin. Treatment with eribulin should only be initiated in patients with ANC values ≥ 1500/µl and platelets > 100000/µl.The dose reduction recommendations shown below are also shown as the dose of eribulin to be administered based on the strength of the ready to use solution.

Method of administration
The dose may be diluted in up to 100 ml of sodium chloride 9 mg/ml (0.9%) solution for injection. It should not be diluted in glucose 5% infusion solution. Good peripheral venous access, or a patent central line, should be ensured prior to administration. There is no evidence that eribulin mesilate is a vesicant or an irritant. In the event of extravasation, treatment should be symptomatic.
Women of childbearing potential must be advised to avoid becoming pregnant during and up to 3 months after treatment.
All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF.

8.4.2 Dose Adjustments

In General
Doses may be held or reduced for hematologic and other adverse events (see section 8.3.2.1). Dose adjustments are to be made according to the system showing the greatest degree of toxicity. Once dose is reduced due to adverse events, patients should not be re-challenged to a higher dose level.

Dose reductions and delays of treatment should follow general GCP criteria for the application of antineoplastic drugs (Repetto et al. 2003, NCCN-Guidelines) and should be made according to the SPC and/or investigator's brochure. The latest versions of the SPCs ("Fachinformation" according to German Drug Law) of the standard therapy will be provided on the DETECT IV website or by contact the DETECT Study Office in Ulm (Univeristäts-frauenklinik Ulm, Studienzentrale, Prittwitzstr. 43, 89075 Ulm, Tel: 0731/500 58520, Fax: 0731/500 58526, Email: studienzentrale.ufk@uniklinik-ulm.de).

The antineoplastic treatment of a patient can be postponed for up to 4 weeks to allow resolution of toxicity. The investigator must consult the DETECT IV Study Office prior continuing therapy for any patient requiring a delay of study treatment of more than two weeks for any reason.

All patients who require permanent discontinuation of eribulin will be withdrawn from the treatment intervention schedule (start of the 2-year follow-up period).

8.4.2.1 Permitted study drug dose adjustments and interruptions

Treatment with eribulin should continue as long as clinical benefit is observed or until unacceptable toxicity occurs. Management of severe and/or intolerable suspected adverse events may require temporary dose reduction and/or interruption.

For patients who do not tolerate the protocol-specified eribulin dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study. The following guidelines need to be applied: Dose adjustments are permitted for any adverse event suspected to be related to eribulin in those patients unable to tolerate the normal dose. If administration of eribulin must be interrupted because of unacceptable toxicity, eribulin dosing will be interrupted or modified according to the guidelines in TABLE 15 below.
All study drug interruptions or dose modifications must be recorded on the Dosage Administration Record page of the eCRF.
For patients who are unable to tolerate the protocol-specified dosing scheme, dose adjustments and interruptions are permitted in order to keep the patient on the study drugs. The following guidelines should be followed.

The administration of eribulin should be delayed on Day 1 or Day 8 for any of the following:

- Absolute neutrophil count (ANC) < 1000/µl
- Platelets < 75000/µl
- Grade 3 or 4 non-hematological toxicities.

Patients who interrupt eribulin therapy for more than 4 weeks must be discontinued from the study.

Dose reduction during therapy
Dose reduction recommendations for retreatment are shown in the following TABLE 24.

Adverse reaction after previous eribulin administration	**Recommended dose of eribulin**
Haematological:	
ANC < 0.5 x 10^9/l lasting more than 7 days	**0.97 mg/m²**
ANC < 1 x 10^9/l neutropenia complicated by fever or infection	
Platelets < 25 x 10^9/l thrombozytopenia	
Platelets < 50 x 10^9/l thrombozytopenia complicated by haemorrhage or requiring blood or platelet transfusion	
Non-haematological:	
Any Grade 3 or 4 in the previous cycle	
Reoccurrence of any haematological or non-haematological adverse reactions as specified above:	
Despite reduction to 0.97 mg/ m²	**0,62 mg/m²**
Despite reduction to 0.62 mg/m²	**Consider discontinuation**

Table 24: Eribulin dose modification guidelines for toxicities.

Do not re-escalate the eribulin dose after it has been reduced.

Patients with hepatic impairment
Impaired liver function due to metastases:
The recommended dose of eribulin in patients with mild hepatic impairment (Child-Pugh A) is 0.97 mg/m² administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle. The recommended dose of eribulin in patients with moderate hepatic impairment (Child-Pugh B) is 0.62 mg/m2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.
Severe hepatic impairment (Child-Pugh C) has not been studied but it is expected that a more marked dose reduction is needed if eribulin is used in these patients.

Impaired liver function due to cirrhosis:
This patient group has not been studied. The doses above may be used in mild and moderate impairment but close monitoring is advised as the doses may need readjustment.

Patients with renal impairment
Patients with severely impaired renal function (creatinine clearance <40 ml/min) may need a reduction of the dose (see section 3.3.7). The optimal dose for this patient groups remains to be established.
Caution and close safety monitoring as advised. No specific dose adjustments are recommended for patients with mild to moderate renal impairment.

Elderly patients
No specific dose adjustments are recommended based on the age of the patient.

These changes must be recorded on the Dosage Administration Record eCRF.

8.4.3 Known undesirable effects of the study drugs

Unless otherwise noted, the TABLE shows the incidence rates of adverse reactions observed in 827 breast cancer patients who received the recommended dose in two Phase 2 and one Phase 3 study. Frequency categories are defined as: very common (≥ 1/10), common (≥ 1/100 to < 1/10), uncommon (≥ 1/1,000 to < 1/100), rare (≥ 1/10,000 to < 1/1,000) and very rare (< 1/10,000).
Within each frequency grouping, undesirable effects are presented in order of decreasing frequency. Where Grade 3 or 4 reactions occurred with a frequency of ≥ 1%, the actual total frequency and the frequency of Grade 3 or 4 reactions are given.

System Organ Class	**Adverse Reactions – all Grades**			
	Very Common (Frequency %)	**Common (Frequency %)**	**Uncommon**	**Rare**
Infections and infestations		Urinary tract infection Oral candidiasis Upper respiratory tract infection Nasopharyngitis Rhinitis	Pneumonia Neutropenic sepsis Oral herpes Herpes zoster	
Blood and lymphatic disorders	Neutropenia (54.5%) (G3/4: 48.3%) Leukopenia (22.1%) (G3/4: 14%) Anaemia (20.3%) (G3/4: 1.4%)	Febrile neutropenia (4.7%) (G3/4: 4.6%)[a] Thrombocytopenia Lymphopenia		Disseminated intravascular coagulation[d]
Metabolism and nutrition disorders	Decreased appetite	Hypokalaemia Hypomagnesaemia Dehydration Hyperglycaemia Hypophosphataemia		
Psychiatric disorders		Insomnia Depression		
Nervous system disorders	Peripheral neuropathy [b] (32.0%) (G3/4: 6.9%) Headache	Dysgeusia Dizziness Hypoaesthesia Lethargy Neurotoxicity		

System Organ Class	**Adverse Reactions – all Grades**			
	Very Common (Frequency %)	**Common (Frequency %)**	**Uncommon**	**Rare**
Eye disorders		Lacrimation increased Conjunctivitis		
Ear and labyrinth disorders		Vertigo	Tinnitus	
Cardiac disorders		Tachycardia		
Vascular disorders		Hot flush	Deep vein thrombosis Pulmonary embolism	
Respiratory, thoracic and mediastinal disorders		Dyspnoea Cough Oropharyngeal pain Epistaxis Rhinorrhoea	Interstitial lung disease	
Gastrointestinal disorders	Nausea (35.1%) (G3/4: 1.1%) Constipation Diarrhoea Vomiting	Abdominal pain Stomatitis Dry mouth Dyspepsia Gastrooesophageal reflux disease Mouth ulceration Abdominal distension		Pancreatitis[d]

Hepatobiliary disorders		Alanine aminotransferase increased (3.0%) (G3/4: 1.1%)[c] Aspartate aminotransferase increased	Hyperbilirubinaemia	
Skin and subcutaneous tissue disorders	Alopecia	Rash Pruritus Nail disorder Night sweats Palmar plantar erythrodysaesthesia Dry skin Erythema Hyperhidrosis	Angioedema	
Musculoskeletal and connective tissue disorders	Arthralgia and Myalgia	Pain in extremity Muscle spasms Musculoskeletal pain and musculoskeletal chest pain Muscular weakness Bone pain Back pain		
Renal and urinary disorders			Dysuria Haematuria Proteinuria Renal failure	
General disorders and administration site conditions	Fatigue/Asthenia (52.8%) (G3/4 : 8.4%) Pyrexia	Mucosal Inflammation (9.8%) (G3/4: 1.3%)[c] Peripheral oedema Pain Chills Influenza like illness Chest Pain		

System Organ Class	**Adverse Reactions – all Grades**			
	Very Common (Frequency %)	**Common (Frequency %)**	**Uncommon**	**Rare**
Investigations		Weight decreased		

Table 25: Known undesirable effects of eribulin

Eribulin may cause adverse reactions such as tiredness and dizziness which may lead to a minor or moderate influence on the ability to drive or use machines. Patients should be advised not to drive or use machines if they feel tired or dizzy.

8.4.3.1 Management of specific toxicities

Neutropenia

The neutropenia observed was reversible and not cumulative; the mean time to nadir was 13 days and the mean time to recovery from severe neutropenia (< 500/µl) was 8 days. Neutrophil counts of < 500/µl that lasted for more than 7 days occurred in 13% of breast cancer patients treated with eribulin. Neutropenia resulted in discontinuation in < 1% of patients receiving eribulin.

Severe neutropenia may be managed by the use of G-CSF or equivalent at the physician's discretion in accordance with relevant guidelines. 18% of breast cancer patients treated in a phase 3 study with eribulin received G-CSF.

Peripheral neuropathy
In the 827 breast cancer patients the most common adverse reaction resulting in discontinuation of treatment with eribulin was peripheral neuropathy (4%). The median time to Grade 2 peripheral neuropathy was 85 days (post 4 cycles). Development of Grade 3 or 4 peripheral neuropathy occurred in 7% of eribulin treated breast cancer patients. In clinical trials, patients with pre-existing neuropathy were as likely to develop new or worsening symptoms as those who entered the study without the condition. In patients with pre-existing Grade 1 or 2 peripheral neuropathy the frequency of treatment-emergent Grade 3 peripheral neuropathy was 10%.
In case of Neuropathy > grade 2 treatment with eribulin should be withheld until recovery to grade 0-2. Continue therapy with a reduced dose of 1 dose level.

8.5 Concomitant Therapy

The investigator should instruct the patient to notify the study site about any new medications she takes after the start of the study drug. The electronic case report forms (eCRFs) will capture the use of all drugs, over-the-counter medications, or alternative therapies including herbal supplements, taken by the patient and significant non-drug therapies (including physical therapy and blood transfusions) administered from 2 weeks prior to recruitment and during the Follow up assessments.

8.5.1 Ancillary treatments

In addition to receiving study treatment, all patients should receive best supportive care (BSC), as per standard local practice for the treatment of pre-existing medical conditions or adverse events that may arise during the study. BSC is defined as drug or non-drug therapies, nutritional support, physical therapy or any other treatment alternative that the investigator believes to be in the patient's best interest, but excluding other antineoplastic treatments. Patients with bone metastasis should be treated additionally according to local guidelines.

All medications and non-drug therapies (including physical therapy, oxygen and blood transfusions) administered to the patient after the first dose of study treatment must be reported on the appropriate Concomitant Medication/Significant Non-Drug Therapy CRF pages throughout the study.

Patients should be instructed not to take additional medications (including over-the-counter products and herbal/alternative medications during the study without prior consultation with the investigator).

8.5.2 Palliative radiotherapie

Palliative radiation is permitted. It should not be delivered to a target lesion.

If palliative radiotherapy is initiated, the reason for its use must be clearly documented and progression as per RECIST 1.1 must be ruled out.

No dose modification of study treatment is needed during palliative radiotherapy.

8.5.3 Permitted concomitant therapy

The patient must be told to notify the investigational site about any new medications she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered during the study and for up to 28 days after study drug discontinuation must be listed on the Concomitant Medications or the Procedures and Significant Non-Drug Therapies eCRF pages, respectively.

Use of supportive therapy for protocol treatment induced toxicities is permitted. Patients should receive full supportive care and palliative care (e.g. pain control) as clinically indicated during the trial, including transfusion of blood products, and treatment with antibiotics, antiemetics, antidiarrheals and analgesics when appropriate, *with the exception of* the therapies mentioned in section 8.4.3.

Palliative radiotherapy is permitted excepting extended radiatio compromising bone marrow capacity.

The first regimen of antineoplastic medication/treatments received after study treatment discontinuation must also be recorded in the eCRF antineoplastic treatment modules.

8.5.4 Prohibited concomitant therapy

Use of the following treatments is NOT allowed during the study:

- Investigational or commercial anticancer agents, such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers, or endocrine therapy other than the once listed must not be given to patients.
- Hormone replacement therapy, topical estrogens (including any intra-vaginal preparations), Megestrol acetate and selective estrogen-receptor modulators (e.g. Raloxifene) are prohibited.
- Patients receiving concomitant immunosuppressive agents or chronic corticosteroids use, after taking the first dose of study drug in cases outlined below: Topical applications (e.g. rash), inhaled sprays (e.g. obstructive airways diseases), eye drops or local injections (e.g. intra-articular) are allowed. Long term low dose therapy, i.e. ≤10 mg/d prednisolone equivalent is permissible.
- Hematopoietic growth factors (e.g. Erythropoietins, G-CSF and GM-CSF) are not to be administered prophylactically. Use of these must be reserved to cases of Grade 3 or 4 neutropenia and anemia as per the labeling of these agents.
- Where possible, it is recommended to avoid drugs or substances known to be inhibitors or inducers of the isoenzyme CYP3A:
  - Co-administration with strong CYP3A inhibitors (e.g. Ketoconazole, Itraconazole, Ritonavir), and strong inducers (e.g. Rifampin, Rifabutin) should be avoided.
  - Co-administration with moderate CYP3A inhibitors (e.g. erythromycin, fluconazole, calcium channel blockers, benzodiazepines) and moderate inducers (e.g., carbamazepine, phenobarbital, phenytoin) should also be avoided if possible or otherwise subject to caution (e.g. increased frequency of safety monitoring).
    - Inhibitors – prohibited 7 days before dosing (6 months for amiodarone) and during protocol treatment
    - Inducers – prohibited 14 days before dosing and during protocol treatment

  Sevilla orange, grapefruit and grapefruit juice affect cytochrome P450 and PgP activity and must therefore be avoided during study drug intake.
  Because the composition, pharmacokinetics and metabolism of many herbal supplements are unknown, concurrent use of all herbal supplements is prohibited during protocol treatment.
  APPENDIX VI – PROHIBITED MEDICATIONS lists clinically relevant CYP3A inhibitors, inducers and the definition of strong and moderate inhibitors/inducers.
- It is to be noted that everolimus may affect the response to vaccinations making it less effective. Live vaccines must be avoided while a patient is treated with everolimus within this study.

8.6 Study Completion and post-study treatment

8.6.1 In General

Patients will be treated with the respective study drug until progression of disease, unacceptable toxicity, death, or discontinuation for any other reason. On the Conclusion Visit the treatment under study conditions ends. Therapy can be continued as clinical indicated during the follow up.

Everolimus/ribociclib cohort

Duration of everolimus/ribociclib therapy in combination with tamoxifen, anastrozole or letrozole is 12 month or until disease progression or other criteria for premature discontinuation (see section 12.1.1) occur. Patients receiving the ribociclib therapy are not allowed to be treated with tamoxifen in combination. Everolimus/ribociclib will be supplied by Novartis on study terms for the duration of the individual study participation within the off-lable use. If medically indicated everolimus/ribociclib treatment may be continued during follow up and will be (within the off-lable use) supplied by Novartis as well. Treatment after completion or premature discontinuation of everolimus/ribociclib (i.e. treatment in the follow-up period) is described in section 12.1.3.

Eribulin cohort

Duration of eribulin treatment is 12 months or until disease progression or other criteria for premature discontinuation (see section 12.1.1) occur. Eribulin will be supplied by Eisai on study terms for the duration of the individual study participation. If medically indicated eribulin treatment may be continued and will be (within the off-lable use) supplied by Eisai as well. Treatment after completion or premature discontinuation of eribulin (i.e. treatment in the follow-up period) is described in section 12.1.3.

8.6.2 Definition of end of the study

The study end is planned for a maximum of three years after recruitment of the last patient (LPLV). Study will be

terminated after death or complete two year follow-up off all patients. Patients will be followed for safety for 28 days after the individually last dose of everolimus/ribociclib/ eribulin study drug. At the end of the study the current progression and survival status for all patients will be updated finally and recorded in the eCRF.

8.6.3 Early study termination

The sponsor can terminate the study for reasons stipulated in the clinical trial agreement. Should this be necessary, the patient should be seen as soon as possible for the final visit and the same assessments should be performed as described for a prematurely withdrawn patient. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the patient's interests. The sponsor will be responsible for informing the ethics committee of the early termination of the trial.

8.6.4 Follow-up for toxicities

Patients whose everolimus/ribociclib treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to study treatment must be followed at least weekly until the adverse event or abnormal laboratory values resolves or returns to grade 1. If a patient requires an everolimus/ribociclib/ eribulin dose delay of > 4 weeks from the intended day of the next scheduled dose, then the patient must be discontinued from the study.

9 EVALUATION

(cf. APPENDIX II – PATIENT EVALUATION FLOW SHEET)

9.1 Evaluation during Treatment Period

In General
With the exception of the blood samplings for circulating tumor cells and translational medical investigations, assessment of adverse events, survival, quality of life and tablet count all procedures performed during treatment period are part of the clinical routine usual in metastatic breast cancer.

Scheduled Control Visits
For all patients there is a visit every 2 – 4 weeks for Everolimus/ribociclib cohort and every 6 weeks for Eribulin cohort. The date of each Control Visit is not determined with respect to the preceding one but with regard to time since first dose of protocol treatment (±3, etc. days after first dose). On each visit the following procedures are carried out:

- Assessment of vital signs (heart rate, blood pressure, temperature)
- Physical examination
- Standard 12-lead ECG for ribociclib cohort
- Assessment of adverse events (see section 11)
- Documentation of protocol treatment
- Tablet count, further supply of everolimus/ribociclib if necessary (everolimus/ribociclib cohort only)
- Pregnancy test (eribulin cohort only)
- Assessment of concomitant medication (denomination, start date/end date, specification if ongoing, dosage, route)
- Blood sampling for hematology and chemistry. Analyses are to include the parameters given in TABLE 26 below.
- Assessment of quality of life by means of the EORTC QLQ-C30 and EORTC QLQ-BR23 questionnaires
- Assessment of survival

Hematology	- Hemoglobin - Hematocrit - Red blood cell count - Differential white blood cell count - Platelet count
Biochemistry	- Total and direct bilirubin - ALT/AST - GGT, AP, and LDH (ribociclib cohort only) - Serum creatinine - Albumin - BUN or urea - Sodium, potassium, calcium, phosphorous (ribociclib cohort only) - Fasting cholesterol and triglycerides (everolimus/cohort only) - Fasting glucose (everolimus cohort only) - Potassium and Magnesium (eribulin cohort only)

Table 26: Standard hematology and biochemistry

During treatment CTC determination should be performed with ***Analysis Kit*** at week 3 - 4 and 9 -12 (that means for the eribulin cohort after cycle 1 and 3) only in patients who have given informed consent for translational medical investigations (**TraFo-Project**).

Thereafter, CTC determination should be performed together with evaluation of therapy response (every 3 month) in the everolimus/ribociclib cohort since the correlation between CTC count and therapy response will be investigated. This procedure is part of the usual clinical routine in metastatic breast cancer.

- Tumor evaluation according to section 10.2.1
- Blood sampling with ***Analysis Kit*** (everolimus/ribociclib cohort only)

Assessments independent of scheduled Control Visits:
- Hematology and chemistry assessments are carried out whenever clinically indicated and when monitoring toxicities in particular (cf. section 8.2). The parameters listed in TABLE 26 are reported on the eCRF laboratory pages.
- Adverse events (cf. section 11) are assessed on each patient visit to the study site whether scheduled or not.

Conclusion Visit of the treatment period
The conclusion visit of the treatment period is performed:
- As soon as possible in case of disease progression
- As soon as possible in case of other criteria for premature discontinuation of protocol treatment (as defined in section 12.1.1.)
- After 12 months in case of no tumor progression or other criteria for premature discontinuation of protocol treatment occur

The following actions are taken:
- Assessment of vital signs (heart rate, blood pressure and body temperature)
- Physical examination
- Standard 12-lead ECG
- Assessment of adverse events (cf. section 11)
- Assessment of concomitant medication
- Documentation of end of protocol treatment and of planned therapy after end of protocol treatment
- Blood sampling for hematology and chemistry, analyses are to include the parameters given in TABLE 26.
- Blood sampling with ***Analysis Kit*** in all patients
- Assessment of quality of life by means of the EORTC QLQ-C30 and the EORTC QLQ-BR23 questionnaires
- Collection of unused everolimus/ribociclib, tablet count (everolimus/ribociclib cohort only)
- Pregnancy test (eribulin cohort only)
- Assessment of survival
- Reminding the patient of the planned follow-up procedures.

Protocol treatment ends with this visit and subsequent therapy is as described in section 12.1.3 “Therapy after end of protocol treatment”.

9.2 Evaluation during Follow–Up Period (after Treatmenet Period)

Follow-up procedures are study specific and not part of the usual routine in case of metastasizing breast cancer.

Between two and four weeks after conclusion visit of the treatment period and then at least every three months the following is assessed during routine visits in the clinical trial center or via telephone call:
- Adverse events which are ≥ grade 3 NCI CTCAE and/or serious
- Concomitant medication
- Survival

Therapy during Follow-Up Period is as described in section 12.1.3 “Therapy after end of protocol treatment”.

10 CRITERIA FOR MEASUREMENT OF STUDY ENDPOINTS

10.1 Defintions

For definition of “target lesion”, “progressive disease” (PD), “partial response” (PR) and “complete response” (CR) please refer to section 10.2.1.

10.1.1 CTC clearance rate

The CTC clearance rate is defined as the 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).

10.1.2 Progression Free Survival (PFS)

PFS is defined as the time interval between the date of recruitment and the date of PD or death from any cause, whichever comes first.

10.1.3 Overall Survival

Overall survival is defined as the time interval between the date of recruitment and the date of death from any cause.

10.1.4 Overall Response Rate

The overall objective response rate applies only to patients with whom target lesions were defined at baseline. It is defined as the rate of CR and PR.

10.1.5 Clinical Benefit Rate

The clinical benefit rate applies to all patients. It is defined as the sum of the total number of patients with whom target lesions were defined and who achieve a complete or partial response and those patients who had stable disease for at least 6 months divided by the total number of patients in a treatment group.

10.1.6 New metastasis-free survival

New metastasis-free survival (nMFS), defined as time from recruitment to death or progression due to appearance of a new metastasis, whichever comes first.

10.1.7 Quality-adjusted survival

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.

10.2 Evaluation of Endpoints

10.2.1 Tumor evaluation (Evaluation of response and progression of metastatic disease)

Examinations regarding tumor evaluation:
On each tumor evaluation (during pre-treatment period and regularly 3 month during randomized treatment period) the **same method** of assessment and **the same technique** should be used to characterize each identified and reported lesion at baseline and during follow-up. The following examinations have to be done:

- Clinical assessment of all lesions is mandatory. For tumor evaluation clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm diameter. Documentation by color photography including a ruler is suggested.
- Imaging is performed as follows:
    - CT, MRI (Chest, Abdomen, Pelvis): CT is the preferred method of tumor evaluation. In certain circumstances MRI may be used instead, for details please refer to the RECIST-Guideline 1.1 (Eisenhauer 2009).

- CT, MRI (Head): A CT/MRI of the head is performed only if clinically indicated or if subject has a history of central nervous system metastases.
- Chest X-Ray: Chest X-Ray measurement of lesions is feasible, but not preferable. Lesions on chest x-ray are only acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. In case of performing CT-Scans there is no additional Chest X-Ray assessment necessary.
- Bone Scan and PET: Are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions (Eisenhauer 2009).
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised (described in greater detail in Appendix II of the RECIST-guideline (Eisenhauer 2009). If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.
- PET/CT: Should not be substituted for dedicated diagnostic contrast enhanced CT scans for anatomically based RECIST measurements. However, if the CT performed as a part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with i.v. and oral contrast) then the CT portion of the PET-CT can be used for RECIST measurements.
- Additional imaging is to be performed for assessment of further known lesions or if required by clinical suspicion of new lesions.

- Tumor markers are assessed on each tumor evaluation: CA15-3 is mandatory, CA125 and CEA are optional.
- Endoscopy, laparoscopy: These techniques must not be used for objective tumor evaluation. However biopsies obtained may be used to confirm complete response or assess relapse after complete response
- Cytology: The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

Definitions and guidelines regarding tumor evaluation

- At baseline during pretreatment period tumor lesions and/or lymph nodes are categorized measurable/non-measurable and target/non-target as follows:

  Measurability:

  A lesion is considered measurable if the longest diameter in the plane of measurement is at least
  - 10 mm in case of CT scan
  - 10 mm caliper measurement in case of clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
  - 20 mm in case of X-ray

  Lymph nodes are considered pathologically enlarged and measurable if ≥ 15 mm in short axis when assessed by CT scan.

  Any other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥10 to < 15 mm short axis) as well as truly non-measurable lesion are considered non-measurable. Truly non-measurable lesions include: leptomeningeal disease, ascites, pleural and pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

  Target/non-target lesions:

  If on baseline tumor evaluation there are at least two measurable lesions a maximum of five in total and a maximum of two per organ are selected as target lesions. In case of lymph nodes the length of the short axis, in case of other lesions the longest diameter is recorded. Target lesions should be selected on the base of their size (lesions with the longest diameter) and be representative of all involved organs, but in addition should be those which lend themselves to reproducible repeated measurements. The sum of the lesion diameters (LD) assessed for all target lesions is calculated and reported as baseline sum LD. All other lesions are considered non-target and reported without diameter whether measurable or not.
- On the regular tumor evaluations during treatment period, which are performed every 3 months LD is re-assessed. All non-target lesions are reported "present", "absent" or "unequivocal progression.
- Generally:
  Response and progression of metastatic disease is evaluated using the international guidelines Version 1.1 proposed by the RECIST committee (Eisenhauer 2009).
  For detailed instruction on lesion measurements, follow-up of non-target lesions and special considerations regarding lymph nodes, bone lesions, cystic lesions, lesions not observed at baseline and lesions with prior

radiologic treatment please refer to the **RECIST guideline Version 1.1** (Eisenhauer 2009).

Assessment of tumor response

Tumor response is assessed as follows:

- *Evaluation of target lesions:*

  Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm

  Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters

  Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest sum on study). In addition to the relative increase of 20% the sum must also demonstrate an absolute increase of at least 5 mm. (Note: The appearance of one or more lesions is also considered progression)

  Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

- *Evaluation of non-target lesions:*

  Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

  Non-CR/Non-PD: Persistence of one or more non-target lesions above normal limits

  Progressive Disease (PD): Unequivocal progression of existing non-target lesions

- *Evaluation of Overall Response:*

  From evaluations of target and non-target lesions and accounting for newly observed lesions Overall Response is assessed as described in the following tables:

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not at all evaluated	No	PR
SD	Non-PD or not at all evaluated	No	SD
Not at all evaluated	Non-PD	No	Not evaluable
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 27: Evaluation of patients with target disease

Non-Measurable Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not at all evaluated	No	Not evaluable
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Table 28: Evaluation of patients with non-target disease only

Data to be reported regarding tumor evaluations:
On each tumor evaluation the following data are reported (definitions see above):

- Concerning each lesion/lymph node:
  - Location
  - Measurable/non-measurable
  - Target/non-target
- Concerning non-target lesions: Present/absent/unequivocal progression
- Concerning target lesions only:
  - Lesion diameter (LD) (short axis in case of lymph nodes, longest axis in other cases according to RECIST guidelines)
  - Sum of lesion diameters (LD) assessed for all target lesions
- Tumor marker level

Beyond, the following information is provided on tumor evaluations during randomized treatment period:

- Response of target lesion (CR, PR, PD, SD)
- Response of non-target lesion (Present/absent/unequivocal progression: CR, Non-CR/Non-PD, PD)

At conclusion of the randomized treatment period the following information is provided:

- Overall Response

10.2.2 Evaluation of Circulating Tumor Cells (CTC)

Count of CTC
For counting CTCs are isolated by means of the CellSearch® Circulating Tumor Cell Kit (Veridex LLC, Raritan, USA).

Assessment of HER2 Status of Circulating Tumor Cells
Blood samples will be collected into CellSave tubes (Veridex Inc.). The CellSearch Epithelial Cell Test (Veridex Inc.) will be applied for CTC enrichment and enumeration. The method has been described in detail elsewhere (Riethdorf et al. 2007). In brief, CTCs are captured from peripheral blood by anti-epithelial cell adhesion molecule (EpCAM)-antibody-bearing ferrofluid and subsequently identified by cytokeratin-positivity/negativity for the leukocyte common antigen CD45 and 4',6-diamidino-2-phenylindole (DAPI) staining to ensure the integrity of the nucleus. A blood sample is considered positive when at least one CTC is present based on the prognostically relevant cut-off as previously published (Cristofanilli et al. 2004, Budd et al. 2006). HER2 expression of CTCs will be characterized within the CellSearch assay by addition of a fluorescein isothiocyanate (FITC)-labeled anti-HER2 antibody (CellSearch tumor phenotyping reagent HER2, Veridex Inc.), as described previously (Hayes et al. 2002, Meng et al. 2004, Riethdorf et al. 2010). The intensity of the HER2-specific immunofluorescence will be categorized into negative (0), weak (1+), moderate (2+), and strong (3+). CTCs are considered HER2 negative, if all measured CTCs have a moderate HER2 staining (2+) or less based on the cut-off level published by Riethdorf et al. 2010.

10.2.3 Evaluation of Quality of Life

The EORTC QLQ-C30 and EORTC QLQ-BR23 questionnaires are used (see APPENDIX V – QUALITY OF LIFE ASSESSMENTS). Data are analyzed according to the respective EORTC manuals.

11 ADVERSE EVENT REPORTING

11.1 Definitions, Collection and Recording of Adverse Events by the Investigator

An adverse event (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. In particular this includes new findings or changes from baseline in laboratory test results or any other safety assessments (e.g. ECGs, radiological scans, vital signs assessments) felt to be clinically significant in the medical and scientific judgment of the investigator.

During treatment period AEs are collected by the investigator at least on every visit of the patient at the study site, whether it is a scheduled study visit or not.

During the follow-up period (after conclusion of the treatment period) only adverse events are collected by the investigator which are $\geqslant$ grade 3 NCI CTCAE and/or serious.

Every adverse event is followed up until it has subsided or stabilized.

On each AE the following data are raised and documented by the investigator in the respective electronic case report form:

- Diagnosis or each single symptom if diagnosis is not available,
- Date and time of onset and end of AE,
- Whether onset was after the first administration of study medication (yes/no),
- Course (continuous / intermittent, if intermittent: number of episodes),
- Whether the AE is a reportable serious adverse event (serious/ non-serious, for the definition of "reportable serious event" cf. section 1.3),
- Intensity (grade 1 to 5 according to the NCI Common Terminology Criteria for Adverse events (CTCAE) version 4.03),
- Causal relationship with investigational medicinal product (no (not related) / yes (reasonable possibility)),
- Counter-measures (none/ dose reduced/ drug withdrawn/ other drug treatment/ other measures),
- Outcome (recovered/ recovering/ not recovered/ recovered with sequelae/ fatal/ unknown),

To allow for a more appropriate evaluation of adverse events, toxicities due to previous anticancer medication which occurred before inclusion visit are also reported specifying the kind of toxicity, start date, end date / specification if ongoing, and severity according to NCI CTCAE Version 4.03.

11.2 Definitions, Collection and Expedited Recording of Serious Adverse Events by the Investigator

A serious adverse event (SAE) is any adverse event that at any dose

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization (excluding hospital admissions for study drug administration, transfusional support for the purposes of decreasing hematological toxicity (e.g. GCSF), elective surgery and admissions for palliative or terminal care)
- Results in persistent or significant disability or incapacity and/or
- Is a congenital anomaly/birth defect.

- Is a medically important event or reaction:

  Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. In Addition any suspected transmission of an infectious agent via a medicinal product is considered an important medical event

The investigator shall report all serious adverse events (including serious adverse reactions and suspected serious adverse reactions) to the sponsor immediately (within 24 hours) after becoming aware of them. Each serious adverse event must be documented on the electronically available "**Serious adverse events report**" form. The completed form is automatically faxed (fax no. is filed automatically in the system) to the sponsor and Alcedis GmbH. In the event that electronic reporting is not possible, paper SAE forms in the investigator's file handed out at the beginning of the study are at the doctor's disposal for notification by conventional fax.

11.3 Collection and Recording of Other Events by the Investigator

The investigator shall report misuse and abuse of an IMP, other medication errors and uses outside of what is foreseen in the protocol (irrespective if a clinical event has occurred).

SPONSOR'S STUDY OFFICE:

Universitätsklinikum Ulm
Studienzentrale
Prittwitzstr.43
D-89075 Ulm
Germany

Physician: Dr. F. Schochter; Dr. S. Albrecht, Dr. A. De Gregorio, A. Polasik, T. Romashova, Prof. J. Huober, Prof. W. Janni
Studycoordinators: Evelyn Jäckel, Jessica D'Andrea, Heike Karl

Phone: +49 (0) 731 500 58520
Fax: +49 (0) 731 500 58526
Email: studienzentrale.ufk@uniklinik-ulm.de

Complete information on the SAE may not be available initially. Initial SAE reports should be submitted as soon as the following minimum information has been obtained:

- EudraCT Number
- Patient identification number
- The investigational medicinal product
- An AE assessed as serious
- Name and address of investigator and clinical trial center

In case of incomplete information at the time of initial SAE reporting, the initial report must be followed by further relevant information immediately after receipt. In certain cases it may be appropriate to follow-up the initial reports by several times.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the events listed above.

Please Note:

- An event which is part of the natural course of the disease under study (e.g. hospitalization for signs/symptoms of the disease or disease progression or death due to disease progression) does not need to be reported as a SAE even though serious criteria are met. However, if the progression of the underlying disease is greater than that which would normally be expected for the subject, or if the investigator considers that there was a causal relationship between protocol treatment or protocol design/procedures and the disease progression, then this must be reported as a SAE.
- For each serious adverse event a corresponding AE must be documented within the eCRF.

Every serious adverse event is followed up until it has subsided or stabilized.

11.4 Sponsor Responsibilities

General Remarks:
When patient data are transferred as described below they must be pseudonymous. This means instead of data allowing patient identification the unique patient identification numbers are specified only.

AE Reporting:
On request of the competent authority detailed records have to be submitted on all adverse events which have been reported by the investigators

Annual Safety Report (ASR) with Serious Adverse Reaction (SAR) Listing:
All noxious and unintended responses to a medicinal product related to any dose are to be considered as adverse drug reaction (AR). "Response to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. Accordingly a Serious Adverse Reaction (SAR) is an AR which is serious according to the criteria given in section

Once a year throughout the clinical trial as well as on request, the sponsor has to provide the competent authority and the competent ethics committee with a listing of all suspected serious adverse reactions (SARs) which have occurred over this period and with a report concerning the clinical trial participant's safety (annual safety report, ASR).

SUSAR and other Safety Issue Reporting:
A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a suspected adverse reaction related to an investigational medicinal product (IMP) or comparators which occurs in the clinical trial, and which is both unexpected and serious. Adverse reactions are considered unexpected if not listed in the latest version of the "Fachinformation" (German SPC).

Suspected unexpected serious adverse reactions (SUSAR) have to be reported to the competent authority and to the competent ethics committees immediately but within a maximum of fifteen days of first knowledge by the sponsor. In this case patient data are passed pseudonymous to the federal and European authority.

This also holds in case of any other issues which might require re-assessment of the risk-benefit ratio of the IMP. Such safety issues may be:

- single case reports of an expected serious adverse reactions with an unexpected outcome (e.g.: a fatal outcome),
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important,
- post-study SUSARs that occur after the patient has completed the clinical trial and are reported by the investigator to the sponsor,
- events relating to the conduct of the trial or the development of the investigational medicinal product likely to affect the safety of the subjects.

SUSARs which are fatal or life threatening must be reported within a time limit of 7 days. Relevant follow-up information must be provided within additional 8 days.

The sponsor also informs the investigators involved in the study immediately on any SUSARs which occur. The time limit also is 15 days. In case of fatal or life-threatening SUSARs the time limit is 7 days, further relevant information must be provided within additional 8 days.

Reporting of safety measures taken for patient protection:
Sponsor and investigator take immediate measures to protect trial participants from immediate hazard, if new or unforeseen conditions might compromise the participants' safety. In that case, the sponsor informs the competent authority and the ethics committee immediately about the measures taken and the conditions which caused their origination.

Reporting of clinical safety information to Novartis
All serious adverse events occurring in a patient who has been treated with the IMP everolimus/ribociclib of Novartis shall be reported by the sponsor to Novartis within 24 hours after becoming aware of them.

The sponsor will notify all SAEs reporting to Novartis Pharma GmbH (Abteilung Arzneimittelsicherheit; Roonstraße 25; 90429 Nürnberg; Fax-Nr.: 0911 -273 12 985) on the appropriate report forms.

Reporting of clinical safety information to Eisai
All serious adverse events occurring in a patient who has been treated with the IMP eribulin of Eisai shall be reported by the sponsor to Eisai within 24 hours after becoming aware of them.

The sponsor will notify all SAEs reporting to Eisai GmbH (Abteilung Arzneimittelsicherheit; Lyonerstr. 36; D-60528 Frankfurt/Main; Fax-Nr.: 069 -66585 45) on the appropriate report forms.

Reporting of clinical safety information to TEVA

All serious adverse events occurring in a patient who has been treated with the IMP Lipegfilgastrim of TEVA shall be reported by the sponsor to TEVA within 24 hours after becoming aware of them.

The sponsor will notify all SAEs reporting to TEVA GmbH (Abteilung Arzneimittelsicherheit; Graf-Arco-Str. 3 D - 89079 Ulm, Fax-Nr.: +49 (0) 731 402 44 57 77) on the appropriate report forms.

12 PREGNANCY

All pregnancies that occur during the study, or within 14 days of completion of the study, are to be reported immediately to the sponsor, and the investigator must provide the sponsor with the pregnancy form. The process for reporting a pregnancy is the same as that for reporting a serious adverse event.

Any patient becoming pregnant during the study will be withdrawn. All patients who become pregnant will be monitored to the completion or termination of the pregnancy. If the pregnancy continues to term, the outcome (health of the infant up to 8 weeks of age), including spontaneous or voluntary termination, details of birth, and presence or absence of any birth defect, congenital abnormalities, or maternal and newborn complications, will be reported to the sponsor. Any complication of pregnancy will be reported as an adverse event or serious adverse event, as appropriate.

If the pregnancy does not continue to term, 1 of the following actions will be taken:

- For a spontaneous abortion, report as a serious adverse event.
- For an elective abortion due to developmental anomalies, report as a serious adverse event.
- For an elective abortion **not** due to developmental anomalies, report on the pregnancy form.

13 TREATMENT DISCONTINUATION AND PREMATURE STUDY DISCONTINUATION

13.1 Protocol Treatment Discontinuation

13.1.1 Criteria for Discontinuing Protocol Treatment

Study drug discontinuation

Study drug interruption refers to a patient stopping either study drug during the course of the study, but then re-starting it at a later time in the study. If the study drug everolimus/ribociclib is interrupted for more than 4 weeks, the patient will be permanently discontinued from the study.

If the administration of study drug must be interrupted because of an unacceptable toxicity, dosing will be interrupted or modified according to rules described in TABLE 14, TABLE 15, TABLE 16, TABLE 17 for everolimus-cohort and TABLE 19, TABLE 20, TABLE 21, TABLE 22 and TABLE 23 for ribociclib-cohort. A patient who requires dose interruption (regardless of the reason for the interruption) lasting > 4 weeks (counting from the first day when a dose was missed) must discontinue study treatment. All study drug interruptions must be recorded on the appropriate Dosage Administration CRF.

Patients whose treatment is interrupted due to an adverse event or abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4 weeks intervals, until resolution or stabilization of the event, whichever comes first.

Study discontinuation refers to a patient's withdrawal from study drug. The reason for discontinuation from treatment must be recorded on the End of treatment CRF.

Patients who discontinue study drug > 4 weeks should be scheduled for an End of treatment Visit, as soon as possible, after discontinuing study drug, at which time all of the assessments listed for the End of treatment Visit will be performed. The complete End of treatment CRF documentation should be done within one week. The date and reason for stopping the study treatment should be recorded on the CRF.

Study drug will be discontinued for any of the following reasons:

- Tumor progression (as defined in section 10.2.)
- Toxicity/ Unacceptable adverse events
- Consent withdrawal/ Request by the patient
- Dose interruption of > 4 weeks
- Intercurrent illness that prevent further administration of study drug
- Need for any other types of anticancer therapy, except for palliative radiotherapy for bone lesions
- General or specific changes in the patient's condition which render the patient unacceptable for further treatment at the discretion of the investigator
- Pregnancy (applicable for eribulin cohort only)

If study drug is permanently discontinued, the patient will be considered to have completed study treatment. **All patients must have safety evaluations for 28 days after the last dose** of study drug.

Once the last dose of study drug is taken, no further AEs/SAEs will be collected on this protocol beyond the 28 day follow-up safety interval. After the last patient in the trial has taken the last dose of study drug, the current progression and survival status for all patients will be updated finally and recorded in the CRF.

13.1.2 Duration of Protocol Treatment

Duration of endocrine therapy:

- Depends on the occurrence of tumor progression or other criteria for discontinuation (see section 12.1.1.)
- The treatment can be extended in case of missing progress. Conclusion Visit should be performed 12 month after recruitment (End of treatment period under study conditions) or as soon as possible if criteria for discontinuation occur.

Duration of treatment with everolimus/ribociclib:

- Depends on the occurrence of tumor progression or other criteria for discontinuation (see section 12.1.1.)
- The treatment with everolimus/ribociclib can be extended if medically indicated. Conclusion Visit should be performed 12 month after recruitment even if therapy with everolimus is continued or as soon as possible if criteria for discontinuation occur.

Duration of treatment with eribulin:

- Depends on the occurrence of tumor progression or other criteria for discontinuation (see section 12.1.1.)
- The treatment with eribulin can be extended if medically indicated. Conclusion Visit should be performed 12 month after recruitment even if therapy with eribulin is continued or as soon as possible if criteria for discontinuation occur.

13.1.3 Therapy after End of Protocol Treatment

Therapy during the follow-up period will be according to current guidelines and is at the discretion of the investigator, regardless of whether the protocol treatment could be completed as planned or had to be discontinued prematurely). The treatment with everolimus/ribociclib can be extended in case of missing progress. The drug will be supplied by Novartis on study terms for the duration of the individual study participation (including 2-year-period of follow-up assessments). In case of clinical benefit the treatment with eribulin can be continued until progression of disease: the study drug will be (within the off-lable use) supplied by Eisai as well.

13.2 Premature Termination of Study Participation in Single Patients

Study participation is terminated prematurely with single patients

- On the patient's request
- If in the investigator's opinion further participation would jeopardize the patient in an unjustifiable way.
- If a pregnancy is ascertained (applicable for eribulin cohort only)

If participation is terminated during the treatment period every effort should be taken to perform the conclusion visit of the randomized treatment period (cf. section 9.1). The obtained patient informations will be analysed. Data which are not necessary for study analysis will be destroyed.

13.3 Discontinuation or Premature Termination of Study in Single Sites

The sponsor, the coordinating investigator, the principal investigators, and the concerned competent authorities are reserved the right to initiate discontinuation or premature termination of the clinical trial in a single study site for the following reasons:

- Identification of a safety risk for the clinical trial participants of a single clinical trial center
- Non-compliance with the principles of GCP
- Insufficient recruitment
- Financial problems

If such action is considered, it is discussed with the principal investigator in advance.

If such action is taken the investigators and the principal investigator at the concerned trial center, the coordinating investigator and the sponsor must be informed immediately. It is the duty of the sponsor to notify the competent authority and the competent ethics committee within 15 days after premature termination or discontinuation. The principal investigator at the concerned center is to inform the local authority within the same time frame.

Furthermore, the clinical trial participants at the concerned trial center must be informed immediately about the discontinuation or termination. The investigator must ensure that the participants of the concerned study site are treated and followed-up appropriately. Date and time of the last application of the investigational drug as well as date and reason for the discontinuation or premature termination are to be documented in the participants' medical records and on the eCRFs.

In case of permanent discontinuation, all study materials (e.g. completed, partly completed and empty eCRFs) must be sent to the sponsor.

13.4 Premature Discontinuation of the Entire Study

The sponsor, coordinating investigator and the competent authorities may discontinue or terminate the entire study for the following reasons:

- Identification of a safety risk for the clinical trial participants
- Identification of problems with the trial design
- Non-compliance with the principals of GCP
- Insufficient recruitment of trial participants
- Financial problems

In consultation with the DSMB and the principle investigator the study should be discontinued by the sponsor, represented by Prof. Dr. W. Janni, due to safety concerns if any of the following reasons are present:
If due to unexpected events the continuation of the study is not acceptable (ethical, medical or pharmaceutical legal aspects). This will be particularly the case,

- If any grade 4 toxicity occurs in > 20 % of the patients.
- If febrile neutropenia (defined as grade 3 or 4 neutropenia + fever > 38.5 C°) occurs in > 20 % of the patients.
- If grade 4 neutropenia occurs in > 20 % of the patients.
- If an unexpected amount of SUSARs occurs.
- If hand-foot-syndrome grade 4 occurs in > 30 % of the patients.

In the event of study termination, steps will be taken to ensure subjects transition smoothly off study, and if the risk/benefit profile is deemed to be appropriate, subject may continue to receive study treatments at the discretion of the investigator.

All investigators and the sponsor must immediately be informed about the discontinuation or premature termination. The principal investigator at each study site must notify the local competent authority within 15 days after premature termination. The sponsor is to notify the competent authority and the competent ethics committee within the same time interval.

Furthermore, all clinical trial participants must be informed immediately about discontinuation or premature termination. The responsible investigators must ensure that the clinical trial participants are treated and followed up appropriately. Date and time of the last application of the investigational medicinal product as well as date and reason for the discontinuation or premature termination are to be documented in the participants' medical records and on the eCRFs.

In case of permanent discontinuation, all study materials must be sent to the sponsor.

14 CENTRAL REVIEW PROCEDURES AND TISSUE COLLECTION

14.1 CTC Counts and Assessment of HER2 Status on CTCs

CTC counts and assessments of HER2 status are carried out by the laboratories named on page 3 of this protocol. For further information refer to section 2.2.

14.2 Tumor Tissue Sampling

If a biopsy of a metastatic site is carried out as part of the routine diagnostic work-up or a tumor block of the primary tumor is available, a sample of tumorous tissue will be investigated for further translational research.

15 STATISTICAL CONSIDERATIONS

15.1 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.

15.2 Statistical Methods

Statistical analysis of experimental data will be performed at the end of the studies, and there is no pre-planned interim analysis. For all parameters, descriptive statistics will be provided (absolute and relative frequencies for categorial data; number of valid and missing observations, means, standard deviations, medians, interquartiles, ranges, and confidence intervals – as appropriate – for continuous variables). Time-to-event data will be analysed using the Kaplan-Meier method and summarized using medians, 95% confidence limits, and Kaplan-Meier survival plots.

The analysis regarding the primary objectives will be based on the patients in the ITT set, with supportative analyses being conducted with the PP set. All other analyses regarding secondary objectives and endpoints will be performed based on the ITT set, the PP set, or subsets thereof, whatever is deemed appropriate.

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).

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. 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.

In the eribulin cohort, preplanned explorative subgroup analyses will be performed to evaluate PFS in the prospectively defined subgroups of hormone-receptor positive and triple-negative patients, as well as in the subgroups of patients receiving the first or second line of cytostatic treatment for metastatic breast cancer and patients receiving the third or fourth line of cytostatic treatment for metastatic breast cancer.

All analyses regarding the secondary objectives will have exploratory character only. The statistical methods described in the following section are appropriate for the data and distributions usually expected in this type of trials. The suitability of the statistical methods will be checked after data entry, and - if necessary - the methods will be modified accordingly.

The presence and number of circulating tumor cells (CTCs) measured at different time points will be evaluated in a descriptive way. The temporal changes in the number of CTCs will be described and analysed using appropriate generalized linear mixed models. In addition, different measures of CTC dynamics (based on various threshold values, relative or absolute changes in CTC counts) and their value for evaluating therapy efficacy or as a prognostic tool will be examined in detail by explorative data analyses.

All secondary endpoints and other outcomes that are calculated based on frequencies/rates (ORR, DCR, CR, PR, SD) will be summarized using frequency tables presenting absolute and relative frequencies together with appropriate confidence intervals. Comparisons between groups will be performed using suitable non-parametric statistical tests such as Fisher's exact test, χ^2-test, or Cochran-Mantel-Haenszel test.

Overall survival will be estimated by the Kaplan Meier product limit method, and median values, 95% confidence intervals and survival plots will be provided. When appropriate, overall survival will be compared between groups using the logrank test, and additional multivariate analyses may be performed by suitable regression models (proportional hazard regression model, logistic regression).

Quality of life (QoL) will be assessed using the EORTC QLQ-C30 and BR23 questionnaires. The raw scores will be transformed to scores ranging from 0 to 100 following the instructions of the respective manuals. Descriptive statistics will be used to summarize both the single-item measures and multi-item scales at each scheduled assessment time point. Additionally, changes in QoL data between baseline scores and the scores obtained at the scheduled time points after start of the study treatment will be summarized for all patients with an evaluable baseline score and at least one evaluable post-baseline score. Longitudinal changes in QoL will be assessed using appropriate generalized linear mixed models with individual patients being fitted as random factors.

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

15.3 Sample Size Assumptions

In the DETECT IV trial, the primary objective 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.

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

- anticipated number of at least 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:

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.

An important secondary objective for the everolimus/ribociclib cohort of the DETECT IV trial is the assessment of the dynamic of CTCs by longitudinal comparisons of CTC counts before during and after treatment and the evaluation of the prognostic value of different measures of CTC dynamics. Based on published studies that used a threshold of ≥ 5 CTCs, it is planned to compare PFS between patients that had ≥ 5 CTCs at baseline (start of treatment, T1) and ≥ 5 CTCs at the time of first radiological tumor evaluation after 12 weeks (T2) and patients that had ≥ 5 CTCs at T1 and < 5 CTCs at T2. Assuming that a total of 160 CTC-positive patients can be enrolled and that 68% of all CTC-positive patients have ≥ 5 CTCs (Fehm et al. 2010, Pierga et al. 2012), 109 patients are expected to have levels of CTCs ≥ 5 at T1. With this sample size, we can detect a 39-40% decreased hazard of progression or death for the group of patients with CTCs decreasing from ≥ 5 CTCs at T1 to < 5 CTCs at T2 as compared to the group of patients with CTCs remaining above the threshold level of ≥ 5 CTCs at T2 (assuming the median PFS being in the range of 5.0 and 10.0 months for this group) with a power of 80% (one-sided test with Type I error of 5%). Please note that this analysis refers to a secondary endpoint and thus has explorative character only.

Eribulin cohort:

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.

15.4 Safety Analysis

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. For all safety analyses, the safety set will be used. A safety analysis will be performed by the coordinating investigator and the DSMB after recruitment of 15 patients. Steps will be taken to preserve data confidentiality and to ensure the scientific integrity of the ongoing study. The safety analysis will be conducted on all patients who received at least one dose of the study treatment. Recruitment will not be suspended while this safety analysis is undertaken, except if the stopping thresholds specified above (cf. Premature Discontinuation) are exceeded or on recommendation of the safety review committee (DSMB) for extenuating circumstances (e.g. delay in data retrieval or analysis, emerging unforeseen safety concerns). Every effort will be made to minimize the time from the analysis cut-off date to data retrieval, analysis, and safety review decision. In case of unexpected toxicity the advisory board and the DSMB will be informed and give their advisory vote. Based on the recommendations of this team, a protocol amendment may be implemented to modify dose or study procedures. The safety review is not limited to these adverse events for which stopping rules are established; in the event that other safety concerns are identified, the study could also be closed. All SAEs which are reported to the Sponsor as required by the protocol will be reviewed during this safety analysis. In addition to this safety analysis of the first cohort, ongoing individual events will be reviewed on an ongoing basis, according to routine protocol and pharmacovigilance procedures.

16 PUBLICATION POLICY

The results of this study will be published. The responsibility is with the coordinating investigator.

17 ETHICAL, REGULATORY AND ADMINISTRATIVE ISSUES

17.1 Regulatory Issues

This study is conducted in compliance with the Declaration of Helsinki (1996), the GCP-Guidelines of the International Conference on Harmonization and applicable German Drug Law.

17.2 Institution Eligibility for Participation

The sponsor acquires information on the potential study site including:

- The potential principal investigator's interest in study participation
- His general experience in the conduct of clinical trials and his experience in similar studies (number of trials performed, number of patients attended in clinical trials)
- Previous audits and inspections
- Suitability of the site facility for study purposes with special regard to radiology
- Emergency equipment, accessibility of the emergency unit of a hospital

17.3 Favorable Opinion of the Competent Ethics Committee and Official Study Approval

On behalf of the sponsor the coordinating investigator will take care, that a favorable opinion of the competent ethics committee on study conduct and approval of the study by the competent authority is obtained. No patient can be included before both have been granted.

Contact data:

Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)
Probandenkontakttelefon: Frau Kovac
Kurt-Georg-Kiesinger-Allee 3
D-53175 Bonn
Tel.: +49 (0) 228-207-4318 //
Stichwort „Fachgebiet Klinische Prüfung / Inspektionen“

17.4 Informed Consent

Patients are only requested to participate in the study, if they suffer from metastasizing breast cancer, if their primary tumor and/or a biopsy from a metastatic site was examined and found to be HER2-negative, and if there is the indication to initiate either a standard endocrine or chemotherapy. Since such patients are expected to be under extreme emotional stress they are intended not to be unduly bothered with detailed information on the study before HER2 status on CTC is known. Therefore informed consent is obtained in two steps. At first patients are asked to consent in blood sampling for CTC count and assessment of HER2 status on CTC (patient information and consent form – part 1). If HER2 status on CTC is negative, their informed consent in study participation is requested (patient information and consent form – part 2). In addition, when both consents have been obtained, patients are also requested to consent in blood sampling for translational medical investigations (patient information and consent form – part 3).

Before signing a consent form, patients are informed about the study orally and in writing. Oral information is to be given by an investigator or by a health professional member of the panel and must include the information given in the respective information leaflets used for written information (patient information – parts 1 to 3). Patients are given ample opportunity to enquire details of the trial and discuss all questions they have. They are left sufficient time to decide on whether to give their consent or not.

The consent forms must be personally signed and dated by the patient and by the investigator who conducted the informed consent discussion. The signed consent forms are retained as part of the trial files. A copy of the consent forms and copy of the information leaflets used are left to the patient. Patient's general practioner will be informed in case the patient agrees to this procedure.

Before the first informed consent has been obtained no study specific procedure may be carried out. Thereafter only blood sampling for CTC count and assessment of HER2 status is allowed. Further study specific procedures may only be carried out after the second informed consent has been given. Blood samplings for translational medical investigations will only take place if informed consent to these additional examinations is obtained (part 3). If a patient objects, no blood sample will be taken for this purpose, but may nevertheless participate in the study.

The written informed consent forms and any other written information to be provided to patients are revised whenever important new information becomes available that may be relevant to the patient's consent. The sponsor obtains the competent ethics committee's favorable opinion on any revised written informed consent form or and written information material in advance of use. The investigator informs patients already included in a timely manner if new information becomes available that may be relevant to their willingness to continue participation. The communication of this information is documented. In case of monitoring, audits and inspevtions the medical confidentiality might be revoked.

17.5 Data Protection

All national and local legal requirements regarding data protection will be adhered to. All study findings and documents will be regarded as confidential. The investigator and other authorized persons will not disclose such information without prior written approval from the sponsor.

Pseudonymity of patient data is assured by means of a patient identification number that will be allocated via eCRF. Each patient will be clearly identified through the patient number and inclusion number given in the enrolment procedure. At the center site the investigator compiles a confidential list, in which the patient name and address is assigned to the patient number.

Throughout documentation, evaluation and notification procedures, the participants will be identified on eCRFs and other documents by their unique participant identification number. If the name, the initials or the year of birth of a participant appear on a document (e.g. laboratory report), that has to be transferred within the notification duties (e.g. to the sponsor or to competent authorities), these data will be obliterated before a copy of the document is transferred. Documents which identify the trial participants (e.g. patient identification log and the signed informed consent forms) will be maintained in confidence by the principal investigator. The participants will be told that all study data will be stored on computer and handled in strictest confidence.

The trial participants are informed that monitors, representatives of the sponsor, the ethics committee and the

concerned competent authorities including the local competent authority may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with legal data protection requirements. Patients' agreement to this is part of the informed consent forms parts 1-3.

17.6 Data Management

17.6.1 Collection of Data

Study data are captured by means of the eCRF. Data are only entered by members of the site personal who

- Have been assigned this competency in the site delegation log (a document to be kept in the ISF, defining the responsibilities of each member of the site personal)
- Have participated in a required training on handling the eCRF
- Have been provided password protected access to the eCRF.

The personal password a member of the site personal has been provided must not be disclosed to any other person whether involved in the study or not.

Data is to be entered in a timely manner.

17.6.2 Data Management

The data management for this study will be performed by Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.
The following sections describe the software employed and measures applied for data security.
Data are recorded, processed and stored using the following software tools:

a) eCRF database (Location: Alcedis)
b) SAE database (Location: Alcedis)

Wherever applicable, current GCP guidelines, actual technical standards and guidelines are observed.

Employed Software eCRF database:
For data capturing and data management of this clinical trial, a web-based validated software (WBDC) based on a relational database will be employed. The software consists of the following modules:

a) Administration:	Administration of sites (clinics/office based physicians) by system administrator and project management. Within the individual sites the following system users are defined: Investigator and study nurse. All access rights are administered in a role-based security system.
b) Forms / Form validator:	Electronic Case Report Forms (eCRFs) for data capture including online validation of eCRFs during data capture, e.g. check on range, plausibility, type mismatch. In addition to the system based plausibility checks, a formal query process will be implemented to resolve inconsistencies in data.
c) Reports:	Dynamic report generator, e.g. reports for investigators on CRF status.
d) Query management:	After completion of data entry checks for plausibility, consistency and completeness of the data will be performed. Based on these checks, queries will be produced combined with the queries generated by manual checks. All missing data or inconsistencies will be reported back to the center(s) through the eCRFs and clarified by the responsible investigator. If no further corrections are to be made in the database it will be declared closed. With the export engine of Alcedis MED, data sets for the statistical analysis were generated.

The employed technology and technical requirements for data entry on site are as follows:

a) The used software is completely server-based, i.e. all programme processes are executed centrally on a web or database server.
b) Data are saved exclusively in the central database server. This server is located in the facilities of Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.
c) For system access, users require a conventional desktop computer with internet access.

Data Security and Storage
For client / server communication via the internet only encrypted transmissions are applied. State of the art encryption technology is used exclusively. For data transmission in this clinical trial an encryption level (128-bit) is employed by means of the Secure Socket Layer Algorithm (SSL).

In addition, the server identifies itself to the client workstation by means of a digital server certificate issued by an authorised certification authority. This ensures that data are sent only to the server of Alcedis GmbH.

Data are protected from potential virtual attacks and physical damage.

Views on data or reports as well as edit or read only rights are controlled with individual passwords. Access authorisation to the eCRF databases is granted individually to investigators and programme personnel by means of user accounts.

The project management of the CRO has a read-only access on all patient data stored in the eCRF database.

Assurance of data will be made by RAID-Systems (Redundant Array of Independent Disks), thereby ensuring data security even if one hard disc failed.

Furthermore a back up onto magnetic tape is performed according to the following scheme:

- Daily back-up over a period of 7 days
- Weekly back-up over a period of 5 weeks
- Monthly back-up over a period of continuance of the clinical trial

Investigators will get a CD-Rom after the end of the trial containing the data of the patients they have documented.

17.6.3 Data Processing

The study personnel must care that any data transferred outside the trial center do not allow identification of the patient. Data on clinical trial participants which are to be transferred to the sponsor, the data management, the competent ethics committee, the competent authority or investigators from other sites must identify the patient only by means of the unique patient identification number.

Data management checks the data entered in the eCRF for completeness and plausibility. In case of findings queries are issued for clarification.

17.6.4 Archiving

Essential study documents are archived for at least 10 years after completion of the trial. The trial master file (TMF) is kept by the sponsor. The ISF is to be archived at the respective site.

The source documents which are created in the clinical trial center are to be filed in the participant's medical records. They are archived at the site in accordance with local requirements.

17.7 Quality Assurance

17.7.1 Responsibilities

The sponsor assumes the responsibility that this clinical trial is planned and conducted in accordance with the quality requirements of the principles of GCP.

17.7.2 Monitoring

Study monitoring is undertaken by monitors appointed by Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.

The responsible monitor will be allowed, on request, to inspect the various records of the trial (Case Report Forms, patient file and other pertinent data). The monitor will visit the site and check to completeness of patient records, the accuracy of entries on the eCRFs, the adherence to protocol of GCP and the control of stored and dispensed study medication by random samples. This will be done according to the monitoring manual agreed with Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.

Due to the electronic documentation system checks for range and plausibility are performed during data entry. The monitor gets an access to read the data only.

In line with ICH GCP guidelines, monitoring will include verification of data entered in the eCRF against original patient records. This verification will be performed by direct access to the original subject records, and the sponsor guarantees that patient confidentiality will be respected at all times. Participation in this study will be taken as agreement to permit direct source data verification.

17.7.3 Audits and Inspections

Independent of and separate from routine monitoring the sponsor and authorities may perform audits and inspections respectively. The investigator must allow specially trained representatives of the sponsor and of the authorities to accede the site and to inspect all relevant facilities and documents.

17.7.4 Notification of the Local Authority

The CRO will notify the local authority as required by §67 AMG (German Drug Law) in conjunction with § 12 GCP-V. The principal investigator of each site will inform the CRO about any change in study personnel, so that the notifications can be done timely.

17.7.5 Definition of Source Documents

The hospital patient records are the only source documents. No data are entered directly into the eCRF.

17.8 Insurance Cover

As required by the principles of GCP and §40(1) of the German Drug Law the sponsor takes out an insurance policy for all clinical trial participants via the annual contract of the University Hospital Ulm.

Insurance provider:
HDI-Gerling Versicherung AG
Police-No.: 57 010315 03015 (Anmeldenummer 1302 2013 110)

Contact data:
HDI-Gerling Industrie Versicherung AG
Riethorst 2; 30659 Hannover
Tel. 0511-645-0
Fax 0511-645-4545
Web: www.hdi-gerling.de
The insurance policy covers all injuries patients suffer from due to the entire study participation. Compensation is limited to 500 000 Euro at most.

The investigator informs each patient on his responsibilities resulting from the terms of insurance. Patients participating in the study must neither undergo any other medical treatment during the clinical trial without prior information of the investigator (except for cases of emergency) nor participate in any other trial. In case of damnification which might be due to trial participation, a patient must inform investigator and insurance immediately and cooperate in the clarification of whether there is a causal relationship between damnification and study participation.

17.9 Financing

This clinical trial is supported by an unrestricted grant of Novartis. The company also provides the study medication free of charge. This clinical trial is supported by Eisai. The company also provides the study medication Eribulin free of charge. This clinical trial is also supported by TEVA GmbH. The study is supported by Janssen Diagnostics (Raritan, NJ, USA) with study supply free of charge.

17.10 Honorarium for Clinical Trial Participants

Patients are not remunerated for study participation. There will be no extra appointments during the treatment according to the treatment plan; therefore travelling expenses are not reimbursed.

17.11 Patient ID Cards

Patients are provided an ID card as specified in APPENDIX VIII – PATIENT ID CARD.

This card also includes all information required according to § 5(8) GCP-V (German directive on GCP), that is not specified on the labels of everolimus/ribociclib or eribulin.

17.12 Data Safety Montitoring Board (DSMB)

A DSMB will be utilized in this study to ensure external objective medical and/or statistical review of safety and/or efficacy issues in order to protect the ethical and safety interests of subjects and to protect the scientific validity of the study. Exact operating procedures are set up before the start of the study.

18 REFERENCES

18.1 Publications

1 AARONSON, N.K., AHMEDZAI, S., BERGMAN, B., BULLINGER, M., CULL, A., DUEZ, N.J., FILIBERTI, A., FLECHTNER, H., FLEISHMAN, S.B. and DE HAES, J.C., 1993. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *Journal of the National Cancer Institute,* **85**(5), pp. 365-376.

2 ADDO S, YATES RA, LAIGHT A. (2002) A phase I trial toasses the pharmacology of the new oestrogen receptor antagonist fulvestrant on the endometrium of healthy postmenopausal volunteers. *BR J Cancer*. **87**(12): 1354-9

3 ABRAHAM, R.T. and GIBBONS, J.J., 2007. The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **13**(11), pp. 3109-3114.

4 AKTAS, B., TEWES, M., FEHM, T., HAUCH, S., KIMMIG, R. and KASIMIR-BAUER, S., 2009. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. *Breast cancer research : BCR,* **11**(4), pp. R46.

5 ASAITHAMBY, A. and CHEN, D.J., 2009. Cellular responses to DNA double-strand breaks after low-dose gamma-irradiation. *Nucleic acids research,* **37**(12), pp. 3912-3923.

6 ATKINS, M.B., HIDALGO, M., STADLER, W.M., LOGAN, T.F., DUTCHER, J.P., HUDES, G.R., PARK, Y., LIOU, S.H., MARSHALL, B., BONI, J.P., DUKART, G. and SHERMAN, M.L., 2004. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **22**(5), pp. 909-918.

7 AWADA, A., CARDOSO, F., FONTAINE, C., DIRIX, L., DE GREVE, J., SOTIRIOU, C., STEINSEIFER, J., WOUTERS, C., TANAKA, C., ZOELLNER, U., TANG, P. and PICCART, M., 2008. The oral mTOR inhibitor RAD001 (everolimus) in combination with letrozole in patients with advanced breast cancer: results of a phase I study with pharmacokinetics. *European journal of cancer (Oxford, England : 1990),* **44**(1), pp. 84-91.

8 BACHELOT, T., BOURGIER, C., CROPET, C., RAY-COQUARD, I., FERRERO, J.M., FREYER, G., ABADIE-LACOURTOISIE, S., EYMARD, J.C., DEBLED, M., SPAETH, D., LEGOUFFE, E., ALLOUACHE, D., EL KOURI, C. and PUJADE-LAURAINE, E., 2012. Randomized phase II trial of everolimus in combination with tamoxifen in patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer with prior exposure to aromatase inhibitors: a GINECO study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **30**(22), pp. 2718-2724.

9 BAI, R.L., PAULL, K.D., HERALD, C.L., MALSPEIS, L., PETTIT, G.R. and HAMEL, E., 1991. Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. *The Journal of biological chemistry,* **266**(24), pp. 15882-15889.

10 BAKER DJ, WIJSHAKE T, TCHKONIA T, LeBRASSEUR NK, CHILDS BG, VAN DE SLUIS B, KIRKLAND JL, van DEURSEN JM. (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature.* 2011 Nov 2;**479**(7372):232-6. doi: 10.1038/nature10600.

11 BARONE, I., CUI, Y., HERYNK, M.H., CORONA-RODRIGUEZ, A., GIORDANO, C., SELEVER, J., BEYER, A., ANDO, S. and FUQUA, S.A., 2009. Expression of the K303R estrogen receptor-alpha breast cancer mutation induces resistance to an aromatase inhibitor via addiction to the PI3K/Akt kinase pathway. *Cancer research,* **69**(11), pp. 4724-4732.

12 BARONE, I., IACOPETTA, D., COVINGTON, K.R., CUI, Y., TSIMELZON, A., BEYER, A., ANDO, S. and FUQUA, S.A., 2010. Phosphorylation of the mutant K303R estrogen receptor alpha at serine 305 affects aromatase inhibitor sensitivity. *Oncogene,* **29**(16), pp. 2404-2414.

13 BARTKOVA J, LUKAS J, MÜLLER H, LÜTZHOFT D, STRAUSS M, BARTEK J. (1994). Cyclin D1 protein expression and function in human breast cancer. *Int J Cancer.* 1994 May 1;**57**(3):353-61.

14 BUCKLEY MF, SWEENEY KJ, HAMILTON JA, SINI RL, MANNING DL, NICHOLSON RI, deFAZIO A, WATTS CK, MUSGROVE EA, SUTHERLAND RL (1993). Expression and amplification of cyclin genes in human breast cancer. *Oncogene*. 1993 Aug;**8**(8):2127-33.

15 BASELGA, J., CAMPONE, M., PICCART, M., BURRIS, H.A.,3RD, RUGO, H.S., SAHMOUD, T., NOGUCHI, S., GNANT, M., PRITCHARD, K.I., LEBRUN, F., BECK, J.T., ITO, Y., YARDLEY, D., DELEU, I., PEREZ, A., BACHELOT, T., VITTORI, L., XU, Z., MUKHOPADHYAY, P., LEBWOHL, D. and HORTOBAGYI, G.N., 2012. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *The New England journal of medicine,* **366**(6), pp. 520-529.

16 BASELGA, J., SEMIGLAZOV, V., VAN DAM, P., MANIKHAS, A., BELLET, M., MAYORDOMO, J., CAMPONE, M., KUBISTA, E., GREIL, R., BIANCHI, G., STEINSEIFER, J., MOLLOY, B., TOKAJI, E., GARDNER, H., PHILLIPS, P., STUMM, M., LANE, H.A., DIXON, J.M., JONAT, W. and RUGO, H.S., 2009. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **27**(16), pp. 2630-2637.

17 BEUVINK, I., BOULAY, A., FUMAGALLI, S., ZILBERMANN, F., RUETZ, S., O'REILLY, T., NATT, F., HALL, J., LANE, H.A. and THOMAS, G., 2005. The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition of p21 translation. *Cell,* **120**(6), pp. 747-759.

18 BJORNSTI, M.A. and HOUGHTON, P.J., 2004. The TOR pathway: a target for cancer therapy. *Nature reviews.Cancer,* **4**(5), pp. 335-348.

19 BOTTERI, E., SANDRI, M.T., BAGNARDI, V., MUNZONE, E., ZORZINO, L., ROTMENSZ, N., CASADIO, C., CASSATELLA, M.C., ESPOSITO, A., CURIGLIANO, G., SALVATICI, M., VERRI, E., ADAMOLI, L., GOLDHIRSCH, A. and NOLE, F., 2010. Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer. *Breast cancer research and treatment,* **122**(1), pp. 211-217.

20 BOTTOMLEY, A., BIGANZOLI, L., CUFER, T., COLEMAN, R.E., COENS, C., EFFICACE, F., CALVERT, H.A., GAMUCCI, T., TWELVES, C., FARGEOT, P., PICCART, M. and EUROPEAN ORGANIZATION FOR RESEARCH AND TREATMENT OF CANCER BREAST CANCER GROUP, 2004. Randomized, controlled trial investigating short-term health-related quality of life with doxorubicin and paclitaxel versus doxorubicin and cyclophosphamide as first-line chemotherapy in patients with metastatic breast cancer: European Organization for Research and Treatment of Cancer Breast Cancer Group, Investigational Drug Branch for Breast Cancer and the New Drug Development Group Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **22**(13), pp. 2576-2586.

21 BOUWMAN, P., ALY, A., ESCANDELL, J.M., PIETERSE, M., BARTKOVA, J., VAN DER GULDEN, H., HIDDINGH, S., THANASOULA, M., KULKARNI, A., YANG, Q., HAFFTY, B.G., TOMMISKA, J., BLOMQVIST, C., DRAPKIN, R., ADAMS, D.J., NEVANLINNA, H., BARTEK, J., TARSOUNAS, M., GANESAN, S. and JONKERS, J., 2010. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nature structural & molecular biology,* **17**(6), pp. 688-695.

22 BRODIE, K.M. and HENDERSON, B.R., 2012. Characterization of BRCA1 protein targeting, dynamics, and function at the centrosome: a role for the nuclear export signal, CRM1, and Aurora A kinase. *The Journal of biological chemistry,* **287**(10), pp. 7701-7716.

23 BUDD, G.T., CRISTOFANILLI, M., ELLIS, M.J., STOPECK, A., BORDEN, E., MILLER, M.C., MATERA, J., REPOLLET, M., DOYLE, G.V., TERSTAPPEN, L.W. and HAYES, D.F., 2006. Circulating tumor cells versus imaging--predicting overall survival in metastatic breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **12**(21), pp. 6403-6409.

24 BUNTING, S.F., CALLEN, E., KOZAK, M.L., KIM, J.M., WONG, N., LOPEZ-CONTRERAS, A.J., LUDWIG, T., BAER, R., FARYABI, R.B., MALHOWSKI, A., CHEN, H.T., FERNANDEZ-CAPETILLO, O., D'ANDREA, A. and NUSSENZWEIG, A., 2012. BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair. *Molecular cell,* **46**(2), pp. 125-135.

25 CAMPBELL, R.A., BHAT-NAKSHATRI, P., PATEL, N.M., CONSTANTINIDOU, D., ALI, S. and NAKSHATRI, H., 2001. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. *The Journal of biological chemistry,* **276**(13), pp. 9817-9824.

26 CHAN, S., 2004. Targeting the mammalian target of rapamycin (mTOR): a new approach to treating cancer. *British journal of cancer,* **91**(8), pp. 1420-1424.

27 CHAPUIS, N., TAMBURINI, J., GREEN, A., WILLEMS, L., BARDET, V., PARK, S., LACOMBE, C., MAYEUX, P. and BOUSCARY, D., 2010. Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. *Leukemia,* **24**(10), pp. 1686-1699.

28 CHEUNG, A.M., TILE, L., CARDEW, S., PRUTHI, S., ROBBINS, J., TOMLINSON, G., KAPRAL, M.K., KHOSLA, S., MAJUMDAR, S., ERLANDSON, M., SCHER, J., HU, H., DEMARAS, A., LICKLEY, L., BORDELEAU, L., ELSER, C., INGLE, J., RICHARDSON, H. and GOSS, P.E., 2012. Bone density and structure in healthy postmenopausal women treated with exemestane for the primary prevention of breast cancer: a nested substudy of the MAP.3 randomised controlled trial. *The lancet oncology,* **13**(3), pp. 275-284.

29 CONROY, T., MERCIER, M., BONNETERRE, J., LUPORSI, E., LEFEBVRE, J.L., LAPEYRE, M., PUYRAVEAU, M. and SCHRAUB, S., 2004. French version of FACT-G: validation and comparison with other cancer-specific instruments. *European journal of cancer (Oxford, England : 1990),* **40**(15), pp. 2243-2252.

30 CONWAY, K., PARRISH, E., EDMISTON, S.N., TOLBERT, D., TSE, C.K., GERADTS, J., LIVASY, C.A., SINGH, H., NEWMAN, B. and MILLIKAN, R.C., 2005. The estrogen receptor-alpha A908G (K303R) mutation occurs at a low frequency in invasive breast tumors: results from a population-based study. *Breast cancer research : BCR,* **7**(6), pp. R871-80.

31 CRISTOFANILLI, M., BUDD, G.T., ELLIS, M.J., STOPECK, A., MATERA, J., MILLER, M.C., REUBEN, J.M., DOYLE, G.V., ALLARD, W.J., TERSTAPPEN, L.W. and HAYES, D.F., 2004. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *The New England journal of medicine,* **351**(8), pp. 781-791.

32 CRISTOFANILLI, M., HAYES, D.F., BUDD, G.T., ELLIS, M.J., STOPECK, A., REUBEN, J.M., DOYLE, G.V., MATERA, J., ALLARD, W.J., MILLER, M.C., FRITSCHE, H.A., HORTOBAGYI, G.N. and TERSTAPPEN, L.W., 2005. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **23**(7), pp. 1420-1430.

33 CRISTOFANILLI M, TURNER NC, BONDARENKO I, RO J, IM SA, MASUDA N, COLLEONI M, DeMICHELE A, LOI S, VERMA S, IWATA H, HARBECK N, ZHANG K, THEALL KP, JIANG Y, BARTLETT CH, KOEHLER M, SLAMON D, 2016. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicenter, double-blind, phase 3 randomised controlled trial. *Lancet Onology, 2016 Apr 17 (4): 425-39.*

34 DI LEO A*.,* JERUSALEM G, PETRUZELKA L, ET AL. (2010) Results of the CONFIRM phase III trialcomparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. *J. Clin Oncol.* Oct 20; **28** (30): 4594-600.

35 DI LEO A*.,* JERUSALEM G, PETRUZELKA L, TORRES R, ET AL. (2013) Final Overall Survivak: Fulvestrant 500 mg vs 250 mg in the Randomized CONFIRM Trial. *JNCI J Natl Cancer Inst* 106(1).

36 DOUMA, S., VAN LAAR, T., ZEVENHOVEN, J., MEUWISSEN, R., VAN GARDEREN, E. and PEEPER, D.S., 2004. Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. *Nature,* **430**(7003), pp. 1034-1039.

37 EISENHAUER, E.A., THERASSE, P., BOGAERTS, J., SCHWARTZ, L.H., SARGENT, D., FORD, R., DANCEY, J., ARBUCK, S., GWYTHER, S., MOONEY, M., RUBINSTEIN, L., SHANKAR, L., DODD, L., KAPLAN, R., LACOMBE, D. and VERWEIJ, J., 2009. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England : 1990),* **45**(2), pp. 228-247.

38 ELLARD, S.L., CLEMONS, M., GELMON, K.A., NORRIS, B., KENNECKE, H., CHIA, S., PRITCHARD, K., EISEN, A., VANDENBERG, T., TAYLOR, M., SAUERBREI, E., MISHAELI, M., HUNTSMAN, D., WALSH, W., OLIVO, M., MCINTOSH, L. and SEYMOUR, L., 2009. Randomized phase II study comparing two schedules of

everolimus in patients with recurrent/metastatic breast cancer: NCIC Clinical Trials Group IND.163. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **27**(27), pp. 4536-4541.

39 ELLIS MJ, COOP A, SINGH B, MAURIAC L, LLOMBERT-CUSSAC A, JÄNICKE F, MILLER WR, EVANS DB, DUGAN M, BRADY C, QUEBE-FEHLING E, BORGS M. (2001). Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol.* 2001 Sep 15;**19**(18):3808-16

40 ELLIS MJ, LLOMBART-CUSSAC A, FELTL D, DEWAR JA, JASIOWKA M, HEWSON N, RUKAZENKOV Y, ROBERTSON JF (2015). Fulvestrant 500 mg vs Anastrazole 1 mg for the First-Line Treatment of Advanced Breast Cancer: Overall Survival Analysis from the phase II FIRST study. *J Clin Oncol.* 10; **33** (32): 3781-7.

41 FEHM, T., MULLER, V., AKTAS, B., JANNI, W., SCHNEEWEISS, A., STICKELER, E., LATTRICH, C., LOHBERG, C.R., SOLOMAYER, E., RACK, B., RIETHDORF, S., KLEIN, C., SCHINDLBECK, C., BROCKER, K., KASIMIR-BAUER, S., WALLWIENER, D. and PANTEL, K., 2010. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. *Breast cancer research and treatment,* **124**(2), pp. 403-412.

42 FINN RS, DERING J, CONKLIN D, KALOUS O, COHEN DJ, DESAI AJ, GINTHER C, ATEFI M, CHEN I, FOWST C, LOS G, SLAMON DJ. (2009). PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res*. 2009;**11**(5):R77. doi: 10.1186/bcr2419.

43 FINN RS, CROWN JP, LANG I, BOER K, BONDARENKO IM, KULYUK SO, ETTL J, PATEL R, PINTER T, SCHMIDT M, SHPARYK Y, THUMMALA AR, VOYTKO NL, FOWST C, HUANG X, KIM ST, RANDOLPH S, SLAMON DJ. (2014). The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol.* 2015 Jan;**16**(1):25-35. doi: 10.1016/S1470-2045(14)71159-3. Epub 2014 Dec 16.

44 FOULKES, W.D., SMITH, I.E. and REIS-FILHO, J.S., 2010. Triple-negative breast cancer. *The New England journal of medicine,* **363**(20), pp. 1938-1948.

45 FRISCH, S.M. and RUOSLAHTI, E., 1997. Integrins and anoikis. *Current opinion in cell biology,* **9**(5), pp. 701-706.

46 FUQUA, S.A., WILTSCHKE, C., ZHANG, Q.X., BORG, A., CASTLES, C.G., FRIEDRICHS, W.E., HOPP, T., HILSENBECK, S., MOHSIN, S., O'CONNELL, P. and ALLRED, D.C., 2000. A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions. *Cancer research,* **60**(15), pp. 4026-4029.

47 GAGOU, M.E., ZUAZUA-VILLAR, P. and MEUTH, M., 2010. Enhanced H2AX phosphorylation, DNA replication fork arrest, and cell death in the absence of Chk1. *Molecular biology of the cell,* **21**(5), pp. 739-752.

48 GIANNAKAKOU, P., SACKETT, D.L., KANG, Y.K., ZHAN, Z., BUTERS, J.T., FOJO, T. and PORUCHYNSKY, M.S., 1997. Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization. *The Journal of biological chemistry,* **272**(27), pp. 17118-17125.

49 GIORDANO, A. and CRISTOFANILLI, M., 2012. CTCs in metastatic breast cancer. *Recent results in cancer research.Fortschritte der Krebsforschung.Progres dans les recherches sur le cancer,* **195**, pp. 193-201.

50 GIORDANO, C., CUI, Y., BARONE, I., ANDO, S., MANCINI, M.A., BERNO, V. and FUQUA, S.A., 2010. Growth factor-induced resistance to tamoxifen is associated with a mutation of estrogen receptor alpha and its phosphorylation at serine 305. *Breast cancer research and treatment,* **119**(1), pp. 71-85.

51 GILLET C, FANTL V, SMITH R, FISCHER C, BARTEK J, DICKSON C, BARNES D, PETERS G. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res. 1994 Apr 1;54(7):1812-7.

52 GILLET C, SMITH P, GREGORY W, RICHARDS M, MILLIS R, PETERS G, BARNES D. Cyclin D1 and prognosis in human breast cancer. Int J Cancer. 1996 Apr 22;69(2):92-9. Juric et al. Ribociclib (LEE011) and letrozole in estrogen receptor-positive (ER+), HER2-negative (HER2–) advanced breast cancer (aBC): Phase Ib safety, preliminary efficacy and molecular analysis. J Clin Oncol 34, 2016 (suppl; abstr 568)

53 GIULIANO, M., GIORDANO, A., JACKSON, S., HESS, K.R., DE GIORGI, U., MEGO, M., HANDY, B.C., UENO, N.T., ALVAREZ, R.H., DE LAURENTIIS, M., DE PLACIDO, S., VALERO, V., HORTOBAGYI, G.N., REUBEN, J.M. and CRISTOFANILLI, M., 2011. Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line systemic treatment. *Breast cancer research : BCR,* **13**(3), pp. R67.

54 GONZALEZ-ANGULO, A.M., STEMKE-HALE, K., PALLA, S.L., CAREY, M., AGARWAL, R., MERIC-BERSTAM, F., TRAINA, T.A., HUDIS, C., HORTOBAGYI, G.N., GERALD, W.L., MILLS, G.B. and HENNESSY, B.T., 2009. Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **15**(7), pp. 2472-2478.

55 HAYES, D.F., CRISTOFANILLI, M., BUDD, G.T., ELLIS, M.J., STOPECK, A., MILLER, M.C., MATERA, J., ALLARD, W.J., DOYLE, G.V. and TERSTAPPEN, L.W., 2006. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **12**(14 Pt 1), pp. 4218-4224.

56 HAYES, D.F., WALKER, T.M., SINGH, B., VITETTA, E.S., UHR, J.W., GROSS, S., RAO, C., DOYLE, G.V. and TERSTAPPEN, L.W., 2002. Monitoring expression of HER-2 on circulating epithelial cells in patients with advanced breast cancer. *International journal of oncology,* **21**(5), pp. 1111-1117.

57 HERMAN, S., VARGA, D., DEISSLER, H.L., KREIENBERG, R. and DEISSLER, H., 2012. Medium-sized deletion in the BRCA1 gene: Limitations of Sanger sequencing and MLPA analyses. *Genetics and molecular biology,* **35**(1), pp. 53-56.

58 HERYNK, M.H., PARRA, I., CUI, Y., BEYER, A., WU, M.F., HILSENBECK, S.G. and FUQUA, S.A., 2007. Association between the estrogen receptor alpha A908G mutation and outcomes in invasive breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **13**(11), pp. 3235-3243.

59 HJERMSTAD, M.J., FOSSA, S.D., BJORDAL, K. and KAASA, S., 1995. Test/retest study of the European Organization for Research and Treatment of Cancer Core Quality-of-Life Questionnaire. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **13**(5), pp. 1249-1254.

60 HORTOBAGYI, G., PICCART, M., RUGO, H., BURRIS, H., CAMPONE, M., NOGUCHI, S., GNANT, M., PRITCHARD, K., VITTORI, L. and MUKHOPADHYAY, P., 2011. Everolimus for postmenopausal women with advanced breast cancer: Updated results of the BOLERO-2 phase III trial, *2011 San Antonio Breast Cancer Symposium. Presentation of late-breaking abstract* 2011.

61 HORTIBAGYI GN, STEMMER SM, BURRIS HA, YAP YS, SONKE GS, PALUCH-SHIMON S, CAMPONE M, BLACKWELL KL, ANDRE F, WINER EP, JANNI W, VERMA S, CONTE P, ARTEAGA CL, CAMERON DA, PETRAKOVA K, HART LL, VILLANUEVA C, CHAN A, JAKOBSEN E, NUSCH A, BURDAEVA O, GRISCHKE EM, ALBA E, WIST E, MARSCHNER N, FAVRET AM, YARDLEY D, BACHELOT T, TSENG LM, BLAU S, XUAN F, SOUAMI F, MILLER M, GERMA C, HIRAWAT S, O'SHAUGHNESSY J 2016. *Ribociclib as First-line Therapy for HR-positive, advanced breast cancer. N Engl J Med 2016 Nov 3; 375 (18): 1738-1748.*

62 HORTOBAGYI GN ET AL. American Society of Clinical Oncology, June 2-6, 2017 Chicago, IL, USA. Poster 1038

63 HOWELL, A., CUZICK, J., BAUM, M., BUZDAR, A., DOWSETT, M., FORBES, J.F., HOCTIN-BOES, G., HOUGHTON, J., LOCKER, G.Y., TOBIAS, J.S. and ATAC TRIALISTS' GROUP, 2005. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet,* **365**(9453), pp. 60-62.

64 HURST, C.D., ZUIVERLOON, T.C., HAFNER, C., ZWARTHOFF, E.C. and KNOWLES, M.A., 2009. A SNaPshot assay for the rapid and simple detection of four common hotspot codon mutations in the PIK3CA gene. *BMC research notes,* **2**, pp. 66-0500-2-66.

65 HUSCHKA, M.M., MANDREKAR, S.J., SCHAEFER, P.L., JETT, J.R. and SLOAN, J.A., 2007. A pooled analysis of quality of life measures and adverse events data in north central cancer treatment group lung cancer clinical trials. *Cancer,* **109**(4), pp. 787-795.

66 JENSEN, E.V. and JORDAN, V.C., 2003. The estrogen receptor: a model for molecular medicine. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **9**(6), pp. 1980-1989.

67 JIA, J., ZHANG, W., LIU, J.Y., CHEN, G., LIU, H., ZHONG, H.Y., LIU, B., CAI, Y., ZHANG, J.L. and ZHAO, Y.F., 2012. Epithelial mesenchymal transition is required for acquisition of anoikis resistance and metastatic potential in adenoid cystic carcinoma. *PloS one,* **7**(12), pp. e51549.

68 JIANG, Y., PALMA, J.F., AGUS, D.B., WANG, Y. and GROSS, M.E., 2010. Detection of androgen receptor mutations in circulating tumor cells in castration-resistant prostate cancer. *Clinical chemistry,* **56**(9), pp. 1492-1495.

69 JOOSSE, S.A., BRANDWIJK, K.I., MULDER, L., WESSELING, J., HANNEMANN, J. and NEDERLOF, P.M., 2011. Genomic signature of BRCA1 deficiency in sporadic basal-like breast tumors. *Genes, chromosomes & cancer,* **50**(2), pp. 71-81.

70 KASIMIR-BAUER, S., HOFFMANN, O., WALLWIENER, D., KIMMIG, R. and FEHM, T., 2012. Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells. *Breast cancer research : BCR,* **14**(1), pp. R15.

71 KEIMLING, M., KAUR, J., BAGADI, S.A., KREIENBERG, R., WIESMULLER, L. and RALHAN, R., 2008. A sensitive test for the detection of specific DSB repair defects in primary cells from breast cancer specimens. *International journal of cancer.Journal international du cancer,* **123**(3), pp. 730-736.

72 KOVARIK, J.M., KAHAN, B.D., KAPLAN, B., LORBER, M., WINKLER, M., ROUILLY, M., GERBEAU, C., CAMBON, N., BOGER, R., RORDORF, C. and EVEROLIMUS PHASE 2 STUDY GROUP, 2001. Longitudinal assessment of everolimus in de novo renal transplant recipients over the first post-transplant year: pharmacokinetics, exposure-response relationships, and influence on cyclosporine. *Clinical pharmacology and therapeutics,* **69**(1), pp. 48-56.

73 KUZNETSOV, G., TOWLE, M.J., CHENG, H., KAWAMURA, T., TENDYKE, K., LIU, D., KISHI, Y., YU, M.J. and LITTLEFIELD, B.A., 2004. Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. *Cancer research,* **64**(16), pp. 5760-5766.

74 LEMIEUX, J., BEATON, D.E., HOGG-JOHNSON, S., BORDELEAU, L.J., HUNTER, J. and GOODWIN, P.J., 2007. Responsiveness to change [corrected] due to supportive-expressive group therapy, improvement in mood and disease progression in women with metastatic breast cancer. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation,* **16**(6), pp. 1007-1017.

75 LIANG, X., WANG, P., GAO, Q., XIANG, T. and TAO, X., 2010. Endogenous LKB1 knockdown accelerates G1/S transition through p53 and p16 pathways. *Cancer biology & therapy,* **9**(2), pp. 156-160.

76 LILJA, H. and SCHER, H.I., 2010. Detection of androgen receptor mutations in circulating tumor cells: highlights of the long road to clinical qualification. *Clinical chemistry,* **56**(9), pp. 1375-1377.

77 LINN, S.C. and VAN 'T VEER, L.J., 2009. Clinical relevance of the triple-negative breast cancer concept: genetic basis and clinical utility of the concept. *European journal of cancer (Oxford, England : 1990),* **45 Suppl 1**, pp. 11-26.

78 LIOTTA, L.A. and KOHN, E., 2004. Anoikis: cancer and the homeless cell. *Nature,* **430**(7003), pp. 973-974.

79 LIU, M.C., SHIELDS, P.G., WARREN, R.D., COHEN, P., WILKINSON, M., OTTAVIANO, Y.L., RAO, S.B., ENG-WONG, J., SEILLIER-MOISEIWITSCH, F., NOONE, A.M. and ISAACS, C., 2009. Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **27**(31), pp. 5153-5159.

80 LLOMBART-CUSSAC, A., RUIZ, A., ANTON, A., BARNADAS, A., ANTOLIN, S., ALES-MARTINEZ, J.E., ALVAREZ, I., ANDRES, R., GARCIA SAENZ, J.A., LAO, J., CARRASCO, E., CAMARA, C., CASAS, I. and MARTIN, M., 2012. Exemestane versus anastrozole as front-line endocrine therapy in postmenopausal patients with hormone receptor-positive, advanced breast cancer: final results from the Spanish Breast Cancer Group 2001-03 phase 2 randomized trial. *Cancer,* **118**(1), pp. 241-247.

81 LUNDGREN K, BROWN M, PINEDA S, CUZIK J, SALTER J, ZABAGLO L, HOWELL A, DOWSETT M, LANDBERG G; TransATAC investigators. Effects of cyclin D1 gene amplification and protein expression on time to recurrence in postmenopausal breast cancer patients treated with anastrozole or tamoxifen: a TransATAC study. *Breast Cancer Res*. 2012 Apr 4;**14**(2):R57.

82 LUO, J., MANNING, B.D. and CANTLEY, L.C., 2003. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer cell,* **4**(4), pp. 257-262.

83 MA, Y., FAN, S., HU, C., MENG, Q., FUQUA, S.A., PESTELL, R.G., TOMITA, Y.A. and ROSEN, E.M., 2010. BRCA1 regulates acetylation and ubiquitination of estrogen receptor-alpha. *Molecular endocrinology (Baltimore, Md.),* **24**(1), pp. 76-90.

84 MABUCHI, S., ALTOMARE, D.A., CHEUNG, M., ZHANG, L., POULIKAKOS, P.I., HENSLEY, H.H., SCHILDER, R.J., OZOLS, R.F. and TESTA, J.R., 2007. RAD001 inhibits human ovarian cancer cell proliferation, enhances cisplatin-induced apoptosis, and prolongs survival in an ovarian cancer model. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **13**(14), pp. 4261-4270.

85 MABUCHI, S., ALTOMARE, D.A., CONNOLLY, D.C., KLEIN-SZANTO, A., LITWIN, S., HOELZLE, M.K., HENSLEY, H.H., HAMILTON, T.C. and TESTA, J.R., 2007. RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. *Cancer research,* **67**(6), pp. 2408-2413.

86 MANEGOLD, P.C., PARINGER, C., KULKA, U., KRIMMEL, K., EICHHORN, M.E., WILKOWSKI, R., JAUCH, K.W., GUBA, M. and BRUNS, C.J., 2008. Antiangiogenic therapy with mammalian target of rapamycin inhibitor RAD001 (Everolimus) increases radiosensitivity in solid cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **14**(3), pp. 892-900.

87 MCINTOSH GG, ANDRESON JJ, MILTON I, STEWARD M, PARR AH, THOMAS MD, HENRY JA, ANGUS B, LENNARD TW, HORNE CH.; Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene. 1995 Sep 7;11(5):885-91

88 MCLACHLAN, S.A., DEVINS, G.M. and GOODWIN, P.J., 1998. Validation of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (QLQ-C30) as a measure of psychosocial function in breast cancer patients. *European journal of cancer (Oxford, England : 1990),* **34**(4), pp. 510-517.

89 MEGO, M., GAO, H., LEE, B.N., COHEN, E.N., TIN, S., GIORDANO, A., WU, Q., LIU, P., NIETO, Y., CHAMPLIN, R.E., HORTOBAGYI, G.N., CRISTOFANILLI, M., UENO, N.T. and REUBEN, J.M., 2012. Prognostic Value of EMT-Circulating Tumor Cells in Metastatic Breast Cancer Patients Undergoing High-Dose Chemotherapy with Autologous Hematopoietic Stem Cell Transplantation. *Journal of Cancer,* **3**, pp. 369-380.

90 MEGO, M., MANI, S.A., LEE, B.N., LI, C., EVANS, K.W., COHEN, E.N., GAO, H., JACKSON, S.A., GIORDANO, A., HORTOBAGYI, G.N., CRISTOFANILLI, M., LUCCI, A. and REUBEN, J.M., 2012. Expression of epithelial-mesenchymal transition-inducing transcription factors in primary breast cancer: The effect of neoadjuvant therapy. *International journal of cancer.Journal international du cancer,* **130**(4), pp. 808-816.

91 MENG, S., TRIPATHY, D., SHETE, S., ASHFAQ, R., HALEY, B., PERKINS, S., BEITSCH, P., KHAN, A., EUHUS, D., OSBORNE, C., FRENKEL, E., HOOVER, S., LEITCH, M., CLIFFORD, E., VITETTA, E., MORRISON, L., HERLYN, D., TERSTAPPEN, L.W., FLEMING, T., FEHM, T., TUCKER, T., LANE, N., WANG, J. and UHR, J., 2004. HER-2 gene amplification can be acquired as breast cancer progresses. *Proceedings of the National Academy of Sciences of the United States of America,* **101**(25), pp. 9393-9398.

92 MILLER TW, BALKO JM, FOX EM, GHAZOUI Z, DUNBIER A, ANDERSON H, DOWSETT M, JIANG A, SMITH RA, MAIRA SM, MANNING HC, GONZALEZ-ANGULO AM, MILLS GB, HIGHAM C, CHANTHAPHAYCHITH S, KUBA MG, MILLER WR, SHYR Y, ARTEAGA CL. ERα-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. *Cancer Discov.* 2011 Sep;**1**(4):338-51. doi: 10.1158/2159-8290.CD-11-0101. Epub 2011 Jul 20.

93 MIYAMOTO, D.T., LEE, R.J., STOTT, S.L., TING, D.T., WITTNER, B.S., ULMAN, M., SMAS, M.E., LORD, J.B., BRANNIGAN, B.W., TRAUTWEIN, J., BANDER, N.H., WU, C.L., SEQUIST, L.V., SMITH, M.R., RAMASWAMY, S., TONER, M., MAHESWARAN, S. and HABER, D.A., 2012. Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. *Cancer discovery,* **2**(11), pp. 995-1003.

94 MOORE, N.L., BUCHANAN, G., HARRIS, J.M., SELTH, L.A., BIANCO-MIOTTO, T., HANSON, A.R., BIRRELL, S.N., BUTLER, L.M., HICKEY, T.E. and TILLEY, W.D., 2012. An androgen receptor mutation in the MDA-MB-453 cell line model of molecular apocrine breast cancer compromises receptor activity. *Endocrine-related cancer,* **19**(4), pp. 599-613.

95 MOZZETTI, S., FERLINI, C., CONCOLINO, P., FILIPPETTI, F., RASPAGLIO, G., PRISLEI, S., GALLO, D., MARTINELLI, E., RANELLETTI, F.O., FERRANDINA, G. and SCAMBIA, G., 2005. Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **11**(1), pp. 298-305.

96 MUSGROVE EA, CALDON CE, BARRACLOUGH J, et al (2011) Cyclin D as a therapeutic target in cancer. Nat *Rev Cancer* 11:558-72.

97 MUKHOPADHYAY, A., ELATTAR, A., CERBINSKAITE, A., WILKINSON, S.J., DREW, Y., KYLE, S., LOS, G., HOSTOMSKY, Z., EDMONDSON, R.J. and CURTIN, N.J., 2010. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **16**(8), pp. 2344-2351.

98 MULLER, V., RIETHDORF, S., RACK, B., JANNI, W., FASCHING, P.A., SOLOMAYER, E., AKTAS, B., KASIMIR-BAUER, S., PANTEL, K., FEHM, T. and ON BEHALF OF THE DETECT STUDY GROUP, 2012. Prognostic impact of circulating tumor cells assessed with the CellSearch System and AdnaTest Breast in metastatic breast cancer patients: the DETECT study. *Breast cancer research : BCR,* **14**(4), pp. R118.

99 O'DONNELL, A., FAIVRE, S., BURRIS, H.A.,3RD, REA, D., PAPADIMITRAKOPOULOU, V., SHAND, N., LANE, H.A., HAZELL, K., ZOELLNER, U., KOVARIK, J.M., BROCK, C., JONES, S., RAYMOND, E. and JUDSON, I., 2008. Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **26**(10), pp. 1588-1595.

100 ONDER, T.T., GUPTA, P.B., MANI, S.A., YANG, J., LANDER, E.S. and WEINBERG, R.A., 2008. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer research,* **68**(10), pp. 3645-3654.

101 OPLUSTILOVA, L., WOLANIN, K., MISTRIK, M., KORINKOVA, G., SIMKOVA, D., BOUCHAL, J., LENOBEL, R., BARTKOVA, J., LAU, A., O'CONNOR, M.J., LUKAS, J. and BARTEK, J., 2012. Evaluation of candidate biomarkers to predict cancer cell sensitivity or resistance to PARP-1 inhibitor treatment. *Cell cycle (Georgetown, Tex.),* **11**(20), pp. 3837-3850.

102 OSOBA, D., RODRIGUES, G., MYLES, J., ZEE, B. and PATER, J., 1998. Interpreting the significance of changes in health-related quality-of-life scores. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **16**(1), pp. 139-144.

103 PAUL, N., PATER, J., WHITEHEAD, M. and SADURA, A., 1991. Methods of toxicity data collection: an evaluation of the relative effectiveness of the case report flow sheet (FS), the patient symptom diary (SD), and the quality of life questionnaire (QLQ). *Controlled clinical trials,* **12**(5), pp. 648.

104 PICCART M, BASELGA J, NOGUCHI S, BURRIS H, GNANT M, HORTOBAGYI G, MUKHOPADHYAYA P, TARAN T, SAHMOUD T and RUGO H, 2012. Final progression-free survival analysis of BOLERO-2: a phase III trial of everolimus for postmenopausal women with advanced breast cancer, *Thirty-Fifth Annual CTRC-AACR San Antonio Breast Cancer Symposium*, Dec 4–8. 2012 2012.

105 PIERGA, J.Y., HAJAGE, D., BACHELOT, T., DELALOGE, S., BRAIN, E., CAMPONE, M., DIERAS, V., ROLLAND, E., MIGNOT, L., MATHIOT, C. and BIDARD, F.C., 2012. High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer patients. *Annals of Oncology : Official Journal of the European Society for Medical Oncology / ESMO,* **23**(3), pp. 618-624.

106 REDON, C.E., NAKAMURA, A.J., ZHANG, Y.W., JI, J.J., BONNER, W.M., KINDERS, R.J., PARCHMENT, R.E., DOROSHOW, J.H. and POMMIER, Y., 2010. Histone gammaH2AX and poly(ADP-ribose) as clinical pharmacodynamic biomarkers. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **16**(18), pp. 4532-4542.

107 REPETTO, L. and DI BARTOLOMEO, C., 2010. The Use of Chemotherapy in Elderly Cancer Patients: Dose Adjusting, Drug Interactions, and Polypharmacy. *Management of Breast Cancer in Older Women.* Springer, pp. 315-330.

108 RIETHDORF, S., FRITSCHE, H., MULLER, V., RAU, T., SCHINDLBECK, C., RACK, B., JANNI, W., COITH, C., BECK, K., JANICKE, F., JACKSON, S., GORNET, T., CRISTOFANILLI, M. and PANTEL, K., 2007. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **13**(3), pp. 920-928.

109 RIETHDORF, S., MULLER, V., ZHANG, L., RAU, T., LOIBL, S., KOMOR, M., ROLLER, M., HUOBER, J., FEHM, T., SCHRADER, I., HILFRICH, J., HOLMS, F., TESCH, H., EIDTMANN, H., UNTCH, M., VON MINCKWITZ, G. and PANTEL, K., 2010. Detection and HER2 expression of circulating tumor cells: prospective monitoring in breast cancer patients treated in the neoadjuvant GeparQuattro trial. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **16**(9), pp. 2634-2645.

110 ROBERTSON JF; HARRISON M. (2004) Fulvestrant: pharmacokinetics and pharmacology. *Br J Cancer*. Mar;90 Suppl 1: S7-10

111 ROSE, C., VTORAYA, O., PLUZANSKA, A., DAVIDSON, N., GERSHANOVICH, M., THOMAS, R., JOHNSON, S., CAICEDO, J.J., GERVASIO, H., MANIKHAS, G., BEN AYED, F., BURDETTE-RADOUX, S., CHAUDRI-ROSS, H.A. and LANG, R., 2003. An open randomised trial of second-line endocrine therapy in advanced breast cancer. comparison of the aromatase inhibitors letrozole and anastrozole. *European journal of cancer (Oxford, England : 1990),* **39**(16), pp. 2318-2327.

112 RUSNAK, D.W., LACKEY, K., AFFLECK, K., WOOD, E.R., ALLIGOOD, K.J., RHODES, N., KEITH, B.R., MURRAY, D.M., KNIGHT, W.B., MULLIN, R.J. and GILMER, T.M., 2001. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. *Molecular cancer therapeutics,* **1**(2), pp. 85-94.

113 SANTEN, R.J., SONG, R.X., ZHANG, Z., KUMAR, R., JENG, M.H., MASAMURA, S., LAWRENCE, J.,JR, MACMAHON, L.P., YUE, W. and BERSTEIN, L., 2005. Adaptive hypersensitivity to estrogen: mechanisms and clinical relevance to aromatase inhibitor therapy in breast cancer treatment. *The Journal of steroid biochemistry and molecular biology,* **95**(1-5), pp. 155-165.

114 SARBASSOV, D.D., ALI, S.M., KIM, D.H., GUERTIN, D.A., LATEK, R.R., ERDJUMENT-BROMAGE, H., TEMPST, P. and SABATINI, D.M., 2004. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Current biology : CB,* **14**(14), pp. 1296-1302.

115 SARRIO, D., RODRIGUEZ-PINILLA, S.M., HARDISSON, D., CANO, A., MORENO-BUENO, G. and PALACIOS, J., 2008. Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer research,* **68**(4), pp. 989-997.

116 SCHNECK, H., BLASSL, C., MEIER-STIEGEN, F., NEVES, R.P., JANNI, W., FEHM, T. and NEUBAUER, H., 2013. Analysing the mutational status of PIK3CA in circulating tumor cells from metastatic breast cancer patients. *Molecular oncology,* **7**(5), pp. 976-986.

117 SHACKELFORD, D.B. and SHAW, R.J., 2009. The LKB1–AMPK pathway: metabolism and growth control in tumour suppression. *Nature Reviews Cancer,* **9**(8), pp. 563-575.

118 SHEN, Z., WEN, X., LAN, F., SHEN, Z. and SHAO, Z., 2002. The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma. *Clinical cancer research,* **8**(7), pp. 2085-2090.

119 SHINOHARA, E.T., CAO, C., NIERMANN, K., MU, Y., ZENG, F., HALLAHAN, D.E. and LU, B., 2005. Enhanced radiation damage of tumor vasculature by mTOR inhibitors. *Oncogene,* **24**(35), pp. 5414-5422.

120 SMITH, I.E. and DOWSETT, M., 2003. Aromatase inhibitors in breast cancer. *The New England journal of medicine,* **348**(24), pp. 2431-2442.

121 SMITH, T.J., KHATCHERESSIAN, J., LYMAN, G.H., OZER, H., ARMITAGE, J.O., BALDUCCI, L., BENNETT, C.L., CANTOR, S.B., CRAWFORD, J., CROSS, S.J., DEMETRI, G., DESCH, C.E., PIZZO, P.A., SCHIFFER, C.A., SCHWARTZBERG, L., SOMERFIELD, M.R., SOMLO, G., WADE, J.C., WADE, J.L., WINN, R.J., WOZNIAK, A.J. and WOLFF, A.C., 2006. 2006 Update of Recommendations for the use of White Blood Cell Growth Factors: an Evidence-Based Clinical Practice Guideline. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **24**(19), pp. 3187-3205.

122 STRATTON, M.R. and RAHMAN, N., 2008. The emerging landscape of breast cancer susceptibility. *Nature genetics,* **40**(1), pp. 17-22.

123 SUNG, M. and GIANNAKAKOU, P., 2014. BRCA1 regulates microtubule dynamics and taxane-induced apoptotic cell signaling. *Oncogene,* **33**(11), pp. 1418-1428.

124 SUTTON, L.M., CAO, D., SARODE, V., MOLBERG, K.H., TORGBE, K., HALEY, B. and PENG, Y., 2012. Decreased androgen receptor expression is associated with distant metastases in patients with androgen receptor-expressing triple-negative breast carcinoma. *American Journal of Clinical Pathology,* **138**(4), pp. 511-516.

125 TABERNERO, J., ROJO, F., CALVO, E., BURRIS, H., JUDSON, I., HAZELL, K., MARTINELLI, E., RAMON Y CAJAL, S., JONES, S., VIDAL, L., SHAND, N., MACARULLA, T., RAMOS, F.J., DIMITRIJEVIC, S., ZOELLNER, U., TANG, P., STUMM, M., LANE, H.A., LEBWOHL, D. and BASELGA, J., 2008. Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* **26**(10), pp. 1603-1610.

126 TALIAFERRO-SMITH, L., NAGALINGAM, A., ZHONG, D., ZHOU, W., SAXENA, N. and SHARMA, D., 2009. LKB1 is required for adiponectin-mediated modulation of AMPK–S6K axis and inhibition of migration and invasion of breast cancer cells. *Oncogene,* **28**(29), pp. 2621-2633.

127 THIKE, A.A., YONG-ZHENG CHONG, L., CHEOK, P.Y., LI, H.H., WAI-CHEONG YIP, G., HUAT BAY, B., TSE, G.M., IQBAL, J. and TAN, P.H., 2014. Loss of androgen receptor expression predicts early recurrence in triple-negative and basal-like breast cancer. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc,* **27**(3), pp. 352-360.

128 TIAINEN, M., VAAHTOMERI, K., YLIKORKALA, A. and MÄKELÄ, T.P., 2002. Growth arrest by the LKB1 tumor suppressor: induction of p21WAF1/CIP1. *Human molecular genetics,* **11**(13), pp. 1497-1504.

129 TIWARI, N., GHELDOF, A., TATARI, M. and CHRISTOFORI, G., 2012. EMT as the ultimate survival mechanism of cancer cells. *Seminars in cancer biology,* **22**(3), pp. 194-207.

130 TOKUNAGA, E., KATAOKA, A., KIMURA, Y., OKI, E., MASHINO, K., NISHIDA, K., KOGA, T., MORITA, M., KAKEJI, Y., BABA, H., OHNO, S. and MAEHARA, Y., 2006. The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. *European journal of cancer (Oxford, England : 1990),* **42**(5), pp. 629-635.

131 TOWLE, M.J., SALVATO, K.A., BUDROW, J., WELS, B.F., KUZNETSOV, G., AALFS, K.K., WELSH, S., ZHENG, W., SELETSKY, B.M., PALME, M.H., HABGOOD, G.J., SINGER, L.A., DIPIETRO, L.V., WANG, Y., CHEN, J.J., QUINCY, D.A., DAVIS, A., YOSHIMATSU, K., KISHI, Y., YU, M.J. and LITTLEFIELD, B.A., 2001. In vitro and in vivo anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. *Cancer research,* **61**(3), pp. 1013-1021.

132 TURNER NC, et al (2015) Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med; 373(3): 209-219

133 VOLCIC, M., KARL, S., BAUMANN, B., SALLES, D., DANIEL, P., FULDA, S. and WIESMULLER, L., 2012. NF-kappaB regulates DNA double-strand break repair in conjunction with BRCA1-CtIP complexes. *Nucleic acids research,* **40**(1), pp. 181-195.

134 WALLWIENER, M., HARTKOPF, A.D., BACCELLI, I., RIETHDORF, S., SCHOTT, S., PANTEL, K., MARME, F., SOHN, C., TRUMPP, A., RACK, B., AKTAS, B., SOLOMAYER, E.F., MULLER, V., JANNI, W., SCHNEEWEISS, A. and FEHM, T.N., 2013. The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer. *Breast cancer research and treatment,* **137**(2), pp. 503-510.

135 WALSH, T., LEE, M.K., CASADEI, S., THORNTON, A.M., STRAY, S.M., PENNIL, C., NORD, A.S., MANDELL, J.B., SWISHER, E.M. and KING, M.C., 2010. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proceedings of the National Academy of Sciences of the United States of America,* **107**(28), pp. 12629-12633.

136 YU, M., BARDIA, A., WITTNER, B.S., STOTT, S.L., SMAS, M.E., TING, D.T., ISAKOFF, S.J., CICILIANO, J.C., WELLS, M.N., SHAH, A.M., CONCANNON, K.F., DONALDSON, M.C., SEQUIST, L.V., BRACHTEL, E., SGROI, D., BASELGA, J., RAMASWAMY, S., TONER, M., HABER, D.A. and MAHESWARAN, S., 2013. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science (New York, N.Y.),* **339**(6119), pp. 580-584.

137 YU Q, SICINSKA E, GENG Y, AHNSTRÖM M, ZAGOZDZON A, KONG Y, GARDNER H, KIYOKAWA H, HARRIS LN, STåL O, SICINSKY P.: Requirement for CDK4 kinase function in breast cancer. *Cancer Cell*. 2006 Jan;**9**(1):23-32.

138 YUE, W., FAN, P., WANG, J., LI, Y. and SANTEN, R.J., 2007. Mechanisms of acquired resistance to endocrine therapy in hormone-dependent breast cancer cells. *The Journal of steroid biochemistry and molecular biology,* **106**(1-5), pp. 102-110.

139 ZHANG, Q.X., BORG, A., WOLF, D.M., OESTERREICH, S. and FUQUA, S.A., 1997. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer research,* **57**(7), pp. 1244-1249.

140 ZHUANG, Z., DI, G., SHEN, Z., DING, J. and SHAO, Z., 2006. Enhanced expression of LKB1 in breast cancer cells attenuates angiogenesis, invasion, and metastatic potential. *Molecular cancer research,* **4**(11), pp. 843-849

18.2 LEGISLATION AND GUIDELINES

1 Gesetz über den Verkehr mit Arzneimitteln (AMG): Medicinal Products Act (The Drug Law), in force since January 1978, english translation provided by the Language Service of the Federal Ministry of Health, 2012 including amendment(s) to the Act by Article 1 of the Act of 25th May 2011 (Federal Law Gazette I p. 946)

2 Guideline for good clinical Practice, ICH Harmonised Trirpartite Guideline, topic E6 (R1), International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, Step 4 version, 10 June 1996, in operation since January 1997, last amendments July 2002; German directive of GCP, in force since August 2004 (Federal Law Gazette I S. 2081), last amendment(s) to the Act by Article 8 on 19th October 2012 (Federal Law Gazette I p. 2192, 2220 f.)

3 NCCN Guidelines - Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network, http://www.nccn.org

4 WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1996; last amendments by the 59th WMA General Assembly, Seoul, Korea, October 2008

EUDRACT-NR.: 2013-001269-18	**Protokoll-Nr.:** D-IV

Sponsor: Universitätsklinikum Ulm, Albert-Einstein-Allee 29, D-89081 Ulm
Wissenschaftliche Leitung: Prof. Dr. Wolfgang Janni, Universitätsklinik Ulm

Leiterin der klinischen Prüfung:
Prof. Dr. Tanja Fehm, Heinrich-Heine-Universität Düsseldorf, Moorenstraße 5, 40225 Düsseldorf

Titel: DETECT IV – Multizentrische, prospektive, offene Phase II Studie bei Patintinnen mit HER2-negativem metastasiertem Brustkrebs und persisitierenden HER2-negativen zirkulierenden Tumorzellen (CTCs).

Studienmedikation: DIVa: Everolimus (Tabletten 5 mg), Ribociclib (Tabletten 3 x 200 mg) 1-21 d q28d oder **DIVb:** Eribulin mesylate 1.23 mg/m² d1+8 q3w

Indikation

Everolimus/Ribociclib Kohorte (DIVa)
Postmenopausale Patientinnen mit hormonrezeptorpositiven, HER2-negativen metastasiertem Brustkrebs mit HER2-negativen zirkulierenden Tumorzellen (CTCs) und Indikation für eine endokrine Standardtherapie.

Eribulin Kohorte (DIVb)
Patientinnen mit hormonrezeptorpositiven, HER2-negativen metastasiertem Brustkrebs und Indikation zur Chemotherapie oder Patientinnen mit triple-negativen metastasiertem Brustkrebs, jeweils beide mit HER2-negativen zirkulierenden Tumorzellen (CTCs).

Studiendesign
Multizentrische, prospektive, offene Phase II Studie.

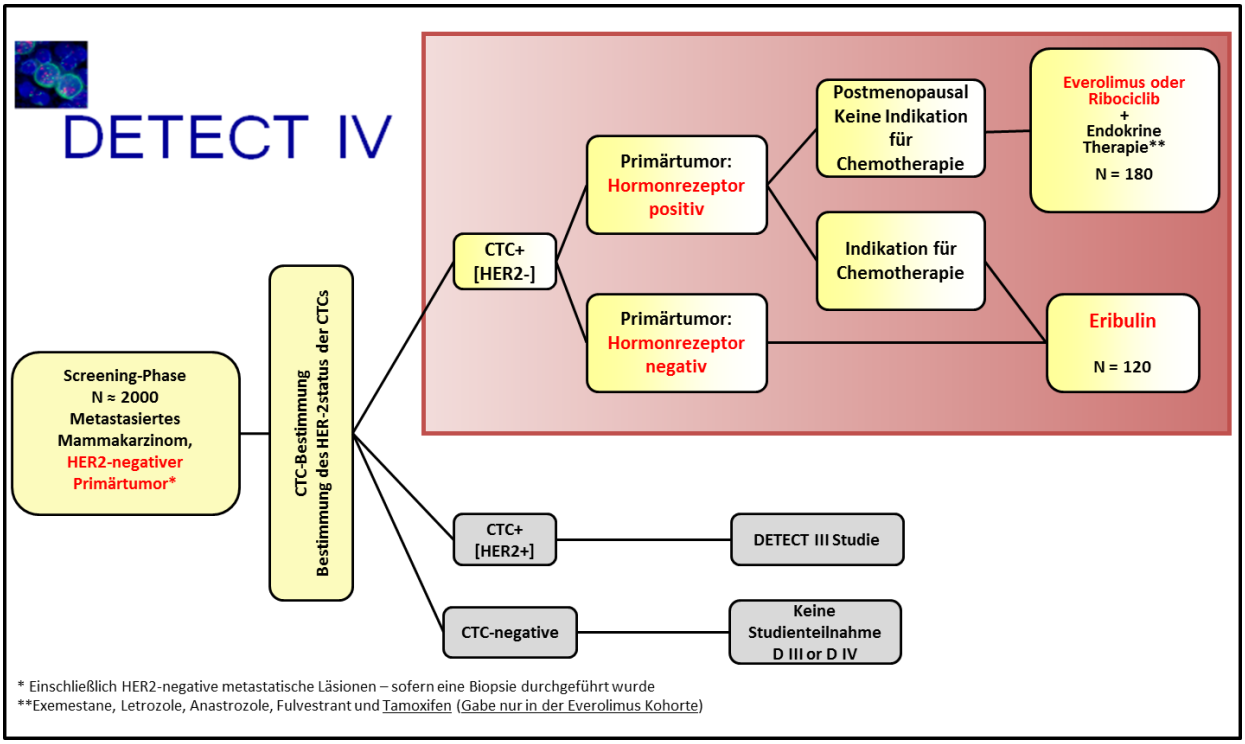


Abbildung: Klinisches Studiendesign

Gesamtstudiendauer:
Maximale Studiendauer: 72 Monate und 3 Wochen

Individuelle Studiendauer:
Die individuelle Studienbeteiligung beginnt mit dem Screening-Besuch und endet mit dem Tod der Patientin bzw. Abschluss der 24-monatigen Follow-up-Phase.

- maximale Dauer der Evaluierungsphase (von Screening bis Einschluss): 3 Wochen
- maximale Dauer der Behandlungsphase im Rahmen der Studienteilnahme: 12 Monate
- geschätzte Maximaldauer der Follow-Up-Phase: 24 Monate

Die geschätzte Maximaldauer der individuellen Studienteilnahme beträgt 36 Monate und 3 Wochen.

Einschlusskriterien:

Allgemeine Einschlusskriterien für beide Kohorten

1. Metastasiertes Mammakarzinom, das einer Operation oder der Strahlentherapie allein nicht zugänglich ist. Histopathologische Sicherung des primären Mammakarzinoms oder einer metastatischen Läsion des Mammakarzinoms.
2. Bestimmung des HER2-Status des primären Mammakarzinoms und/oder einer metastatischen Läsion. HER2-Negativität aller untersuchten Gewebeproben, d.h. Immunhistochemie 0-1+ oder 2+ und Fluoreszenz in situ Hybridisierung (FISH) negativ oder nur FISH negativ.
3. Nachweis zirkulierender Tumorzellen (CTCs) (mindestens eine CTC/7.5 ml Blut CellSearch® Circulating Tumor Cell Kit)
4. Negativer HER2-Status bei allen detektierten CTCs.
5. Adäquate Knochenmarksreserve und Organfunktion 7 Tage vor dem Zeitpunkt der Rekrutierung, durch folgende Laborparameter bestätigt:
   - Absolute Neutrophile ≥ 1500/µL,
   - Thrombozyten ≥ 100000/µL,
   - Hämoglobin ≥ 9g/dL,
   - ALT (SGPT) /AST (SGOT)≤ 3.0 × ULN,
   - Bilirubin (gesamt) ≤ 2.0 × ULN
   - Kreatinin ≤ 2.0 × ULN
6. Schriftliches Einverständnis zur Studienteilnahme.
7. Eine Rebiopsie zur Gewebegewinnung von gut zugänglichen Metastasen ist wünschenswert aber nicht notwendig.
8. Tumorevaluation innerhalb von 6 Wochen vor Studienrandomisierung.
9. Mindestens eine nach RECIST auswertbare metastatische Läsion, entsprechend den RECIST Leitlinien Version 1.1. Patienten mit messbaren und nicht-messbaren Läsionen können eingeschlossen werden. (Eisenhauer 2009).
10. Alter ≥ 18 Jahre.
11. ECOG ≤ 2.

Everolimus/Ribociclib cohort (DIVa)	Eribulin cohort (DIVb)
Beide Kohorten: • Indikation zur endokrinen Therapie: (Histologisch gesicherter positiver Östrogenrezeptorstatus (ER+) und/oder Progesteronrezeptorstatus (PgR+) des Mammakarzinoms) • Nicht mehr als zwei vorrangegangene Chemotherapielinien • Jede endokrine Vortherapie ist erlaubt • Krankheitsprogression unter vorhergehender endokrinen Therapie (endokrine Therapie muss nicht die letzte Therapie vor Studieneinschluss sein)	• Entweder hormonrezeptornegativer metastasierter Brustkrebs oder hormonrezeptorpositiver metastasierter Brustkrebs mit Indikation zur Chemotherapie • Bis zu drei vorangegangene Chemotherapielinien in der metastasierten Situation • Bei gebärfähigen Patientinnen gilt: - Negativer Schwangerschaftstest (minimale Sensitivität 25 IU/L oder äquivalente Einheiten des HCG) innerhalb von 7 Tagen vor Rekrutierung

• Der postmenopausale Status muss vom Prüfer zuvor gesichert werden. Die Postmenopause ist definiert als: - Alter ≥ 55 Jahre und ein Jahr oder länger mit Amenorrhoe - Alter < 55 Jahre und ein Jahr oder länger mit Amenorrhoe und postmenopausalen Serumspiegeln von FSH und LH entsprechend der Referenzwerte der jeweiligen Institution - Frühere Hysterektomie und postmenopausale Serumspiegel von FSH und LH entsprechend der Referenzwerte der jeweiligen Institution - Menopause durch beidseitige Adnexektomie **Everolimus-Arm** • Cholesterin ≤ 2.0 × ULN **Ribociclib-Arm** • INR ≤ 1,5 • Unauffällige Laborwerte für: Natrium, Kalium, Kalzium • 12-Kanal-EKG: -QTcF Interval bei der Einschlussvisite < 450 msec -Ruheherzfrequenz 50-90 s/min	- Sichere Kontrazeption (d.h. nicht-hormonelle Kontrazeption, IUP, Anwendung einer Doppelbarriere-Methode, Vasektomie des Geschlechtspartners, komplette sexuelle Abstinenz) andauernd über mindestens 3 Monate nach Komplettierung der Studientherapie.

Ausschlusskriterien:

Allgemeine Ausschlusskriterien für beide Kohorten

1. Behandlung mit Prüfsubstanzen oder andere antineoplastische Therapie während der Studie oder innerhalb von 2 Wochen vor Randomisierung.
2. Unerwünschte Nebenwirkungen aufgrund einer vorhergehenden Antikrebs-Therapie > Grad 1 (NCI CTCAE), die zum Zeitpunkt des Therapiebeginns therapeutisch relevant ist.
3. Bekannte HIV-Infektion.
4. Aktive Hepatitis B oder C, Einschränkung der Leberfunktion nach der Child Pugh Klassifikation (Grad B und C) oder aktuelle Leber- oder Gallenwegserkrankung (mit Ausnahme von Patientinnen mit Gilberts-Syndrom, mit asymptomatischen Gallensteinen, Lebermetastasen oder stabiler chronischer Lebererkrankung).
5. Vorliegen einer Erkrankung, die die adäquate Einschätzung oder Evaluation der Studiendaten stören könnte, oder Vorliegen anderer Gründe, bei denen die Patientin durch eine Studienteilnahme unverhältnismäßig gefährdet wird.
6. Zweitkarzinom in den letzten 3 Jahre (außer in-situ-Karzinom der Cervix uteri oder Basaliom der Haut).
7. Demenz, veränderter mentaler Status oder andere psychiatrische oder soziale Einflüsse, die das Verständnis der informierten Einwilligung oder welche die Einhaltung des Studienprotokolls verhindern.
8. Lebenserwartung < 3 Monate.
9. Männliche Patienten.

Everolimus/Ribociclib Kohorte (DIVa)	**Eribulin Kohorte (DIVb)**
• Anamnestisch bekannte Überempfindlichkeit gegenüber Everolimus/Ribociclib oder chemisch verwandten Substanzen • Unverträglichkeit gegen Soyalecithin und Erdnüsse (Ribociclib-Kohorte). • Unfähigkeit der oralen Aufnahme der Studienmedikation (z.B. bei Malabsorptionssyndrom, parenteraler Ernährung, vorangegangenen chirurgischen Eingriffen, die die Absorption beeinflussen (z.B. Dünndarm- oder Magenresektionen), oder bei unzureichend therapierten entzündlichen Darmerkrankungen (z.B. M. Crohn, Colitis ulcerosa).	• Anamnestisch bekannte Überempfindlichkeit gegenüber Eribulin. • Vorbestehende Neuropathie größer Grad 2. • Schweres kongenitales Long-QT-Syndrom. • Schwangerschaft oder Stillzeit.

Behandlung und Dosierung

Therapie mit Everolimus in Kombination mit einer endokrinen Standardtherapie (DIVa):
Die Indikation zur endokrinen Therapie erfolgt gemäß den Empfehlungen der Arbeitsgemeinschaft Gynäkologische Onkologie (AGO). Die individuelle Therapieentscheidung erfolgt nach Ermessen des verantwortlichen Prüfarztes unabhängig von der klinischen Studie.

Die Therapie mit Everolimus wird den Patientinnen zusammen mit der indizierten endokrinen Therapie verabreicht. Die Einnahme erfolgt einmal täglich möglichst zur gleichen Tageszeit. Die Behandlung mit Everolimus sollte - wenn medizinisch indiziert – über die randomisierte Phase hinaus verlängert werden. Die Everolimus-Dosierung erfolgt in Abhängigkeit von der vorgesehenen endokrinen Therapie. Es dürfen nur die in der Tabelle aufgeführten Behandlungspläne verwendet werden.

Endokrine Therapie		**+Everolimus**
Exemestan (Baselga et al. 2012 – BOLERO 2-trial) Vorangegangene Therapie mit Letrozol/ Anastrozol	25 mg/d	10 mg/d*
Tamoxifen (Bachelot et al. 2011 – TAMRAD-trial) Vorangegangene Therapie mit AI	20 mg/d	10mg/d*
Letrozol (Baselga et al. 2009 [neo-adjuvant, Phase II]; Awada et al. 2008 [MBC, Phase I])	2,5 mg/d	10mg/d*
Anastrozol	1 mg/d	10mg/d*

* Everolimus wird entsprechend der Zulassung verschrieben. Der behandelnde Prüfarzt entscheidet über eine Dosisanpassung, die der individuellen medizinischen Notwendigkeit der Patientin entspricht. Eine Start-Dosis von 5mg pro Tag ist erlaubt, sofern diese medizinisch indiziert ist. Die Entscheidung über die Behandlung der Patientin mit Everolimus wird unabhängig von der Studie getroffen.

Tabelle 29: Empfohlene Dosierung der endokrinen Therapie in Kombination mit Everolimus

Nach Beginn der Behandlung mit Everolimus wird die Dosierung je nach Dosierung der verabreichten Standard-Therapie und in Abhängigkeit der aufgetretenen Nebenwirkungen angepasst. Die tägliche Maximaldosis von Everolimus beträgt 10 mg, die Minimaldosis ist 5 mg alle zwei Tage. Die Gabe von Everolimus erfolgt über 12 Monate und kann nach der Behandlungsphase bei entsprechender medizinischer Indikation verlängert werden. Everolimus wird außerhalb der Zulassung mit Exemestan unter Studienbedingungen von Novartis für die Dauer der individuellen Studienteilnahme (inklusive 2 Jahre Follow-Up-Phase) zur Verfügung gestellt. Ein vorzeitiger Abbruch der Therapie erfolgt bei Progress der Erkrankung oder aus anderen Gründen für einen vorzeitigen Therapieabbruch. Eine Beendigung oder Modifikation der Therapie ist entsprechend der Einschätzung des behandelnden Arztes auch aus anderen Gründen möglich.

Therapie mit Ribociclib in Kombination mit einer endokrinen Standardtherapie (DIVa):
Die Indikation zur endokrinen Therapie erfolgt gemäß den Empfehlungen der Arbeitsgemeinschaft Gynäkologische Onkologie (AGO). Die individuelle Therapieentscheidung erfolgt nach Ermessen des verantwortlichen Prüfarztes unabhängig von der klinischen Studie.

Die Therapie mit Ribociclib wird den Patientinnen zusammen mit der indizierten endokrinen Therapie verabreicht. Die Einnahme erfolgt einmal täglich möglichst zur gleichen Tageszeit. Die Behandlung mit Ribociclib sollte - wenn medizinisch indiziert – über die randomisierte Phase hinaus verlängert werden. Es dürfen nur die in der Tabelle aufgeführten Behandlungspläne verwendet werden.

Endokrine Therapie		+Ribociclib
Exemestan	25 mg/d	600 mg/d*
Letrozol (Hortobagyi et al., 2016 MonaLEEsa-2)	2,5 mg/d	600 mg/d*
Anastrozol	1 mg/d	600 mg/d*
Fulvestrant	500 mg q4/w	600 mg/d*

*1-21d q28d

Tabelle 30: Empfohlene Dosierung der endokrinen Therapie in Kombination mit Ribociclib

Nach Beginn der Behandlung mit Ribociclib wird die Dosierung je nach Dosierung der verabreichten Standard-Therapie und in Abhängigkeit der aufgetretenen Nebenwirkungen angepasst. Die tägliche Maximaldosis von Ribociclib beträgt 600 mg, die Minimaldosis ist 200 mg. Die Gabe von Ribociclib erfolgt über 12 Monate und kann nach der Behandlungsphase bei entsprechender medizinischer Indikation verlängert werden. Ribociclib wird unter Studienbedingungen von Novartis für die Dauer der individuellen Studienteilnahme (inklusive 2 Jahre Follow-Up-Phase) zur Verfügung gestellt. Ein vorzeitiger Abbruch der Therapie erfolgt bei Progress der Erkrankung oder aus anderen Gründen für einen vorzeitigen Therapieabbruch. Eine Beendigung oder Modifikation der Therapie ist entsprechend der Einschätzung des behandelnden Arztes auch aus anderen Gründen möglich.

Therapie mit Eribulin (DIVb):
Eribulin wird als Fertiglösung in einer Dosierung von 1,23 mg/m² intravenös über zwei bis fünf Minuten am Tag 1 und 8 eines 21-tägigen Zyklus verabreicht. Die Therapie mit Eribulin erfolgt über 12 Monate bzw. bis zum Progress, unzumutbarer Toxizität, Tod oder Therapieabbruch aus anderen Gründen. Bei entsprechender medizinischer Indikation kann die Gabe nach der Behandlungsphase verlängert werden.

Behandlungsende („End of treatment“ (EOT))
Das Behandlungsende („End of tratment“ (EOT)) ist der Zeitpunkt, an dem die Patientin die letzte Studienmedikation erhalten hat, ausgenommen sind Unterbrechungen von weniger als 4 Wochen. Bei der Follow-up-Visite 4 Wochen nach der letzten Studienmedikationsgabe sollen alle unerwünschten Ereignisse während dieser erfasst werden.

Behandlung in der Follow-Up-Phase:
Die Therapie in der Follow-Up-Phase, d.h. nach Abschluss der Studientherapie erfolgt gemäß den Empfehlungen des Prüfarztes.

Rationale der Studie

Everolimus Kohorte (DIVa)

Evaluation der Effektivität des m-TOR-Inhibitors Everolimus zur Verhinderung des Krankheitsprogresses bei Patientinnen mit metastasiertem HER2 negativem Brustkrebs, welche HER2-negative zirkulierende Tumorzellen (CTCs) aufweisen.

Eribulin Kohorte (DIVb)

Evaluation der Effektivität von Eribulin zur Verhinderung des Krankheitsprogresses bei Patientinnen mit sowohl metastasiertem hormonrezeptorpositiven, HER2 negativem Brustkrebs mit Indikation zur Chemotherapie oder triple-negativen metastasiertem Brustkrebs, welche HER2-negative zirkulierende Tumorzellen (CTCs) aufweisen.

Primäre Zielkriterien:

Everolimus/Ribociclib Kohorte (DIVa)

CTC Clearance Rate: Anteil an Patientinnen mit mindestens einer vor Behandlungbeginn in 7.5ml peripherem Blut nachgewiesenen zirkulierenden Tumorzelle (CTC), bei denen nach Behandlung keine CTCs im Blut mehr nachgewiesen werden können (CTC-Nachweis erfolgt mit dem CellSearch® System; Veridex LLC, Raritan, USA).

Eribulin Kohorte (DIVb)

Progressionsfreies Überleben (PFS)

Sekundäre Zielkriterien:

- *Allgemeine Ansprechrate (ORR): Komplettremission (CR), Teilremission (PR)*
- *Klinische Erfolgsrate (DCR)*
- *Gesamtüberleben (OS)*

- *Dynamik der zirkulierenden Tumorzellen*
- *Lebensqualität (quantifiziert anhand der EORTC QLQ-C30 und EORTC QLQ-BR23 Fragebögen)*
- *"Qualitäts-adjustierte" Überlebenszeiten (quantifiziert anhand der Q-TWiST-Methodologie).*
- *Toxizitätsanalyse der Studienmedikation (Everolimus (DIVa) und Eribulin (DIVb)): Sicherheit und Verträglichkeit*
- *Compliance*

Everolimus/Ribociclib cohort (DIVa)	Eribulin cohort (DIVb)
• Progressionsfreies Überleben (PFS) • Änderung der pS6- Levels in CTCs in Folge der Behandlung und Zusammenhang dieser Änderungen mit Überlebenszeiten • Änderungen in der PI3K/Akt/mTOR-Signalwegs-Aktivierung auf CTCs (Bestimmung des PI3KCA Mutationsstatus mittels SNaPshot-Technologie) • Mutationsstatus des Östrogenrezeptors auf zirkulierenden Tumorzellen • Expression von Transskriptionsfaktoren welche epithelial-mesenchymale Transition in CTCs induzieren • Ausprägung der Resistenz von CTCs gegenüber durch Verlust von Zell-Zell Adhäsion verursachtem Zelltod (Anoikis) • Expression des Tumorsuppressors LKB1 auf CTCs • Quantifizierung der zirkulierenden microRNAs miR-125a, miR-125b, miR-18a und miR18b im Serum	• Effektivität von Eribulin hinsichtlich der Neu-Metastasen-freien Überlebenszeiten • Androgenrezeptor (AR) Expression und deren Mutationsstatus auf CTCs • PIK3CA Mutationsstatus auf CTCs (SNaPshot technology) • Charakterisierung von molekularen Signalwegen welche epithelial-mesenchymale Transition und die Resistenz gegenüber durch Verlust von Zell-Zell Adhäsion verursachtem Zelltod (Anoikis) in Krebszellen induzieren • Expression des Proteins 53BP1 (Marker für DNA Schäden und DNA Reparaturaktivitäten in der Zelle) auf CTCs

Patientinnenkollektive, statistische Methoden und Fallzahlberechnung

Patientinnenkollektive

Folgende Patientinnenkollektive werden ausgewertet:

Intention to Treat (ITT) Kollektiv: Alle in die Studie aufgenommene Patientinnen, welche die Studienmedikation (Everolimus/Ribociclib plus endokrine Therapie oder Eribulin) mindestens einmal erhalten haben.

Safety-Analysis (SA) Kollektiv: Alle in die Studie aufgenommene Patientinnen, welche die Studienmedikation (Everolimus/Ribociclib plus endokrine Therapie oder Eribulin) mindestens einmal erhalten haben und für die mindestens eine Sicherheitsauswertung nach Studienbeginn durchgeführt wurde.

Per Protocol (PP) Kollektiv: Alle Patientinnen des ITT-Kollektivs, welche weder Ein- noch Ausschlusskriterien verletzt haben und welche nach Studienplan behandelt wurden.

Statistische Methoden

Die statistische Analyse wird nach Beendigung der Studie durchgeführt und es sind keine Zwischenauswertungen geplant. Für alle Parameter werden beschreibende Statistiken präsentiert, Ereignisdaten werden mit der Kaplan-Meier Methode analysiert.

Für die Analysen zum Primärziel der Studie wird das ITT Patientinnenkollektiv zu Grunde gelegt, wobei weiterführende unterstützende Analysen auch mit dem PP Patientinnenkollektiv durchgeführt werden. Alle die sekundären Studienziele betreffenden Analysen werden basierend auf dem ITT Kollektiv, dem PP Kollektiv, oder auch Subkollektiven hiervon durchgeführt.

Das Primärziel der DETECT IV Studie ist eine Schätzung der Effektivität der Behandlung von Patientinnen mit HER2-negativen, metastasierten Brustkrebs und HER2-negativen zirkulierenden Tumorzellen. Bei Patientinnen, welche mit Everolimus/Ribociclib und einer endokrinen Therapie behandelt werden (Everolimus/Ribociclib-Kohorte) wird die Effektivität der Behandlung anhand der CTC clearance rate gemessen; bei Patientinnen, welche mit Eribulin behandelt werden (Eribulin-Kohorte) wird die Effektivität der Behandlung anhand des progressionfreien Überlebens gemessen. Alle statistischen Analysen welche die sekundären Studienziele betreffen haben nur explorativen Charakter.

Eine Sicherheitsauswertung wird durchgeführt sobald 15 Patientinnen für die Studie rekrutiert worden sind.

Fallzahlberechnung

Das primäre Ziel der DETECT IV Studie ist die Schätzung der Effektivität der Behandlung von Patientinnen mit HER2-negativem, metastasiertem Brustkrebs und HER2-negativen zirkulierenden Tumorzellen, welche mit Everolimus/Ribociclib und einer endokrinen Therapie oder Eribulin behandelt werden. Die Everolimus-Kohorte umfasst postmenopausale Patientinnen mit HER2-negativem, hormonrezeptor-positivem metastasiertem Brustkrebs ohne Indikation zur Chemotherapie, während die Eribulin-Kohorte sowohl Patientinnen mit HER2-negativem, hormonrezeptor-positivem metastasiertem Brustkrebs ohne Indikation zur Chemotherapie und Patientinnen mit triple-negativem metastasiertem Brustkrebs umfasst. Es gibt keine explizite statistische Hypothese für die Primäranalyse.

Folgende Annahmen liegen der Fallzahlkalkulation für die DETECT IV Studie zu Grunde:

- Mindestens 2000 Patientinnen mit HER2-negativen metastasierten Brustkrebs werden für DETECT IV und die verwandte Studie DETECT III (welche für Patientinnen mit HER2-positiven zirkulierenden Tumorzellen konzipiert ist) auf das Vorhandensein von zirkulierenden Tumorzellen getestet
- Zirkulierende Tumorzellen (≥ 1) in 65% aller Patientinnen mit metastasierten Brustkrebs (konservative Schätzung basierend auf Ergebnissen von Botteri et al. 2010[1], Fehm et al. 2010a, Pierga et al. 2012)
- Ausschließlich HER2-negative zirkulierende Tumorzellen in 70% aller Patientinnen mit zirkulierenden Tumorzellen im Blut (konservative Schätzung basierend auf Erfahrungen aus der DETECT III Studie)

Basierend auf diesen Annahmen wird davon ausgegangen, dass von den 2000 gescreenten Patientinnen ca. 910 Patientinnen HER2-negativen metastasierten Brustkrebs und ausschliesslich HER2-negative zirkulierende Tumorzellen aufweisen.

Everolimus/Ribociclib-Kohorte: Basierend auf der Annahme dass 70% der Brustkrebspatientinnen einen hormonrezeptor-positiven Primärtumor aufweisen, von denen wiederum ca. 75% postmenopausal sind, wird davon ausgegangen, dass das Screening ca. 480 postmenopausale Patientinnen mit HER2-negativen, hormonrezeptor-positiven, metastasierten Brustkrebs und ausschliesslich HER2-negativen zirkulierenden Tumorzellen ergibt. Allerdings ist zu erwarten – basierend auf Erfahrungen aus der DETECT III Studie – dass für einen beträchtlichen Anteil dieser Patientinnen nicht alle Einschlusskriterien für die DETECT IVa Studie erfüllt sind,einige Ausschlusskriterien zutreffen, oder eine Indikation zur Chemotherapie vorliegt. Daher wird hier von der konservativen Annahme ausgegangen, dass nur 180 der 480 Patientinnen letztendlich in die Studie eingeschlossen werden können.

Eribulin-Kohorte:

Basierend auf der Annahme dass 30% der Brustkrebspatientinnen einen hormonrezeptor-negativen Primärtumor aufweisen, wird davon ausgegangen, dass das Screening ca. 270 Patientinnen mit triple-negativen metastasierten Brustkrebs und ausschliesslich HER2-negativen zirkulierenden Tumorzellen ergibt. Weiterhin wird geschätzt, dass für etwa 20% der hormonrezeptor-positiven Brustkrebspatientinnen eine Indikation für Chemotherapie gegeben ist, womit insgesamt etwa 390 Patientinnen für die Eribulin-Kohorte der DETECT IV Studie zur Verfügung stehen würden. Da jedoch anzunehmen ist, dass dieses Patientinnenkollektiv (triple-negativ oder hormonrezeptor-positiv mit Indikation zur Chemotherapie) insgesamt einen deutlich schlechteren Allgemeinzustand aufweist als die Patientinnen der Everolimus-Kohorte, ist davon auszugehen, dass ein beträchtlicher Anteil dieser Patientinnen nicht alle Einschlusskriterien erfüllt, bzw. zusätzliche Komorbiditäten aufweist, welche einen Einschluss in den Eribulin-Arm der DETECT IV Studie verhindert. Unter der konservativen Annahme, dass dies für etwa zwei Drittel der Patientinnen zutrifft, wird mit etwa 120 Patientinnen gerechnet, die tatsächlich in den Eribulin-Arm der Studie eingeschleust werden können.

STUDIENABLAUF DETECT IV

Visiten	**Screening-visite**	**Einschluss-visite**	**Kontroll-visiten**	**Abschluss-visite**	**Follow-up-visiten**
Zeitpunkt	≤ 21 Tage vor Einschluss-visite	≤ 21 Tage nach Screening-visite	Alle 3-4 Wochen (Everolimus/Ribociclib-Kohorte) Alle 6 Wochen (Eribulin-Kohorte)	12 Monate nach 1. Dosis/ nach 12 Montaten oder bei Therapieabbruch (z.B. wegen Progress)	2 bis 4 Wochen nach Abschluss-visite, dann alle 3 Monate
Zeitraum	**Vorbehandlung – Evaluierungsphase**		**Behandlunsphase**		**Follow-Up-phase**
Pat.-Einverständnis Nr. 1 (Blutentnahme für CTC-Bestimmung und Bestimmung des HER2- Status auf den CTCs[1])	X				
Pat.-Einverständnis Nr. 2[1]		X			
Pat.-Einverständnis Nr. 3 (Blutentnahmen i.R. des translationalen Forschungsprojekts)[1,2]		X			
Vergabe der Patienten-Identifikationsnummer	X				
Demographische Daten (Geburtsjahr)*	X				
Datum der primären Tumordiagnose*	X				
Informationen über die primäre Brustkrebserkrankung (TNM*, Histologie*, Grading*, Lokalisation**, Operation**)	X				
Informationen über die Metastasen (Datum der Diagnose*, Lokalisation*, Operation**)	X				
HER2 Status des Primärtumors und/oder der Metastasen	X				
Hormonrezeptorstatus des Tumorgewebes[4]*	X				
Adjuvante/Neoadjuvante Therapie**	X				
Anzahl vorangegangener Chemotherapien in palliativer Situation*, Art der Therapien im metastasierten Stadium**	X				
Blutentnahme für die CTC-Bestimmung im Rahmen der klinischen Studie (Patientinneneinverständnis Teil 3 nicht erforderlich)	X[5] *Screening-Kit*		X[17] *(Analyse Kit);* nur Everolimus/Ribociclib Kohorte (alle 12 Wochen)	X[17] (*Analyse Kit*)	
Blutentnahmen i.R. des translationalen Forschungsprojekts	X[6] *(im Screening-Kit enthalten)*	X[19] *Analyse-Kit*	X[19] (*Analyse Kit*; Woche 3-4 und 9–12)		
Größe und Gewicht der Patientin		X			
Anamnese / Voroperationen		X			
Begleiterkrankungen		X			
Andauernde Toxizitäten aufgrund vorangegangener antineoplastischer Therapien[3]		X			
Vorangegangene antineoplastische Medikation, andere relevante Vor-Medikation		X			
Begleitmedikation		X	X	X	X
		X			

Visiten	Screening-visite	Einschluss-visite	Kontroll-visiten	Abschluss-visite	Follow-up-visiten
Dokumentation und Prüfung der Eignung der geplanten endokrinen Therapie in Kombination mit Everolimus/Ribociclib					
Behandlung gemäß Prüfplan		X	X	X[16]	
Vitalparameter (Herzfrequenz, Blutdruck, Körpertemperatur)		X	X	X	
Körperliche Untersuchung		X	X	X	
Unerwünschte Ereignisse		X	X	X	X
Blutbild[7]		X[9,20]	X[20, 21]	X[20]	
Klinische Chemie[8]		X[9]	X[20]	X	
Schwangerschaftstest (nur Eribulinkohorte)		X[9]	X	X	
Tumorbeurteilung gemäß der RECIST Leitlinien (Version 1.1)[12] (siehe Protokoll Abschnitt 10.2.1.)		X[15]	X[11]		
Tumormarker[18]		X	X		
12-Kanal EKG		X[10]	X[20]	X	
Lebensqualität (EORTC QLQ-C30 und -BR23)		X[13]	X	X	
Durchsicht der Ein- und Ausschlusskriterien		X			
Ausgabe von Everolimus/Ribociclib		X			
Zusätzliche Ausgabe von Everolimus/Ribociclib falls notwendig			X		
Tablettenzählung (Everolimus/Ribociclib)			X	X	
Einsammeln der ungebrauchten Everolimus/Ribociclib-Tabletten				X	
Erinnerung der Patientin an die geplanten Follow-up Visiten				X	
Überleben			X	X	X

1 vgl. Abschnitt 16.4

2 Die Patientin kann auch an der Studie teilnehmen, wenn sie ihr Einverständnis hierfür nicht gegeben hat. In diesem Fall darf kein Material für das translationale Forschungsprojekt gesammelt und verwendet werden.

3 vgl. Abschnitt 11.1

4 Östrogen- und Progesteronstatus jeweils als positiv oder negativ eingestuft

5 Das Ergebnis muss vor dem Einschlussbesuch vorliegen. Die Blutentnahme zur Bestimmung von zirkulierenden Tumorzellen und Bestimmung des HER2-Status auf den CTCs sollte mit einem Mindestabstand von 1 Woche zu einer vorangegangenen antineoplastischen Therapie stattfinden.

6 ggf.: zusätzliche Blutentnahme bei Patientinnen, die ihre Einwilligung zur Teilnahme am translationalen Forschungsprojekt gegeben haben (Bestandteil der Pat.-Info und der Einverständniserklärung Teil 1). Die dazugehörigen Blutröhrchen sind im Screening-Kit enthalten. Wenn eine Patientin diese Blutentnahme ablehnt, kann sie dennoch an der Studie teilnehmen.

7 muss beinhalten: Hämoglobin, Hämatokrit, Zählung der Erythrozyten, Differentialblutbild, Zählung der Thrombozyten

8 muss beinhalten: Bilirubin (total und direkt), ALT, AST, Albumin, Serum Kreatinin, Harnstoff, Kalium, Magnesium, Nüchternblutzucker, Triglyzeride (nüchtern) und Cholesterin (nüchtern); Ribociclib-Arm: Natrium, Kalium, Calcium, Phosphat, GGT, AP und LDH; bei der Einschlussvisite und klinischer Indikation: Gesamtcholesterin, LDL, HDL und Triglyzeride. Leberfunktionstests: vor der Einleitung der Behandlung und jeweils vor Beginn der ersten 6 Zyklen und danach, falls klinisch erforderlich.

9 Ergebnisse dürfen nicht älter als 7 Tage sein

10 Ergebnisse dürfen nicht älter als 3 Wochen sein

11 Alle 12 Wochen nach Beginn der palliativen Behandlung (je nach individuellem Behandlungsschema) oder falls medizinisch indiziert. Generell soll die Kontrolle des Therapieansprechens zusammen mit der Bestimmung der CTCs erfolgen.

12 Bei jeder Beurteilung muss dieselbe Methode verwendet werden.

13 Ergebnisse dürfen nicht älter als 1 Woche sein

14 nur wenn medizinisch indiziert

15 Ergebnisse dürfen nicht älter als 6 Wochen sein

16 Dokumentation der Behandlung am Ende der Behandlungsphase und Dokumentation der geplanten Therapie im Anschluss

17 CTC Bestimmung mittels Analyse Kit sollte im Everolimus/Ribociclib-Arm im Rahmen der klinischen Studie alle 3 Monate erfolgen

(entfällt beim Eribulin-Arm). Generell soll die Kontrolle des Therapieansprechens (z.B. CT) zusammen mit der Bestimmung der CTCs erfolgen. Reguläre Bestimmung der CTCs in beiden Kohorten bei Studienende (End of treatment, Progress etc.) mittels Analyse-Kits.

[18] Tumormarker werden bei jeder Tumorevaluation bestimmt: CA15-3 obligat, CA125 und CEA optional

[19] CTC Bestimmung im Rahmen der translationalen Forschungsprojekte (nur bei Vorliegen der Einverständniserklärung Teil 3) mittels Analyse-Kit in Woche 3-4 sowie in Woche 9 – 12 (In der Eribulin-Kohorte sollte die CTC-Bestimmung nach Zyklus 1 und 3 erfolgen, bei der Everolimus-Kohorte sollte der Zeitpunkt im o.g. Zeitraum liegen)

[20] Ribociclib-Kohorte:
EKG: Zyklus 1 d14, dann zu Beginn des zweiten Zyklus und anschließend wie klinisch erforderlich.
Leberfunktionstest: vor der Einleitung der Behandlung, während der ersten 2 Zyklen alle 2 Wochen, danach zu Beginn jeder der folgenden 4 Zyklen und anschließend wie klinisch erforderlich.

[21] Eribulin-Kohorte: Differential-Blutbild vor jeder Gabe.

*Diese Daten müssen beim Screening-Visit erhoben werden. Eine Teilnahme am Screening ist ohne diese Daten nicht möglich.

**Die Patientin kann auch ohne Dokumentation dieser Daten gescreent werden. Die Erhebung und Dokumentation wird zusätzlich vergütet.

Visits	**Screening Visit**	**Inclusion Visit**	**Control Visits**	**Conclusion Visit of Treatment Period**	**Follow-up Assessments**
Time	⩽ 21 days prior to end of Inclusion Visit	⩽ 21 days after Screening Visit	Every 3 to 4 weeks (Everolimus/ribociclib cohort) and every 6 weeks (eribulin cohort)	12 months after first dose or at premature termination of protocol treatment	2 to 4 weeks after Conclusion Visit, then q 3 months
Periods	**Pre-Treatment Evaluation Period**		**Treatment Period**		**Follow-Up Period**
Informed consent - part 1 in blood sampling for CTC count and HER2 status on CTC[1]	X				
Informed consent - part 2 in study participation[1]		X			
Informed consent - part 3 in blood sampling for translational medical investigations[1,2]		X			
Allocation of the patient identification number	X				
Demography (YOB)*	X				
Date of primary tumor diagnosis*	X				
Information on primary breast cancer (TNM*, histology*, grading*, localisation**, surgery**)	X				
Information on metastases (date of diagnosis*, localization*, surgery**)	X				
HER2 status on primary tumor tissue and/or biopsies from metastatic sites	X				
Hormone receptor status on primary tumor tissue and/or biopsies from metastatic sites[4]	X				
Adjuvant/Neoadjuvant Therapy**	X				
Number of prior chemotherapy lines for metastatic disease*, type of therapies for metastatic disease**	X				
Blood sampling for CTC within the clinical trial	X[5] *Screening Kit*		X[17](every 12 weeks) with Analysis Kit everolimus/ribociclib cohort only	X[17]*(Analysis Kit)*	
Blood sampling for translational medical investigations	X[6] (part of Screening Kit)	X[19] *Analysis Kit*	X[19]*(Analysis Kit)* Only at visits week 3-4 and 9-12		
Patient height + weight		X			
Medical/surgical history		X			
Concomitant diseases		X			
Ongoing toxicities attributed to prior anticancer therapies[3]		X			
Prior anticancer medication, other relevant prior medication		X			
Concomitant medication		X	X	X	X

Visits	Screening Visit	Inclusion Visit	Control Visits	Conclusion Visit of Treatment Period	Follow-up Assessments
Documentation of and check for compatibility of planned endocrine therapy in combination with everolimus		X			
Protocol treatment		X	X	X[16]	
Vital signs (heart rate, blood pressure, temperature)		X	X	X	
Physical examination		X	X	X	
Adverse Events		X	X	X	X
Hematology[7]		X[9, 20]	X[20, 21]	X	
Biochemistry[8]		X[9]	X[20]	X	
Pregnancy test (eribulin cohort only)		X[9]	X	X	
Tumor evaluation according to RECIST guidelines (version 1.1)[12] (see protocol section 10.2.1.)		X[15]	X[11]		
Tumor markers[18]		X	X		
12-lead ECG		X[10]	X[20]	X[20]	
Quality of life (EORTC QLQ-C30 and -BR23)		X[13]	X	X	
Review of inclusion and exclusion criteria		X			
Dispense of everolimus/ribociclib		X			
Additional supply of everolimus/ribociclib if necessary			X		
Tablet count (everolimus/ribociclib cohort only)			X	X	
Collection of unused everolimus				X	
Reminding the patient of the follow-up procedures planned				X	
Survival			X	X	X

1 Cf. section 16.4
2 Patient may participate in the study if this consent was not granted. However, in this case body material must not be sampled for the purpose of translational medical investigations
3 Cf. section 11.1
4 Estrogen and progesterone status each graded positive or negative
5 Results must be obtained prior inclusion visit. Blood sampling for CTC count and assessment of their HER2 status should be scheduled at least one week after last application of investigational agents of any type or anticancer therapy.
6 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.
7 Must include: hemoglobin, hematocrit, red blood cell count, differential white blood cell count, platelet count
8 Must include: total and direct bilirubin, ALT, AST, albumin, serum creatinine, BUN or urea, potassium, magnesium, fasting glucose, fasting triglycerides and fasting serum cholesterol, Ribociclib-cohort: sodium, potassium, calcium, phosphorous. GGT, AP, and LDH; at baseline and if clinically indicated: total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Monitor electrolytes at the beginning of each cycle for 6 cycles, and as clinically indicated.
9 Results obtained within the preceding 7 days may be employed
10 Results obtained within the 3 preceding weeks may be employed
11 The therapy response evaluation should be performed every 3 months or if medically indicated. Treatment response evaluation should be performed together with the determination of CTCs
12 The same method should be used on every assessment
13 Results obtained within the preceding week may be employed
14 Only if medically indicated

[15] Results obtained within the 6 preceding week may be employed

[16] Documentation of end of protocol treatment and documentation of planned therapy after end of protocol treatment

[17] CTC count with Analysis Kit should be performed every 3 months (not in the eribulin cohort). Generally treatment response evaluation should be performed together with the determination of CTCs as part of clinical trial. CTC count at conclusion visit (i.e. end of treatment or progress) should be performed for both cohorts with Analysis Kit.

[18] Tumor markers are assessed on each tumor evaluation: CA15-3 is mandatory, CA125 and CEA are optional

[19] Blood sampling with Analysis Kit as part of translational research projects (only with informed consent part 3) at week 3 - 4 and at week 9 – 12 (in the eribulin cohort CTC count should be performed after cycle 1 and 3, in the everolimus/ribociclib cohort CTC count should be performed in the mentioned period).

[20] Ribociclib-cohort:
ECG:Cycle 1 d14, and at the beginning of the second cycle, and as clinically indicated.
Liver Function Test (LFT): monitor LFTs every 2 weeks for the first 2 cycles, at the beginning of each subsequent 4 cycles, and as clinically indicated.

[21] Eribulin-cohort: monitoring of complete blood counts should be performed on all patients prior to each dose of eribulin.

*Data must be obtained before screening

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

APPENDIX III - PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA

Karnofsky and Lansky performance scores are intended to be multiples of 10.

ECOG (Zubrod)		Karnofsky		Lansky*	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
		90	Able to carry on normal activity; minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly.
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

* The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

In this clinical trial the ECOG-Score will be used only.

APPENDIX IV – NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS VERSION 4.03 (CTCAE)

This study will utilize the NCI Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE) for adverse events and serious adverse event reporting. A copy of the CTCAE Version 4.03 can be downloaded from the CTEP home page: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

APPENDIX V – QUALITY OF LIFE ASSESSMENTS

Introduction

Possible prolongation of survival due to additional treatment with everolimus must be appraised in the light of contingent side effects, which may lower patients' quality of life. To evaluate eventual efficacy of everolimus, quality of life assessments are indispensable.

Instructions for Administration of a Quality of Life Questionnaire

The instructions below are intended as a guide for the administration of the Quality of Life questionnaire.

1. Preamble

   Quality of life data are collected for research purposes, and will usually not be used for the patient's individual medical care. The assessment is in the form of a self report questionnaire. Therefore, it must be completed by the patient only, without translation, coaching or suggestions as to the "correct" answer by relatives or health care personnel.

   If a particular question has not been answered, please document the reason(s) in the appropriate space on the questionnaire. If the whole questionnaire has not been completed, please document the reason(s) on the appropriate case report forms.

2. Pretreatment Assessment

   It should be explained to the patient that the purpose of the questionnaire is to assess the impact of treatment on different areas of the patient's life, e.g.: psychological distress, social disruption, side-effects, et cetera.

   The center CRA should collect the questionnaire as soon as it has been completed, check to see that each question has been answered and gently remind the patient to answer any inadvertently omitted questions. If a patient states that she prefers not to answer some questions and gives a reason(s), the reason(s) should be noted on the questionnaire. If a specific reason is not given, this also should be noted on the questionnaire.

3. Assessments During Treatment

   The quality of life questionnaire should be given to the patient before being seen by the doctor, and prior to treatment on the day of treatment, as required by the schedule in the protocol. If the patient does not have a doctor visit scheduled, or if it was not possible for the patient to complete the questionnaire before being seen by the doctor, she should still complete the questionnaire prior to treatment.

   A patient may, on occasion, be reluctant to complete the questionnaire because they feel unwell. In that case, you may express sympathy that things are below par, but state that this is exactly the information we require if we are to understand more about how quality of life is affected. You may also remind them that it takes only a few minutes to complete.

   It defeats the whole purpose of the assessment if it is delayed until the patient feels better!

4. What If . . .

   The patient should complete the questionnaires at the clinic. The exception is that the design of some trials may require the patient to take the questionnaire home with them after leaving the clinic, and complete it on the specific day, because a return visit to the clinic is not scheduled.

   There may be circumstances when the patient does not complete the questionnaire as required in the clinic. Three situations are described below. In these cases, it is beneficial if quality of life data can still be collected.

   A. The patient leaves the clinic before the questionnaire could be administered, or someone forgets to give the questionnaire to the patient.

      Contact the patient by phone informing him or her that the questionnaire was not completed. Ask the patient if she is willing to complete one:

      If yes, mail a blank questionnaire to the patient, and make arrangements for return of the questionnaire in a timely fashion. Record the date it was mailed and the date received on the questionnaire.

      If this is not feasible, then ask the patient if s/he is willing to complete a questionnaire over the phone. If the patient agrees, read out the questions and range of possibilities, and record the answers. Make a note on the questionnaire that the questionnaire was completed over the phone.

      If no, note the reason why the questionnaire was not completed on the appropriate case report form.

   B. The patient goes on an extended vacation for several months and won't attend the clinic for regular visit(s).

Ensure that the patient has a supply of questionnaires, with instructions about when to complete them, and how to return them. If it is known beforehand, give the patient blank questionnaires at the last clinic visit; if the extended absence is not known in advance, mail the blank questionnaires to the patient. Written instructions may help ensure that the patient stays on schedule as much as possible.

C. The patient does not want to complete the questionnaire in clinic.

Should the patient not wish to answer the questionnaire in the clinic but insists on taking it home, and failing to comply with the patient's wishes is likely to result in the questionnaire not being completed at all, then the patient may take the questionnaire home with instructions that it is to be completed the same day. When the questionnaire is returned, the date on which the questionnaire was completed should be noted and a comment made on the questionnaire as to why the patient took it away from the clinic before completion.

5. Waiving the Quality of Life Component
The only time that we will not require a patient to complete the quality of life questionnaires is if she is not literate in either English or French (or other languages that the questionnaire may be available in). In other words, if the assistance of a translator is required to comprehend the questions and reply, the questionnaires should not be completed. Translation of the questions is not acceptable. Please indicate on questionnaire.

6. Unwillingness to Complete Quality of Life Questionnaire
If a patient speaks one of the languages that the questionnaire may be available in, but does not wish to complete the questionnaires then she is NOT eligible and should NOT be put on study.

7. Inability to Complete Quality of Life Questionnaire (for reason other than illiteracy in English or French)
An eligible patient may be willing but physically unable to complete the questionnaires, because of blindness, paralysis, etc. If the patient is completing the QOL assessment in the clinic, the questionnaire should be read to them and the answers recorded by a health care professional (e.g. preferably the clinical research associate assigned to the trial, but another clinic nurse, a doctor or social worker who is familiar with the instructions for administering the questionnaires would be acceptable). If the patient is completing the questionnaire at home, and a telephone interview by the center clinical research associate is not possible, then a spouse or friend may read the questions to the patient and record the answers. However, this method should be a last resort, and the spouse or friend should be instructed to not coach or suggest answers to the patient. Whichever method is used, it should be recorded on the questionnaire.

If these special arrangements are not possible or feasible, then the patient would not be required to complete the questionnaires, and this should be reported on the appropriate case report form.

The Quality of Life Questionnaires

The Quality of Life Questionnaire to be applied in this clinical trial is the EORTC QLQ-C30, Version 3.0. In addition, the Breast Module EORTC QLQ-BR23, Version 1.0 is to be used. A German translation of both documents can be found below.

Patientin-Nr.: | | | | | | Zentrums-Nr.: | | | Geburtsjahr der Patientin: | | | |

Erhebung der Lebensqualität:

Das heutige Datum (Tag, Monat, Jahr): | | | | | | | | |

Vor Studieneinschluss ☐ **Vor jeder Kontrollvisite** ☐

Nach Abschluss der Studientherapie (Abschlussvisite) ☐

EORTC QLQ-C30

Wir sind an einigen Angaben interessiert, die Sie und Ihre Gesundheit betreffen. Bitte beantworten Sie die folgenden Fragen selbst, indem Sie die Zahl ankreuzen, die am besten auf Sie zutrifft. Es gibt keine "richtigen " oder "falschen" Antworten. Ihre Angaben werden streng vertraulich behandelt.

		Überhaupt nicht	Wenig	Mäßig	Sehr
1	Bereitet es Ihnen Schwierigkeiten, sich körperlich anzustrengen (z. B. eine schwere Einkaufstasche oder einen Koffer zu tragen)?	1	2	3	4
2	Bereitet es Ihnen Schwierigkeiten, einen längeren Spaziergang zu machen?	1	2	3	4
3	Bereitet es Ihnen Schwierigkeiten, eine kurze Strecke außer Haus zu gehen?	1	2	3	4
4	Müssen Sie tagsüber im Bett liegen oder in einem Sessel sitzen?	1	2	3	4
5	Brauchen Sie Hilfe beim Essen, Anziehen, Waschen oder Benutzen der Toilette?	1	2	3	4
Während der letzten Woche:		Überhaupt nicht	Wenig	Mäßig	Sehr
6	Waren Sie bei Ihrer Arbeit oder bei anderen tagtäglichen Beschäftigungen eingeschränkt?	1	2	3	4
7	Waren Sie bei Ihren Hobbys oder anderen Freizeitbeschäftigungen eingeschränkt?	1	2	3	4
8	Waren Sie kurzatmig?	1	2	3	4
9	Hatten Sie Schmerzen?	1	2	3	4
10	Mußten Sie sich ausruhen?	1	2	3	4
11	Hatten Sie Schlafstörungen?	1	2	3	4
12	Fühlten Sie sich schwach?	1	2	3	4
13	Hatten Sie Appetitmangel?	1	2	3	4
14	War Ihnen übel?	1	2	3	4

DETECT IV

Patientin-Nr.: | | | | | | Zentrums-Nr.: | | | | Geburtsjahr der Patientin: | | | |

Während der letzten Woche:	Überhaupt nicht	Wenig	Mäßig	Sehr
15 Haben Sie erbrochen?	1	2	3	4
16 Hatten Sie Verstopfung?	1	2	3	4
17 Hatten Sie Durchfall?	1	2	3	4
18 Waren Sie müde?	1	2	3	4
19 Fühlten Sie sich durch Schmerzen in Ihrem alltäglichen Leben beeinträchtigt?	1	2	3	4
20 Hatten Sie Schwierigkeiten, sich auf etwas zu konzentrieren, z. B. auf das Zeitunglesen oder das Fernsehen?	1	2	3	4
21 Fühlten Sie sich angespannt?	1	2	3	4
22 Haben Sie sich Sorgen gemacht?	1	2	3	4
23 Waren Sie reizbar?	1	2	3	4
24 Fühlten Sie sich niedergeschlagen?	1	2	3	4
25 Hatten Sie Schwierigkeiten, sich an Dinge zu erinnern?	1	2	3	4
26 Hat Ihr körperlicher Zustand oder ihre medizinische Behandlung Ihr Familienleben beeinträchtigt?	1	2	3	4
27 Hat Ihr körperlicher Zustand oder Ihre medizinische Behandlung Ihr Zusammensein oder Ihre gemeinsame Unternehmungen mit anderen Menschen beeinträchtigt?	1	2	3	4
28 Hat Ihr körperlicher Zustand oder Ihre medizinische Behandlung für Sie finanzielle Schwierigkeiten mit sich getragen?	1	2	3	4

Bitte kreuzen Sie bei den folgenden Fragen die Zahl zwischen 1 und 7 an, die am besten auf Sie zutrifft

29. Wie würden Sie insgesamt Ihren Gesundheitszustand während der letzten Wochen einschätzen?

1	2	3	4	5	6	7
sehr schlecht						ausgezeichnet

30. Wie würden Sie insgesamt Ihre Lebensqualität während der letzten Wochen einschätzen?

1	2	3	4	5	6	7
sehr schlecht						ausgezeichnet

DETECT IV

Patientin-Nr.: | | | | | | Zentrums-Nr.: | | | Geburtsjahr der Patientin: | | | |

Erhebung der Lebensqualität:

Das heutige Datum (Tag, Monat, Jahr): | | | | | | | | |

Vor Studieneinschluss ☐ **Vor jeder Kontrollvisite** ☐

Nach Abschluss der Studientherapie (Abschlussvisite) ☐

EORTC QLQ-BR23

Patientinnen berichten manchmal die nachfolgend beschriebenen Symptome oder Probleme. Bitte beschreiben Sie wie stark Sie diese Symptome oder Probleme währen der letzten Woche empfunden haben.

Während der letzten Woche:	Überhaupt nicht	Wenig	Mäßig	Sehr
31 Hatten Sie einen trockenen Mund?	1	2	3	4
32 War Ihr Geschmacksempfinden beim Essen oder Trinken verändert?	1	2	3	4
33 Schmerzten Ihre Augen, waren diese gereizt oder tränten sie?	1	2	3	4
34 Haben Sie Haarausfall?	1	2	3	4
35 *Nur bei Haarausfall ausfüllen:* Hat Sie der Haarausfall belastet?	1	2	3	4
36 Fühlten Sie sich krank oder unwohl?	1	2	3	4
37 Hatten Sie Hitzewallungen?	1	2	3	4
38 Hatten Sie Kopfschmerzen?	1	2	3	4
39 Fühlten Sie sich wegen Ihrer Erkrankung oder Behandlung körperlich weniger anziehend?	1	2	3	4
40 Fühlten Sie sich wegen Ihrer Erkrankung oder Behandlung weniger weiblich?	1	2	3	4
41 Fanden Sie es schwierig, sich nackz anzusehen?	1	2	3	4
42 Waren Sie mit Ihrem Körper unzufrieden?	1	2	3	4
43 Waren Sie wegen Ihres künftigen Gesundheitszustandes besorgt?	1	2	3	4

DETECT IV

Patientin-Nr.: | | | | | | Zentrums-Nr.: | | | Geburtsjahr der Patientin: | | | |

Während der letzten vier Wochen:	Überhaupt nicht	Wenig	Mäßig	Sehr
44 Wie sehr waren Sie an Sex interessiert?	1	2	3	4
45 Wie sehr waren Sie sexuell aktiv?	1	2	3	4
46 *Nur ausfüllen, wenn Sie sexuell aktiv waren*: Wie weit hatten Sie Freude am Sex?	1	2	3	4

Während der letzten Woche:	Überhaupt nicht	Wenig	Mäßig	Sehr
47 Hatten Sie Schmerzen in Arm oder Schulter?	1	2	3	4
48 War Ihr Arm oder Ihre Hand geschwollen?	1	2	3	4
49 War das Heben oder Seitwärtsbewegen des Arms erschwert?	1	2	3	4
50 Hatten Sie im Bereich der betroffenen Brust Schmerzen?	1	2	3	4
51 War der Bereich Ihrer betroffenen Brust angeschwollen?	1	2	3	4
52 War der Bereich der betroffenen Brust überempfindlich?	1	2	3	4
53 Hatten Sie Hautprobleme im Bereich der betroffenen Brust (z.B. juckende, trockende oder schuppende Haut)?	1	2	3	4

DETECT IV

Drug class	Agent	**Wash-out** (period of time that the medication should be discontinued prior to administration of the first dose of protocol treatment)*
Inducers of CYP3A4		
Antibiotics	All rifamycin class agents (rifampicin, rifabutin, rifapentine)	14 days
Anticonvulsants	Phenytoin, carbamazepine, barbiturates (phenobarbital)	14 days
Antiretrovirals	Efavirenz, nevirapine, tipranivir, etravirine	14 days
Glucocorticoids (oral) (premedication before the administration of taxanes is allowed)	Chronic use of cortisone (> 50mg), hydrocortisone (> 40 mg), prednisone or prednisolone (> 10 mg), methylprednisolone or triamcinolone (> 8 mg), betamethasone or dexamethasone (> 1.5 mg). Glucocorticoid daily doses (oral) ≤ 1.5 mg dexamethasone (or equivalent) are allowed. Short term steroid use (up to 2 weeks) is allowed**.** Premedication for taxane treatment is permitted.	14 days
Other	St. John's Wort, modafinil	14 days
Inhibitors of CYP3A4		
Antibiotic	clarithromycin, erythromycin, troleandomycin, flucloxacillin	7 days
Antifungals	itraconazole, ketoconazole, fluconazole (> 150 mg daily), voriconazole	7 days
Antiretrovirals, Protease Inhibitors	delaviridine, nelfinavir, amprenavir, ritonavir, indinavir, saquinavir, lopinivir, atazanavir	7 days
Calcium channel blockers	verapamil, diltiazem	7 days
Antidepressants	nefazodone, fluvoxamine	7 days
GI Agents	Cimetidine**, aprepitant	7 days
Other	Grapefruit, grapefruit juice, star fruit, papaw amiodarone	7 days 6 months
Substrates of CYP2C8, Pgp, BCRP and OATP1B1		
Antidiabetics	Repaglinide	1 day
Statins	Rosuvastatine	1 week
Cardiac glycosides	Digoxin, digitoxin	2 weeks
Miscellaneous		
Antacids	Magnesium and aluminium hydroxide, simethicone, calcium carbonate, magnesium carbonate	1 hour before and after dosing
Herbal or dietary supplements and traditional Chinese medicines	Ginkgo biloba, kava, grape seed, valerian, ginseng, *Echinacea*, evening primrose oil.	14 days

* All patients must have observed the specified washout period for all prohibited drugs prior to inclusion.

** Note: cimetidine may be used as taxane pre-medication if this is the local institutional practice

Active substance by interaction	Interaction – Change in everolimus AUC/C_{max} Geometric mean ratio (observed range)	Recommendations concerning co-administration
Potent CYP3A4/PgP inhibitors		
Ketoconazole	AUC ↑ 15.3-fold (Range 11.2-22.5) C_{max} ↑ 4.1-fold (Range 2.6-7.0)	Concomitant treatment of everolimus and potent inhibitors is not recommended.
Itraconazole, posaconazole, voriconazole	Not studied. Large increase in everolimus concentration is expected.	
Telithromycin, clarithromycin		
Nefazodone		
Ritonavir, atazanavir, saquinavir, darunavir, indinavir, nelfinavir		
Moderate CYP3A4/PgP inhibitors		
Erythromycin	AUC ↑ 4.4-fold (Range 2.0-12.6) C_{max} ↑ 2.0-fold (Range 0.9-3.5)	Use caution when co-administration of moderate CYP3A4 inhibitors or PgP inhibitors cannot be avoided. If patients require co-administration of a moderate CYP3A4 or PgP inhibitor, dose reduction to 5 mg daily or 5 mg every other day may be considered. However, there are no clinical data with this dose adjustment. Due to between subject variability the recommended dose adjustments may not be optimal in all individuals, therefore close monitoring of side effects is recommended.
Verapamil	AUC ↑ 3.5-fold (Range 2.2-6.3) C_{max} ↑ 2.3-fold (Range 1.3-3.8)	
Ciclosporin oral	AUC ↑ 2.7-fold (Range 1.5-4.7) C_{max} ↑ 1.8-fold (Range 1.3-2.6)	
Fluconazole	Not studied. Increased exposure expected.	
Diltiazem		
Amprenavir, fosamprenavir	Not studied. Increased exposure expected.	
Grapefruit juice or other food affecting CYP3A4/PgP	Not studied. Increased exposure expected (the effect varies widely).	Combination should be avoided.
Potent CYP3A4 inducers		
Rifampicin	AUC ↓ 63% (range 0-80%) C_{max} ↓ 58% (range 10-70%)	Avoid the use of concomitant potent CYP3A4 inducers. If patients require co-administration of a potent CYP3A4 inducer, an everolimus dose increase from 10 mg daily up to 20 mg daily should be considered using 5 mg increments applied on Day 4 and 8 following start of the inducer. This dose of everolimus is predicted to adjust the AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment. If treatment with the inducer is discontinued, the everolimus dose should be returned to the dose used prior to initiation of the co-administration.
Corticosteroids (e.g. dexamethasone, prednisone, prednisolone)	Not studied. Decreased exposure expected.	
Carbamazepine, phenobarbital, phenytoin	Not studied. Decreased exposure expected.	
Efavirenz, nevirapine	Not studied. Decreased exposure expected.	
St John's Wort (*Hypericum perforatum*)	Not studied. Large decrease in exposure expected.	Preparations containing St John's Wort should not be used during treatment with everolimus

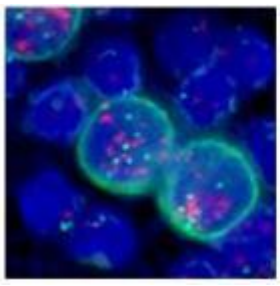
APPENDIX VII – NEW YORK HEART ASSOCIATION CLASSIFICATION OF CARDIAC DISEASE

Clinical Evaluation of Functional Capacity of Patients with Heart Disease in Relation to Ordinary Physical Activity				
Class	Cardiac Symptoms	Limitations	Need for Additional Rest *	Physical Ability to Work **
I	None	None	None	Full time
II	Only moderate	Slight	Usually only slight or occasional	Usually full time
III	Defined, with less than ordinary activity	Marked	Usually moderate	Usually part time
IV	May be present even at rest, and any activity increases discomfort	Extreme	Marked	Unable to work

* To control or relieve symptoms, as determined by the patient, rather than as advised by the physician.
** At accustomed occupation or usual tasks.
Reference: Bruce, RA: Mod Concepts Cardiovasc Dis 25:321, 1956. (Modified from New York Heart Association, 1953)

APPENDIX VIII – PATIENT ID CARD

EudraCT-Nr.: 2013-001269-18 **Protocol Nr.:** D-IV **Sponsor:** Universitätsklinikum Ulm (AöR), Albert-Einstein-Allee 29, 89081 Ulm Prof. Dr. W. Janni, Tel.: +49 (0) 731 500 58501	**Im Notfall oder bei Einweisung in ein Krankenhaus zeigen Sie diese Karte bitte dem behandelnden Arzt** Werden weitere Informationen benötigt, wenden Sie sich bitte an unten stehende Telefonnummer.
Name der Patientin:	**...nimmt an folgender klinischen Prüfung teil:** DETECT IV – Multizentrische, prospektive, offene Phase II Studie bei Patintinnen mit HER2-negativem metastasiertem Brustkrebs und persisitierenden HER2-negativen zirkulierenden Tumorzellen (CTCs).
Everolimus/Ribociclib Kohorte (DIVa) Postmenopausale Patientinnen mit hormonrezeptorpositiven, HER2-negativen metastasiertem Brustkrebs mit HER2-negativen zirkulierenden Tumorzellen (CTCs) und Indikation für eine endokrine Standardtherapie. Die Patientin erhält eine Behandlung mit Everolimus/Ribociclib (DIVa): Standardtherapie: _____ _____ und zusätzlich _____ Tabletten (_____mg) Everolimus/Ribociclib/Tag Einnahmevorschrift für Everolimus/Ribociclib: _____ Tabletten immer zur gleichen Zeit einmal täglich und dann auch gleichbleibend entweder zusammen mit einer Mahlzeit oder zwischen zwei Mahlzeiten, eingenommen werden. Bei Erbrechen kurz nach einer Einnahme diese nur wiederholen, wenn alle Tabletten erbrochen wurden, intakt sind und gezählt werden konnten.Patienten, die mit Everolimus/Ribociclib behandelt werden, dürfen grundsätzlich weder Grapefruits noch Grapefruitsaft zu sich nehmen.	**Eribulin Kohorte (DIVb)** Patientinnen mit hormonrezeptorpositiven, HER2-negativen metastasiertem Brustkrebs und Indikation zur Chemotherapie oder Patientinnen mit triple-negativen metastasiertem Brustkrebs, jeweils beide mit HER2-negativen zirkulierenden Tumorzellen (CTCs). Die Patientin erhält eine Behandlung mit Eribulin (DIVb): Dosierung:
Prüfzentrum:	**Prüfarzt:** **Telefon:**



DETECT IV

Informationsblatt: Behandlung mit Everolimus (Afinitor®)

Sie erhalten im Rahmen der DETECT IV Studie zusätzlich zur Antihormontherapie das Medikament mit dem Handelsnamen Afinitor®. Everolimus (Afinitor®) ist ein Wirkstoff zur Behandlung von Brustkrebs. Es hemmt einen Signalstoff in den Tumorzellen mit der Bezeichnung mammalian Target of Rapamycin (mTOR). Dieser ist für die Steuerung des Zellstoffwechsels und der Zellvermehrung zuständig. Zudem verhindert er die Bildung von neuen Blutgefäßen, die der Tumor für seine Versorgung benötigt.

Anwendung von Everolimus (Afinitor®)
Aus Sicherheitsgründen erhalten Sie nur eine Kombination, die bereits für die Behandlung zugelassen ist, oder die schon einmal in einer anderen klinischen Studie untersucht worden ist. Es kann zudem erforderlich sein, die Dosis im Verlauf der Therapie anzupassen. So kann sie bei Nebenwirkungen verringert werden.
Everolimus wird in Form von ein bzw. zwei Tabletten fortlaufend täglich eingenommen. Eine einzelne Tablette enthält 5 mg Everolimus

Die **Einnahme** sollte folgendermaßen erfolgen:

- einmal täglich fortlaufend
- zur selben Tageszeit
- eine Tablette nach der anderen mit einem Glas Wasser

Bitte beachten Sie folgende **Hinweise**:

- Everolimus-Tagesdosis nicht aufteilen. Die Tabletten nicht zerkauen oder zerstoßen.
- Falls Sie eine Einnahme vergessen haben, setzten Sie die Einnahme beim nächsten Mal wie üblich fort. Nehmen Sie keine doppelte Tablettendosis ein.
- Trinken Sie während der Therapie mit Everolimus keinen Grapefruitsaft. Er kann auf die Medikamente störend einwirken. Achten Sie in diesem Zusammenhang auch auf „versteckte“ Inhaltsstoffe: so kann z.B. in einem FFMultivitaminsaft auch Pampelmuse/Grapefruit enthalten sein.
- Die gemeinsame Behandlung mit Everolimus und bestimmten anderen Medikamenten sollte aufgrund von Wechselwirkungen unbedingt vermieden werden. Besprechen Sie vor Beginn der Therapie mit Everolimus die Einnahme anderer Arzneimittel (inkl. pflanzlicher und/oder nicht verschreibungspflichtiger Präparate) mit Ihrem Prüfarzt.

Bei Ihren Besuchen im Prüfzentrum werden Sie jeweils so mit Everolimus-Tabletten versorgt, dass Sie ausreichend Tabletten bis zum nächsten Besuch haben. Sie erhalten Everolimus in Flaschen mit je 30 Tabletten.

Maßnahmen beim Auftreten von Nebenwirkungen durch Everolimus (Afinitor®)
Wie alle Arzneimittel kann Everolimus Nebenwirkungen hervorrufen, die aber nicht bei jedem auftreten müssen. Hier geben wir Ihnen Anweisungen, was Sie bei Nebenwirkungen tun können:

Entzündungen der Mundschleimhaut
Treffen häufig zu Beginn auf, lassen meist aber im Behandlungsverlauf nach.

Wichtig: Benachrichtigen Sie umgehend Ihren behandelnden Prüfarzt beim ersten Auftreten.

- Achten Sie auf einen guten Zustand ihrer Zähne, konsultieren Sie gegebenenfalls ihren Zahnarzt vor der Therapie.
- Putzen Sie regelmäßig Ihre Zähne mit einer weichen Zahnbürste
- Führen Sie regelmäßige Mundspülungen durch und halten die Schleimhaut feucht; z.B. durch Salbeitee oder gefrorene Ananasstückchen.
- Verzichten Sie auf scharfe Speisen, saure Früchte.

Hautausschläge

- Waschen Sie sich mit lauwarmen Wasser
- Verwenden Sie milde, pH-neutrale Waschlotion
- Achten Sie auf regelmäßige, konsequente Hautpflege, benutzen Sie hierfür harnstoffhaltige Cremes und Salben, die einer Austrocknung entgegenwirken.
- Benutzen Sie Sonnencreme meiden Sie direktes Sonnenlicht und tragen Sie bedeckende Kleidung

Übelkeit und Erbrechen

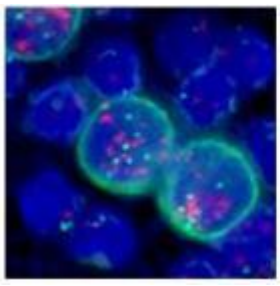
- Essen Sie, wenn Sie Appetit haben, bevor Sie sich hungrig fühlen
- Essen Sie mehrere kleine Mahlzeiten pro Tag
- Vermeiden Sie zu heiße oder zu kalte Nahrung
- Gehen Sie in ein anderes Zimmer, falls Sie Kochgerüche stören
- Vermeiden Sie Fettiges, sehr Süßes oder Scharfes

Durchfall

- Trinken Sie ausreichend Wasser oder milde klare Flüssigkeit 8-10 Gläser z.B. Mineralwasser oder Tee
- Vermeiden Sie fettige und scharf gewürzte Speisen. Stattdessen sollte Ihr Essen aus fettarmen Mahlzeiten mit hohem Eiweißanteil bestehen (z.B. mageres Fleisch oder Eier)
- Vermeiden Sie Milch und Milchprodukte
- Vermeiden Sie sowohl koffeinhaltige als auch alkoholhaltige Getränke
- Essen Sie statt rohem Gemüse gekochtes Gemüse, und entfernen Sie die Schale von Obst

Atemwegsprobleme

- Bitte informieren Sie Ihren Prüfarzt beim Auftreten von Luftnot, Fieber oder Husten.



DETECT IV

Informationsblatt: Behandlung mit Ribociclib

Sie erhalten im Rahmen der DETECT IV Studie zusätzlich zur Antihormontherapie das Medikament mit dem Prüfmedikament Ribociclib (LEE011). Ribociclib (LEE011) hemmt die Funktion spezieller Eiweiße, die die natürliche Zellteilung kontrollieren und wahrscheinlich auch an der Vermehrung von Brustkrebszellen beteiligt sind. Ribociclib ist ein Arzneimittel in der klinischen Erprobung, d.h., es ist von der Brhörde für die Behandlung Ihrer Krankheit noch nicht zugelassen und deshalb auf dem Arzneimittelmarkt nicht käuflich zu erwerben. Die MONALEESA-2 Studie hat gezeigt, dass Ribociclib (LEE011) in Kombination mit Letrozol das Fortschreiten der metastasierten-Brustkrebserkrankung stärker verhindert als Letrozol alleine. Es wurde bis zum Stichtag 06. August 2015 bisher bei mehr als 900 Personen weltweit in Phase I, II und III-Studien geprüft.

Anwendung von Ribociclib (LEE011)
Aus Sicherheitsgründen erhalten Sie nur eine Kombination, die bereits für die Behandlung zugelassen ist, oder die schon einmal in einer anderen klinischen Studie untersucht worden ist. Es kann zudem erforderlich sein, die Dosis im Verlauf der Therapie anzupassen. So kann sie bei Nebenwirkungen verringert werden.
Ribociclib wird in Form von drei Tabletten 21 Tage eingenommen, danach Pause. Eine einzelne Tablette enthält 200 mg Ribociclib.

Die **Einnahme** sollte folgendermaßen erfolgen:

- Einnahme 21 Tage, danach 7 Tage Pause. Dann wieder Einnahme 21 Tage, 7 Tage Pause usw.
- zur selben Uhrzeit am Morgen
- eine Tablette nach der anderen mit einem Glas Wasser
- Einnahme zum Essen oder unabhängig von einer Mahlzeit möglich

Bitte beachten Sie folgende **Hinweise**:

- Ribociclib-Tagesdosis nicht aufteilen. Die Tabletten nicht zerkauen oder zerstoßen.
- Falls Sie eine Einnahme vergessen haben, sich aber innerhalb von 6 Stunden daran errinnern, nehmen Sie die Medikamente bitte nachträglich ein. Fällt es ihnen erst nach Ablauf der 6 Stunden ein, muss die Einnahme an diesem Tag ausgelassen werden. Die nächste Einnahme erfolgt planmäßig am nächsten Tag.
- Sollten Sie nach Tabletteneinnahme Erbrechen müssen, nehmen Sie bitte an diesem Tag keine weitere Tablette mehr ein.
- Das Essen von Orangen und das Trinken von Orangen Saft sind erlaubt. Andere exotische Früchte und deren Säfte (z.B. Bitterorangen, alle Arten von Grapefruit, Sternfrucht) dürfen ab sieben Tage vor der ersten Einnahme der Studienmedikation und währen der gesamten Studie nicht gegessen oder getrunken werden. Der Grund für diese Beschränkung ist ein veränderter Abbau der Prüfpräte durch Ihren Körper.
- Die Einnahme von Vitaminen ist erlaubt. Vermeiden Sie jedoch alle pflanzlichen Präparate/Medikamente und Nahrungergänzungsmittel.

- Bitte bringen Sie zu jedem Kontrollbesuch Ihre Prüfmedikamente (leer, angebrochen und voll) ans Prüfzentrum mit.

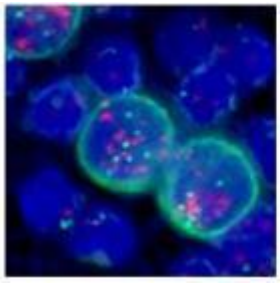
Bei Ihren Besuchen im Prüfzentrum werden Sie jeweils so mit Ribociclib-Tabletten versorgt, dass Sie ausreichend Tabletten bis zum nächsten Besuch haben. Sie erhalten Ribociclib in Flaschen mit je 30 Tabletten.

Maßnahmen beim Auftreten von Nebenwirkungen durch Ribociclib (LEE011)
Wie alle Arzneimittel kann Ribociclib Nebenwirkungen hervorrufen, die aber nicht bei jedem auftreten müssen. Hier geben wir Ihnen Anweisungen, was Sie bei Nebenwirkungen tun können:

Übelkeit und Erbrechen
- Essen Sie, wenn Sie Appetit haben, bevor Sie sich hungrig fühlen
- Essen Sie mehrere kleine Mahlzeiten pro Tag
- Vermeiden Sie zu heiße oder zu kalte Nahrung
- Gehen Sie in ein anderes Zimmer, falls Sie Kochgerüche stören
- Vermeiden Sie Fettiges, sehr Süßes oder Scharfes

Durchfall
- Trinken Sie ausreichend Wasser oder milde klare Flüssigkeit 8-10 Gläser z.B. Mineralwasser oder Tee
- Vermeiden Sie fettige und scharf gewürzte Speisen. Stattdessen sollte Ihr Essen aus fettarmen Mahlzeiten mit hohem Eiweißanteil bestehen (z.B. mageres Fleisch oder Eier)
- Vermeiden Sie Milch und Milchprodukte
- Vermeiden Sie sowohl koffeinhaltige als auch alkoholhaltige Getränke
- Essen Sie statt rohem Gemüse gekochtes Gemüse, und entfernen Sie die Schale von Obst

Hautausschläge
- Waschen Sie sich mit lauwarmen Wasser
- Verwenden Sie milde, pH-neutrale Waschlotion
- Achten Sie auf regelmäßige, konsequente Hautpflege, benutzen Sie hierfür harnstoffhaltige Cremes und Salben, die einer Austrocknung entgegenwirken.
- Benutzen Sie Sonnencreme meiden Sie direktes Sonnenlicht und tragen Sie bedeckende Kleidung



DETECT IV

Informationsblatt: Behandlung mit Eribulin (HALAVEN®)

Sie erhalten im Rahmen der DETECT IV Studie das Medikament mit dem Handelsnamen HALAVEN®. Eribulin (HALAVEN®) ist ein Wirkstoff zur Behandlung von lokal fortgeschrittenem oder metastasiertem Brustkrebs (d.h. ein Brustkrebs, der sich über den ursprünglichen Tumor hinaus ausgebreitet hat). Es hemmt in den Krebszellen die Dynamik der sog. Mikrotubuli, die als Bestandteil des Zellskeletts für die Bewegung und den Transport innerhalb der Zelle, sowie bei der Zellvermehrung nötig sind. Eribulin stoppt somit das Wachstum und die Ausbreitung der Krebszellen.

Anwendung von Eribulin (HALAVEN®)
Eribulin ist bereits für die Behandlung von lokal fortgeschrittenem oder metastasiertem Brustkrebs nach bestimmten Vorbehandlungen zugelassen und wurde bereits in anderen klinischen Studien untersucht.
Eribulin wird Ihnen von einem Arzt oder einer Kankenpfegekraft für eine Dauer von 2 bis 5 Minuten über einen venösen Zugang gegeben. Die Dosis, die Sie erhalten, richtet sich nach Ihrer Körpergröße (ausgedrückt in Quadratmeter (m^2)). Und wird nach Ihrem Körpergewicht und Ihrer Körpergröße berechnet. Die übliche Dosis Eribulin beträgt 1,23 mg/m^2, es kann jedoch erforderlich sein, die Dosis im Verlauf der Therapie anzupassen. So kann sie bei Nebenwirkungen verringert werden. Eribulin wird in der Regel an Tag 1 und 8 eines 21-Tage-Zyklus gegeben. Ihr Arzt wird festlegen, wie viele Behandlungszyklen Sie erhalten sollen. Je nach den Ergebnissen Ihrer Blutuntersuchungen muss der Arzt die Gabe des Eribulin hinauszögern bis sich die Ergebnisse normalisiert haben und/ oder die Dosis reduzieren.
Um für eine Behandlung mit Eribulin zugelassen werden zu können, dürfen Sie nicht schwanger sein. Sie müssen sich außerdem bereit erklären, während der Behandlungsphase sowie bis einschließlich 3 Monate nach der Behandlung eine wirksame Methode der Empfängnisverhütung anzuwenden.

Maßnahmen beim Auftreten von Nebenwirkungen durch Eribulin (HALAVEN®)
Wie alle Arzneimittel kann Eribulin Nebenwirkungen hervorrufen, die aber nicht bei jedem auftreten müssen. Die Nebenwirkungen sind vor allem auf die Hemmung der Zellteilung zurückzuführen.

Zu den häufigsten unerwünschten Wirkungen gehören:

- Blutbildungsstörungen (Blutarmut, Abnahme der weißen Abwehrzellen)
- Appetitverlust, Müdigkeit, Schwäche
- Taubheitsgefühl, Kribbeln oder Prickeln
- Fieber
- Gelenk- und Muskelschmerzen, Kopfschmerzen
- Haarausfall

Weitere Nebenwirkungen sind möglich. Informieren Sie Ihren Arzt, wenn eine der aufgeführten Nebenwirkung Sie erheblich beeinträchtigt oder Sie Nebenwirkungen bemerken, die hier nicht aufgeführt sind.