Prospective, randomized, pharmacological intervention study; evaluating the effect of the angiotensin II-receptor (AT1) blocker candesartan versus placebo in prevention of trastuzumab-associated cardiotoxicity in patients with primary breast cancer treated with trastuzumab

Protocol code: M06HER

Protocol Date: 19 August 2010

Protocol Version: 8.0
Including Amendment 1 dd. 12 July 2007
Amendment 2 dd. 12 November 2007
Amendment 3 dd. 26 March 2008
Amendment 4 dd. 11 August 2008
Amendment 5 dd. 02 October 2008
Amendment 6 dd. 06 May 2009
Amendment 7 dd. 19 August 2010

Principal investigators
J.H.M. Schellens, MD PhD & E.G.E. de Vries, MD PhD

Research centers:
Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital A.H. Boekhout
University Medical Center Groningen, Groningen J.H.M. Schellens, MD PhD
J.A.Gietema, MD PhD
W. Smit, MD PhD
E.G.E. de Vries, MD PhD
P.B. Ottevanger, MD PhD
W.E. Fiets, MD PhD
M. Soesan, MD
C.M.P.W. Mandigers, MD PhD

Wilhelmina Ziekenhuis, Assen
Jeroen Bosch Hospital, ’s Hertogenbosch
Medisch Centrum Alkmaar, Alkmaar
Martini Ziekenhuis, Groningen
Antonius Ziekenhuis, Nieuwegein
Ziekenhuis de Tjongerschans, Heerenveen
Streekziekenhuis Koningin Beatrix, Winterswijk
Beth Israel Deaconess Medical Center, Boston, USA
VieCuri Medisch Centrum voor Noord-Limburg, Venlo
Flevziekenhuis, Almere

J.D. Chang, MD
A.J. van der Wouw, MD PhD
V.Lustig, MD
Other personnel involved:

Netherlands Cancer Institute
Antoni van Leeuwenhoek Hospital
O. Dalesio

University Medical Center Groningen
P.J. Perik, MD PhD

Slotervaart Hospital
J.H. Beijnen, PhD
R.A.M. van Liebergen, MD PhD

Drug Safety Monitoring Board:

D.J. van Veldhuisen MD PhD
University Medical Center Groningen

D. Richel, MD PhD
Academic Medical Center Amsterdam

G. Hart, MSc
Statistician

Data management:

Trial office and data management center
The Netherlands Cancer Institute

Contact

J.H.M. Schellens, MD PhD
E.G.E. de Vries, MD PhD
The Netherlands Cancer Institute
University Medical Center Groningen
Department of Medical Oncology
Department of Medical Oncology
Plesmanlaan 121
Hanzeplein 1
1066 CX Amsterdam
9713 GZ Groningen
Tel + 31(0)20 5122446
Tel + 31(0)50 3612821
Fax + 31(0)20 5122572
Fax + 31(0)50 3614862
E-mail: jhm@nki.nl
E-mail: e.g.e.de.vries@int.umcg.nl

Eudract registration number: 2006-001707-11

CONFIDENTIAL: Information and data included in this protocol contain trade secrets and privileged or confidential information which is the property of the Netherlands Cancer Institute. No person is authorized to make it public without written permission of the Netherlands Cancer Institute. These restrictions on disclosure will apply equally to all future information supplied to you, which is indicated as privileged or confidential.
<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sponsor:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands Cancer Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsterdam, Netherlands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prof. S. Rodenhuis, MD, Ph.D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical Director</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands Cancer Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsterdam, Netherlands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prof. J.H.M. Schellens, MD, PhD</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University Medical Center Groningen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prof. E.G.E. de Vries, MD, PhD</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Co-investigator:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands Cancer Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsterdam, Netherlands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.H. Boekhout</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Co-investigator:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University Medical Center Groningen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.A. Gietema, MD, PhD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# List of Abbreviations and Definition of Terms

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

## 1. Study Summary: Prevention of Trastuzumab-Associated Cardiotoxicity

### Study Assessments and Treatment of Study Patients

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

## 2. Introduction and Background

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

### Primary Objectives

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

## 3. Rationale

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
</tr>
</tbody>
</table>

### Rationale for Administration Schedule and Dosage of Candesartan

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
</tr>
</tbody>
</table>

## 4. Study Objectives

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

### Secondary Objectives

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

### Primary Endpoint

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

## 5. Study Design and Study Population

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

### Registration and Randomization

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

### Expected Duration

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

### Study Population

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

### Inclusion Criteria

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

### Exclusion Criteria

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
</tr>
</tbody>
</table>

### Withdrawal Criteria

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

### Procedures for Handling Withdrawn Patients

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

### Concomitant Medication and Treatment

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

## 6. Treatment of Study Patients

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

### Study Treatment

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

### Candesartan (Atacand®)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
</tr>
</tbody>
</table>

### Placebo

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
</tr>
</tbody>
</table>

### Packaging and Dispensing of the Study Medication and Description of the Double Blind Procedure

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
</tr>
</tbody>
</table>

### Unblinding Procedure

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

### Missing of Doses of Study Medication

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

## 7. Study Assessments and Schedules

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

### Medical History, Physical Examination, Haematological & Chemistry Studies

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

### New York Heart Association Score (NYHA) and Cardiac Questionnaire

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
</tr>
</tbody>
</table>

### Electrocardiogram

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
</tr>
</tbody>
</table>

---

Protocol M06HER version 8.0, 19 – August - 2010  Page 4 of 48
7.4 MUGA SCAN .................................................................................................................... 26
7.5 SCHEDULE OF ASSESSMENTS..................................................................................... 26
7.5.1 Screening schedule........................................................................................................ 26

8. MANAGEMENT OF TOXICITY ......................................................................................... 27
8.1 MANAGEMENT OF TOXICITY, DELAY OF CANDESARTAN ADMINISTRATION DUE TO TOXICITY .... 27
8.2.1 Congestive Heart Failure.............................................................................................. 28
8.2.2 Early discontinuation of trastuzumab........................................................................... 29
8.2.3 Trastuzumab in combination with other anticancer drugs........................................... 29

9. SAFETY REPORTING ....................................................................................................... 29
9.1 SECTION 10 WMO EVENT ............................................................................................ 29
9.2.1 Adverse event and assessing severity of adverse events............................................. 29
9.2.2 Assessing relationship to study treatment .................................................................... 30
9.2.3 Classification of causality of an adverse event and study treatment........................... 30
9.2.4 Serious adverse event (SAE)........................................................................................ 31
9.2.5 Suspected Unexpected Serious Adverse Reaction (SUSAR) ........................................ 31
9.2.6 Reporting of serious adverse events (SAEs).............................................................. 31
9.2.7 Reporting of a SUSAR................................................................................................ 32
9.2.8 Reporting of a cardiac failure...................................................................................... 32
9.2.9 Data Safety Monitoring Board.................................................................................... 32

10. STATISTICS .................................................................................................................... 33
10.1 STATISTICAL CONSIDERATIONS .............................................................................. 33

TABLE A .................................................................................................................................. 33

TABLE B .................................................................................................................................. 34

11. ETHICAL CONSIDERATIONS ......................................................................................... 34
11.1 DECLARATION OF HELSINKI AND WMO ................................................................. 34
11.2 DOCUMENTATION OF ESSENTIAL DOCUMENTS DURING THE STUDY ......................... 34
11.3 ETHICS COMMITTEE .................................................................................................... 34
11.4 INSURANCE OF LIABILITIES ...................................................................................... 35
11.5 PATIENT INFORMATION AND INFORMED CONSENT .............................................. 35

12. ADMINISTRATIVE ASPECTS ......................................................................................... 35
12.1 DOCUMENTATION OF PATIENT’S PARTICIPATION .................................................. 35
12.2 DATAMANAGEMENT .................................................................................................... 35
12.3 PATIENT IDENTIFICATION AND CONFIDENTIALITY ................................................ 35
12.4 ANNUAL PROGRESS REPORT ..................................................................................... 35
12.5 ANNUAL SAFETY REPORT .......................................................................................... 36
12.6 END OF STUDY REPORT AND FINAL STUDY REPORT ............................................. 36
12.7 FINANCING OF THE TRIAL ........................................................................................ 36
12.8 PROCEDURES FOR PROTOCOL AMENDMENTS ...................................................... 36
12.9 PUBLICATION .............................................................................................................. 36

13. REFERENCES .................................................................................................................. 38

APPENDIX 1: ALGORITHM FOR CONTINUATION AND DISCONTINUATION OF TRASTUZUMAB BASED ON LVEF ASSESSMENTS ......................................................... 42
APPENDIX 2: TABLE 1 FOLLOW-UP M06HER .................................................................... 43
APPENDIX 3: GUIDELINES FOR THE TREATMENT OF CHF ............................................ 44
### LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABR</td>
<td>Angiotensin II-receptor (AT1) blocker (ABR)</td>
</tr>
<tr>
<td>ABRs</td>
<td>Angiotensin II-receptor (AT1) blockers (ABRs)</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme inhibitors (ACE)</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>BNP</td>
<td>Brain Natriuretic Peptide</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>CREC</td>
<td>Cardiac Review and Evaluation Committee</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CTC</td>
<td>Common Toxicity Criteria version 3.0</td>
</tr>
<tr>
<td>DSMC</td>
<td>Data Safety Monitoring Committee</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>HF</td>
<td>Heart Failure</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SNPs</td>
<td>Single nucleotide polymorphisms</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
</tr>
</tbody>
</table>
1. **STUDY SUMMARY: PREVENTION OF TRASTUZUMAB-ASSOCIATED CARDIOTOXICITY**

<table>
<thead>
<tr>
<th>Study title</th>
<th>Prospective, randomized, pharmacological intervention study evaluating the effect of the angiotensin II-receptor (AT1) blocker candesartan versus placebo in prevention of trastuzumab-associated cardiotoxicity in patients with primary breast cancer treated with trastuzumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study code</td>
<td>M06HER</td>
</tr>
</tbody>
</table>
| Research centers & investigators | Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital
A.H. Boekhout
J.H.M. Schellens
University Medical Center Groningen, Groningen
J.A. Gietema
E.G.E. de Vries
Medisch Spectrum Twente, Enschede
W. Smit
University Medical Center St. Radboud, Nijmegen
P.B. Ottevanger
Isala klinieken, Zwolle
A. Honkoop
Medisch Centrum Leeuwarden, Leeuwarden
W.E. Fiets
Slotervaart Hospital, Amsterdam
M. Soesan
Canisius Wilhelmina Hospital, Nijmegen
C.M.P.W. Mandigers
Wilhelmina Ziekenhuis, Assen
P. Nieboer
Deventer Ziekenhuis, Deventer
L. Kessels
Onze Lieve Vrouwe Gasthuis, Amsterdam
B. De Valk
Jeroen Bosch Hospital, ’s Hertogenbosch
T. Smilde
Medisch Centrum Alkmaar, Alkmaar
C.H. Smorenburg
Martini Ziekenhuis, Groningen
A.W.G. van der Velden
Antonius Ziekenhuis, Nieuwegein
M. Los
Ziekenhuis de Tjongerschans, Heerenveen
J. De Boer
Streekziekenhuis Koningin Beatrix, Winterswijk
P.P.J.B.M.M. Schiphorst
Beth Israel Deaconess Medical Center, Boston, USA
J.D. Chang
Viecuri Medisch Centrum voor Noord-Limburg, Venlo
A.J. van der Wouw
Flevoziekenhuis, Almere
V. Lustig |

**Methodology**

Prospective, randomized pharmacological intervention study

**Objectives**

**Primary objectives:**

1) To determine whether concurrent ATII-antagonist treatment can prevent trastuzumab-related cardiotoxicity, defined as a decline in LVEF of more than 15% or a decrease to an absolute value <45%

**Secondary objectives:**

1) To determine if ‘Brain Natriuretic Peptide’ (NT-proBNP) and troponin T can be used as surrogate marker in the monitoring of trastuzumab-associated cardiotoxicity
2) To determine genetic variability in relevant genes such as the HER2 gene (by assessing single nucleotide polymorphisms [SNPs] in the kinase domain) and explore any correlations with trastuzumab induced cardiotoxicity
3) To determine the reversibility of a decrease in left ventricular ejection fraction (LVEF) associated with trastuzumab treatment
**Primary endpoint:**
The primary endpoint of the study is the occurrence of cardiotoxicity during the one-year trastuzumab therapy and during the 40 weeks after discontinuation of trastuzumab treatment, defined as a decline in LVEF (MUGA) of more than 15% or a decrease to an absolute value below 45%.

<table>
<thead>
<tr>
<th>Arm I</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm II</td>
<td>AT1 blocker candesartan (32 mg/day; run in 16 mg during week 1)</td>
</tr>
</tbody>
</table>

**Study duration**
Registration: before chemotherapy treatment period. Randomization: before start of trastuzumab treatment. Study period: chemotherapy period, trastuzumab treatment period 26 weeks follow up after discontinuation of trastuzumab treatment and thereafter 3 months follow-up after end of candesartan or placebo. Candesartan treatment will start the same day as the first infusion of trastuzumab and will continue up to 26 weeks after the end of treatment with trastuzumab. The last study assessment will take place 3 months after discontinuation of candesartan or placebo.

**Type of patients**
Women with primary HER2 positive breast cancer who are considered for adjuvant systemic treatment with anthracycline containing (neo-) chemotherapy and trastuzumab.

**Inclusion and Exclusion criteria**

### Inclusion criteria
1. Women aged ≥18 years
2. WHO: ≤ 2
3. Strongly HER2-positive breast cancer, defined as an immunohistochemistry score of 3+ using the HercepTest™, or gene amplification by fluorescence in situ hybridization, or chromogenic in situ hybridization (CISH).
4. Serum creatinine <140 umol/l or creatinine clearance > 50 ml/min (by Cockcroft-Gault formula)
5. Thyroid stimulating hormone between 0.5-3.9 MU/l. If the thyroid stimulating hormone value is < 0.5 MU/l or > 3.9 mU/l; thyroid hormone FT4 should be 8 – 26 pmol/l
6. Blood pressure systolic ≥ 140 mmHg and diastolic ≥ 90 mmHg is acceptable at registration. However at randomization, prior to the first administration of trastuzumab blood pressure should be regulated and should be systolic ≥ 100 mmHg and ≤ 180 mmHg and diastolic ≥ 60 mmHg and ≤ 100 mmHg. (blood pressure should be regulated according to the guidelines of appendix 5)
7. LVEF ≥ 50% assessed by multigated angiography (MUGA) or cardiac ultrasound
8. (Neo-) adjuvant regimen: trastuzumab start ≥ 3 weeks after day 1 of the last anthracycline chemotherapy cycle
9. Must have received at least an approved anthracycline based (neo-) adjuvant chemotherapy regimen
10. Trastuzumab treatment according to standard medical care
11. Written informed consent to participate in the study

### Exclusion criteria
1. Prior anthracycline chemotherapy regimen or anti-HER2 therapy, or other prior biologic or immunotherapy for breast cancer treatment or any malignancy
2. Previous malignancy requiring chemotherapy or mediastinal...
### Radiotherapy

3. Uncontrolled serious concurrent illness
4. Patients with New York Heart Association (NYHA) class II/III/IV congestive heart failure
5. Myocardial infarction < 6 months before registration
6. Treatment with ACE inhibitor, ATII blocker, or lithium. Patients treated with ACE inhibitor, or ATII blocker can switch (after registration and during the chemotherapy period) to alternative antihypertensive therapy; see appendix 5.
7. History of hypersensitivity to the study medication
8. Pregnancy or breast feeding

### Instruments

| - New York Heart Association (NYHA) scale |
| - Cardiac questionnaire |
| - Electrocardiogram |
| - MUGA scan (Common Toxicity Criteria 3.0) |
| - NT-proBNP and troponin T analysis |
| - Genotype analysis |

### Measurements

#### Before start of anthracycline treatment:

- Medical history, physical examination
- New York Heart Association (NYHA) score
- Cardiac questionnaire
- Electrocardiogram
- MUGA scan
- Laboratory assessments; hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, sodium, potassium, calcium, thyroid stimulating hormone, glucose, cholesterol, bilirubin, alkaline phosphatase, ASAT/ALAT, LDH, albumin, NT-proBNP, troponin T analysis
- Pregnancy test
- Genotype analysis

#### Every chemotherapy cycle

- Laboratory assessments; hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, sodium, potassium, calcium, glucose, bilirubin, alkaline phosphatase, ASAT/ALAT, LDH, albumin

#### Before start of trastuzumab treatment:

- Physical examination
- New York Heart Association (NYHA) score
- Cardiac questionnaire
- Electrocardiogram
- MUGA scan
- Laboratory assessments; hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, sodium, potassium, calcium, glucose, bilirubin, alkaline phosphatase, ASAT/ALAT, LDH, albumin, NT-proBNP, troponin T analysis

#### After 3, 6 and 9 months trastuzumab:

- Physical examination
- New York Heart Association (NYHA) score
- Cardiac questionnaire
- MUGA scan
- Laboratory assessments; hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, sodium, potassium,
calcium, glucose, bilirubin, alkaline phosphatase, ASAT/ALAT, LDH, albumin, NT-proBNP, troponin T analysis

**After 1 year trastuzumab, 26 and 40 weeks after the last trastuzumab administration (= 3 months after stopping of study medication):**
- Physical examination
- New York Heart Association (NYHA) score
- Cardiac questionnaire
- Electrocardiogram
- MUGA scan
  - Laboratory assessments; hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, sodium, potassium, calcium, glucose, bilirubin, alkaline phosphatase, ASAT/ALAT, LDH, albumin, NT-proBNP, troponin T analysis

**Statistics**

The primary endpoint of the study is the deterioration of the cardiac function defined as a decline in LVEF of more than 15% from baseline or a decrease of less than 15% to an absolute value below 45%, during the year with treatment and during the 40 weeks after discontinuation of trastuzumab treatment.

From previous studies it is estimated that about 30% of the patients treated with trastuzumab will show deterioration of LVEF. A total of 200 patients will receive trastuzumab and candesartan or trastuzumab and placebo in this double blind placebo-controlled study. The number of patients registered (= before chemotherapy period) for this trial shall be more than 200 as a small number of patients might drop out before start of therapy with trastuzumab. This number cannot exactly be determined beforehand.

A two group chi² test with alpha 0.050 two-sided significance level will have in excess of 80% power to detect the difference between a Group 1 proportion, $p_1$, of 0.300 and a Group 2 proportion, $p_2$, of 0.130 (odds ratio of 0.349) when the sample size in each group is 100.

To closely monitor the safety of the outlined intervention 3 interim looks will be performed before the final analysis. Cardiovascular failures (according to the definition) need to be reported actively and immediately. The interim analyses will take place at equally spaced information fractions with the final look at about 200 patients. The interim analyses are planned after 10, 20 and 30 LVEF failures have occurred. The final analysis will be after the last patient has been followed for 92 weeks. At each interim analysis we will use the logrank test on all available patient data. The final analysis will use the Chi square test with the squared boundary from the logrank test. We will use the EAST software package to calculate the stopping boundaries for the interim tests. For the efficacy side, we choose the overall one-sided significance level of the tests to be 0.025. We will use the Lan and DeMets error spending function resembling the O'Brien-Fleming boundary, to find the stopping boundaries. At the same time points, we will also perform analyses of safety. We choose the overall one-sided significance level of the safety tests to be 0.05. We will use the error spending function proposed by Lan and DeMets resembling the Pocock boundary.

Importantly, only the DSMB will have access to unblinded data. They will report whether the trial may be continued or should be stopped for safety reasons.
2. INTRODUCTION AND BACKGROUND

2.1 Introduction

Breast cancer is the most common life-threatening cancer diagnosis in women. In one year (the year 2000) in the Netherlands 11,000 new cases of breast cancer were diagnosed [1].

Surgery is the main modality of local treatment for breast cancer. Surgery and/or radiotherapy can control local-regional disease in the majority of patients. In order to decrease the risk of distant relapse, the addition of adjuvant systemic hormonal and/or chemotherapy is necessary to increase the cure rate. Adjuvant chemotherapy involves a combination of cytotoxic anticancer drugs; adjuvant hormonal therapy deprives cancer cells of the hormone estrogen, which some breast cancer cells need to grow. These therapeutic modalities are complementary and are often used in combination.

It has been recognized that a subset of approximately 20-30% of patients with breast cancer have tumors with HER2 neu gene amplification. The human epidermal growth factor receptor HER2 is a member of the epidermal growth factor receptor (EGFR) family of transmembrane tyrosine kinases and is normally involved in the regulation of cell proliferation. Breast cancer patients in whom the HER2 neu gene is overexpressed in their tumor generally experience a less favorable response to traditional chemotherapy, including anthracyclines. They have poorly differentiated tumors with high proliferative rate, positive axillary lymph nodes, decreased expression of estrogen and progesterone receptors. These markers are associated with shorter overall and disease-free survival [2, 3].

Trastuzumab (Herceptin®), a humanized monoclonal antibody against the extracellular domain of HER2, has been shown to benefit patients with HER2-positive metastatic breast cancer when administered weekly or every three weeks, alone or in combination with chemotherapy [4-7]. Trastuzumab is in the management of metastatic disease commonly used in combination with taxanes, vinorelbine and platinum salts. Recently, promising publications reported that the addition of trastuzumab in the adjuvant setting of primary breast cancer (e.g. surgery, radiotherapy, chemotherapy) is effective for HER2 positive breast cancer, as measured by reductions in recurrence and death. The results of these trials show highly significant reductions in the risk of recurrence. The unadjusted hazard ratio for recurrence of breast cancer, contralateral breast cancer, second non-breast malignant disease, or death in the trastuzumab group for one year, as compared with the observation group, was 0.54 (95 % confidence interval, 0.43 to 0.67; P<0.0001 by the log-rank test) in the HERA trial [8]. An absolute benefit in the disease-free survival at two years of 8.4 %, the overall survival in the two groups was not significantly different. Another trial reported an absolute difference in disease-free survival between the trastuzumab group and the control group of 12 % at three years. Trastuzumab was associated with 33 % reduction in the risk of death (P=0.015) [9]. On the basis of these results, patients with HER2-positive breast cancer receive one year of trastuzumab as part of optimal standard adjuvant systemic therapy.

2.2 Trastuzumab

A strategy to antagonize the function of over-expressed HER2 has been developed. Murine monoclonal antibodies were produced against the extracellular domain of the HER2 protein. One such antibody (muMAb 4D5) was found to inhibit markedly the proliferation of human tumor cells over-expressing HER2. Efficacy was observed in non-clinical in vivo studies using the antibody alone and in combination with cytotoxic chemotherapy. Since chronic administration of murine monoclonal antibodies to humans is limited by immune responses to the non-human protein, the antibody was “humanized” (i.e. the regions of muMAb 4D5 that determine anti-HER2 binding specificity were engineered into the framework of a generic human antibody). The resulting antibody, rhuMAb HER2 (trastuzumab, Herceptin®), binds specifically to the HER2 protein extracellular domain with
high affinity. An important property of trastuzumab is its ability to mediate profound antibody-dependent cellular cytotoxicity (ADCC) specifically against HER2 over-expressing tumor cell lines in the presence of human peripheral blood mononuclear cells. Thus trastuzumab may not only block the activity of HER2 on the cell-surface, thereby inhibiting tumor cell proliferation, but may also have direct tumor cell killing potential.

A large, randomized, controlled phase III trial (H0648g), was conducted in women with metastatic breast cancer whose tumors overexpress HER2. The objectives were to evaluate the efficacy and safety of trastuzumab in combination with first line chemotherapy regimens. Women who had received anthracyclines as adjuvant treatment were randomized to paclitaxel with or without trastuzumab and women who had not been treated with anthracyclines before received doxorubicin and cyclophosphamide (AC) with or without trastuzumab. The study enrolled 469 patients and the results demonstrated that the combination of trastuzumab and chemotherapy significantly prolongs the time to disease progression compared with chemotherapy alone. The addition of trastuzumab also increased the response rate in patients receiving paclitaxel or AC as well as increasing median survival [10].

Recently, interim analyses of three large randomized trials, evaluating the use of trastuzumab after adjuvant standard chemotherapy in HER2-positive breast cancer patients, showed beneficial effects of the addition of trastuzumab to standard adjuvant treatment. In the NSABP-B31 trial, patients with HER2-positive breast cancer with one or more axillary lymph node metastases, were randomized between 4 cycles of AC chemotherapy, followed by 4 cycles of paclitaxel (175 mg/m² every 3 weeks) with or without trastuzumab (4 mg/kg loading dose, followed by weekly doses of 2 mg/kg). Trastuzumab treatment was continued for 1 year. The NCCTG N9831 trial also enrolled patients with a HER2-positive breast tumor. In this Canadian study, patients with high-risk breast cancer, but without axillary lymph node metastases were also eligible. Patients were randomized between 3 treatment arms: 4 cycles AC, followed by 12 cycles of weekly paclitaxel (80 mg/m²) (control arm); the same chemotherapy regimen plus weekly trastuzumab (2 mg/kg) started concurrently with the start of paclitaxel chemotherapy (trastuzumab concurrent arm) or after paclitaxel chemotherapy (trastuzumab sequential arm). Combined analysis of both trials (N = 3,351), disregarding the trastuzumab sequential arm of the Canadian trial, showed beneficial effects of the addition of trastuzumab in terms of disease free (87% versus 75%) and overall survival (94% versus 92%), after a median follow-up of 3 years [11, 12].

A large European trial evaluating the use of trastuzumab for the adjuvant treatment of patients with HER2-positive breast cancer also showed benefit of trastuzumab. In this trial, patients with HER2-positive breast cancer, with or without lymph node metastases were randomized between observation, 1 or 2 years 3-weekly trastuzumab (8 mg/kg loading, 6 mg/kg maintenance dose) after standard chemotherapy. The comparison between the observation (N = 1,693) and the 1-year trastuzumab (N = 1,694) arms, showed that patients treated with trastuzumab had higher 2-year disease-free survival than patients in the observation arm (87.2% versus 78.6%). After 2 years, 89.7% of the patients in the trastuzumab arm were free of distant metastases, compared to 81.8% in the observation arm. No marked difference in overall survival was observed at this time.

In a smaller study, women with primary breast cancer with axillary-node-positive or high-risk node-negative cancer, were randomized between three cycles of docetaxel or vinorelbine, followed by three cycles of fluorouracil, epirubicin and cyclophosphamide. Women with HER2-positive breast cancer assigned to receive or not to receive nine weekly trastuzumab infusions. Patients who received trastuzumab had better three-year recurrence-free survival than those who did not receive trastuzumab (89 percent versus 78 percent; hazard ratio for recurrence or death, 0.42; 95 percent confidence interval, 0.21 to 0.83; P=0.01) [13].
2.3 **Cardiotoxicity and mechanisms of trastuzumab-related cardiotoxicity**

Treatment with trastuzumab can coincide with as side effect the development of clinically manageable left ventricular systolic dysfunction, and occasionally advanced congestive heart failure in a small proportion of patients. Cardiotoxicity has been reported in women who received trastuzumab as single agent of in combination with chemotherapy for metastatic disease of in primary breast cancer.

In embryonic wild-type mice, HER2 is immunohistochemically present in myocardial and endocardial cells [14, 15]. Cardiomyocyte HER2 expression is mostly restricted to the T-tubular network, indicating a non-random cardiac distribution pattern [16]. It is therefore likely that HER2 regulates circumscribed processes in cardiac physiology. The first evidence indicative of HER2 involvement in the heart, stems from HER-deficient, and neuregulin deficient mice, which die early (before day 10.5) in embryonic development as a result of severe cardiac and neurologic abnormalities [17-20]. HER2 is crucial for the prevention of dilated cardiomyopathy. This was demonstrated in conditional mutant mice with a cardiac-restricted HER2 gene deletion. These mice were born at normal Mendelian frequencies and showed no overt phenotypic abnormalities at birth. However, shortly after birth they developed a dilated cardiomyopathy, with severely attenuated myocardial contractility [21, 22, 23]. HER2 appears to play an important role in compensatory cardiac hypertrophy. Hypertrophic growth can serve as a compensatory mechanism for different mechanical, hemodynamic, hormonal and pathologic stimuli. Aortic banding in conditionally mutated mice with a cardiac-restricted HER2 deficiency, did not result in a hypertrophic growth response. The precise role of HER2 in human cardiac physiology and disease is still unknown. Myocardial HER2 mRNA expression was studied in LV biopsies (RT-PCR) from 36 patients with severe CHF due to ischemic or non-ischemic cardiomyopathy, undergoing left ventricular assist device implantation. HER2 was upregulated after implantation of the device, whereas HER2 prior to implantation was comparable to healthy controls [24]. Recently, in six of 60 severe CHF patients, immunohistochemical expression of HER2 (and HER4) was shown in myocardial biopsies [25].

2.4 **Retrospective data of cardiac side effects in trastuzumab treated patients**

Analysis of a retrospective review of seven phase II and III trastuzumab clinical trials by an independent Cardiac Review and Evaluation Committee (CREC) evaluated the risk and severity of cardiac dysfunction, baseline risk factors and the role of cumulative doses of anthracyclines and trastuzumab [26]. The CREC defined cardiac dysfunction as 1) a decline in LVEF of 5% to < 55% without accompanying signs or symptoms, 2) symptoms of congestive heart failure, 3) associated signs of congestive heart failure including S3 gallop and/or tachycardia or 4) cardiomyopathy characterized by a decline in LVEF that either was global or more severe in the septum. Cardiac events were also categorized by means of the New York Heart Association (NYHA).

When patients were treated with trastuzumab alone as the first therapy for metastatic breast cancer the observed rate of cardiac dysfunction was 3% compared with 5% when patients were treated as second or third treatment with trastuzumab alone. An increased incidence of cardiac dysfunction was also seen in patients treated with paclitaxel and trastuzumab at a rate of 13% compared with paclitaxel alone at a rate of 1% respectively. When patients treated with concurrent trastuzumab and anthracyclines, cardiotoxicity is increased in a rate of 27% compared with a rate of 8% in patients treated with anthracyclines alone. Seventy-five % of all patients, the CREC screened 1,219 patient records, developed symptomatic cardiac dysfunction. Eighty-two of these patients received standard treatment for congestive heart failure (CHF).

Statistically significant predictive risk factors for trastuzumab-related cardiac dysfunction were older age and pre-treatment with anthracyclines. The cumulative dysfunction risk began to increase with doxorubicin exposure of approximately 300mg/m².
Other factors as hypertension and prior radiotherapy were not found to be independent predictors of cardiac dysfunction.

Another retrospective review was performed over a period of 4 years, in which 38 patients were referred for suspected trastuzumab-related cardiotoxicity. All patients had previously received anthracycline based chemotherapy. All patients underwent an initial cardiac evaluation that included a baseline left ventricular ejection fraction (LVEF) measurement before trastuzumab. Before trastuzumab the mean LVEF was 0.61 ± 0.13, and the LVEF decreased to 0.43 ± 0.16 after trastuzumab (P< .0001). After withdrawal of trastuzumab, the LVEF increased to 0.56 ± 0.11. Mean time to recovery of LVEF was 1.5 months and was associated with medical treatment in 32 (84%) of the 38 patients but occurred without treatment in six patients (16%). Increases of LVEF were noted in 37 of the 38 patients. Twenty-five of these patients were re-treated with trastuzumab; 3 patients had recurrent left ventricular dysfunction, 22 patients (88%) not [27].

In a study in which trastuzumab plus vinorelbine was administered as second-line or third-line therapy to 40 women with metastatic breast cancer, no patients developed clinical congestive heart failure. Three patients (all had prior doxorubicin exposure of > 240 mg/m²) developed grade 2 cardiac toxicity (defined as a decline in LVEF of > 20% or < 50%) and a fourth patient experienced a grade 1 toxicity (defined as a decline in LVEF of 10-20%) [28].

2.5 Prospective data of cardiac side effects in trastuzumab treated patients

Most of recent adjuvant clinical trials have adopted more stringent cardiac monitoring. Studies mandate formal baseline measurement of LVEF before initiating trastuzumab therapy and exclude patients with abnormal cardiac function or high-doses of cumulative anthracyclines. In the HERA trial, prior mediastinal irradiation and cumulative doses of anthracycline exceeding 360 mg/m² of body-surface area for doxorubicin, or 720 mg/m² for epirubicin were exclusion criteria of the study. Only patients with a LVEF ≥ 55% were eligible. Patients with cardiac dysfunction were excluded from the study. Trastuzumab was discontinued if symptomatic congestive heart failure, LVEF ≤ 45%, or a drop in LVEF of at least 10% from baseline and a LVEF of less than 50% developed. Trastuzumab was discontinued if, in asymptomatic patients, LVEF did not return to a level above the criteria for withholding treatment after the therapy was stopped for three weeks.

In the HERA trial 0.54% of the women who were treated with trastuzumab developed severe congestive heart failure (defined as NYHA functional class III or IV and a decrease of LVEF of ≥ 10% from baseline or an LVEF < 50%). Symptomatic CHF occurred in 1.7% of patients in the trastuzumab group and 0.06% of patients in the observation group. A decrease in LVEF (a decrease of LVEF of ≥ 10% from baseline or an LVEF < 50%) was noted by 7.1% of trastuzumab treated patients compared with 2.2% of the observation group.

Trastuzumab was stopped before completion of the planned 1 year treatment in 8.5% of the patients for reasons other than relapse. In 5.5% was stopping with the treatment necessary with as reason an adverse event. This percentage included the adverse events of cardiotoxicity [8].

Publications of the NSABP B-31 trial reported an incidence of class III or IV CHF or death from cardiac causes at three years of 4.1% in the trastuzumab group and 0.8% in the control group. Criteria of CHF in this trial mirrored the CREC criteria: NYHA class III or IV symptoms with a decrease from baseline in LVEF of more than 10 percentage points to < 55% or a decrease of more than 5 percentage points to less than the lower limit of normal. Thirty-one percent of available patients temporarily held or permanently discontinued trastuzumab because of asymptomatic decline in LVEF (24%) and/or symptomatic cardiotoxicity (8%). Twenty-seven of the 31 patients have been followed for ≥ 6 months after diagnoses of cardiac dysfunction; 26 were asymptomatic and 18 remained on medical therapy.
The trial N9831, reported an incidence of 2.9% of class III or IV CHF in the trastuzumab group versus 0 percent in the control group. Twenty patients had CHF, one of whom died of cardiomyopathy [9].

In the study of Joensuu, trastuzumab was not associated with decreased left ventricular ejection fraction or cardiac failure [13].

2.6 Interpreting data of cardiotoxicity in trastuzumab treated patients

The association between trastuzumab and cardiomyopathy was not predicted by the preclinical screens and was not observed in the early clinical trials. Cardiac monitoring was not mandated in many trastuzumab trials and even among studies in which cardiac monitoring was performed, measurements were not consistent from one study to another. This makes it difficult to compare results between studies and to identify predisposing risk factors.

Based on currently available information about the incidence of trastuzumab-associated cardiac dysfunction some questions can be answered. Trastuzumab is associated with an increased risk of cardiac dysfunction. Statistically significant predictive factors for trastuzumab-related cardiac dysfunction are older age and the combination of trastuzumab with concurrent anthracyclines. Factors as a history of hypertension, overweight, smoking, diabetes mellitus and hypercholesterolemia are also associated with an increased risk of cardiac dysfunction. Decrease of LVEF and cases of congestive heart failure have been detected. Congestive heart failure is mostly responsive to medical therapy. Trastuzumab-related cardiotoxicity seems to be largely reversible when trastuzumab is withdrawn.

Several questions are unanswered such as the pathogenesis of cardiotoxicity associated with trastuzumab, long-term clinical cardiac course, long-term effects of treatable CHF, the safety of continuation of trastuzumab in patients who develop cardiotoxicity, the clinical significance of an asymptomatic drop in LVEF, and early markers to detect cardiotoxicity. If LVEF identified by MUGA scan or echocardiogram can be used as surrogate marker and if cardiac dysfunction normalize in the absence of cardiac medication, and strategies how to prevent or limit trastuzumab-associated cardiotoxicity are unknown.

In the detection and management of trastuzumab-related cardiotoxicity and the combination of trastuzumab and anthracyclines we must learn lessons from the anthracycline-related cardiotoxicity and the non anti-cancer drug related cardiotoxicity.

Cardiotoxicity is a major side effect of anthracyclines. In the vast majority of patients receiving anthracyclines, there are no clinical symptoms, but patients who do not develop cardiotoxicity within the first year, CHF is rarely diagnosed later [29]. So it is possible that when trastuzumab is used widely in clinical practice and trastuzumab-related cardiotoxicity is followed for some years the rate of cardiac dysfunctions could be higher than that observed in previous trials.

In non-trastuzumab treated patients asymptomatic left ventricular (LV) dysfunction progresses to overt heart failure over time. Patients with asymptomatic LV dysfunction not treated with an angiotensin converting enzyme (ACE) inhibitor progressed to symptomatic heart failure at a rate of 9.7 % per year [30]. This data indicate that every trastuzumab treated patient with decreases of LVEF must be carefully monitored, pharmacologically treated or trastuzumab must be discontinued.

Optimizing screening methods prior, during and long after the treatment of drug therapy are important steps in decreasing the morbidity and mortality from potentially curative interventions.

2.7 Medical treatment of trastuzumab-associated cardiotoxicity

The majority of patients who developed clinical cardiac dysfunction during trastuzumab treatment responded well to standard medical management for CHF or trastuzumab discontinuation [31-33]. Mostly the standard treatment according the guidelines
of the American College of Cardiology/American Heart Association and the Heart Failure Society of America is given. The Memorial Sloan-Kettering Cancer Center guidelines recommend the use of angiotensin-converting enzyme (ACE) inhibitors and β-blockers, with or without digoxin and diuretics to treat trastuzumab-associated CHF [24]. Pharmacological treatment should be similar to that in patients with systolic dysfunction of any other aetiology [34].

2.8 ACE inhibition & Angiotensin II receptor blockers

ACE inhibitors are a standard therapy for heart failure because multiple, large, prospective, randomized trials have consistently demonstrated a significant reduction in mortality. In a placebo-controlled trial evaluating the effects of enalapril in patients with heart failure, an increase in LVEF from 25 (± 7%) before treatment to 29 (± 8%) one year after the start of treatment was observed in the enalapril-treated group (P < 0.001)[35]. ACE inhibition can also ameliorate symptoms in patients with anthracycline-induced congestive heart failure [36]. Recently, a randomized, double blind, controlled clinical trial compared enalapril treatment to placebo in 135 survivors of childhood malignancy, who had one or more cardiac abnormalities at any time during follow-up after anthracycline-based chemotherapy. The enalapril group showed markedly reduced left ventricular end-systolic wall stress, compared to the patients receiving placebo [37]. Cardiotoxicity by trastuzumab however, is dissimilar to anthracycline-induced cardiotoxicity, regarding the fact that trastuzumab cardiotoxicity is not clearly dose-dependent, does not appear to occur in all patients, is expressed in a broad range of severity when it does occur, and is not associated with identifiable ultrastructural abnormalities [38]. Currently, no evidence is available with regard to the use of ACE inhibitors for the prevention or treatment of trastuzumab-related cardiotoxicity. In the clinical trials however, most patients with cardiac dysfunction responded to appropriate medical therapy for heart failure, which includes ACE inhibitors.

In addition to the established effects of ACE inhibition for the treatment of cardiac dysfunction, i.e. cardiac pre- and afterload reduction, ACE inhibitors might also directly influence HER2-mediated signaling. HER1, the archetype of the HER family of receptors, can be transactivated through activation of the angiotensin II type 1 receptor, which leads to myocardial hypertrophy [39]. Thus, a connection exists between (activation of) the renin-angiotensin system and the HER-related signaling in myocardial hypertrophy. In prostate cancer cells, Western blot analysis showed that angiotensin II can transactivate both HER1 and HER2 [40]. Also based on the observation that HER2 is involved in left ventricular hypertrophy in mice, it can be postulated that the renin-angiotensin system is involved in compensatory HER2 signaling in the heart.

The angiotensin II-receptor (AT1) blockers are as effective as the ACE inhibitors in the treatment of hypertension. Among hypertensive patients who develop left ventricular hypertrophy, angiotensin II-receptor blockers (ABRs), calcium channel blockers, and ACE inhibitors appear to decrease left diastolic dysfunction [41, 42]. Approximately 10% of patients with CHF are intolerant to ACE inhibitors, with as the most common limiting side effect the development of cough. This side effect does not appear to occur with the angiotensin receptor blockers [43]. Angiotensin II receptor antagonists may induce a more complete inhibition of the renin-angiotensin system than ACE inhibitors, they do not affect the response to bradykinin, and are less likely to be associated with nonrenin-angiotensin effects such as cough and angioedema [44]. Clinical studies showed that a large population of patients who are intolerant of ACE inhibitors tolerate the angiotensin-receptor blockers (ABRs), candesartan or losartan [45, 46].

2.9 Angiotensin II-receptor (AT1) blocker ‘candesartan (Atacand®)’

Candesartan (Atacand®) is an angiotensin receptor antagonist. Angiotensin II acts as a vasoconstrictor. In addition to causing direct vasoconstriction, angiotensin II also stimulates
the release of aldosterone. Once aldosterone is released, sodium as well as water are reabsorbed. The end result is an elevation in blood pressure. As therapeutic agent in the management of cardiac diseases, candesartan is a frequently used angiotensin II-receptor blocker.

3. **RATIONALE**

3.1 **Rationale**

The addition of trastuzumab to the standard adjuvant chemotherapy in HER2-positive breast cancer patients markedly improves treatment outcome. Cardiac dysfunction is an important side effect observed with the use of trastuzumab for the treatment of patients with HER2-positive breast cancer. In recent clinical trials evaluating the addition of trastuzumab to the adjuvant treatment of these patients, trastuzumab treatment had to be discontinued in approximately 20% due to the occurrence of a significant decrease in LVEF.

In a fraction of the patients trastuzumab was stopped before completion of the treatment, or patients were excluded of trastuzumab treatment because of reasons like decreased LVEF or baseline LVEF < 50-55%. These patients with HER2 positive breast cancer are excluded from the best adjuvant systemic treatment at this moment. It is imperative in practice to follow the stringent criteria for eligibility and cardiac monitoring used by the HERA and NSABP B-31 trials. However, cardio-protective drugs and optimizing screening methods are important measurements to investigate. It is important to decrease the morbidity and mortality of trastuzumab treatment to enable optimal therapy with trastuzumab in the adjuvant setting, but also in patients with metastatic disease. The median survival time has traditionally been shorter for patients with visceral disease (6-13 months) than for those with bone-only disease (18-30 months) [47]. Furthermore, the 1-year mortality of patients with severe heart failure (NYHA class III and IV) is lower than for breast cancer patients with distant metastases (approximately 9.0-12% versus 16%) [48-49].

Therefore, methods to avoid this toxicity are of major relevance. ACE inhibitors are standard of care in the treatment of patients with congestive heart failure. In addition, ACE inhibition has beneficial effects in attenuating symptoms of anthracycline-induced heart failure. However, ACE inhibitors are increasingly being replaced by ATII blockers, because of intolerance to ACE inhibitors. Short-term treatment with ARBs is well tolerated in CHF patients and may improve symptoms, exercise tolerance and reduced the risk of incident hypertension. We hypothesize that the concurrent use of ARBs in patients treated with adjuvant trastuzumab for HER2-positive breast cancer can limit the development of trastuzumab-related cardiotoxicity. Furthermore, if this is the case, more patients can probably be treated longer with trastuzumab since cardiac function remains better.

3.2 **Rationale for administration schedule and dosage of candesartan**

The duration of study medication in this protocol includes the whole trastuzumab treatment period and 26 weeks after stopping trastuzumab.

Candesartan treatment will start the same day (onset of action of candesartan is 6-8 hours) as the first infusion of trastuzumab and will continue up to 26 weeks after the end of treatment with trastuzumab. The half-life of trastuzumab, is approximately 28 days (95% confidence interval, 25-33 days) and trastuzumab may persist in the circulation for up to 24 weeks after stopping trastuzumab treatment (95% confidence interval, 18-24 weeks). Also during continued candesartan treatment up to 26 weeks after stopping trastuzumab cardiac function will carefully be followed. However, the exact duration of useful candesartan treatment as cardioprotecting agent is unknown.

The usual dosage of candesartan in the treatment of cardiac diseases is 2-32 mg/day [50]. In randomized placebo controlled trials of candesartan in the treatment of diabetic retinopathy, patients with chronic heart failure and reduced left-ventricular systolic function a dose of 32 mg candesartan daily is well tolerated. The optimal dose of candesartan in the
treatment of heart failure is 32 mg daily [51, 52]. The candesartan dosage in this study is 32 mg/day. There will be a run-in period of 16 mg candesartan for one week.

### 3.3 Rationale for placebo intervention

A limitation of uncontrolled trials is the possible effect of the treatment intervention itself on the outcome of the trial, the so-called placebo effect. The knowledge of being included in a study may be sufficient to cause people to change the effect of the variable of interest. It is precisely for this reason that placebo controlled studies are so powerful. In this study, objective, but also subjective data will be collected, for example the cardiac questionnaire. Especially in case of subjective measurements double-blind experiments are essential to prevent systematically distorted data, or to prevent that participants respond in a biased way. The once a day oral placebo capsule is therefore considered an important part of the study design limiting as much as possible bias in the outcome of the trial.

### 3.4 Cardiac monitoring

#### 3.4.1 Reversibility of drops in LVEF

In the adjuvant trials, trastuzumab was discontinued in patients with asymptomatic drops in LVEF. The measurements in these cases were medical history, physical examination, MUGA scan or cardiac ultrasound and the NYHA classification. Trastuzumab-associated cardiotoxicity seems to be frequently reversible when trastuzumab is withdrawn. More information about the reversibility of a decrease in LVEF in trastuzumab treated patients is needed.

#### 3.4.2 Markers to detect cardiotoxicity

The cardiac troponins I and T, proteins that are exclusively present in myocardial cells, are examples of plasma markers of cardiac injury. The troponin T plasma concentration is a well-established specific and sensitive marker of myocardial injury, with both high diagnostic and prognostic value. In a recent report, the troponin T release pattern was measured soon after chemotherapy (early) and one month later (late), in patients with different types of malignancies who received treatment with high-dose chemotherapy, consisting of regimens with or without an anthracycline derivative. Patients who had a positive troponin T value both early and late, had a high risk (84%) of a cardiovascular event (i.e. cardiovascular death, pulmonary edema, (a)symptomatic left ventricular dysfunction, rhythm disturbances), compared to patients with a positive early, but negative late troponin T (37%) and patient in the negative troponin T group (1%).

Plasma natriuretic peptides are increased in patients with cardiac dysfunction. The release of NT-proBNP is increased in heart failure, as ventricular cells are recruited to secrete both ANP and NT-proBNP in response to the high ventricular filling pressures [46]. The plasma concentrations of both hormones are increased in patients with asymptomatic and symptomatic left ventricular dysfunction, permitting their use in diagnosis. In high-risk breast cancer patients, higher NT-proBNP levels have been observed one year after adjuvant anthracyclines and chest wall irradiation, compared to before treatment [53].

In a study of Perik, pre-treatment plasma NT-proBNP levels were higher in patients with heart failure during trastuzumab treatment (n=3) than in patients without heart failure (n=12) [54]. This preliminary finding suggests that plasma NT-proBNP may be a measurement to detect or predict trastuzumab induced cardiotoxicity. More evidence is needed for the usefulness of measuring NT-proBNP levels in relation to trastuzumab-associated cardiotoxicity during trastuzumab treatment. Measuring plasma natriuretic peptide levels for identifying patients at risk for trastuzumab-associated cardiotoxicity is simple and deserves further investigation.
3.5 Rationale for genotype analysis

The aim of the genotype analysis is to determine if there is a correlation between genetic polymorphism (as assessed by analysis of single nucleotide polymorphisms (SNPs) in the kinase domain) such as in the HER2 gene and the development of trastuzumab-associated cardiotoxicity. Interindividual variation of drug toxicity and efficacy is at least partly determined by genetic polymorphisms. Most of these variations are SNPs. For example, a recent publication reported a significant relationship between polymorphisms in the DPYD gene and 5-fluorouracil-related toxicity [55]. An objective of pharmacogenetic studies is to search for genetic variants that enable individualized treatment with low toxicity and maximum benefit [56, 57]. Therefore, SNPs in the Her2 gene will be determined.

The identification of genetic factors predisposing patients to the development of cardiotoxicity is an important secondary goal of the study. The analysis of SNPs in genes potentially in development of cardiotoxicity may give more insight to identify patients at risk for significant cardiac morbidity in trastuzumab anticancer treatment. In view of the planned chemotherapy (doxorubicin, epirubicin, cyclophosphamide, paclitaxel) SNPs in drug metabolizing (CYP), conjugating (GST, UGT) and transporting (ABC-transporters) genes are of interest and will be determined in this study.

4. Study objectives

4.1 Primary objectives

1. To determine whether concurrent ATII-antagonist treatment can prevent trastuzumab-related cardiotoxicity, defined as a decline in LVEF of more than 15% or a decrease of less than 15% to an absolute value below 45%

4.2 Secondary objectives

1. To determine if 'Brain Natriuretic Peptide' (NT-proBNP) and troponin T can be used as surrogate marker in the monitoring of trastuzumab-associated cardiotoxicity

2. To determine genetic variability in the HER2 gene (by assessing single nucleotide polymorphisms [SNPs] in the kinase domain) and explore any correlations with trastuzumab induced cardiotoxicity

3. To determine the reversibility of a decrease in left ventricular ejection fraction (LVEF) associated with trastuzumab treatment

4.3 Primary endpoint

The primary endpoint of the study is the occurrence of cardiotoxicity during the one year trastuzumab therapy and during the 40 weeks after discontinuation of trastuzumab treatment, defined as a decline in LVEF (MUGA) of more than 15% or a decrease of less than 15% to an absolute value below 45%.
5. STUDY DESIGN AND STUDY POPULATION

5.1 Registration and randomization

This is a prospective, randomized, double blind controlled pharmacological intervention study. After informed consent has been obtained, eligibility criteria will be checked and --when eligible- patients will be registered. Preferably obtaining informed consent and registration will take place before start of chemotherapy. However, it is also allowed to obtain informed consent and register when the patient has already started chemotherapy, under the condition that an LVEF assessment has been performed before start of chemotherapy. Registration will be performed centrally by the NKI Data Centre phone number 020-5122668 or fax: 020-5122679. The following information will be requested:

- Protocol number
- Institution name
- Caller’s name
- Local investigator’s name
- Patient’s identification (first letter of first name and first two letters of surname)
- Patient’s chart number (optional)
- Patient’s date of birth (day/month/year)
- Performance status
- Verification of all inclusion and exclusion criteria with values of hematologic and biochemical assessments, radiological results and dates of all examinations performed

At the end of the treatment period with anthracycline-containing chemotherapy the following criteria will be checked:

- LVEF
- Blood pressure

A randomization form will be completed and faxed to the NKI Data Centre. When the patient is still eligible, the Data Centre will randomize the patient to receive trastuzumab and candesartan or trastuzumab and placebo by assigning a box number. A confirmation of randomization will be sent (by fax or e-mail) to the investigator, the responsible data manager and the study coordinators of the study.

5.2 Expected duration

The study duration should be the chemotherapy treatment period, trastuzumab treatment period and 40 weeks follow up after discontinuation of trastuzumab treatment (= 3 months follow-up after end of candesartan or placebo). The treatment of candesartan and placebo will start the same day as the first infusion of trastuzumab and will continue for 26 weeks after the end of treatment with trastuzumab. The last study assessment will take place 3 months after discontinuation of candesartan or placebo.

5.3 Study population

Female patients with HER2-positive breast cancer who are indicated to receive (neo-) adjuvant chemotherapy are eligible for this study.

5.4 Inclusion criteria

1. Women aged ≥18 years
2. WHO: ≤ 2

3. Strongly HER2-positive breast cancer, defined as an immunohistochemistry score of 3+ using the HercepTest™ or gene amplification by fluorescence in situ hybridization, or chromogenic in situ hybridization (CISH)

4. Serum creatinine <140 umol/l or creatinine clearance > 50 ml/min (by Cockcroft-Gault formula)

5. Thyroid stimulating hormone between 0.5-3.9 MU/l. If the thyroid stimulating hormone value is < 0.5 MU/l or > 3.9 mU/l; thyroid hormone FT4 should be 8 – 26 pmol/l

6. Blood pressure systolic ≥ 140 mmHg diastolic ≥ 90 mmHg is acceptable at registration. However at randomization, prior to the first administration of trastuzumab blood pressure should be regulated and should be systolic ≥ 100 mmHg and ≤ 180 mmHg and diastolic ≥ 60 mmHg and ≤ 100 mmHg (blood pressure should be regulated according to the guidelines of appendix 5).

7. LVEF ≥ 50% assessed by multigated angiography (MUGA) or cardiac ultrasound.

8. (Neo-) adjuvant regimen: trastuzumab start ≥ 3 weeks after day 1 of the last anthracycline chemotherapy cycle

9. Must have received at least an approved anthracycline based (neo-)adjuvant chemotherapy regimen

10. Trastuzumab treatment according to standard medical care

11. Written informed consent to participate in the study

5.5 Exclusion criteria

1. Prior anthracycline chemotherapy regimen or anti-HER2 therapy, or other prior biologic or immunotherapy for breast cancer treatment or any malignancy

2. Previous malignancy requiring chemotherapy or mediastinal radiotherapy

3. Uncontrolled serious concurrent illness

4. Patients with New York Heart Association (NYHA) class II/III/IV congestive heart failure

5. Myocardial infarction < 6 months before registration

6. Treatment with ACE inhibitors, ATII blockers, or lithium. Patients treated with ACE inhibitors or ATII blockers can switch (after registration and during the chemotherapy period) to alternative antihypertensive therapy; see appendix 5.

7. History of hypersensitivity to the study medication

8. Pregnancy or breast feeding
5.6 Withdrawal criteria

Patients are free to withdraw at any time without giving reasons and without prejudice to their subsequent care. The investigator can also withdraw the patient from the study for any of the following reasons:
- occurrence of unacceptable toxicity
- pregnancy
- use of non-permitted concomitant treatment
- unacceptable non-compliance with the protocol

Patients who meet cardiotoxicity for discontinuation of trastuzumab as outlined in section 8.2 or toxicity for discontinuation of placebo or candesartan outlined in section 8.1 should still be followed according to their treatment allocation and assessments described in table 1 (appendix 2), unless they withdraw their consent to further participation in the study.

5.7 Procedures for handling withdrawn patients

If a patient has failed to attend scheduled study assessments, the investigator must make every attempt to contact the patient and determine the reasons and the circumstances as completely and accurately as possible.

5.8 Concomitant Medication and Treatment

Only the following concomitant medication must be recorded in the CRF: antihypertensive therapies, diuretics, anti-thrombotic therapies, glucose control therapies, anti-arrhythmia therapies, beta-blocker therapies, analgesic medication, calcium-channel blockers, cholesterol-lowering therapies, asthma and/or chronic obstructive pulmonary disease (COPD) therapies.

6. TREATMENT OF STUDY PATIENTS

6.1 Study Treatment

ARM I: placebo
ARM II: angiotensin II-receptor (AT1) blocker candesartan (32 mg/day; run in 16 mg during week 1)

The treatment of candesartan and placebo will start the same day as the first infusion of trastuzumab. Patients will start at a dose of candesartan of 16 mg daily (one capsule) for 1 week. From week two until the end of the treatment period of candesartan patients will take 32 mg daily (one capsule twice daily), i.e. dose-doubling of candesartan compared to the starting dose.

A patient will be treated with candesartan 32 mg daily if the patient does not experience disabling toxicity associated with candesartan (see paragraph 8.1).

6.2 Candesartan (Atacand ®)

Approximately 100 patients in this study will receive candesartan 32 mg/daily for 78 weeks

Therapeutic indication

Treatment of hypertension, treatment of heart failure (NYHA class II-IV).

Dosing adults

Hypertension 4-32 mg once daily. Usual recommended starting dose, 16 mg once daily. Target dose of candesartan in the treatment of congestive heart failure, 16 mg twice daily.
Contraindications
Hypersensitivity to candesartan of any component of the formulation; hypersensitivity to other A-II receptor antagonists; bilateral renal artery stenosis.

Drug interactions
Substrate of CYP2C8/9 inducers (carbamazepine, phenobarbital, phenytoin, rifampin, rifapentine, secobarbital). Lithium: risk of toxicity may be increased by candesartan.

Pharmacodynamics/kinetics
Metabolism: intestinal wall cells.

Administration of candesartan
May be administered with or without food.

Side effects
Often (1-10%): dizziness and headache.
Uncommon (<1%): hyperkalemia when used for the treatment of essential hypertension, hyponatremia, myalgia, low back pain, allergic reactions like urticaria, nausea, leucocytopenia, neutropenia, liver dysfunction, renal dysfunction.
    In the treatment of congestive heart failure, often (1-10%): hypotension, hyperkalemia and reduced kidney function [51].

Drug information
Candesartan: light pink colour tablets of 6 or 7 mm
Dosing forms: oral
Dosing: one capsule of 16 mg twice daily
        (run in of one week of one capsule daily)
Duration: 78 weeks (1.5 years): 52 weeks during trastuzumab therapy and 26 weeks immediately post trastuzumab

6.3 Placebo
Placebo capsules will be the same as the candesartan supplies apart from the active ingredient. Pharmaceutical information about the placebo can be found in the Investigational Medicinal Product Dossier.

Drug information
Placebo: light pink colour tablets of 6 or 7 mm
Dosing form: oral
Dosing: one capsule twice daily (run in of one week of one capsule daily)
Duration: 78 weeks (1.5 years): 52 weeks during trastuzumab therapy and 26 weeks immediately post trastuzumab

6.4 Packaging and dispensing of the study medication and description of the double blind procedure
At the pharmacy of AstraZeneca the study medication will be labelled to ensure adequate blinding of active drug and placebo. The labelled study medication will be supplied by AstraZeneca to the pharmacy of the Slotervaart hospital. Each bottle of investigational
product includes an investigational-use label, box-code and space for patient name. A sealed envelope containing the code will be included in each individual package.

The complete code list will be provided by AstraZeneca to the pharmacist of the Slotervaart hospital and will be provided for the analyses. The pharmacy of the Slotervaart hospital will supply each participating centre with study medication for patients to be enrolled in this trial. It is the centre’s responsibility to keep accurate documentation of drug receipt and use. The Data Centre of the Netherlands Cancer Institute will conduct the registration and randomization procedure and thus assign box numbers to individual patients.

6.5 Unblinding procedure

Treatment allocations will only be unblinded at the time of trial analysis and planned interim analyses. For the planned interim analysis (after each new cohort of 40 patients will have been entered into the trial) data will be made available to the drug safety monitoring board (DSMB). Only the DSMB will have access to unblinded data during the study. However, the box-code for an individual patient while on study should only be opened in case of an imperative need to identify the actual treatment given to a certain patient such as in medical emergencies. The pharmacy of each study centre is responsible for breaking the codes. Any breaking of the treatment code must be documented. In case of code breaking, the centre will fax a letter to the Data Centre Netherlands Cancer Institute (fax 020-5122679) which must include the identification of the patient, the date and detailed description of the reason for code breaking. Warning: the actual treatment should not be written on this letter.

6.6 Missing of doses of study medication

If a dose or doses are missed, the reason(s) and the number of doses not taken should be noted and recorded in the medical records. Patients will be instructed to return any unused capsules prior to the start of the next cycle (every 3 months) which must be returned to the pharmacy. The pharmacy will maintain a complete drug accountability record for each lot number of drugs received, including the number of capsules dispensed to each patient (appendix 7). This should also be recorded in the patient’s medical records. At the end of the study, the investigator will be require submission of a drug accountability record providing a complete accounting of the receipt and disposition of the study drug, lot numbers and total number of capsules dispensed to each patient.

7. STUDY ASSESSMENTS AND SCHEDULES

7.1 Medical history, physical examination, haematological & chemistry studies

These and other appropriate diagnostic testing methods may be done at more frequent intervals if clinically indicated. Medical history, physical examination, haematology and chemistry (including BNP analysis), bodyweight, performance status, vital signs (blood pressure and heart rate) should be obtained at baseline, three weeks after starting the study medication and should be monitored throughout the study. A minimum follow up frequency of once every three months for the first year during trastuzumab treatment as well as three and seven months after the end of trastuzumab treatment should be done. The last follow-up visit will be 6 months after the last trastuzumab treatment. At baseline the medical history will include the family cardiac history and individual cardiac history or risk factors. Blood samples will be taken for haematological and serum biochemical monitoring. These analyses will be performed by the local lab. Shipment of blood samples for genotyping and blood samples for NT-proBNP and troponin T (depending of the particular centre) should be done according appendix 6.
7.2 New York Heart Association Score (NYHA) and Cardiac questionnaire

The New York Heart Association published a classification of patients with cardiac disease based on clinical severity and prognosis. The NYHA is frequently used in clinical research and a valid measure of functional status, a concept that is distinct from functional capacity and functional performance [58-60]. The NYHA is summarized in the cardiac questionnaire (appendix 4).

7.3 Electrocardiogram

The electrocardiogram (ECG) is a graphical record of electric potentials generated by the heart muscle during each cardiac cycle. The ECG is the most important test for interpretation of cardiac rhythm, conduction system abnormalities, and for the detection of myocardial ischemia. The ECG is also of great value in the evaluation of other types of cardiac abnormalities including valvular heart disease, cardiomyopathy, pericarditis, and hypertensive disease. The identification of ECG is standard practice.

7.4 MUGA scan

MUGA scans for determination of the LVEF will be performed 7-14 days before the planned start of trastuzumab treatment and subsequently every 3 months for the duration of 1.5 year. Therefore, 400 MBq Tc-99m labeled autologous red blood cells will be injected and acquisition will be done in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. A LVEF value between 45-50% is classified as borderline, values below 45% as abnormal. A decline of LVEF of more than 15% from baseline is called a significant decline.

7.5 Schedule of assessments

The schedule of assessments is summarized in table 1 (appendix 2).

7.5.1 Screening schedule

The screening assessments must be completed before the start of the study treatment. Informed consent must be given before any study-specific screening procedures are performed.

Before start of chemotherapy

- Informed consent
- Medical history and physical examination
- NYHA score
- Cardiac questionnaire
- ECG
- MUGA
- Blood chemistry
- Hematology
- NT-proBNP analysis
- Troponin T measurement
- Pregnancy test
- Genotype analysis

Every chemotherapy cycle

- Blood chemistry
- Hematology

Before start of trastuzumab treatment
All visits should be made on the scheduled day whenever possible. When the patient cannot attend on this day the visit should occur within 5 days of the planned day.

- Concomitant medications and treatments
- Physical examination, blood pressure, heart rate, bodyweight
- Adverse event recording
- NYHA score
- Cardiac questionnaire
- ECG
- MUGA scan
- Blood chemistry
- Hematology
- NT-proBNP analysis
- Troponin T

**Trastuzumab treatment**
Visits 3, 12, 24, 36, 52 weeks after starting trastuzumab

- Concomitant medications and treatments
- Physical examination, blood pressure, heart rate, bodyweight
- Adverse event recording
- NYHA score
- Cardiac questionnaire
- MUGA scan
- Blood chemistry
- Hematology
- NT-proBNP analysis
- Troponin T

**End of trastuzumab treatment, 12, 26 and 40 weeks after stopping of trastuzumab**

- Concomitant medications and treatments
- Physical examination, blood pressure, heart rate, bodyweight
- Adverse event recording
- NYHA score
- Cardiac questionnaire
- ECG (26 weeks after stopping trastuzumab)
- MUGA scan
- Blood chemistry
- Hematology
- NT-proBNP analysis
- Troponin T

8. **MANAGEMENT OF TOXICITY**
8.1 **Management of toxicity, delay of candesartan administration due to toxicity**

Patients must be monitored for toxicity. All toxicities should be recorded and graded according to the CTC index version 3 [61]. Based on large-scale available clinical data it is very unlikely that patients will suffer from disabling (> CTC grade 1) side-effects from
candesartan at the dose of 16 mg or 32 mg. However, if a patient would develop > CTC grade 1 side-effects at 16 mg the patient will go off study and will further be treated in the best interest for breast cancer as well as heart failure. If a patient tolerated 16 mg, but shows > CTC grade 1 toxicity associated with candesartan at 32 mg, then the dose will be reduced to 16 mg (i.e. to one capsule per day).

As this is a double-blinded randomized trial also patients on placebo who develop > CTC grade 1 toxicity when dosed at two capsules per day will receive dose-reduction from two capsules per day to one capsule per day. In the unlikely case that the patient would develop these toxicities at one capsule per day the patient will go off study and will be treated in the best interest as described above.

If patients present with an acute worsening or new onset of angioedema or hypersensitivity of the study medication, the treatment should be interrupted and the patient promptly investigated.

Asymptomatic (NYHA class I) and mildly symptomatic (NYHA class II) drops in LVEF must be provided on a CRF form and should be graded for intensity according to CTC version 3.0 (NYHA class I CHF corresponds with CTC grade 1). However, trastuzumab must be discontinued in all patients for whom a drop of LVEF to below 45% is documented and confirmed with a subsequent repeat assessment approximately 3 weeks after the first assessment (algorithm figure 1, appendix 1). For patients whose LVEF drops to values between 45-49% the decision to stop or continue trastuzumab is based on the algorithm (figure 1). If trastuzumab must be discontinued due to a drop in LVEF confirmed by a MUGA scan, LVEF assessment must be followed by an cardiac ultrasound. The decision to stop or continue trastuzumab should be based on the result of LVEF confirmed by MUGA or cardiac ultrasound. Confirmation of the reduced LVEF by MUGA or cardiac ultrasound will be obtained as soon as possible, but ultimately within 3 weeks. If trastuzumab is held for 2 consecutive cycles of in total 6 weeks, it must be discontinued. The patient will go off study and will be treated in the best interest of the patient.

For all patients experiencing this outlined drop in LVEF the frequency of cardiac monitoring (LVEF assessment) will be repeated after 3 weeks (appendix 1) including also BNP analysis, NYHA score, cardiac questionnaire, laboratory assessments shown in figure 1 (appendix 1). LVEF measurement must also be performed at all patient visits shown in table 1 (appendix 2) for a total duration of 1.5 year (after starting of the treatment) following the initial diagnosis. In addition, any change in the symptoms of the patients including the NYHA class should be provided on a CRF form.

Study treatment (candesartan or placebo) will be given as long as trastuzumab will be administrated plus 26 weeks after the last dose of trastuzumab, unless the patient went off study (i.e. candesartan or placebo study medication was discontinued).

### 8.2.1 Congestive Heart Failure

Symptomatic cardiac failure should be reported as a syndrome and not the individual signs and symptoms and should be graded according to the New York Heart Association (NYHA). In such cases, CTC grade 2, 3 and 4 would mean NYHA class II, III and IV.

In case of development of CHF of NYHA class III or IV during trastuzumab treatment, trastuzumab will be discontinued in that patient. The diagnosis will be confirmed by a drop in LVEF by MUGA scan (or cardiac ultrasound) and corresponding clinical findings. The patient will be followed according to the assessments described in table 1. The CHF should be confirmed, treated and followed according to standard treatment according to the guidelines of the American College of Cardiology/American Heart Association and the Heart Failure Society of America (appendix 3) [62,63]. For all patients experiencing CHF the frequency of cardiac monitoring will be increased. Cardiac assessments will be repeated after 3 weeks.
(appendix 1) including BNP analysis, NYHA score, cardiac questionnaire, laboratory assessments shown in figure 1 (appendix 1). Assessments must be performed at all patient visits shown in table 1 for a total of 1.5 year and 1 month (after starting of the treatment) following the initial diagnosis of CHF. In addition, any change in the symptoms of the patients including the NYHA class should be provided on a CRF form.

Each patient who is suspected of having developed symptomatic congestive heart failure (CHF) should promptly be evaluated by a cardiologist (preferably within two working days, but maximally within one week). Only after confirmation of symptomatic CHF by the consulted cardiologist should symptomatic CHF be reported as a study related serious adverse event (SAE). Further see paragraph 9.2.4. about SAE reporting.

8.2.2 Early discontinuation of trastuzumab

All cases of asymptomatic drop in LVEF or symptomatic cardiac heart failure have to be treated according paragraph 8.2.1 and 8.2.2. Patients who experience serious adverse reactions to trastuzumab as judged by the responsible medical oncologist must discontinue treatment with trastuzumab. If trastuzumab for safety reasons, other than cardiac toxicity, is discontinued study treatment will be given 26 weeks after discontinuation of trastuzumab. Assessments must be performed at all patient visits shown in table 1 for the duration of 26 weeks after discontinuation of trastuzumab. Study medication will not be unblinded, the patient will not go off study.

8.2.3 Trastuzumab in combination with other anticancer drugs

According to standard practice trastuzumab is commonly used in combination with taxanes. The combination of trastuzumab and a taxane is an acceptable chemotherapy regimen for participation in this trial. In patients with an ER and/or PR positive breast tumor aromatase inhibitors (AI) start concurrently with the treatment of trastuzumab, but after the end of the treatment with the taxane. AI may be given in combination with trastuzumab and candesartan, which currently is common clinical practice.

Other medication, which is considered necessary for the patient’s safety and well being, may be given at the discretion of the investigator(s). Co-medication must be recorded in the CRF.

9. SAFETY REPORTING

9.1. Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects’ health. The investigator will take care that all subjects are kept informed.

9.2.1 Adverse event and assessing severity of adverse events

An adverse event (AE) is any undesirable event associated with the use of a drug, whether or not considered drug related, and includes any side effect, injury, toxicity, or sensitivity reactions. It also includes any undesirable clinical or laboratory change, which does not commonly occur in the patients. AEs (grade 3, 4 and 5) occurring during the treatment period of candesartan or placebo with or without trastuzumab must be reported AEs will be
graded according to CTCAE v3 criteria where possible [61]. All signs and symptoms of CHF must be recorded on the CRF. Symptomatic cardiac failure should be reported as a syndrome and not the individual signs and symptoms and should be graded according to the New York Heart Association (NYHA). AEs not covered by CTCAE v3 criteria or NYHA will be graded on a 5-point scale (mild, moderate, severe; see below) and reported in the detail indicated on the CRF.

The definitions are as follows;

**Mild:** discomfort noticed, but no disruption of normal daily activity

**Moderate:** discomfort sufficient to reduce or affect normal daily activity

**Severe:** incapacitating, with inability to work or to perform normal daily activity

**Life-threatening:** represents an immediate threat to life

**Death:** any death occurring during the study

### 9.2.2 Assessing relationship to study treatment

Items to be considered when assessing the relationship of an AE to the study treatment are:

- Temporal relationship of the onset of the event to the initiation of the study treatment candesartan or the treatment of trastuzumab, or other given anticancer therapy as outlined in paragraph 8.2.4.

- The course of the event, considering especially the effect of discontinuation of study treatment or reintroduction of study treatment, as applicable

- Whether the event is known to be associated with the study treatment, or with other similar treatments

- The presence of risk factors in the study subject known to increase the occurrence of the event

- The presence of non-study treatment related factors which are known to be associated with the occurrence of the event

### 9.2.3 Classification of causality of an adverse event and study treatment

The relationship of an AE to study medication will be reported on the CRF and defined as ‘unrelated’, ‘unlikely’, ‘possible’, ‘probable’ or ‘definite’.

**Unrelated:** the event is clearly related to other factors such as the patient’s clinical state, other therapeutic interventions or concomitant drugs administered to the patient

**Unlikely:** toxicity is doubtfully related to investigational agent (S). The event was most likely related to other factors such as the patient’s clinical state, other therapeutic interventions, or concomitant drugs

**Possible:** the event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient’s clinical state, other therapeutic interventions or concomitant drugs.

**Probable:** the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The toxicity cannot be reasonably explained by other factors such as the patient’s clinical state, therapeutic interventions or concomitant drugs.

**Definite:** the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient’s condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves on stopping the drug, or reappears on re-exposure.
9.2.4 Serious adverse event (SAE)

A serious adverse event (SAE) is any event that is fatal, life-threatening, requires or prolongs hospitalization, results in persistent or significant disability or incapacity, a congenital anomaly or birth defect, an important medical event. Important medical events are those that may not be immediately life-threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes.

A SAE is defined as:
- Results in death
- Is life threatening
- Requires hospitalization or prolongation of existing inpatients’ hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Hospitalization: any adverse event leading to hospitalization or prolongation of hospitalization will be considered as ‘serious’, UNLESS at least one of the following exceptions are met:
- the admission is pre-planned (e.g. elective or scheduled surgery, documented in the patient’s file);
- prolonged hospitalization for technical, practical or social reasons, in the absence of an adverse event.

Serious adverse events should be reported from the time the patient starts the study treatment until the end of the study period. Each SAE must be followed up until resolution or stabilization, which has to be recorded and reported.

9.2.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. The investigational products in this protocol are candesartan and placebo. Unexpected adverse reactions are adverse reactions of which the nature or severity is not consistent with the applicable product information (the Summary of Product Characteristics (SPC) for trastuzumab and candesartan).

9.2.6 Reporting of serious adverse events (SAEs)

All SAEs, irrespective of relationship to study treatment, must be reported within 1 working day by telephone and/or fax to: NKI Data Center, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam. Phone: 020-5122668. Fax: 020-5122679. All SAEs occurring during the treatment period of candesartan or placebo with or without trastuzumab must be reported.

Deaths and life-threatening events should be reported immediately by telephone 020-512 2668 NKI Data Center, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam. All deaths should be reported on the death report form section of the CRF regardless of cause.

All SAEs will be recorded on the SAE form by the NKI Data Center. If the relationship to the study medication is possible, probable or definite the investigator will record on the SAE form if the event is expected or unexpected according to the definition given in section 9.6. In case the SAE is unexpected a SUSAR will be reported (see section 9.2.7).

The safety report should include:
- detailed information (see SAE form in CRF) regarding the nature and severity of the event,
- patient initials and study number,
- date and time of treatment,
- start and stop date,
- maximum intensity of the event (CTCTCv3 grading),
- likelihood of its relationship to the study medication,
- treatment administered as a result of the event,
- any concomitant medication taken before or as a result of the event.

All SAEs will be reported to the accredited METC that approved the protocol, according to the requirements of that METC.
All SAE reports will be filed in the Investigator Study File.

9.2.7 Reporting of a SUSAR

SUSARs that are fatal or life-threatening will be reported within 7 days for a preliminary report, with another 8 days for completion of the report to:
- the accredited METC,
- the CCMO (Centrale Commissie Mensgebonden Onderzoek)
- the Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen).

All other SUSARs have to be reported not later than 15 days after the investigator has first knowledge of the SUSAR. Additional information can be send as soon as possible.
SUSARs have to be reported unblinded. Blinding of candesartan/placebo will be broken by the Pharmacy of the Slotervaart Hospital. The study medication part of the SUSAR reporting form will be completed at the Pharmacy and the form will be send by the Pharmacy as mentioned above. SUSAR reports of SUSARs related to trastuzumab will be sent by the Trial Office.

9.2.8 Reporting of a cardiac failure

All cardiac failures, defined as a decrease in LVEF of more than 15% compared to baseline (absolute %) or an absolute value of LVEF below 45%, must be reported within 2 weeks by telephone and/or fax to: NKI Data Center, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam. Phone: 020-5122668. Fax: 020-5122679.

The safety report should include:
- patient initials and study number,
- date and time of treatment,
- date of LVEF measurement
- Result of LVEF (in %)

9.2.9 Data Safety Monitoring Board

An external independent Data Safety Monitoring Committee (DSMC) will be formed to review accumulating safety data. Efficacy data may be provided upon request of the DSMC if this is considered necessary for accurate risk-benefit assessment. The DSMC will meet to review data when 10, 20 and 30 LVEF failures have. The DSMC will advise the investigators of the study regarding the safety of current participants and those yet to be recruited, as well as the continuing scientific validity of the trial. The DSMC will be composed of one statistician and two medical doctors, one specialized in the field of medical oncology and one specialized in the field of cardiology.
Based upon the type and severity of toxicities observed during the study and reviewed during the study may be modified.

The mission of the DSMC is to ensure the safety of patients entered on the study and to guarantee the ethical conduct of this study.

10. STATISTICS

10.1 Statistical considerations

The primary endpoint of the study is deterioration of the cardiac function defined as a decline in LVEF of more than 15% from baseline or a decrease of less than 15% to an absolute value below 45%, during the year of treatment and during the 40 weeks after discontinuation of trastuzumab treatment. From previous studies it is estimated that about 30% of the patients treated with trastuzumab will show cardiac deterioration.

A total of 200 patients will receive trastuzumab and candesartan or trastuzumab and placebo in this double blind, placebo-controlled study. The number of patients registered (=before chemotherapy period) for this trial shall be more than 200 as a small number of patients might drop out before start of therapy with trastuzumab. This number cannot be exactly determined beforehand.

A two group Chi square test with alpha 0.050 two-sided significance level will have in excess of 80% power to detect the difference between a Group 1 proportion, \( p_1 \), of 0.300 and a Group 2 proportion, \( p_2 \), of 0.130 (odds ratio of 0.349) when the sample size in each group is 100.

To closely monitor the safety of the outlined intervention 3 interim looks will be performed before the final analysis. Cardiovascular failures (according to the definition) need to be reported actively and immediately. The interim analyses will take place at equally spaced information fractions with the final look at about 200 patients. The interim analyses are planned after 10, 20 and 30 LVEF failures have occurred. The final analysis will be after the last patient has been followed for 92 weeks. At each interim analysis we will use the logrank test on all available patients data. The final analysis will use the Chi square test with the squared boundary from the logrank test. We will use the EAST software package to calculate the stopping boundaries for the interim tests. For the efficacy side, we choose the overall one-sided significance level of the tests to be 0.025. We will use the Lan and DeMets error spending function resembling the O'Brien-Flemming boundary, to find the stopping boundaries. The use of the O'Brien-Flemming boundary is conservative in the sense that it will give a relatively low probability of stopping the trial early for efficacy. These boundaries are depicted in table A. The calculation of the analysis time assumes an accrual of 40 patients per year and an exponentially distributed time to event.

Table A

<table>
<thead>
<tr>
<th>Information Fraction</th>
<th>Cumulative Events</th>
<th>Alpha Spent</th>
<th>Boundary to Reject H0</th>
<th>Boundary Crossing Probabilities</th>
<th>Analysis Time in years Under H0</th>
<th>Under H1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.250</td>
<td>10</td>
<td>0.000</td>
<td>4.333</td>
<td>0.000</td>
<td>0.002</td>
<td>1.701</td>
</tr>
<tr>
<td>0.500</td>
<td>20</td>
<td>0.002</td>
<td>2.963</td>
<td>0.002</td>
<td>0.166</td>
<td>2.463</td>
</tr>
<tr>
<td>0.750</td>
<td>30</td>
<td>0.010</td>
<td>2.359</td>
<td>0.008</td>
<td>0.372</td>
<td>3.129</td>
</tr>
<tr>
<td>1.000</td>
<td>41</td>
<td>0.025</td>
<td>2.014</td>
<td>0.015</td>
<td>0.260</td>
<td>3.889</td>
</tr>
</tbody>
</table>

At the same time points, we will also perform analyses of safety. We choose the overall one-sided significance level of the safety tests to be 0.05. We will use the error
spending function proposed by Lan and DeMets resembling the Pocock boundary. The Pocock boundary is less conservative than the O’Brien-Flemming boundary as it gives a larger probability to stop early for safety. These boundaries are depicted in table B.

Table B

<table>
<thead>
<tr>
<th>Information Fraction</th>
<th>Alpha Spent</th>
<th>Boundary to Reject H0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.250</td>
<td>0.018</td>
<td>-2.100</td>
</tr>
<tr>
<td>0.500</td>
<td>0.031</td>
<td>-2.077</td>
</tr>
<tr>
<td>0.750</td>
<td>0.041</td>
<td>-2.053</td>
</tr>
<tr>
<td>1.000</td>
<td>0.050</td>
<td>-2.035</td>
</tr>
</tbody>
</table>

If for safety reasons the trial should be stopped, the DSMB will report its findings without delay to the two study coordinators (see first page of protocol) who will then without delay inform all involved investigators. Codes of medication will be broken and patients on study medication (candesartan) will immediately be informed to stop intake of trial medication and they will be given close follow up by their responsible treating medical oncologist. Follow up of LVEF and clinical cardiotoxicity will proceed according to the scheme as outlined in Appendix 2, or more often if deemed necessary for the individual patient. The latter is left to the discretion of the responsible medical oncologist, if necessary after consultation of a cardiologist. Continuation of anticancer therapy including trastuzumab will be left to the responsibility of the treating medical oncologist.

11. ETHICAL CONSIDERATIONS

11.1 Declaration of Helsinki and WMO
This study is to be performed in accordance with the Declaration of Helsinki (Hong Kong amendment) and in accordance with the Medical Research Involving Human Subjects Acts (WMO) [66].

11.2 Documentation of essential documents during the study
The following guidelines, instructions are regarded as relevant supplements: European Note for Guidance on Good Clinical Practice (ICH topic E6, CPMP/ICH/135/95) and the approval of the relevant ethics committee.

11.3 Ethics committee
Before initiation of the study the protocol, the informed consent form (s), the patient information sheet (s), details of the patient recruitment procedures, and any other relevant study documentation will be submitted to the responsible local ethics committee. In particular, change(s) to any aspect of the study, such as modifications to the protocol, the written informed consent form, the written information provided to patients, and/or other procedures must be approved, in writing by the ethics committee.

The investigator will report promptly to the ethics committee any new information that may adversely affect the safety of patients or the conduct of the study. Similarly, the investigator will submit written summaries of the study status to the ethics committee annually, or more frequently if requested by the ethics committee. Upon completion of the study, the investigator will provide the ethics committee with a brief report of the outcome of the study, if required.
11.4 **Insurance of Liabilities**

All subjects participating in the study will be satisfactorily insured by their participating hospital against any injury, caused by the study.

11.5 **Patient information and informed consent**

The investigator is responsible for ensuring that no patient is subject to any study-related examination or activity before the patient has given informed consent. Written informed consent must be given by the patient after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided for the patient.

The investigator will inform the patient of the aims, methods, anticipated benefits, and potential hazards of the study including any discomfort it may entail. The patient may be given time to reflect if this is required, or if the patient requests more time. The patient must be given every opportunity to clarify any points she does not understand and if necessary ask for more information. The patient has the possibility to contact with an independent medical doctor. Patient and/or legal guardian will be required to sign and date the informed consent form. After completion, informed consent forms will be kept an archived in the patient’s medical records.

The patient may withdraw from the study at anytime without prejudicing future medical treatment. Patients who refuse to give, or withdraw, written informed consent may not be included or continued in this study but this will not impact on their subsequent care.

12. **ADMINISTRATIVE ASPECTS**

12.1 **Documentation of patient’s participation**

For all patients who give informed consent, regardless of whether they receive any investigational product, the investigator must record patient identification data. The investigator must keep the list of patient identification codes for a period of at least 15 years after registration or withdrawal of the study treatment. A statement acknowledging the participation of a patient in this clinical study must be documented in the patient’s medical file or notes and where the study physician is not the primary care physician, it is recommended that the patient’s primary care physician be informed of the patient’s participation in this clinical study.

12.2 **Datamanagement**

All Case Report Forms (CRFs) are to be completed in a neat, legible manner to ensure accurate interpretation of data.

12.3 **Patient Identification and confidentiality**

Before the patient’s enrolment in the study, the patient’s consent is required for the data to be used for these purposes and to gain direct access to their medical records for data verification purposes. The patients must be assured that their identity will be protected. Personal and sensitive personal data will be treated as confidential. Each patient will be assigned a patient allocation number on registration. This will be used when reporting AEs, SAEs or other study related data to be entered on the Case Report Form.

All information regarding this study will be maintained in strict confidence. The information obtained during the study period will be used by the investigators, the monitor, the accredited METC and the inspection.

12.4 **Annual progress report**

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject,
numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.5 Annual safety report
The investigator should also submit, once a year throughout the clinical trial or on request, a safety report to the accredited METC, CCMO and Medicines Evaluation Board (CBG). The annual safety report should discuss all suspected serious adverse reactions occurred in the concerned trial. This report has the following contents:
- a report on the subject’s safety in the concerned clinical trial,
- a line listing of all suspected SARs (including all SUSARs) occurred in the concerned trial,
- an aggregated summary tabulation of suspected SARs.

12.6 End of Study report and Final study report
The investigator will notify the competent authority and accredited METC of the end of the study within a period of 90 days. This is done through the Notification of End of Trial form available on the EudraCT public website. The end of the study is defined as the last patient’s last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC and the CCMO within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the CCMO.

12.7 Financing of the trial
AstraZeneca will support this trial according to the Clinical Study Agreement. The company will deliver the trial medication candesartan and placebo free of charge.

12.8 Procedures for protocol amendments
Should any change be required to the approved protocol, a protocol amendment must be prepared. Any amendments must be submitted writing, detailing the reasons necessitating the amendment, and are to be approved by the same personnel approving the original study protocol. Before implementation of any change in the protocol, the amendment must be approved by the ethics committee.

12.9 Publication
Neither the whole nor any part of the results of the study carried out under this protocol will be published or passed on to any third party without prior written consent of the study coordinators.

The final publication of the trial results will be written by the study coordinators on the basis of the final analysis performed at the NKI-AVL Data Centre. After revision by the Data Centre and other co-authors the manuscript will be send to an appropriate peer reviewed and scientific journal. Authors of the manuscript will include at least the study coordinators, the investigators who have included more than 10% of the eligible patients in the trial (by order of inclusion).

All manuscripts will include an appropriate acknowledgement section, mentioning all investigators who have contributed to the trial, the data center staff involved in the study, as well as supporting bodies (cancer leagues, sponsors).

All publications including data from the present trial will be submitted for review to the Data Centre and to all co-authors prior to submission. The study coordinators and the Data Centre Team must approve all publications, abstracts and presentations of data.
pertaining to patients included in this study. This is applicable to any individual patient registered/randomized in the trial, or any subgroup of these. It is the policy of the study coordinators not to release trial results before data maturity has been reached for the primary endpoint(s) of the trial as defined in the protocol.
13. REFERENCES


34. Ewer MS, Lippman SM. Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. J Clin Oncol 2005; 23: 2900-2902.
47. Perez EA. Current management of metastatic breast cancer. Semin Oncol 1999;26:1
Heart Lung 2002;31:262-270.
64. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. Biometrika 1983;70:659-663