F. HOFFMANN-LA ROCHE LTD
CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER BO22227
Ro 045-2317
TRASTUZUMAB IV / TRASTUZUMAB SC

EUDRACT NUMBER 2008-007326-19

PROTOCOL APPROVAL

Protocol Number / Version: C

Date: See last date in electronic signature manifestation below.

Protocol approved by: See electronic signature manifestation below.

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	04-Oct-2012 17:21:44

Table of Contents

List of Tables

List of Figures

List of Appendices

SYNOPSIS OF PROTOCOL NUMBER BO22227C

TITLE	A phase III, randomized, open-label study to compare pharmacokinetics, efficacy and safety of subcutaneous (SC) trastuzumab with intravenous (IV) trastuzumab administered in women with HER2 positive early breast cancer (EBC)
SPONSOR	F. Hoffmann-La Roche Ltd CLINICAL PHASE III
INDICATION	Locally advanced, inflammatory or early stage HER2 positive breast cancer
OBJECTIVES	PRIMARY OBJECTIVE To compare between SC trastuzumab and IV trastuzumab in the neoadjuvant setting • the serum trough concentrations (C_{trough}) observed pre-surgery • the efficacy (pathological complete response, pCR) SECONDARY OBJECTIVES To compare between SC trastuzumab and IV trastuzumab • the observed C_{trough} concentrations post-surgery • the predicted C_{trough} concentrations pre-surgery and post-surgery • the pharmacokinetic profile To evaluate in the SC trastuzumab and IV trastuzumab arm • total pathological complete response (tpCR) • overall response rate (ORR) • time to response (TTR) • event-free survival (EFS) • overall survival (OS) • safety and tolerability • immunogenicity
TRIAL DESIGN	This is a randomized, open label, international, multi-center trial. Patients with operable or locally advanced breast cancer with tumor size of ≥ 1 cm will be randomized 1:1 to pre-operative treatment with 8 cycles of chemotherapy (docetaxel followed by 5-fluorouracil/epirubicin/cyclophosphamide) concurrent with SC trastuzumab or IV trastuzumab. Randomization is stratified by stage of disease and ER status. After surgery patients will receive further 10 cycles of IV or SC trastuzumab as per randomization to complete one year of treatment. Hormone receptor-positive patients may receive hormonal therapy with tamoxifen or aromatase inhibitors as per local practice. Radiotherapy, if applicable, will be given as per local guidelines. Hormonal therapy and radiotherapy may be given concomitantly with SC or IV trastuzumab. *After the end of study treatment* patients will be followed for safety and efficacy *for at least 5* years, or until disease recurrence, whichever is earlier.

NUMBER OF SUBJECTS	Approximately 552 patients, (276 per treatment arm) will be recruited into this study
TARGET POPULATION	Women with HER2-positive early breast cancer clinical stage I to III whose primary tumors are ≥ 1 cm.
LENGTH OF STUDY	Individual patients will be on trial for at least *73* months (from screening through study therapy completion and follow-up) for each subject as follows: • Screening: up to 4 weeks • Total treatment duration: *12 months* • *Safety and efficacy* follow-up: *60* months
END OF STUDY	The end of study will be when the last patient has completed at least *60* months of follow-up after end of treatment.
INVESTIGATIONAL MEDICAL PRODUCT(S) DOSE/ ROUTE/ REGIMEN	**SC trastuzumab** SC trastuzumab (Ro 045-2317/F04) will be injected subcutaneously with a fixed dose of 600 mg throughout the treatment phase
NON-INVESTIGATIONAL MEDICAL PRODUCT(S)	Both study arms receive trastuzumab concurrently with 75 mg/m^2 docetaxel given every 21 days for four cycles followed by four cycles of 5-fluorouracil 500 mg/m^2, epirubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2 given every 21 days. Adjuvant tamoxifen, *luteinizing-hormone-releasing hormone (LHRH) agonists,* or *an* aromatase inhibitor may be administered to *patients with* hormone receptor *(estrogen and/or progesterone receptor)* positive *disease after completion of surgery,* according to local practice.
COMPARATOR "DRUG" (or STANDARD OF CARE) DOSE/ ROUTE/ REGIMEN	**IV trastuzumab** IV trastuzumab (Ro 045-2317/V03 or Ro 045-2317/V01) will be administered with a first dose of 8 mg/kg (loading dose), thereafter it will be given q3w with 6 mg/kg (maintenance dose).
ASSESSMENTS OF: - EFFICACY	Pathological complete response rate (pCR) will be evaluated post-surgery at the clinical site. Tumor size will be measured by caliper and ultrasound. Tumor response (ORR) will be classified according to modified RECIST v1.0. Evaluation of disease recurrence (EFS) will be performed on the basis of protocol-specified criteria.
- SAFETY	Safety of patients will be monitored by physical examination, performance status and vital sign assessment. Cardiac function will be evaluated by echocardiography or MUGA and ECG. Biochemical and hematological laboratory parameters will be measured. Blood samples for testing immunogenicity of treatment will be drawn at different time points in the study.

		Adverse events will be collected and classified according to Common Toxicity Scale (NCI-CTC) version 3.0 with the exception of congestive heart failure which in addition will be graded according to the New York Heart Association (NYHA) functional classification.
-	PHARMACOKINETICS/ PHARMACODYNAMICS	**SC trastuzumab study arm** • pre-dose sample: cycle 1 -13 • post-dose sample : cycle 1 day 2 and 15. Cycle 7 day 2, 4, 8 and 15. Cycle 9 day 2 and 15. Cycle 12 day 2, 4, 8 and 15. **IV trastuzumab study arm** • pre-dose sample: cycle 1 -13 • end of infusion sample: cycle 1-12 • post-dose sample : cycle 1 day 2 and 15. Cycle 7 day 2, 4, 8 and 15. Cycle 9 day 2 and 15. Cycle 12 day 2, 4, 8 and 15
-	PHARMACOECONOMICS/ QUALITY OF LIFE (QOL)	NA
BIOMARKER ANALYSIS/TRANSLATIONAL RESEARCH (OPTIONAL)		**Conversion of HER2 status:** HER2 status will be compared between the tumor sample obtained at initial diagnosis and the tumor sample taken from primary tumor remnants at surgery. **Fcγ-Receptor Polymorphism:** Analysis of Fcγ-receptor polymorphisms will be performed on a blood sample taken at baseline **Exploratory Biomarker Analysis:** Remaining tissue from tumor blocks obtained at initial diagnosis and/or from primary tumor remnants at surgery will be kept at the Roche Clinical Repository for further research purposes.

INCLUSION CRITERIA

1. Patients must have signed and dated an informed consent form
2. Female
3. Age ≥ 18 years
4. Non-metastatic primary invasive adenocarcinoma of the breast which is clinical stage I (T1, N0, M0) to IIIC (any T, N3, M0) including inflammatory and multicentric/multifocal breast cancer
   a. with tumor size ≥ 1 cm by ultrasound or ≥ 2 cm by palpation
   b. histologically confirmed
   c. centrally confirmed HER2 positive (IHC3+ or ISH+)
5. At least one measurable lesion in breast or lymph nodes (≥ 1cm by ultrasound or ≥ 2 cm by palpation), except for inflammatory carcinoma (T4d)
6. Performance status ECOG of 0-1
7. Baseline LVEF ≥ 55% measured by echocardiography or MUGA scan prior to first dose of trastuzumab

EXCLUSION CRITERIA

1. History of any prior (ipsi- and/or contralateral) invasive breast carcinoma
2. Past or current history of malignant neoplasms, except for curatively treated:
   a. Basal and squamous cell carcinoma of the skin
   b. in situ carcinoma of the cervix
3. Metastatic disease
4. Any prior therapy with anthracyclines
5. Prior use of anti-HER2 therapy for any reason or other prior biologic or immunotherapy
6. Concurrent anti-cancer treatment in another investigational trial, including immunotherapy
7. Patients with severe dyspnoea at rest or requiring supplementary oxygen therapy, patients with other concurrent serious diseases that may interfere with planned treatment including severe pulmonary conditions/illness
8. Serious cardiac illness or medical conditions that would preclude the use of trastuzumab, specifically: history of documented CHF, high-risk uncontrolled arrhythmias, angina pectoris requiring medication , clinically significant valvular disease, evidence of transmural infarction on ECG, poorly controlled hypertension,
9. Medical conditions that would preclude the use of 5-fluorouracil, epirubicin, cyclophosphamide or docetaxel, including: cystitis, urinary obstruction, active infections or severe mucositis
10. History of severe allergic and immunological reactions, e.g. difficult to control asthma
11. Known hypersensitivity to any of the study drugs or any of the excipients, known hypersensitivity to murine proteins
12. Known dihydropyrimidine dehydrogenase (DPD) deficiency
13. Any of the following abnormal laboratory tests at baseline:
    Biochemistry:
    i. serum total bilirubin > 1.25 x upper limit of normal (ULN)
    ii. alanine amino transferase (SGPT, ALT) or
    aspartate amino transferase (SGOT, AST) > 2.5 x ULN
    iii. alkaline phosphatase (ALP) > 2.5 x ULN
    iv. serum creatinine > 1.5 x ULN
    Hematology:
    i. absolute neutrophil count (ANC) < 1.5 x 10^9/L
    ii. platelets < 100 x 10^9/L
    iii. haemoglobin < 10 g/dl
14. Pregnant or lactating women
15. Women of childbearing potential or less than one year after menopause (unless surgically sterile) who are unable or unwilling to use adequate contraceptive measures during study treatment
16. Patients unwilling or unable to comply with protocol procedures

PROCEDURES (summary):

Screening (day -28 to -1)
Informed consent, demographics, medical history, physical examination, vital signs, height, weight, ECOG performance status, local determination of HER2 status (core biopsy), local determination of hormone receptor status, confirmation of HER2 status in central laboratory, chest X-ray, liver imaging (optional), bone scan (optional), bilateral mammography, ECG, LVEF measurement (by echocardiogram or MUGA scan)

Baseline (day -7 to -1)
Blood samples for hematology and biochemistry, tumor assessment by caliper and ultrasound, pregnancy test (serum), immunogenicity blood sampling, urinalysis, biomarker blood sample (optional)

Study treatment phase pre-surgery (cycles 1 - 8)
Physical examination, recording of vital signs, ECOG performance status, weight, hematology and biochemistry, immunogenicity blood sampling, urinalysis, tumor assessment by caliper and ultrasound, ECG, LVEF measurement (by echocardiogram or MUGA scan), PK blood sampling

Surgery visit
Tumor assessment by caliper and ultrasound, urinalysis, pathologist post-surgery tumor assessment, biomarker tumor sample (optional)

Study treatment phase post-surgery (cycles 9 - 18)
Physical examination, recording of vital signs, ECOG performance status, chest X-ray (optional), liver imaging (optional), bone scan (optional), bilateral/unilateral mammography, blood samples for hematology, biochemistry and immunogenicity, PK blood sampling, ECG, LVEF measurement (by echocardiogram or MUGA scan).

Post-treatment follow-up period
Physical examination, recording of vital signs, ECOG performance status, chest X-ray (optional), liver imaging (optional), bone scan (optional), bilateral/unilateral mammography, blood samples for hematology and biochemistry, immunogenicity blood sampling, ECG, LVEF measurement (by echocardiogram or MUGA scan), PK blood sampling

STATISTICAL ANALYSES:

The main analysis of PK, efficacy, and safety will be performed after all randomized patients have undergone surgery (unless prematurely withdrawn) and at least 100 patients in each arm have completed cycle 18 of study treatment. There will be a preliminary PK analysis in the first 8 patients in each arm *once they have received the first dose of trastuzumab* to assess if the expected drug exposure for the SC *trastuzumab starting dose* has been achieved. The cohort to be analysed may be expanded to 20 patients per arm in case the variability in PK is too high to allow a conclusion to be drawn. Follow-up analysis for efficacy *and* safety will be performed once *all patients have* completed 24 months *of treatment free* follow-up *and at the end of study (after the last patient has completed a total of 60 months of treatment-free follow-up).*

The study has a PK variable (observed C_{trough}) and an efficacy variable (pCR) as co-primary endpoints.
The primary variable for the pharmacokinetic analysis will be the observed serum trough concentration C_{trough} of trastuzumab at cycle 7 (i.e. the measured pre-dose concentration value at cycle 8). Bio-non-inferiority will be concluded if the lower bound of the confidence interval is equal or greater than 0.8. The predicted C_{trough} will be considered as supportive endpoint of the measured C_{trough}. The same methodology will be used for a secondary analysis performed for the post-surgery period at cycle 12 (i.e the measured pre-dose concentration value at cycle 13). Other PK variables will be regarded as secondary. The analysis will be performed on the per protocol set. A PK model will be used to assess whether the SC dose results in the expected range of concentrations on the first 8 patients in each arm.

Primary analysis of pCR will aim at showing non-inferiority of SC trastuzumab vs IV trastuzumab. Non-inferiority in pCR rate will be established if the one-sided 97.5 % confidence interval for the difference in pCR rate is above -12.5% (the difference is defined as pCR rate in SC trastuzumab arm minus pCR rate in IV trastuzumab arm). Analysis of time to event variables will be descriptive (survival estimates and Kaplan-Meier curves). The analyses will be run on the PPS (primary analysis) and ITT population (supportive analysis).

Safety analysis will be descriptive and will include adverse event frequency, laboratory parameter values, LVEF changes over time, and symptomatic left ventricular dysfunction.

GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
AST (SGOT)	Aspartate aminotransferase
AUC	Area under the serum concentration-time curve
BCIRG	Breast cancer international research group
BP	Blood pressure
CE	Cardiac event
CHF	Congestive heart failure
CI	Confidence interval
C_{max}	Maximum serum concentration
CPU	Clinical Pharmacology Unit
CR	Complete Response
CT	Computer Tomography
CXR	Chest X-Ray
DFS	Disease free survival
DLC	Dose-limiting cardiotoxicity
EBC	Early Breast Cancer
EC_{50}	Plasma concentration associated with half-maximal effect
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
eCRF	electronic Case Report Form
EF	Ejection fraction
eform	Electronic form

GLOSSARY OF ABBREVIATIONS

EFS	Event-free survival
ESF	Eligibility screening form
EU	European Union
FDA	Food and Drug Administration
FFPE	Formalin fixed, paraffin embedded
FISH	Fluorescence in situ hybridization
GCP	Good Clinical Practice
G-CSF	Granulocyte colony-stimulating factor
H_0	Null hypothesis
H_1	Alternative hypothesis
HAHA	human anti–human antibody
HPLC	High performance liquid chromatography
HR	Hazard ratio
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB/IEC	Institutional Review Board/Independent Ethics Committee
ISH	In-situ hybridization
ITT	Intent to treat
IU	International unit
IV	Intravenous
LHRH	luteinizing-hormone-releasing hormone
LVEF	Left ventricular ejection fraction
MBC	Metastatic breast cancer
MedDRA	Medical dictionary for regulatory activities terminology
MRI	Magnetic Resonance Image
MUGA	Multiple gated acquisition

GLOSSARY OF ABBREVIATIONS

NCCTG	North central cancer treatment group
NCI	National Cancer Institute
NCI-CTC	National Cancer Institute-Common Toxicity Criteria
NCI-CTCAE	National Cancer Institute-Common Toxicity Criteria for Adverse Events
NSABP	National Surgical Adjuvant Breast Project
NYHA	New York Heart Association
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cells
pCR	pathologic Complete Response
pCRis	pathologic Complete Response with in situ disease
PD	Progressive disease or Pharmacodynamic
PFS	Progression free survival
PPS	Per Protocol Set
PS	Performance Status
PK	Pharmacokinetic
po	Oral administration
PR	Partial Response
PS	Performance Status
q3w	Every 3 weeks
qw	Once a week
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Stable Disease
SmPC	Summary of Product Characteristics
$t_{1/2}$	Elimination half-life

GLOSSARY OF ABBREVIATIONS

TNM	Primary tumor/regional lymph nodes/distant metastasis
t_{max}	Time to maximum serum concentration
TTP	Time to Tumor Progression
ULN	Upper Limit of Normal

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 Background

Breast cancer is by far the most frequent cancer of women (23% of all cancers), with an estimated 1.15 million new cases in 2002. More than half of the cases are in industrialized countries - about 361,000 in Europe (27.3% of cancers in women) and 230,000 in North America (31.3%) [1]. Breast cancer remains the main cause of death in women aged between 35 and 59.

In 20 % to 30 % of breast cancers, the HER2 (human epidermal growth factor receptor 2) protein is overexpressed. HER2 belongs to a family of four transmembrane receptor tyrosine kinases that mediate the growth, differentiation and survival of cells [2, 3]. HER2 overexpression is associated with aggressive behaviour of the tumor [4, 5].

Surgery is the main modality of local treatment for breast cancer. Systemic chemotherapy or endocrine therapy in hormone receptor-positive patients reduce the risk of relapse and are given either prior to surgery (neoadjuvant therapy) or following surgery (adjuvant therapy).

Neoadjuvant (primary systemic) therapy has become a frequently used treatment option in patients with operable breast cancer who would be candidates for adjuvant therapy. Randomized studies indicated that the survival benefit is similar, regardless of whether treatment is administered pre- or post-operatively [6, 7]. A practical benefit of pre-operative therapy is that it will downstage the primary tumor in most women, allowing a higher rate of breast preservation. It also provides an *in vivo* assessment of tumor response to the particular drug regimen and, therefore, an opportunity to optimize therapy [8].

Achieving a pathological complete response (pCR) of the breast primary has been shown in several trials to be associated with better disease-free and overall survival [6, 9].

1.1.1 Herceptin® (IV Trastuzumab)

Herceptin® (hereafter referred to as IV trastuzumab) is a humanized monoclonal antibody against the extracellular domain of HER2. It is indicated for treatment of patients with HER2-positive metastatic breast cancer (MBC) and commonly given until disease recurrence. Recently it was approved for treatment of patients with HER2-positive early breast cancer (EBC). The recommended duration of IV trastuzumab in early breast cancer is 12 months.

For the regulatory status and approved indications in specific countries please refer to the local label.

1.1.1.1 IV Trastuzumab Pharmacokinetics

Based on a population PK analysis with data primarily from the MBC setting [10] the predicted median AUC (over a period of 3 weeks at steady-state) for the q1w and q3w regimens were 1677 and 1793 mg·day/L, respectively, and the corresponding median C_{min} values were 64.9 and 47.3 mg/L, respectively. A two-compartment model

satisfactorily describes the data, the typical IV trastuzumab PK parameters are clearance (CL) of 0.026 L/day and a volume of distribution of the central compartment (Vc = 3.17 L), which corresponds to human plasma volume which is characteristic of IgG immunoglobulins. The equilibrium half-life is about 26.3 days which is similar to that of endogenous IgG1 immunoglobulin (23 days) which constitutes the backbone of IV trastuzumab.

Please refer to the Herceptin® Investigators' Brochure for a detailed review of the pharmacokinetics of IV trastuzumab.

1.1.1.2 Safety Findings with IV Trastuzumab (Herceptin®)

It is estimated that as of September 25, 2008, approximately 550,000 patients have been treated with IV trastuzumab.

The most common adverse reactions are infusion-associated symptoms such as fever and chills, usually following the first infusion of IV trastuzumab. These symptoms are usually mild to moderate in severity and occur infrequently with subsequent IV trastuzumab infusions. Some adverse reactions to IV trastuzumab infusion can be serious and include dyspnea, hypotension, elevated blood pressure, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, and respiratory distress. In the post-marketing setting, rare occurrences of severe infusion reactions leading to a fatal outcome have been associated with the use of IV trastuzumab.

Severe pulmonary events leading to death have rarely been reported with the use of IV trastuzumab in the post-marketing setting. Signs, symptoms, and clinical findings include dyspnea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, and acute respiratory distress syndrome. These events may or may not occur as sequelae of infusion reactions. Patients with symptomatic intrinsic lung disease or with extensive tumor involvement of the lungs, resulting in dyspnoea at rest, maybe at greater risk of severe reactions. Other severe events reported rarely in the post-marketing setting include pneumonitis and pulmonary fibrosis.

In addition, severe hypersensitivity reactions have been infrequently reported in patients treated with IV trastuzumab. Signs and symptoms include anaphylaxis, urticaria, bronchospasm, angioedema, and/or hypotension. In some cases, the reactions have been fatal. Symptom onset generally occurred during an infusion, but onset after the completion of an infusion has also been reported. Reactions were most commonly reported in association with the initial infusion.

The clinically most relevant adverse event associated with IV trastuzumab is left ventricular cardiac dysfunction (congestive heart failure). In the adjuvant setting the absolute incidence of symptomatic cardiac dysfunction for patients with IV trastuzumab was low (<4%).

Immunogenicity of IV trastuzumab has been investigated in the pre–approval clinical studies. Out of 903 evaluated patients with metastatic breast cancer human anti–human antibodies (HAHA) to IV trastuzumab were detected in one patient, who had no allergic manifestations.

For detailed information on the full safety profile of IV trastuzumab, please refer to the Herceptin® Investigators' Brochure.

1.1.1.3 IV Trastuzumab in Early Breast Cancer (adjuvant setting)

The efficacy and safety of IV trastuzumab was studied in 4 large adjuvant trials.

- HERA (BO16348) [11]
- Joint Analysis of NSABP B31 and NCCTG N9831 trials [12]
- BCIRG 006 [13]

Each study investigated a treatment duration of 12 months with IV trastuzumab, either following completion of an approved (neo)adjuvant chemotherapeutic regimen and radiotherapy, if applicable (HERA), or starting after four cycles of doxorubicin/cyclophosphamide concurrently with four cycles of paclitaxel chemotherapy (B31, N9831, BCIRG 006). All trials demonstrated that the risk of a DFS event was almost halved by one year treatment with IV trastuzumab. Also the risk of death was significantly reduced (see Table 1). Cardiac toxicity in these adjuvant trials was low; the rate of congestive heart failure NYHA class III/IV in the IV trastuzumab study arms was below 4% [13, 14, 15, 16].

For further details please refer to the Herceptin® Investigators' Brochure.

Table 1 Efficacy of IV Trastuzumab in the Adjuvant Setting

	Disease-free Survival DFS	Overall Survival OS	Median FU
HERA	HR 0.64, p< 0.0001	HR 0.66, p= 0.0115	23 months
Joint Analysis	HR 0.48, p<0.00001	HR 0.65, p=0.0007	35 months
BCIRG 006	HR 0.61, p< 0.0001	HR 0.59, p= 0.004	36 months

1.1.1.4 IV Trastuzumab in Early Breast Cancer (neoadjuvant setting)

Primary systemic therapy (PST) or neoadjuvant therapy is used in non-metastatic breast cancer to treat systemic disease shortly after diagnosis, with the aim to decrease tumor bulk ideally to a pathological complete response (pCR; eradication of tumor as assessed at surgery) and thus to reduce the extent of surgery [17]. A meta-analysis of 9 randomized trials with nearly 4000 patients found no difference in overall survival, disease progression and distant disease recurrence between adjuvant and neoadjuvant treatment. In six of these trials the rate of breast conserving surgery was increased in those patients receiving neoadjuvant therapy [7]. In addition, achievement of a pCR was associated with a significantly longer disease free and overall survival compared with patients with residual disease after PST [6, 9].

Many clinical studies have been conducted or are in progress investigating the role of trastuzumab in this setting [18]. The trial of Buzdar and coworkers and the NOAH study are the only clinical trials that investigated the addition of IV trastuzumab to pre-operative chemotherapy in a randomized design [19, 20]. An overview on neoadjuvant trials with IV trastuzumab is displayed in Table 2.

As of today, an optimal neoadjuvant treatment regimen is not yet established. Although patient population and type of chemotherapy regimen investigated vary among trials, it appears that a combination of anthracyclines and taxanes produces the highest pCR rates [18]. The major relevant neoadjuvant trials supporting this conclusion are described below.

Buzdar AU, Ibrahim NK, Francis D et al (2005)

Buzdar and coworkers at the MD Anderson Cancer Center investigated the role of IV trastuzumab when given concurrently with preoperative chemotherapy (paclitaxel 225 mg/m^2 q3w for 4 cycles followed by 5-fluorouracil, epirubicin and cyclophosphamide (500/75/500 q3w for 4 cycles). One-hundred and sixty-four patients were planned to be enrolled into this randomized clinical trial, however the Data Monitoring Committee of the study recommended that accrual be suspended as the trial was considered to have reached its primary objective with only 42 patients enrolled. Pathological complete response rate in breast and nodes in the IV trastuzumab arm was 65% compared to 26% in the control arm (p=0.016) [19]. These efficacy data were further updated by including additional 22 patients into the IV trastuzumab arm who achieved a pCR rate of 55% [21]. No new safety concerns emerged among the altogether 45 patients enrolled into the IV trastuzumab arm of this trial. There was no clinical cardiac dysfunction observed and there were no cardiac deaths. At a median follow-up of 36 months from study entry the 3 year DFS rate in the IV trastuzumab arm was 100% compared to 85% in the control arm [21].

The same regimen was used by Pernas and coworkers, but paclitaxel 225 mg/m^2 q3w for 4 cycles was replaced by 12 cycles of weekly paclitaxel 80 mg/m^2 [22]. Thirty-three patients with clinical stage II to III were enrolled. The pathological complete response rate in breast and nodes was 73%. Transient asymptomatic LVEF decreases were observed in 18% of patients.

Based on the experience of the MD Anderson Cancer Center Dawood et al (2007) presented a retrospective review of the efficacy and safety of the regimen introduced by Buzdar and coworkers [23]. Forty patients were identified from the medical charts, 60% of them had stage III disease, four patients had inflammatory breast cancer. The pCR rate was 55% in this cohort. At a median follow-up of 19 months no symptomatic congestive heart failure was observed.

Table 2 Neoadjuvant Trials with IV Trastuzumab

Study	HER2-positive patients N	Clinical stage	Chemotherapy	Cycles** N	pCR
Pernas (2007) [22]	33	II, III, inflammatory	PH→FECH	8	73%
Buzdar* (2005) [19]	23	T1-3, N0-1	PH→FECH	8	65%
Buzdar (2007) [21]	22	T1-3, N0-1	PH→FECH	8	55%
Coudert (2006) [24]	26	II,III, non-inflammatory	TH	6	47%
Limentani (2007) [25]	31	IIB/III, inflammatory	TH + vinorelbine	4	45%
Lybaert (2007) [26]	25	T4 and/or N2-3	TXH	6	45%
Gianni* (2007) [20]	115	LABC, incl. inflammatory	APH→PH→CMFH	11	43%
Untch (2005) [28]	174	>2cm or Inflammatory	EC→PH	8	41%
Coudert (2007) [27]	70	II, III, non inflammatory	TCpH	6	39%
Untch (2008) [29]	452	>1cm, T1-4, N+if T2, inflammatory	ECH→TH±X	8	32%
Harris (2003) [30]	28	II/III incl. inflammatory	H + vinorelbine	4	29%
Tripathy (2007) [31]	23	T2-3, N0-1, M0	TXH	4	29%
Hurley (2006) [32]	48	LABC or T4d	TH + cisplatin	4	23%
Carey (2002) [33]	22	IIB-IV, inflammatory	AC→PH	8	22%
Burstein (2003) [34]	40	II/III incl. inflammatory	PH	4	18%
Torrisi (2006) [35]	30	T2-4, N0-3, M0	H + vinorelbine	8	11%
Bines (2003) [36]	27	III A/B	TH	5	11%

Adapted from Lazaridis 2008. Only trials with more than 20 patients included. Definition of pCR variable among trials. *randomized trial. **weekly regimen translated into three-weekly.
A, Doxorubicin; C, Cyclophosphamide; P, Paclitaxel; E, Epirubicin; T, Docetaxel; F, 5-Fluorouracil; X, Capecitabine; M, Methotrexate; Cp, Carboplatin, H, IV trastuzumab

NOAH Trial

This ongoing, randomized phase III trial in the neoadjuvant setting enrolled 228 patients with HER2-positive locally advanced breast cancer including inflammatory breast cancer. Patients received 3 cycles of doxorubicin-paclitaxel (AP: A 60 mg/m^2, P 150 mg/m^2 q3w) followed by 4 cycles of paclitaxel (P:175 mg/m^2 q3w) and then 3 cycles of cyclophosphamide/methotrexate/5-fluorouracil (CMF: C 600 mg/m^2, M 40 mg/m^2, F 600 mg/m^2 q4w on day 1 and 8), with (n=115) or without (n=113) concomitant IV trastuzumab. After surgery patients continued on IV trastuzumab monotherapy to complete one year of treatment. Adding IV trastuzumab to AP/P/CMF significantly increased the pCR rate (43% vs. 23%, p=0.002) in the ITT population [20]. IV trastuzumab was also efficacious in the subgroup of patients with inflammatory breast cancer (N=62) showing pCR rates of 55% in the IV trastuzumab arm compared to 19% in the control [37]. Event-free survival at a median follow-up of 3 years was favouring the IV trastuzumab arm (HR 0.56, p=0.0062) and there was a trend towards longer overall survival (HR 0.65, p=0.18) [38]. The cardiac safety profile was acceptable with 2 patients in the IV trastuzumab arm experiencing symptomatic left ventricular dysfunction of NCI-CTC grade 3 and 4, respectively, compared to one patient with a grade 2 event in the control arm [38].

GeparQuattro Trial

The GeparQuattro study enrolled 1510 patients with early breast cancer including inflammatory breast cancer, 453 of whom had HER2-positive disease. In this 3-arm trial, HER2-positive patients were randomized to receive pre-operative IV trastuzumab concurrently with either a) four cycles of epirubicin plus cyclophosphamide (EC 90/600) followed by 4 cycles of docetaxel 100 mg/m^2, or b) four cycles of EC 90/600 followed by 4 cycles of docetaxel 75 mg/m^2 plus concomitant capecitabine 1800 mg/m^2, or c) four cycles of EC 90/600 followed by 4 cycles of docetaxel 75 mg/m^2 followed by 4 cycles of q3w capecitabine 1800 mg/m^2. The pCR rate in the HER2-positive patients (from all three study arms) was 32% [29]. There were no cardiac safety concerns in this trial. LVEF decreases beyond 45% were not observed, neither was congestive heart failure grade 4 [29].

Untch M, Stoeckl D, Koneczy G et al (2005)

This multicenter, single arm phase II study investigated pre-operative epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) for four cycles q3w, followed by four cycles of paclitaxel (175 mg/m^2) q3w concurrently with IV trastuzumab [28]. One hundred and seventy-four patients were evaluable for efficacy. The pCR rate observed in this study was 41%.

1.1.1.5 Co-administration of Anthracyclines with IV Trastuzumab

Data from 115 patients of the NOAH study [38] with a median follow-up of 3 years demonstrated that the concurrent administration of doxorubicin (total cumulative dose 180 mg/m^2) with IV trastuzumab is feasible and shows an acceptable cardiac safety profile (see section 1.1.1.4). Dawood and coworkers reported in their study no symptomatic congestive hear failure at a median follow up of 19 months [23]. Likewise,

no symptomatic cardiac dysfunction was observed in patients participating in the GeparQuattro trial (total cumulative dose of epirubicin 360 mg/m^2) and the trial of Buzdar and coworkers (total cumulative dose of epirubicin 300 mg/m^2) [29, 21].

More data on the concurrent administration of anthracyclines and IV trastuzumab stem from the metastatic setting. The pivotal H0648g trial investigated the efficacy and safety of the combination of either paclitaxel (P) or anthracycline/cyclophosphamide (AC) with IV trastuzumab (H) [39]. This trial included 469 HER2-positive patients with metastatic breast cancer who were randomly assigned to 4 groups: P plus H vs P alone; AC plus H vs AC alone. One hundred and 43 patients were assigned to the AC/IV trastuzumab combination. Chemotherapy was given for six cycles, 36 patients received epirubicin (75 mg/m^2), the others were treated with doxorubicin (60 mg/m^2). Cyclophosphamide was given at 600 mg/m^2. The addition of IV trastuzumab to AC was shown to provide statistically significant benefit for Time to Progression (TTP 7.8 months vs 6.1 months, $p<0.001$) and Time to Treatment Failure (TTF 7.2 months vs 5.6 months, $p<0.001$). A similar benefit was observed for objective tumor response (ORR 56% vs 42%, $p=0.02$). However, the combination of IV trastuzumab with AC was associated with a high incidence of cardiac dysfunction (27% vs 8%) compared to the AC alone arm. More specifically, the incidence of cardiac dysfunction of NYHA class III or IV was 16% in the AC plus trastuzumab arm compared with 3 % in the control arm.

While cumulative doses of $\geq$ 450 mg/m^2 doxorubicin are associated with potential cardiotoxicity, epirubicin is considered generally devoid of cumulative risk of cardiac events up to a threshold of 1,000 mg/m^2 [40, 41, 42]. Therefore epirubicin is investigated for concurrent use with trastuzumab.

The phase I/II study Hercules (M77003) evaluated the cardiac safety of IV trastuzumab (H) in combination with epirubicin/cyclophosphamide (EC) as first line therapy in anthracycline-naive patients with HER2 overexpressing MBC [43]. Two doses of epirubicin were tested (60 mg/m^2 and 90 mg/m^2). The control arm in this study comprised patients who did not overexpress HER2 and received EC only. A total of 180 patients (60 in each of the study arms) were enrolled into the study. The results presented here were based on the main analysis of the study which was performed when all treated patients had completed at least 1 year in the study (see Table 3).

Table 3 Cardiac Safety and Efficacy Results from Study M77003

	HEC-60 (n = 60)	HEC-90 (n = 60)	EC-90* (n = 60)
Cardiac Safety			
• Total patients with ≥ 1 cardiac event, n (%)	9 (15.0)	14 (23.3)	5 (8.3)
• Total patients with LVEF decrease of > 10 pp to < 50%, n (%)	4 (6.7)	9 (15.0)	-
• CHF NYHA class III/IV with LVEF decrease of >10 pp to < 50%, n (%)	1 (1.7)	3 (5.0)	-
Efficacy			
• ORR	57 %	60 %	25 %
• CR	20 %	18 %	3 %
• PR	37 %	42 %	22 %
• Median TTP (months)	12.5	10.1	7.6
• Median PFS (months)	12.5	10.0	7.4

*patients in the chemotherapy alone arm were HER2 negative

The increase in epirubicin dose from 60 mg/m^2 to 90 mg/m^2 was associated in the IV trastuzumab arms with an increase in cardiac events from 15.0% to 23.3%. More specifically, the incidence of congestive heart failure NYHA class III/IV increased from 1.7% to 5.0%. At the same time the lower epirucin dose compared favourably regarding all efficacy variables. The data provide strong evidence that trastuzumab in combination with epirubicin and cyclophosphamide is a feasible and active regimen for patients with HER2-positive MBC.

1.1.2 SC Trastuzumab

The current marketed product is IV trastuzumab. F. Hoffmann-La Roche Ltd is currently developing a subcutaneous (SC) application form of trastuzumab (SC trastuzumab) for three-weekly treatment. It is expected that the administration of SC trastuzumab would be performed over a shorter time period (maximum 5 minutes) leading to improved convenience and compliance which are particularly important when patients are treated for prolonged periods of time (i.e. in the adjuvant setting or the metastatic setting when patients are on IV trastuzumab monotherapy). Treatment fatigue and stopping treatment earlier than the recommended 12 months after completion of adjuvant chemotherapy have indeed been reported in a small proportion of patients. The more convenient SC administration is expected to result in improved compliance due to fewer hospital visits and to offer the potential for home administration or even self administration by the patient via an administration-specific device. Costs associated with IV administration (i.e. nursing costs for IV administration, rental of day-beds, patient travel etc.) are expected to be reduced. Finally, SC administration of trastuzumab may be associated with a reduced frequency/intensity of infusion-related reactions, as it was observed with alemtuzumab (MabCampath®) [44].

A key excipient in the SC solution is the enzyme hyaluronidase. Animal-derived hyaluronidase has been available commercially for over 60 years and is used primarily as a permeation enhancer to increase the dispersion and absorption of other co-administered

drugs. It transiently hydrolyses hyaluronan, a matrix component of the SC matrix. The hydrolysis leads to a decreased viscosity of the SC matrix and, thus, to an improved delivery of SC administered drugs to the systemic circulation. The decreased viscosity is also expected to facilitate SC administration of larger volumes of fluid. In the United States, rHuPH20 (Hylenex® recombinant [45]) is licensed to facilitate the absorption and dispersion of drugs when given SC at doses between 50 IU and 300 IU [46].

1.1.2.1 Non-clinical Studies with rHuPH20

After IV administration in the dose range 0.3 to 30 mg/kg, rHuPH20 demonstrated nonlinear PK, rapid clearance and a half life of around 5 minutes at the lowest dose tested. The bioavailability of rHuPH20 following SC administration was extremely low (not determinable at low doses, 6% to 8% in the dose range 3 to 30 mg/kg). Treatment of various species with rHuPH20 (IV or SC) was generally well tolerated and no major abnormalities were noted in any toxicology studies.

For further details on non-clinical studies with rHuPH20 please refer to the Herceptin® Investigators Brochure.

1.1.2.2 Clinical Studies with rHuPH20

Mammalian hyaluronidase preparations differing in source, species, and manufacturing process have been the subject of multiple investigations and regulatory approvals in Europe, the United States, and Asia. The extent of human administration of these products in the U.S. has been estimated to be in the tens of millions of subjects. The safety and efficacy of hyaluronidase products have been widely established. The most significant safety risk identified is hypersensitivity/allergenicity, which is thought to be related to the lack of purity of the animal-derived preparations [46, 47].

Clinical data are available from four studies with rHuPH20.

- In an allergic sensitivity study (R04-0851), 100 healthy volunteers were injected intradermally with 0.1 mL (15 U) of Hylenex and saline control. The most common side effects were generally mild redness, bruising, swelling, discomfort and itching. No adverse events were serious and none were judged to be related to study treatment.
- A proof-of-concept dose escalation study (HZ2-06-02) with adalimumab and rHuPH20 in 15 patients with rheumatoid arthritis (RA) evaluated the effects of rHuPH20 on the PK, safety and tolerability of adalimumab. A single co-administration of adalimumab with rHuPH20 increased adalimumab exposure by a weighted average of 13% compared to adalimumab alone. The injection was well tolerated with only mild and moderate adverse events.
- HZ2-07-01 was a double-blinded, within-subject-controlled, two way cross-over study comparing the time to inject (flow rate), safety and tolerability of a SC administered 10% (2,000 mg in 20 mL) solution of immunoglobulin G (diluted Carimune® NF) with and without rHuPH20 in 30 healthy volunteers. There was a statistically non-significant trend towards a decrease in time to inject and an increase in flow rate in the presence of rHuPH20 relative to the control group. The most common AEs were injection site reactions, consisting of erythema, pain, oedema,

induration or pruritus (communication Halozyme Therapeutics Inc. on preliminary study results).

- [redacted] investigated the SC injection of different rHuPH20 concentrations in a viscous solution of IgG and adalimumab in healthy volunteers using different volumes of injection (2, 8 and 16 mL). The maximum total enzyme dose administered in this study was 96,000 U.

  The injections were well tolerated with no serious adverse events reported. All injection site reactions such as erythema, pain and induration were mild (98%) or moderate (2%) in severity.

  There was a trend to lower mean time to inject in subjects that received rHuPH20 compared to those without rHuPH20, as well as a trend towards an increase in the exposure to adalimumab in the presence of rHuPH20.

  Pain increased across all volume cohorts after injection, with no clear difference between the presence and absence of rHuPH20.

The highest total rHuPH20 dose administered in the clinical studies was 96,000 U and was well tolerated by healthy volunteers.

For detailed information on the conducted clinical trials with rHuPH20, please refer to the Herceptin® Investigators Brochure.

1.1.2.3 Non-clinical Studies with SC Trastuzumab

In a single dose local tolerance study in rabbits still ongoing, no rHuPH20 related adverse effects were noted 24 or 96 hours post dose.

A 13-week repeat-dose toxicity study in Cynomolgus monkeys is also ongoing. This study covers general systemic, specific systemic (lymphatic drainage), and local tolerance endpoints and will compare and bridge safety endpoints obtained by IV dosing with SC data. The SC trastuzumab dose is expected to result in exposure (AUC) levels that are comparable to those achieved at the highest doses in the corresponding non-clinical safety program for IV trastuzumab. An interim assessment of *in vivo* data after 6 weeks of treatment revealed no test article related local or systemic adverse effects.

For further information, please refer to the Herceptin® Investigators Brochure.

1.1.2.4 Clinical Studies with SC Trastuzumab

The trastuzumab SC dose finding study BP22023 conducted in healthy male volunteers and patients with HER2-positive early breast cancer is ongoing. Meanwhile dosing has been completed. Twenty-four healthy male subjects and 46 female HER2-positive EBC patients received single doses of either IV or SC trastuzumab as outlined in Table 4.

In Part 1 (dose finding) of the trial, male subjects were administered SC trastuzumab either at 6 mg/kg or 10 mg/kg. Following a review of preliminary PK data, a decision was made to administer 8 mg/kg trastuzumab SC which was predicted to result in comparable serum concentrations to 6 mg/kg IV trastuzumab.

In part 2 (dose confirmation) of the trial, patients were administered SC trastuzumab either at 8 mg/kg or 12 mg/kg.

Table 4 Cohorts in study BP22023

Part 1 (Dose finding)

Cohort	Route of administration	Subjects	Dose (mg/kg)
1	IV	6 Male subjects	6
2	IV	6 HER2+ patients	6
3	SC	6 Male subjects	6
4	SC	6 Male subjects	10
5	SC	6 Male subjects	8

Part 2 (dose confirmation)

Cohort	Route of administration	Subjects	Dose (mg/kg)
A	SC	20 HER2+ patients	8
B	SC	20 HER2+ patients	12

1.1.2.4.1 Preliminary Safety Data

Safety and tolerability were assessed by reviewing unblinded individual data from subjects enrolled in both Part 1 and 2 of the study. At the time of this report all 12 subjects of Part 1 and 33 patients of Part 2 (19 patients in Cohort A and 14 patients in Cohort B) were available for analysis.

In Part 1, a total of 84 AEs were observed in 26 subjects. Of these, 72% were considered to be mild, 27% moderate and there was 1 severe AE (infusion related reaction).

In Cohort 1, in which male subjects received IV trastuzumab at 6 mg/kg, the most frequent AEs were headache (3), musculoskeletal pain (2), diarrhea (2), abdominal pain (2) and infusion related reactions (2; one event of severe, the other of moderate intensity). In the severe case, the IRR started 5 hours after infusion initiation and was resolved within 2 days.

In Cohort 2, in which female patients received IV trastuzumab at 6 mg/kg, the most commonly observed AE was headache which occurred in 2 patients (1 mild, 1 moderate).

In Cohorts A and B, there was no apparent dose-related increase in AEs and the SC administration was generally well tolerated. The most commonly observed AEs in these patients were headache (21; 13 mild, 8 moderate), diarrhea (7; 5 mild, 2 moderate), lethargy (6; 4 mild, 2 moderate) and injection site erythema (6; all mild).

Overall the adverse events observed with SC trastuzumab are in line with the safety profile known from IV trastuzumab.

Special attention was given to local tolerability of SC trastuzumab administration. In subjects treated with SC trastuzumab there were 15 AEs that can be classified as administration site conditions. All of these AEs were of mild intensity and include erythema (7), discoloration (5), injection site discomfort (1), injection site pain (1), and injection site reaction (1).

There were no serious adverse events (SAEs) reported in any subject in study BP22023.

With the introduction of a SC administration route, there is a potential that the previously observed very low incidence of human anti-human antibodies (HAHA) formation (one patient out of 903 patients tested) could be increased. Literature evidence suggests that in some cases, where IV and SC administration of the same protein drugs has been compared, no significant increases in the incidence of HAHA have been observed following SC administration [48]. In cases where increased immunogenicity was observed, the magnitude of increase was small [49], e.g., a less than 2-fold increase [50]. The potential formation of anti-bodies against trastuzumab and rHuPh20 is monitored in the ongoing study BP22023.

Plasma concentrations of rHuPH20 were assessed in all subjects who received SC trastuzumab at pre-dose, 0.5 h, 1 h and 24 h post-dose. Concentrations of rHuPH20 were below limit of quantification (10 U/mL) at all time points assessed in all healthy volunteers in Part 1. At the time of this report results from 14 patients in Cohort A were available and concentrations were below limit of quantification at all time points assessed.

At the time of analysis, results of anti-rHuPH20 antibody testing were available for all subjects in Part 1, Cohorts 3 to 5. No antibodies to rHuPH20 were detectable at screening and Day 15 in any subject. The assay for anti-trastuzumab antibodies is currently under development and results will be reported when available.

1.1.2.4.2 Preliminary PK Data

Dosing in BP22023 is completed and preliminary pharmacokinetic results are available from 24 healthy male subjects and 46 female HER2-positive EBC patients who received single doses of either IV or SC trastuzumab (see Table 4).

Figure 1 and Figure 2 show the mean serum concentration time profiles of trastuzumab by dosing cohort for Part 1 and Part 2 respectively. Table 5 summarizes the computed pharmacokinetic parameters in serum of trastuzumab.

Subjects in Cohorts 1 (healthy male volunteers) and 2 (HER2-positive female patients) received a single dose of 6 mg/kg trastuzumab IV. Comparable pharmacokinetics were achieved with a mean C_{max} of 185 µg/mL compared to 154 µg/mL and a mean $AUC_{0\text{-}Day21}$ of 32100 µg.h/mL compared to 28800 µg.h/mL in patients and healthy volunteers, respectively. Serum concentrations at Day 21 were similar in both Cohorts 1 and 2 (25.6 and 27.5 µg/mL respectively.

A dose linear increase in C_{max} and $AUC_{0\text{-}Day21}$ was observed across Cohorts 3 to 5 in healthy volunteers who received 6, 8 or 10 mg/kg SC. C_{Day21} was also dose linear with mean concentrations of 31.6, 39.4, and 51.4 µg/mL with 6, 8, and 10 mg/kg SC, respectively.

In Part 2, patients received a single dose of either 8 or 12 mg/kg SC. Patients who received 8 mg/kg SC in Cohort A showed similar C_{max} (86.6 vs 82.0 µg/mL) and $AUC_{0\text{-}Day21}$ (28100 vs 29600 µg.h/mL) to healthy volunteers who received the same dose in Cohort 5. The CV% for C_{max} and $AUC_{0\text{-}Day21}$ in Cohort A was 38.3% and 28.0%, respectively. In those patients who received 12 mg/kg SC (Cohort B) a mean C_{max} of 151 µg/mL and mean $AUC_{0\text{-}Day21}$ of 47600 µg.h/mL were observed. The mean serum concentration on Day 21 was 65.0 µg/mL. The CV% for C_{max} and $AUC_{0\text{-}Day21}$ in Cohort A was 38.9% and 23.8%, respectively. As in Part 1, pharmacokinetic parameters were dose-linear at the dose levels administered.

The final study results are now available and published [68].

Table 5 Summary of Preliminary Pharmacokinetic Parameters (Mean ± S.D.) of Trastuzumab in Serum

Parameter	Part 1					Part 2	
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort A	Cohort B
	6 mg/kg IV HV	6 mg/kg IV Px	6 mg/kg SC HV	10 mg/kg SC HV	8 mg/kg SC HV	8 mg/kg SC Px	12 mg/kg SC Px
	N = 6	N = 6	N = 6	N = 6	N = 6	N = 20	N = 20
C_{max} (µg/mL)	154 ± 20.7	185 ± 42.9	66.8 ± 11.4	102 ± 17.2	82.0 ± 11.3	86.6 ± 33.2	151 ± 58.7
Range	(136 – 192)	(138 – 251)	(55.7 – 86.2)	(70.2 – 119)	(71.3 – 99.3)	(40.8 – 156)	(77.2 – 340)
CV (%)	13.5	23.2	17.1	16.8	13.8	38.3	38.9
T_{max} (h)	5.25 ± 9.19	6.25 ± 8.72	156 ± 65.7	148 ± 59.6	116 ± 49.0	128 ± 62.5	108± 53.7
Range	(1.5 - 24)	(1.5 - 24)	(96 - 216)	(96 - 216)	(96 - 216)	(48 - 216)	(48 - 216)
CV (%)	175	139	42.1	40.3	42.2	49.0	49.7
$AUC_{0\text{-}inf}$ (µg·h/mL)	38700 ± 7930	44100 ± 4510	32600 ± 7260	60200 ± 12100	46700 ± 6140	38900 ± 12300	64000 ± 15900
Range	(26300 - 46400)	(38500 - 51900)	(25600 - 44600)	(47700 - 81900)	(38700 - 55700)	(17800 - 74400)	(49800 - 91100)
CV (%)	20.5	10.2	22.2	20.2	13.1	31.5	24.9
$AUC_{0\text{-}Day21}$ (µg·h/mL)	28800 ± 4820	32100 ± 3150	21300 ± 3530	34300 ± 4120	29600 ± 3860	28100 ± 7850	47600 ± 11300
Range	(21200 – 33200)	(28800 - 36500)	(18200 - 27700)	(27600 - 40300)	(25100 - 35200)	(14800 - 48700)	(26200 - 73300)
CV (%)	16.7	9.81	16.6	12.0	13.1	28.0	23.8
C_{Day21} (µg/mL)	25.6 ± 12.1	27.5 ± 7.45	31.6 ± 12.0	51.4 ± 15.8	39.4 ± 5.48	36.6 ± 9.30	65.0 ± 20.8
Range	(4.95 – 41.7)	(15.2 – 37.5)	(22.0 – 53.5)	(36.2 – 77.3)	(34.9 – 50.0)	(17.6 – 64.7)	(26.1 – 98.5)
CV (%)	47.1	27.1	38.1	30.8	13.9	25.4	32.1

Figure 1 **Mean Serum Concentration Time Profile of Trastuzumab by Dosing Cohort (Part 1)**

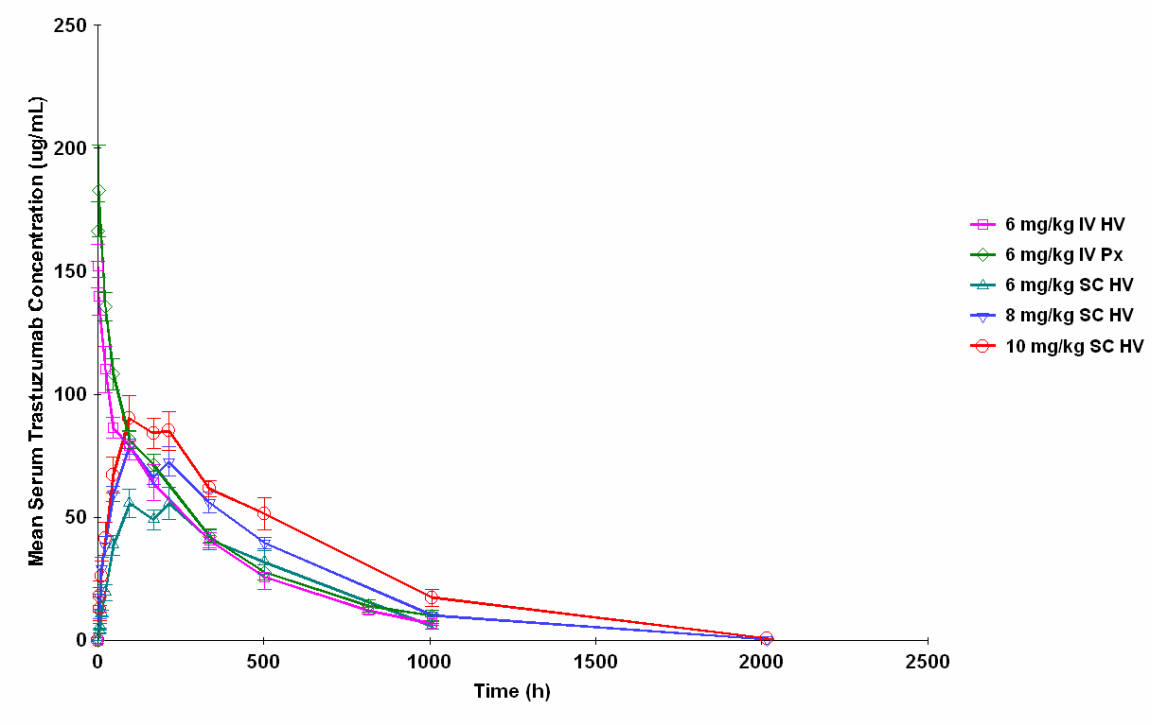
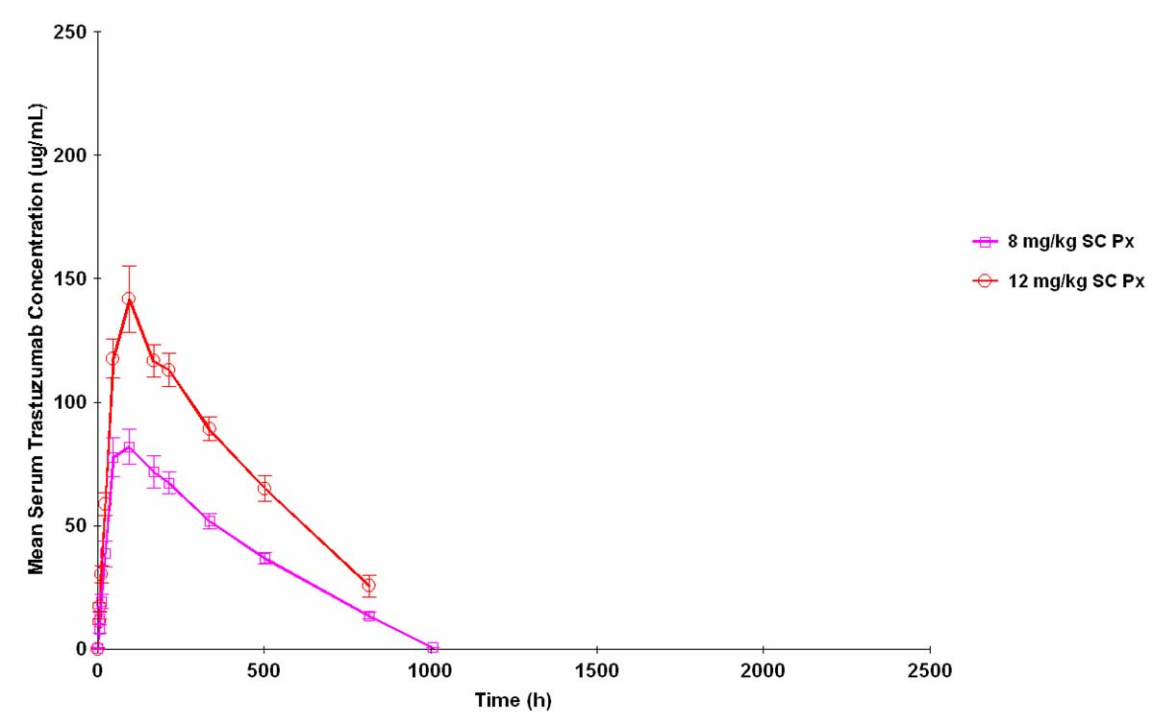


Figure 2 **Mean Serum Concentration Time Profile of Trastuzumab by Dosing Cohort (Part 2)**



Part 2: Last time point results available - 1008 h for 8 mg/kg and 816 h for 12 mg/kg.

1.1.2.4.3 SC Trastuzumab Dose Determination for BO22227

In the dose finding trial BP22023, patients were dosed on a body weight-adjusted basis both for the SC and IV administration of trastuzumab.

The preliminary IV and SC pharmacokinetic data from study BP22023 were integrated into a PK model and model-based simulations were then used to predict C_{trough} and AUC for various SC trastuzumab doses. The simulations showed that a fixed dose of 600 mg would be expected to achieve C_{trough} values at least as high as with the q3w IV regimen (8 mg/kg loading dose followed by 6 mg/kg maintenance dose). The simulations were based on a normal distribution of body weight with a mean of 68 kg and standard deviation of 11 kg.

Figure 3 illustrates the distribution of predicted median C_{trough} and predicted 5th percentile of C_{trough}, respectively, at Cycles 1 and 8, comparing the IV loading and maintenance doses of 8 mg/kg and 6 mg/kg and the fixed SC dose of 600 mg. For both cycles, with the selected fixed dose of 600 mg, median C_{trough} values are predicted to be higher than those achieved with the IV doses.

Figure 4 illustrates the distribution of predicted median AUC_{tau} and predicted 5th percentile of AUC_{tau}, respectively, at Cycles 1 and 8, comparing the IV loading and maintenance doses of 8 mg/kg and 6 mg/kg and the fixed SC dose of 600 mg. For Cycle 8, with the selected fixed dose of 600 mg, median AUC_{tau} values are predicted to be higher than those achieved with the IV 6 mg/kg maintenance dose. For Cycle 1, median AUC_{tau} values are predicted to be lower than those achieved with the IV 8 mg/kg loading dose. It is predicted that with the 600 mg fixed dose - similar to the approved q1w IV regimen - a comparable cumulative AUC to the q3w IV regimen is reached during Cycle 3.

Notably, the predicted C_{max} values after SC administration (95th percentile) do not exceed the maximum exposure previously measured for the IV regimen (BO16348 CSR [63]). Therefore, no unexpected safety issues related to trastuzumab exposure are expected with the SC formulation at the recommended dose.

Figure 3 Distribution of Simulated Median C_{trough} Levels at Cycles 1 and 8

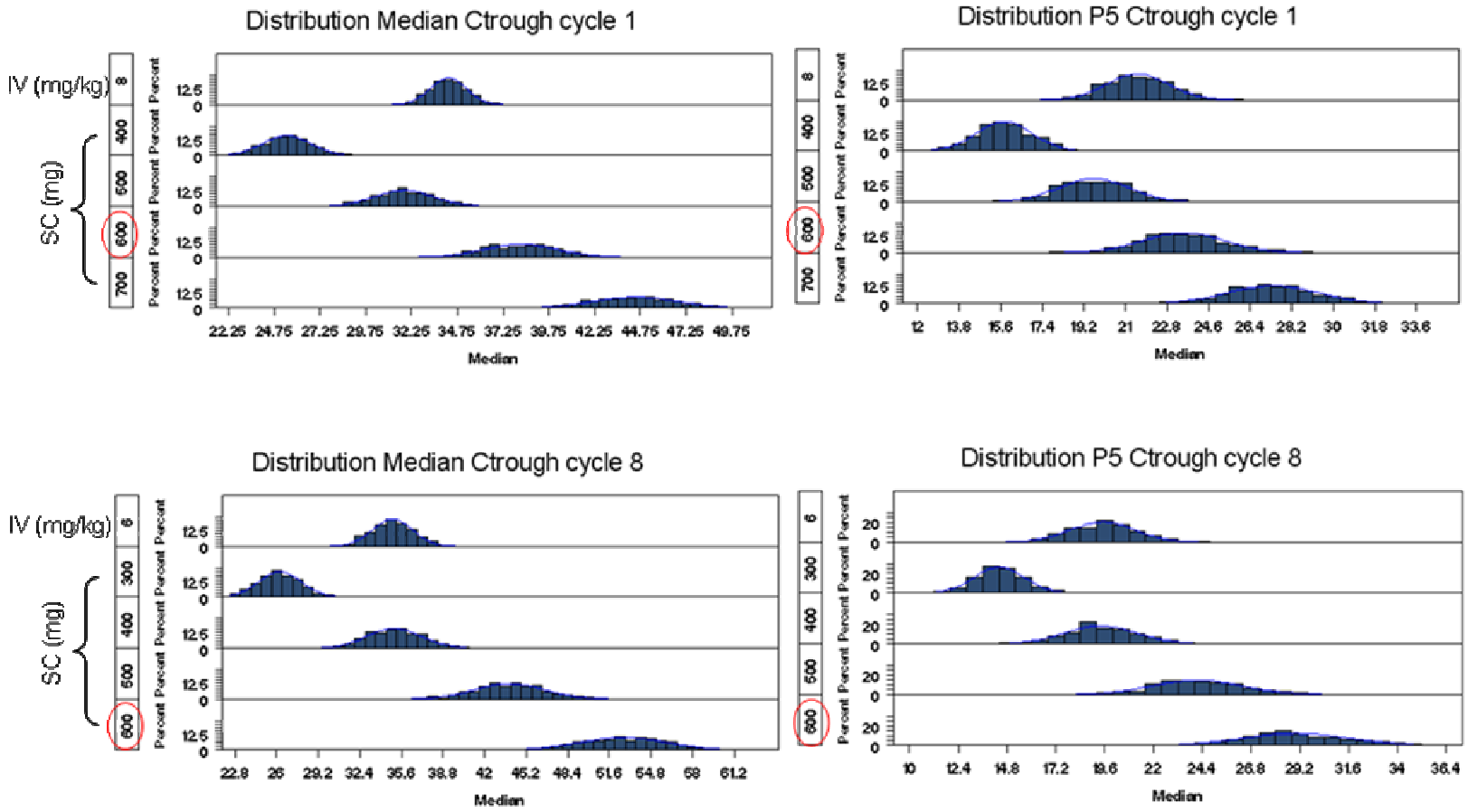
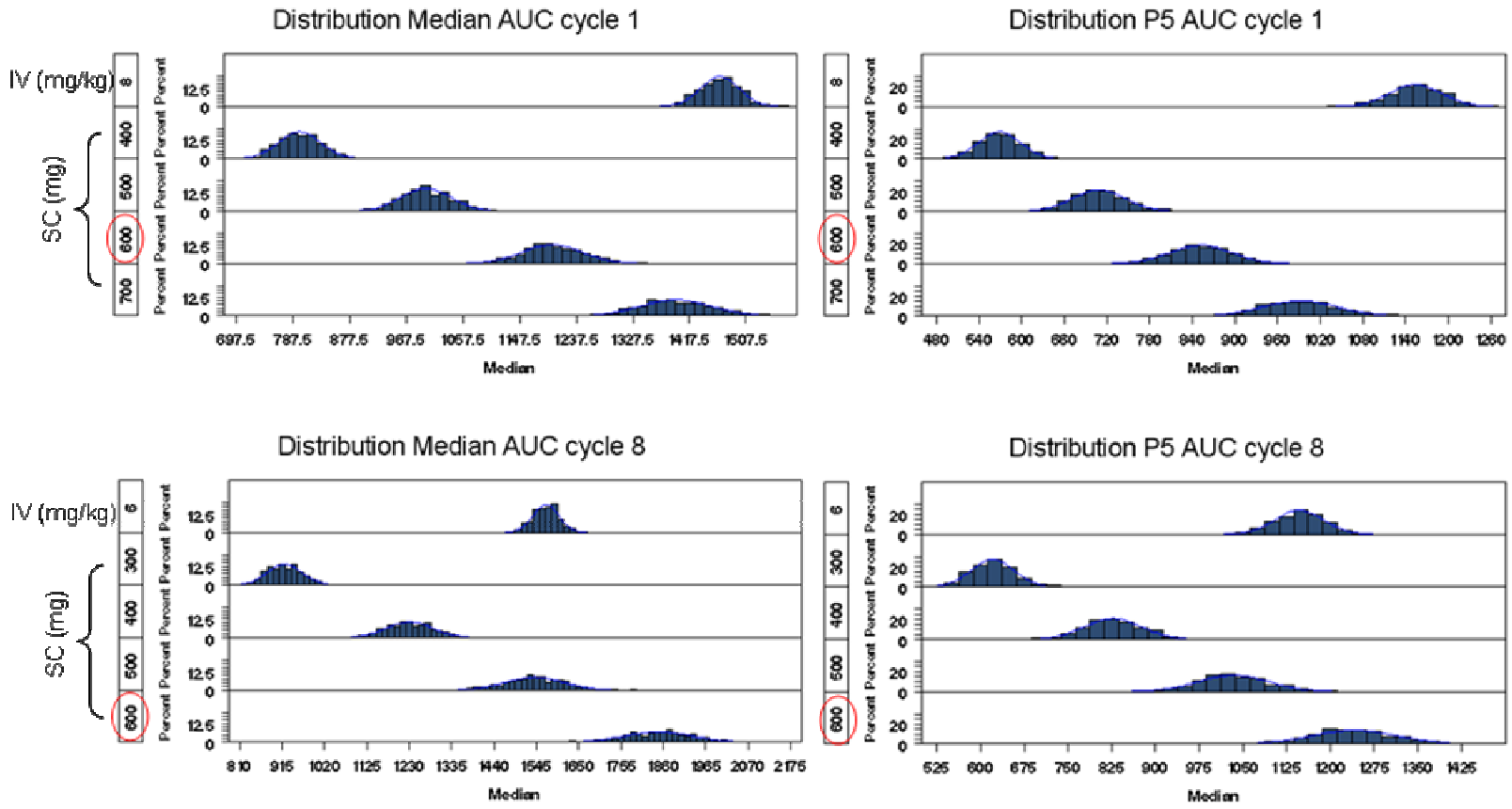


Figure 4 Distribution of Simulated Median AUC_{tau} Levels at Cycles 1 and 8



1.2 Rationale for the Study

IV trastuzumab is indicated for treatment of early breast cancer with a recommended duration of therapy of 12 months. SC administration of trastuzumab is investigated for home administration and possibly self-administration by the patient using a special administration device. Treatment with SC trastuzumab would be more convenient for patients with increased compliance over the entire treatment period. The purpose of this study is to evaluate whether comparable serum concentrations, efficacy and safety can be achieved with SC trastuzumab compared to the already marketed IV trastuzumab.

2. OBJECTIVES

2.1 Primary Objectives

To compare between SC trastuzumab and IV trastuzumab in the neoadjuvant setting

- the serum trough concentrations (C_{trough}) observed pre-surgery
- the efficacy (pathological complete response, pCR)

2.2 Secondary Objectives

To compare between SC trastuzumab and IV trastuzumab

- the observed C_{trough} concentrations post-surgery
- the predicted C_{trough} concentrations pre-surgery and post-surgery
- the pharmacokinetic profile

To evaluate in the SC trastuzumab and IV trastuzumab arm

- total pathological complete response (tpCR)
- overall response rate (ORR)
- time to response (TTR)
- event-free survival (EFS)
- overall survival *(OS)*
- safety and tolerability
- immunogenicity

3. STUDY DESIGN

3.1 Overview of Study Design

This is a phase III, randomized, open label multi-center trial in the neoadjuvant setting to investigate pharmacokinetics, efficacy and safety of SC trastuzumab versus IV trastuzumab in patients with HER2-positive early breast cancer. The study design is shown in Figure 5.

Patients with operable or locally advanced breast cancer with tumor size of ≥ 1 cm will be randomized 1:1 to receive eight cycles of either IV or SC trastuzumab concurrently with chemotherapy. Randomisation will be stratified by stage of disease and ER status. Chemotherapy consists of 75 mg/m^2 docetaxel given every 21 days for four cycles followed by four cycles of 5-fluorouracil 500 mg/m^2, epirubicin 75 mg/m^2 and cyclophosphamide 500 mg/m^2 given every 21 days. Thereafter patients will undergo

surgery without interruption of trastuzumab treatment. After surgery patients will receive additional 10 cycles of IV or SC trastuzumab as per randomization to complete one year of treatment with trastuzumab. Radiotherapy will be administered as per local practice. Hormone receptor-positive patients may receive adjuvant treatment with tamoxifen or aromatase inhibitors. After the end of study treatment, patients will be followed for efficacy and safety *for a total of 5* years, or until disease recurrence, whichever is earlier *(see Section 4.5)*.

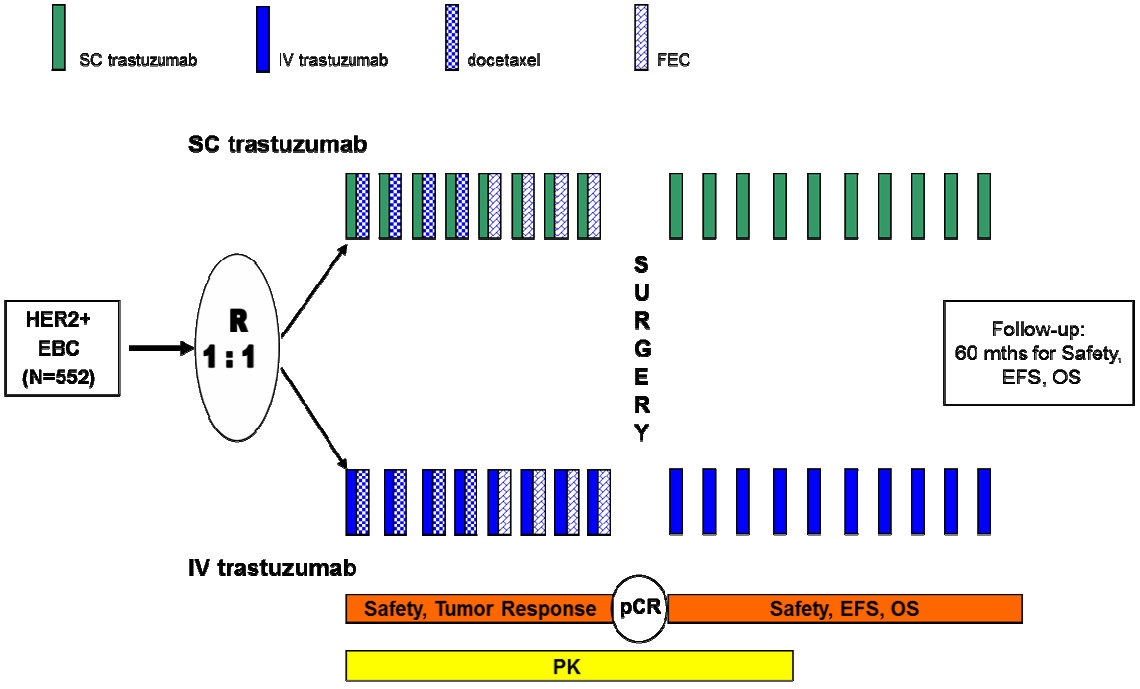
There will be a preliminary PK analysis in the first 8 patients in each arm once they have received the first dose of trastuzumab to assess if the expected drug exposure for the SC trastuzumab starting dose has been achieved. The cohort to be analysed may be expanded to 20 patients per arm in case the variability in PK is too high to allow a conclusion to be drawn.

The main analysis of PK, efficacy and safety will be performed after all randomized patients have undergone surgery (unless prematurely withdrawn) and at least 100 patients in each arm have completed cycle 18 of study treatment.

The clinical cut-off date was on 12 July 2011 and data were published [69].

A follow-up analysis for efficacy *and* safety will be performed once all patients have completed 24 months *of* treatment-free follow-up *and at the end of study (after the last patient has completed a total of 60 months of treatment-free follow-up).*

Figure 5 Study Design Flow Chart



Individual patients will be on trial for at least 73 months (from screening through study therapy completion and follow-up) for each subject as follows:

- Screening: up to 4 weeks
- Total treatment duration: 12 months
- Safety and efficacy follow-up: 60 months

3.1.1 Rationale for Study Design

SC trastuzumab would be most beneficial for patients who receive longer trastuzumab treatment without any other concomitant IV medication. This is predominantly the case in early breast cancer patients where the recommended treatment duration with trastuzumab is one year. Therefore this patient population was chosen to investigate SC trastuzumab.

This trial is a non-inferiority trial which aims to show that serum C_{trough} concentrations and efficacy of SC trastuzumab are comparable to IV trastuzumab. The neoadjuvant setting allows accelerated investigation of the efficacy of an anticancer drug and pathological complete response (pCR) has been repeatedly shown to be a reliable surrogate for long-term benefit (disease-free and overall survival) from neoadjuvant therapy [6, 9].

3.1.2 Rationale for Selection of Chemotherapeutic Regimen

Multiple trials have investigated the efficacy of IV trastuzumab in the neoadjuvant setting. Many different chemotherapeutic regimens were used, based on anthracyclines, taxanes, platinum agents or vinca alkaloids reporting pCR rates from 7% up to 78% [18]. Apart from two trials [20, 19] the available evidence stems mostly from single arm phase II studies involving only a limited number of patients. Although no optimal neoadjuvant chemotherapeutic regimen could be established as of today, it appears nevertheless that a combination of anthracyclines and taxanes is able to produce the highest pCR rates.

The chemotherapy regimen chosen for this study was derived from the combination regimen introduced by Buzdar and coworkers at the MD Anderson Cancer Center [19]. Four cycles of three-weekly paclitaxel 225 mg/m^2 and IV trastuzumab followed by four cycles of FEC (500/75/500) concurrent with IV trastuzumab investigated in a randomised trial yielded a highly significant improvement in pCR rate to 67% for the combination of chemotherapy with IV trastuzumab which led to early stopping of the trial by the DMC. Meanwhile results from 118 patients undergoing this regimen (q3w paclitaxel replaced by weekly paclitaxel with 80 mg/m^2) are available reporting consistently high pCR rates ranging between 55% and 73% with an acceptable cardiac safety profile. Asymptomatic LVEF decreases were reported in individual patients, but no symptomatic cardiac dysfunction was observed [19, 21, 22, 23]. The combination of paclitaxel plus IV trastuzumab followed by FEC concurrent with IV trastuzumab is also recommended as standard in the neoadjuvant setting for patients with HER2-positive early BC in the National Comprehensive Cancer Network (NCCN) treatment guidelines.

Since docetaxel is the registered standard of care in Europe and the three-weekly administration of chemotherapy is the preferred option for the q3w administration of trastuzumab, twelve cycles of weekly paclitaxel will be replaced by four cycles of three-

weekly docetaxel. There is no impact on efficacy expected as data from a large randomised trial in almost 5000 patients have demonstrated that 100 mg/m^2 docetaxel given three-weekly are equivalent to 12 cycles of 80 mg/m^2 paclitaxel weekly [51]. Also, docetaxel 100 mg/m^2 given concurrently with IV trastuzumab was shown to be efficacious in the neoadjuvant setting with pCR rates up to 47% [24, 52].

To increase the tolerability of docetaxel the dose will be reduced from the registered dose of 100 mg/m^2 to 75 mg/m^2 . A comparison of the efficacy and safety of different doses of docetaxel conducted in a randomised clinical trial in metastatic breast cancer demonstrated that time to progression and overall survival were comparable for 60 mg/m^2, 75 mg/m^2 and 100 mg/m^2 whilst the rates of neutropenia, febrile neutropenia or infection were notably decreased by a dose reduction of docetaxel from 100 mg/m^2 to 75 mg/m^2 [53]. Although in this trial a linear relationship between docetaxel dose and tumor response was detected (100 mg/m^2 - ORR 29.8%; 75 mg/m^2 - 22.3%, 60 mg/m^2 - 19.9%) a potential impact on the primary objective (pCR) seems acceptable given the better tolerability of the treatment. Comparability of efficacy (RFS, OS) between docetaxel 100 mg/m^2 and 80 mg/m^2 was also demonstrated by an exploratory analysis in the FinHER trial [54].

Based on the above considerations the chemotherapy regimen in this study will be docetaxel (T) 75 mg/m^2 q3w for 4 cycles followed by 5-fluorouracil (F) (500 mg/m^2), epirucubine (E) (75 mg/m^2) and cyclophosphamide (C) (500 mg/m^2) q3w for 4 cycles.

3.1.3 Rationale for Pharmacokinetic Assessments

Primary endpoint of the study is the comparison of pre-surgery observed C_{trough} levels of SC trastuzumab with those of IV trastuzumab. Sampling timepoints for PK assessments have been selected to gain sufficient data to determine the absorption, distribution and elimination phases of trastuzumab. As the C_{max} upon IV administration is expected to be higher and to occur earlier compared to SC administration, sampling time points have been selected accordingly. Samples obtained during Cycle 1 will be used as part of an early PK assessment in order to confirm the dose selected from study BP22023 for the SC arm. Samples in later cycles will be used to estimate when steady-state is reached. More intensive sampling between cycles will be used to derive the AUC for specific cycles.

3.1.4 Rationale for Dose Selection of SC and IV Trastuzumab

The dose of IV trastuzumab given throughout the full study treatment is the dose registered with the 3-weekly regimen, i.e. an 8 mg/kg loading dose followed by a 6 mg/kg maintenance dose.

SC trastuzumab will be given with a fixed dose of 600 mg with a 3-weekly regimen throughout the treatment phase independent of the patient's weight. The fixed dosing in the pre-surgery phase will allow assessment of the efficacy of SC trastuzumab for the primary efficacy endpoint pCR at the final dosing regimen intended to be used in the clinical setting. A single fixed dose has been applied for other therapeutic monoclonal antibodies, particularly in chronic conditions as rheumatoid arthritis (e.g. adalimumab), but also in oncology, e.g. pertuzumab.

The fixed dose has been calculated based on PK modeling of preliminary BP22023 data which showed that 600 mg of SC trastuzumab were able to achieve a serum C_{trough} level at least as high as the one achieved by the weight adjusted dosing (see section 1.1.2.4.3). Trastuzumab exhibits linear pharmacokinetics in the clinical dose range, which is an indication for saturation of target receptors. Therefore, when achieving C_{trough} levels with the SC formulation that are at least as high as with the IV dose, efficacy is expected to be comparable. Patients with lower body weight may be exposed to higher C_{trough} levels as if they were dosed on a weight adjusted basis. However, available reports from overdosing of IV trastuzumab do not show any detrimental effect on the patient's safety. Also, based on the preliminary data from the BP22023 study, the predicted maximal concentrations following eight three-weekly cycles of 600 mg are expected to be below the maximal serum levels of IV trastuzumab observed in the PK loading trial (MO16982, range of 199-375 mg/L) where no increase in adverse events was observed [55]

3.1.5 Rationale for Biomarker Analyses

Note: Participation in the Biomarker/translational research is optional under this protocol.

3.1.5.1 Conversion of HER2 Status

Whether the initial HER2 status of the tumor is subject to change during neoadjuvant chemotherapy is an intriguing question. The currently available evidence is limited and data are contradictory. Based on findings of a small study involving surgical tissues from 23 patients Mittendorf and coworkers reported that out of 23 patients with early breast cancer not achieving a pCR after neoadjuvant treatment with chemotherapy and concomitant trastuzumab HER2 status in 7 patients had changed from positive to negative [56]. Further publications reported a modification of the HER2-status after conventional neo-adjuvant chemotherapy without the use of trastuzumab [57, 58], which directly contradicts results from similar studies, where the HER2 status of the tumor remained stable under chemotherapy treatment [59]. Further data are warranted to explore this controversial issue.

The re-assessment of the HER2 status after the neoadjuvant treatment phase on available surgical samples is an optional part of the present study and will allow to evaluate the incidence of HER2 conversion.

3.1.5.2 Fcγ Receptor-Polymorphism

There is increasing evidence that the Fc portion of anti-tumor monoclonal antibodies is a major component of their therapeutic activity, through binding to Fcγ receptors expressed by effector cells present in the tumor microenvironment. The antibody-dependent cell-mediated cytotoxicity (ADCC) is mediated by natural killer (NK) cells and macrophages. The Fcγ receptors expressed on their cell surface recognize trastuzumab bound to HER2 molecules of the tumor cells, thus triggering destruction of these tumor cells. Studies investigating whether polymorphisms of the Fcγ-receptor gene influence clinical outcome of breast cancer patients who receive trastuzumab have reported inconsistent results so far. A retrospective analysis of patients with heavily pretreated HER2-positive metastatic breast cancer who received trastuzumab monotherapy did not demonstrate any association between Fcγ -receptor polymorphism and clinical outcome [60]. On the other hand, a recently published trial showed that in the 54 patients with

HER2 positive metastatic breast cancer distinct variants of Fcγ -receptors significantly correlated with response rate and progression-free survival [61].

In order to further explore the relationship of Fcγ-receptor polymorphisms and response to trastuzumab 3 ml of whole blood samples will be taken at baseline.

3.1.6 End of Study

The end of study will be when the last patient has completed at least *60* months of follow-up after end of treatment.

3.2 Number of Patients/ Assignment to Treatment Groups

Approximately 552 patients, (276 per treatment arm) will be recruited over a planned recruitment period of about 16 months.

3.3 Centers

This is a multicentre, multinational trial involving approximately 200 sites.

4. STUDY POPULATION

Under no circumstances are patients who enrol in this study permitted to be re-randomised to this study and enrolled for a second course of treatment.

4.1 Overview

This study will recruit women with HER2-positive early breast cancer clinical stage I to III whose primary tumors are ≥ 1 cm.

4.2 Inclusion Criteria

Patients must meet ALL of the following criteria in order to be eligible for this study.

1. Patients must have signed and dated an informed consent form
2. Female
3. Age ≥ 18 years
4. Non-metastatic primary invasive adenocarcinoma of the breast which is clinical stage I (T1, N0, M0) to IIIC (any T, N3, M0) including inflammatory and multicentric/multifocal a breast cancer
    a. with tumor size ≥ 1 cm by ultrasound or ≥ 2 cm by palpation
    b. histologically confirmed
    c. centrally confirmed HER2 positive (IHC3+ or ISH+)
5. At least one measurable lesion in breast or lymph nodes (≥ 1 cm by ultrasound or ≥ 2 cm by palpation), except for inflammatory carcinoma (T4d)
6. Performance status ECOG of 0-1
7. Baseline LVEF ≥ 55% measured by echocardiography or MUGA scan prior to first dose of trastuzumab

4.3 Exclusion Criteria

Patients meeting any ONE of the following criteria are not eligible for this study.

1. History of any prior (ipsi- and/or contralateral) invasive breast carcinoma [b]
2. Past or current history of malignant neoplasms, except for curatively treated:
    a. Basal and squamous cell carcinoma of the skin
    b. *in situ* carcinoma of the cervix
3. Metastatic disease
4. Any prior therapy with anthracyclines
5. Prior use of anti-HER2 therapy for any reason or other prior biologic or immunotherapy
6. Concurrent anti-cancer treatment in another investigational trial, including immunotherapy
7. Patients with severe dyspnoea at rest or requiring supplementary oxygen therapy, patients with other concurrent serious diseases that may interfere with planned treatment including severe pulmonary conditions/illness
8. Serious cardiac illness or medical conditions that would preclude the use of trastuzumab, specifically: history of documented CHF, high-risk uncontrolled arrhythmias, angina pectoris requiring medication , clinically significant valvular disease, evidence of transmural infarction on ECG, poorly controlled hypertension,
9. Medical conditions that would preclude the use of 5-fluorouracil, epirubicin, cyclophosphamide or docetaxel, including: cystitis, urinary obstruction, active infections or severe mucositis
10. History of severe allergic and immunological reactions, e.g. difficult to control asthma
11. Known hypersensitivity to any of the study drugs or any of the excipients, known hypersensitivity to murine proteins
12. Known dihydropyrimidine dehydrogenase (DPD) deficiency
13. Any of the following abnormal laboratory tests at baseline:
    a. Biochemistry:
        i. serum total bilirubin > 1.25 x upper limit of normal (ULN)
        ii. alanine amino transferase (SGPT, ALT) or aspartate amino transferase (SGOT, AST) > 2.5 x ULN
        iii. alkaline phosphatase (ALP) > 2.5 x ULN
        iv. serum creatinine > 1.5 x ULN;

b. Hematology:

i. absolute neutrophil count (ANC) < 1.5 x 109/L

ii. platelets < 100 x 109/L

iii. haemoglobin < 10 g/dl

14. Pregnant or lactating women c

15. Women of childbearing potential or less than one year after menopause (unless surgically sterile) who are unable or unwilling to use adequate contraceptive measures during study treatment d

16. Patients unwilling or unable to comply with protocol procedures

[a] A patient is considered to have multifocal breast cancer if all lesions are within the same
[b] breast quadrant. Otherwise her breast cancer will be considered as multicentric.
[c] Previous history of ductal carcinoma in situ (DCIS) of the breast is not an exclusion criterion
[d] Women of child bearing potential must have a negative pregnancy test (serum) within 7 days prior to randomisation and/or trastuzumab treatment
[e] Menopause is defined in this study, as patients who have had, at an absolute minimum, 12 consecutive months of amenorrhoea during which time no other biological or physiological cause had been identified as a potential cause of this state [67]. Examples of adequate contraceptive measures are intra-uterine device, barrier method (condoms, diaphragm), also in conjunction with spermicidal jelly, or total abstinence. Oral, injectable, or implant

hormonal contraceptives are not acceptable.

4.4 Concomitant Medication and Treatment

All concomitant medication(s) (including premedication and antiemetic therapy) must be reported in the electronic case report form. In addition, any diagnostic, therapeutic or surgical procedure performed during the study period, must be recorded.

Any medication which is necessary for the management of side effects of chemotherapy may be used at the discretion of the investigator. Paracetamol (acetaminophen), and antihistamines and other supportive medication may be used according to local clinical practice, for the treatment of infusion reactions associated with SC and IV trastuzumab.

Hematopoietic growth factors may be used to treat symptomatic neutropenia. In patients with complicated neutropenia (including prolonged neutropenia, febrile neutropenia, or infection), it is recommended to use G-CSF to provide prophylactic coverage (eg, day 4 to 11) in all subsequent cycles.

Patients with anemia should be treated according to routine clinical practice and local guidelines. It is recommended that hemoglobin be maintained above 10 g/dL.

Anti-emetics should be chosen in compliance with the conventional anti-emetic protocol of each clinical site. Mesna should be given with cyclophosphamide treatment, according to local protocols.

Post-surgery radiotherapy may be administered according to local practice. Trastuzumab treatment post-surgery should be given concomitantly with radiotherapy treatment. In order to minimize cardiac irradiation, all patients who receive internal mammary node

(IMN) irradiation must have the radiotherapy planned using a technique that enables minimization of cardiac irradiation, preferably 3-D conformational CAT scan.

Adjuvant tamoxifen*, luteinizing-hormone-releasing hormone (LHRH) agonists,* or an aromatase inhibitor may be administered to patients with hormone receptor (estrogen and/or progesterone receptor) positive disease after completion of surgery, according to local practice. Tamoxifen or aromatase inhibitors may be given concomitantly with trastuzumab and radiotherapy.

Patients may have started bisphosphonate therapy for their licensed indication before entering the study. Bisphosphonate therapy can also be initiated during the study for the treatment of documented osteoporosis. The use of bisphosphonates for prevention of bone metastases is not allowed before being licensed for this indication.

The following treatments are not permitted:

- Treatment with other systemic anti-cancer agents (e.g. chemotherapy, hormonal therapy different from the above mentioned, immunotherapy) or other treatments not part of protocol-specified anti-cancer therapy
- Concurrent investigational agents of any type

4.5 Criteria for Premature Withdrawal

Patients have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, treatment failure after a prescribed procedure, protocol violation, cure, administrative reasons or for other reasons. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided.

Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. The investigator should contact the patient or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation should be made within 30 days from last study drug (see Table 8) including a physical examination, assessment of hematological and biochemical laboratory parameters (tumor assessment by caliper and ultrasound if prior to surgery) with an explanation of why the patient is withdrawing from the study. If the reason for removal of a patient from the study is an adverse event the principal specific event will be recorded on the eCRF.

In the case that the patient decides to prematurely discontinue study treatment [“refuses treatment”], she should be asked if she can still be contacted for collection of further information. The outcome of that discussion should be documented in both the medical records and in the eCRF.

Patients who withdraw due to progression of disease at any time during the study, will be managed as per local practice and followed for survival only *every 6 months until the end of study*. Patients who discontinue study treatment prematurely due to lack of tolerability prior to surgery will be managed as per local practice and followed for survival only *every 6 months until the end of study***.** Patients who discontinue trastuzumab treatment after surgery prematurely may stay on the trial and will be followed for DFS, OS and safety.

All prematurely withdrawn patients, who have received at least one dose of trastuzumab and are clinically followed outside of the study, should be monitored regarding cardiac function as described in the Herceptin© SmPC.

4.6 Replacement Policy (Ensuring Adequate Numbers of Evaluable Patients)

4.6.1 For Patients

Patients randomized into the study will not be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow or no recruitment
- Poor protocol adherence

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

The complete schedule of assessments is provided in Table 6, Table 7, and Table 8. Table 6 and Table 7 outline the schedule of assessments during the treatment phase of the study starting with first drug administration. Please note that surgery needs to be performed after completion of 8 cycles of chemotherapy without interruption of the trastuzumab administration schedule. Table 8 describes the assessments to be performed in the follow-up phase of *60* months starting with the dose of last study drug.

Please note: this trial has a PK parameter as primary endpoint (observed C_{trough} levels of trastuzumab). It is therefore of utmost importance to adhere to the visiting schedule and to take the PK samples on the exact day scheduled.

Table 6 Schedule of Assessments (Treatment Period prior to Surgery)

	Screening	Baseline	Treatment Period															
Study Week			**1**			**4**	**7**	**10**	**13**	**16**	**19**					**22**	
Cycle			**1**			**2**	**3**	**4**	**5**	**6**	**7**					**8**	**S[13]**
Day of Cycle	-28 to -1	-7 to -1	1	2	15	1	1	1	1	1	1	2	4	8	15	1	1
Informed Consent[0]	X																
Demographics	X																
Medical History	X																
Physical Examination[1]	X		X						X								
Vital signs[1]	X		X						X								
ECOG performance status[1]	X		X						X								
Height	X																
Weight[1]	X		X			X	X	X	X	X	X					X	
Chest X-ray	X																
Liver imaging	(X)																
Bone scan[2]	(X)																
Bilateral mammogram	X																
Clinical tumor assessment by caliper and ultrasound (including lymph nodes)[3]		X					X		X		X						X
ECG	X								X								
LVEF (Echo or MUGA)	X								X								
Hematology and Biochemistry[1,4]		X	X[14]			X	X	X	X	X	X					X	
Serum Pregnancy Test		X															
Urinalysis (Dipstick)		**X**							X[1]								X
HER2 status local lab[5]	X																
Hormone receptor status (ER, PgR) local lab[5]	X																
Pathologist post-surgery tumor assessment																	X

Table 6 Schedule of Assessments (Treatment Period prior to Surgery) (Cont.)

	Screening	Baseline	Treatment period														
Study Week			**1**			**4**	**7**	**10**	**13**	**16**	**19**					**22**	
Cycle			**1**			**2**	**3**	**4**	**5**	**6**	**7**					**8**	**S[13]**
Day of Cycle	-28 to -1	-7 to -1	1	2	15	1	1	1	1	1	1	2	4	8	15	1	1
Blood sample for PK analysis (SC arm), (2 ml)[6]			X[15]	X	X		X[15]	X[15]		X[15]	X[15]	X	X	X	X	X[15]	
Blood sample for anti-trastuzumab analysis (SC arm), (2 ml)		X															
Blood sample for PK and anti-trastuzumab analysis (SC arm) (5 ml)[6]						X[15]			X[15]								
Blood sample for anti-rHuPH20 analysis (SC arm) (2 ml)		X				X[15]			X[15]								
Blood sample for PK analysis (IV arm), (2 ml)[6]			X[16] X[17]	X	X	X[17]	X[16] X[17]	X[16] X[17]	X[17]	X[16] X[17]	X[16] X[17]	X	X	X	X	X[16] X[17]	
Blood sample for anti-trastuzumab analysis (IV arm), (2 ml)		X															
Blood sample for PK and anti-trastuzumab analysis (IV arm) (5 ml)[6]						X[16]			X[16]								
Blood sample (optional) for FcγR polymorphism (3 ml)		X															
Tumor sample at screening (mandatory)[7]	X																
Tumor sample at surgery (optional)[8]																	X
Trastuzumab (SC or IV)			X[18]			X	X	X	X	X	X					X	
5-Fluorouracil[9]									X	X	X					X	
Epirubicin[10]									X	X	X					X	
Cyclophosphamide[11]									X	X	X					X	
Docetaxel[12]			X			X	X	X									
Adverse events			all AEs and SAEs														
Concomitant medication			continuous														

Table 6 Schedule of Assessments (Treatment Period prior to Surgery) (Cont.)

Notes

Cycle 1, Day 1 = first dose of study drug.

Clinical visits must be scheduled within ± 3 days of the day specified. PK samples need to be taken at the exact day of the visiting schedule.

[0] Informed Consent must be obtained before any study-specific procedures

[1] Must be performed pre-dose on dosing days

[2] Plain films (CT scan in the case of vertebral column abnormalities) are required to exclude metastatic disease if a bone scan is positive. In the absence of radioactive isotopes, MRI scan (with gadolinium enhancement if required) or F18 PET scan is an acceptable form of assessment of the skeleton for the presence of bone metastases

[3] If there is suspicion of disease progression based on clinical or laboratory findings before the next scheduled tumor assessment, an unscheduled assessment should be performed. Tumor assessments are to be performed within up to 7 days prior to dosing or surgery. If the response is CR or PR, no additional tumor assessment needs to be performed as the next scheduled tumor assessment is already to be done after 6 weeks.

[4] Hematology: hemoglobin, total WBC, absolute neutrophil count (ANC)/neutrophils, platelet count. Biochemistry: creatinine, urea (BUN), SGPT (ALT), SGOT (AST), total bilirubin, alkaline phosphatase, albumin, sodium, potassium and calcium

[5] Must be performed in tissue from core biopsy or open biopsy (fine needle aspiration not acceptable)

[6] The date/time of PK sampling must be carefully recorded in all cases. On non-dosing days, the timing of PK sampling during the day is left to the investigator (however, the time should be carefully recorded). Fasting is not a requirement

[7] Tumor sample at screening is to be used for local HER2 and hormone receptor status testing. If locally HER2 positive the tumor sample must be sent for central HER2 status confirmation to the central laboratory. This tumor sample is also used for the RCR project (optional)

[8] For all those patients without pCR in the breast the tumor sample taken at surgery will be used for the analysis of HER2 conversion (optional) and the RCR project (optional)

[9] 5-Fluorouracil will be administered at 500mg/m^2 on day 1 of cycles 5 to 8. It may be given as an IV bolus over 3 -5 minutes or as an infusion over 30 minutes. It is to be given after completion of trastuzumab administration.

[10] Epirubicin will be administered at 75mg/m^2 on day 1 of cycles 5 to 8. It may be given as an IV bolus over 3 - 5 minutes or as an infusion over 30 minutes. It is to be given after completion of trastuzumab administration.

[11] Cyclophosphamide will be administered at 500mg/m^2 on day 1 of cycles 5-8. It should be given as an IV bolus over 3 - 5 minutes. It is to be given after completion of trastuzumab administration.

[12] Docetaxel will be administered at 75 mg/m^2 as an IV infusion over 60 (± 10) minutes. It is to be given after completion of trastuzumab administration.

[13] After 8 cycles of chemotherapy the patient undergoes surgery. The SC or IV trastuzumab administration schedule should not be interrupted.

[14] Does not need to be repeated if baseline hematology and biochemistry was performed within 3 days prior to cycle 1

[15] Take sample pre-dose

[16] Take sample pre-infusion

[17] Take additional PK sample at the end of the infusion

[18] Loading dose for IV trastuzumab is 8mg/kg followed by maintenance doses of 6mg/kg. SC trastuzumab is given as a fixed dose of 600 mg.

(X) Parentheses indicate that the item is optional, required only if symptoms or clinical suspicion are present

Table 7 Schedule of Assessments (Treatment period after Surgery)

Study Week	**25**			**28**	**31**	**34**					**37**	**40**	**43**	**46**	**49**	**52**
Cycle	**9**			**10**	**11**	**12**					**13**	**14**	**15**	**16**	**17**	**18**
Day of cycle	1	2	15	1	1	1	2	4	8	15	1	1	1	1	1	1
Physical examination	X										X					X
Vital signs	X										X					X
ECOG performance status	X										X					X
Weight	X										X					X
Chest X-ray																(X)
Liver imaging																(X)
Bone scan[0]																(X)
Bilateral mammogram[1]																X
Hematology and Biochemistry[2]	X										X					X
Blood sample for PK analysis (SC arm), (2 ml)[3]	X[4]	X	X	X[4]	X[4]	X[4]	X	X	X	X						
Blood sample for PK and anti-trastuzumab analysis (SC arm) (5 ml)[3]											X[4]					X[4]
Blood sample for anti-rHuPH20 analysis (SC arm) (2 ml)											X[4]					X[4]
Blood sample for PK analysis (IV arm), (2 ml)[3]	X[5] X[6]	X	X	X[5] X[6]	X[5] X[6]	X[5] X[6]	X	X	X	X						
Blood sample for PK and anti-trastuzumab analysis (IV arm) (5 ml)[3]											X[5]					X[5]
ECG	X										X					X
LVEF (Echo or MUGA)	X										X					X
Trastuzumab (SC or IV)[4]	X			X	X	X					X	X	X	X	X	X
Adverse events	all AEs and SAEs															
Concomitant Medication	continuous															

Table 7 Schedule of Assessments (Treatment period after Surgery) (Cont.)

Notes

Clinical visits must be scheduled within ± 3 days of the day specified. PK samples need to be taken at the exact day of the visiting schedule.

[0] Plain films (CT scan in the case of vertebral column abnormalities) are required to exclude metastatic disease if a bone scan is positive. In the absence of radioactive isotopes, MRI scan (with gadolinium enhancement if required) or F18 PET scan is an acceptable form of assessment of the skeletion for the presence of bone metastases.

[1] Unilateral for patients with mastectomy.

[2] Hematology: hemoglobin, total WBC, absolute neutrophil count (ANC)/neutrophils, platelet count. Biochemistry: creatinine, urea (BUN), SGPT (ALT), SGOT (AST), bilirubin, alkaline phosphatase, albumin, sodium, potassium and calcium

[3] The date/time of PK sampling must be carefully recorded in all cases. On non-dosing days, the timing of PK sampling during the day is left to the investigator (however, the time should be carefully recorded). Fasting is not a requirement

[4] Take sample pre-dose

[5] Take sample pre-infusion

[6] Take additional PK sample at the end of the infusion

(X) Parentheses indicate that the item is optional, required only if symptoms or clinical suspicion are present

Table 8 Schedule of Assessments (Treatment-free Follow-up)

	Final Visit (Completion of Treatment/ premature Withdrawal) [1]	Follow-up Visits[4]										
Month from last dose of study drug	1	3	6	12	18	24	*30*	*36*	*42*	*48*	*54*	*60*
Physical Examination	X	X	X	X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
Vital signs		X	X	X	X	X						
ECOG performance status			X	X	X	X						
Chest X-ray			(X)	(X)		(X)		*(X)*		*(X)*		*(X)*
Liver imaging			(X)	(X)		(X)		*(X)*		*(X)*		*(X)*
Bone scan[2]			(X)	(X)		(X)		*(X)*		*(X)*		*(X)*
Bilateral mammogram[3]				X		X		*X*		*X*		*X*
Clinical tumor assessment by caliper and ultrasound (including lymph nodes)	X[6]											
Hematology and Biochemistry	X	X	X	X	X	X						
ECG and LVEF (Echo or MUGA)[8]			X	X	X	X		*X*		*X*		*X*
Blood sample for PK and anti-trastuzumab analysis (SC arm) (5 ml)		X	X									
Blood sample for anti-trastuzumab analysis (SC arm) (2 ml)				X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
Blood sample for anti-rHuPH20 analysis (SC arm) (2 ml)		X	X	X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
Blood sample for PK and anti-trastuzumab analysis (IV arm) (5 ml)		X	X									
Blood sample for anti-trastuzumab analysis (IV arm) (2 ml)				X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
Concomitant Medication	Continuous[7]											
Adverse events	Continuous[5]											

Notes:

[1] 30 days (± 5 days) after the last dose of study treatment.

[2] Plain films (CT scan in the case of vertebral column abnormalities) are required to exclude metastatic disease if a bone scan is positive. In the absence of radioactive isotopes, MRI scan (with gadolinium enhancement if required) or F18 PET scan is an acceptable form of assessment of the skeletion for the presence of bone metastases.

[3] Unilateral for patients with mastectomy *or breast implant*

[4] Visit to be performed within ± 15 days

[5] See section 7.2.1. and 7.2.2 for timeframes of (S)AE reporting

[6] Only for patients who discontinue treatment prematurely prior to surgery

[7] Concomitant medication only to be collected until 28 days after end of study treatment

[8] *A significant drop in LVEF identified by MUGA scan or echocardiography requires a follow-up LVEF for confirmation approximately three weeks later.*

(X) Parentheses indicate that the item is optional, required only if symptoms or clinical suspicion are present

5.1 Screening Examination and Eligibility Screening Form

All patients must provide written informed consent before any study specific assessments or procedures are performed.

A screening/baseline examination checking all parameters described in the visiting schedule Table 6 should be performed between -28 and -1 days before the first study dose of study drug (Day 1). Patients who fulfill all the inclusion and none of the exclusion criteria can be randomized into the study.

An eligibility screening form (ESF) documenting the patient's fulfillment of the entry criteria for all patients considered for the study and subsequently included or not included, is to be completed by the investigator/designee. Patients who are considered for study entry, but fail to meet the eligibility requirements, should also have an ESF completed with the reason for lack of eligibility given, since this provides information on the selected trial population. This information will not be entered on the clinical trial database but will be collected on the database of the central randomization centre via screen-failure calls with the reason for screen failure. All ESFs should be kept in the study files at the sites.

5.2 Procedures for Randomization of Eligible Patients

Randomization will be conducted by a central randomization center.

To ensure accurate and timely monitoring of patient randomization, the following procedures will be implemented:

- Patients who are candidates for the study will be evaluated for eligibility by the investigator to ensure that the criteria given in Sections 4.2 and 4.3 have been satisfied, and that the patient is eligible for participation in this clinical study
- Eligible patients must be randomized via a central randomization centre

Patients who are eligible for study entry, will be randomly assigned (1:1) to one of the two treatment groups via the central randomization centre.

Randomization will be stratified by

1. breast cancer type (operable versus locally advanced versus inflammatory breast cancer; for staging see Appendix 7)
2. estrogen receptor status (positive, negative, unknown)

The randomization number and eCRF/patient number will be provided to the investigator when he/she contacts the central randomization centre at the time of individual patient enrolment once all eligibility criteria have been fulfilled. The randomization number and eCRF/patient number will be entered in the appropriate section in the eCRF. Patients should receive their first dose of study treatment on the day of randomization.

A Patient Randomization and Identification Code List must be maintained by the investigator.

The password-protected and/or encrypted electronic Master Randomization List will be kept in a central repository by the Biometrics and Drug Safety Departments. No open key to the code will be available at the study center, to the Roche monitors, project statisticians, or to the project team at Roche.

5.3 Clinical Assessments and Procedures

5.3.1 Diagnosis of Breast Cancer and Surgical Assessment

Diagnosis of primary breast cancer will be performed histologically as per local standard of care. The site surgeon will assess the breast tumor during screening and will identify the planned surgical procedure required after completion of cycle 8 treatment.

Following surgery the actual surgery procedure performed will be reported in the eCRF.

HER2-testing at diagnosis will be performed as per local standard of care, and must be confirmed in a central laboratory prior to randomization.

5.3.2 Assessment of Progression of Disease and Disease Recurrence

5.3.2.1 Clinical Tumor Response

Clinical tumor response will be evaluated according to RECIST criteria [62] with the following additional specifications: as imaging equipment and local practice may vary, RECIST guidelines have been adapted to the neoadjuvant setting inasmuch as clinical tumor response will be measured using assessment by caliper and ultrasound in order to achieve consistency in tumor assessments across sites. In addition to the primary breast tumor(s), affected lymph nodes if present, will be considered target lesions, provided they have a size of >= 1cm by ultrasound or >=2 cm by palpation. Inflammatory Breast Cancer will be considered as non-target lesion in this protocol. Response of inflammatory breast cancer will be assessed based on the criteria in Table 9.

All tumor measurements should be performed with the same method of assessment and made by the same investigator/radiologist for each patient during the study to the extent that this is feasible. In case of inflammatory breast cancer and clinically measurable superficial (such as skin) lesions, repeated photographs should be used to document tumor response. These photos must include a ruler for documentation purposes.

Table 9 Assessment of Response for Inflammatory Breast Cancer

Parameter	CR	PR	SD	PD*
Edema	Complete Resolution	Decrease or stable	Decrease or stable	Progression
Erythema	Complete Resolution	Clear decrease	Stable	Progression

If both signs are present the response will be based on evaluation of both of them. PD is defined by the progression of any of the two signs if both are present.
In patients presenting with edema only, decrease will be considered as PR, no change (stable) will be considered SD.

Total tumor burden must be assessed within a maximum of 28 days before first dose of study drug. A screening chest X-ray and bilateral mammogram is mandatory. A screening bone scan and liver imaging are required only if symptoms or clinical suspicion are present.

Before starting treatment, the tumor should be marked using the method which is standard locally (for example skin tattoo or surgical clip) so that the appropriate excision can be made should the patient experience complete clinical regression of the tumor during therapy.

Post-baseline tumor assessments are to be performed every other cycle (see Table 6) and prior to surgery. Tumor assessments will be performed on the day of dosing or surgery, or within 7 days prior to dosing or surgery. If there is suspicion of disease progression based on clinical findings before the scheduled tumor assessment, an unscheduled assessment should be performed. If a lesion shows clear signs of progression the patient will be removed from study treatment and provided with the local standard of care.

If tumor response is observed at any scheduled assessment, no additional tumor assessment will need to be scheduled for confirmation. The next following tumor assessment will be used for response confirmation.

Detection of contralateral ductal carcinoma *in situ* (DCIS) during cycle 1 to 8 will not be considered progressive disease. However, invasive contralateral breast carcinoma will be classified as progressive disease.

5.3.2.2 Pathological Complete Response (pCR)

Pathological complete response of the primary tumor is defined as absence of invasive neoplastic cells at microscopic examination of the primary tumor remnants after surgery following primary systemic therapy. The response should be classified as pCRis in case only in-situ carcinoma is found in the tumor remnants.

pCR will be assessed by the local pathologist following surgery and will not be independently reviewed. Guidelines for assessment of pCR are outlined in Appendix 2.

Total pCR is defined as absence of invasive neoplastic cells at microscopic examination of the primary tumor remnants and the axillary lymph nodes after surgery following primary systemic therapy.

5.3.2.3 Disease Recurrence (Post Surgery)

Event-free survival (EFS) is a secondary endpoint in this study. EFS is defined as time to local, regional or distant recurrence, contralateral breast cancer or death due to any cause.

Post-surgery, screening for relapse/metastatic disease should be performed after completion of trastuzumab treatment and during follow-up phase at months 6, 12, 24*, 36, 48, and 60* (see Table 7, Table 8). The assessments include a mandatory mammogram. Chest X-ray**,** bone scan, and liver imaging are required only if symptoms or clinical suspicion are present.

Diagnosis of breast cancer relapse will be made when clinical, radiological and laboratory findings meet specific criteria as defined below.

a) Local recurrence

- In the ipsilateral breast after surgery
    a) In case of conservative surgery (lumpectomy): Defined as evidence of tumor, except lobular carcinoma *in situ*, in the ipsilateral breast after mass excision. Patients who develop clinical evidence of tumor recurrence in the remainder of the ipsilateral breast should have positive histology or cytology of the suspicious lesion to confirm the diagnosis.

b) In case of mastectomy, local recurrence (other than ipsilateral breast after lumpectomy): Defined as evidence of tumor confirmed by positive histology or cytology in any soft tissue or skin of the ipsilateral chest wall after mastectomy.

b) Regional recurrence: Defined as the development of tumor in the ipsilateral internal mammary and/or ipsilateral axillary lymph nodes, as well as extranodal soft tissue of the ipsilateral axilla confirmed by positive histology or cytology, or by chest-x-ray, CT-scan or MRI. Regional recurrence does not include supraclavicular lymph nodes or tumor in the opposite breast.

c) Distant recurrence: Defined as evidence of tumor in all areas, with the exception of those described in the aforementioned sections a) and b). The following criteria apply:

- Skin, subcutaneous tissue, and lymph nodes (other than local or regional) confirmed by positive cytology, aspirate or biopsy, or radiological (by CT scan or MRI or ultrasound).
- Bone, confirmed by X-ray, CT scan, or MRI evidence of lesions consistent with bone metastasis, or histological/cytological proof of bone metastases.
- Bone marrow, confirmed by positive cytology or histology or MRI scan.
- Lung confirmed by X-ray evidence of multiple pulmonary nodules consistent with pulmonary metastases or positive histology/cytology.
- Liver, positive abdominal CT scan, liver scan, ultrasound, or MRI consistent with liver metastases, or positive liver biopsy/fine needle aspiration.
- Central nervous system, positive MRI or CT scan, or positive cytology of the cerebrospinal fluid in case of meningeal involvement.

d) d) Contralateral invasive breast cancer: confirmed by positive cytology or histology.

5.3.3 Performance status

Performance Status (PS) will be measured using the ECOG Performance Status Scale (see Appendix 3).

5.3.4 Clinical Safety Assessments

The NCI CTCAE version 3 (Appendix 6) will be used to evaluate the clinical safety of the treatment in this study. Symptomatic left ventricular dysfunction will in addition be graded according to the NYHA classification (see Appendix 5). Patients will be assessed for adverse events at each clinical visit and as necessary throughout the study. A complete medical history (including demographics) will be performed at screening. A physical examination will be performed as indicated in the schedule of assessments (Table 6, Table 7 and Table 8).

5.3.4.1 LVEF Assessment

Echocardiography or MUGA scan will be used to assess left ventricular ejection fraction (LVEF). The method of assessment chosen needs to be maintained throughout the study.

The screening LVEF assessment should be performed within ≤ 28 days prior to first drug administration and the LVEF must be ≥ 55 to be eligible for this study.

Echocardiography is the preferred method to evaluate cardiac function and will be conducted as specified in the schedule of assessments and as medically indicated (see Appendix 4). It is recommended that the same echocardiographist perform the cardiac evaluations on a patient throughout the study and that the Simpson method be used.

If MUGA scan is chosen investigators must be aware that there may be local guidelines which govern how many MUGA scans (or amount of irradiation) a patient may have in a year, and must ensure that patients are able to adhere to the cardiac assessment schedule as outlined in Table 6, Table 7, Table 8. In case more than the scheduled LVEF assessments become necessary for medical management of the patient the investigator may use echocardiography instead of MUGA scan to remain within the locally accepted amount of irradiation.

5.3.4.2 ECG

A twelve lead ECG needs to be performed at screening, during the study as specified in the schedule of assessments (see Table 6, Table 7 and Table 8) and as clinically indicated.

5.4 Laboratory Assessments

Biological samples taken from all patients are not known to be infectious and will therefore be classified as "non-infectious/diagnostic specimens" for dispatch purposes.

5.4.1 HER2 Testing for Eligibility

Patients must be HER2-positive in order to be eligible for this study. HER2 status needs to be assessed locally within 28 days before first study drug as initial screen but needs confirmation of HER2 positivity by the central laboratory before the patient can be randomised. Only in exceptional cases where local lab facilities are not available permission may be granted for centers to send tumor samples directly to the central lab.

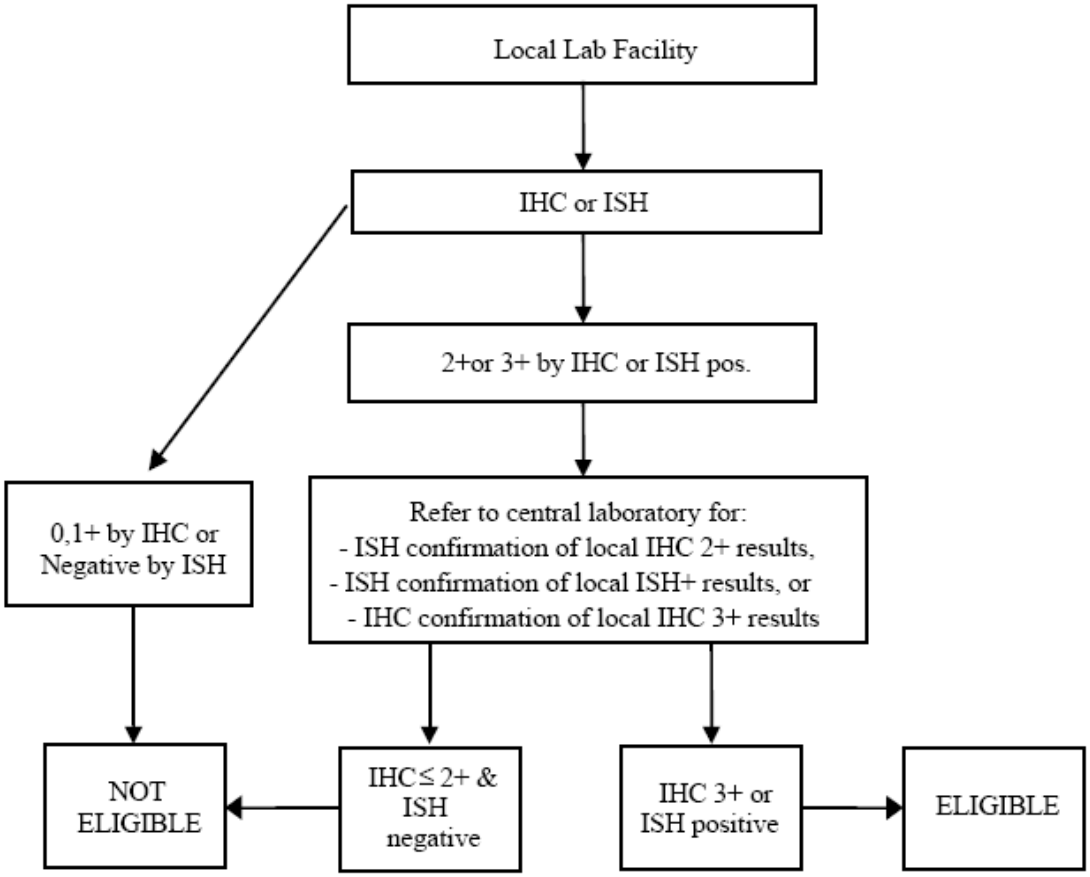
HER2-positivity is either evaluated by measuring HER2 overexpression on a protein level by IHC and/or HER2 gene amplification on a DNA level by an ISH technique.

The following criteria define a HER2 positive status on the invasive part of the tumor:

- a score of 3+ by IHC or
- a score of 2+ by IHC that is also positive by ISH or
- a positive ISH result

Local HER2 testing should be done on validated assays systems and according to recommendations outlined in the SmPC for IV trastuzumab (Herceptin®). Patients with negative local ISH tests or 0 and 1+ scores by IHC are not eligible for participation in the study. Samples from these patients should not be sent to the central lab for re-testing. Patients with local 2+ and 3+ scores by IHC are potentially eligible: tumor samples for such patients have to be sent to the central lab for verification of HER2 status by ISH (if IHC 2+ locally) or by IHC (if IHC 3+ locally). Patients with positive ISH test at the local lab are potentially eligible: tumor samples for such patients require central laboratory confirmation of the local ISH testing result prior to randomization. If ISH confirmation of a local IHC 2+ score was also performed locally and the result is positive, central confirmation of the ISH testing result, however, is still required. A summary of the HER2 screening procedure is provided in Figure 6.

Figure 6 HER2 screening procedure



Note: HER2 or ISH tests must be performed locally as an initial screen. Only in exceptional cases where local lab facilities are not available may permission be granted for centers to send tumor samples directly to the central lab.

The procedure for taking tumor biopsies for central HER2 testing and their storage and shipping are described in the Pathology Manual.

Tumor slides sent to the central laboratory will not be returned. Tumor blocks sent to the central laboratory will be returned to the sender after the end of the screening, unless the patient consented her tissue samples could be kept for further research projects (see section 5.4.4.3)

HER2 test results from the central laboratory will be directly uploaded onto the clinical study database. They will be communicated to the site as central confirmation of a positive HER2 status is essential prior to randomization to the study.

5.4.2 Safety Laboratory Assessments

Normal ranges for the safety laboratory parameters must be supplied to Roche before the study starts.

Hematology and biochemistry will be done as part of regular safety assessments. Specifically

- Hematology: Hemoglobin, White Blood Cells (WBC), absolute neutrophil count (ANC)/neutrophils, platelet count
- Biochemistry: Creatinine, urea (BUN), SGPT (ALT), SGOT (AST), total bilirubin, alkaline phosphatase, albumin, sodium, potassium and calcium

Additional safety laboratory assessments include

- Urinalysis (dipstick)
- pregnancy test in women of child-bearing potential (serum test)

The total volume of blood loss for safety laboratory assessments will be approximately 15 ml per visit. The samples will be destroyed in the local lab after analysis.

5.4.3 Immunogenicity assessments

The purpose of immunogenicity testing is to determine whether human anti-human antibodies (HAHAs) against trastuzumab or rHuPH20 develop, and whether these impact upon safety or efficacy**.** Blood sampling for immunogenicity testing will be done as per visiting schedule (see Table 6, Table 7**,** Table 8).

All patients will be evaluated for antibodies against trastuzumab and rHuPH20 at baseline and during treatment (pre-dose cycle 2, 5, 13, 18) and during post-treatment follow-up phase (month 3, 6, 12, 18, 24*, 30, 36, 42, 48, 54, and 60*). Blood sampling for anti-trastuzumab analysis will be coupled to blood sampling for PK analysis as results on serum trastuzumab concentrations are needed for the evaluation of the anti-trastuzumab assay. The exact volume of blood required at each assessment visit is detailed in the Schedule of Assessments (Table 6, Table 7, and Table 8). Serum samples (used for PK and anti-trastuzumab analysis) and plasma samples (used for anti-rHuPH20 analysis) will be shipped to a central laboratory on a continuous basis. The total blood loss for immunogenicity testing during the study is 46 ml for patients in the SC arm and 26 ml for patients in the IV arm, respectively. Details of sampling, storage and shipping are described in the study's Sample Handling and Logistics Manual.

A three-tiered analytical testing approach will be performed for HAHAs against both trastuzumab and rHuPH20. Screening for the potential emergence of antibodies will use bridging immunoassays. Any samples testing positive will be subsequently re-tested in a confirmatory assay. Finally, confirmed positives will be tested for the presence of neutralizing antibodies.

All immunogenicity samples will be analyzed in a central laboratory. Samples will be kept for re-testing (if required) at the central laboratory, and will be destroyed no later than 12 months after the clinical study report is finalized.

5.4.4 Biomarker Assessments

Biomarker assessments are optional under this protocol and will only be conducted in patients who have provided their respective consent.

5.4.4.1 HER2 conversion

Formalin-fixed paraffin-embedded (FFPE) tissue (tumor blocks) from primary tumor remnants taken at surgery will be sent to a central reference laboratory for determination of HER2 status. Comparison of centrally confirmed HER2 status at screening with HER2 status of the tumor tissue removed at surgery will allow for assessment of HER2 conversion under trastuzumab therapy.

For quality assurance reasons only tumor blocks will be accepted, and no slides, as long-term storage could have a negative impact on the quality of the material (i.e. proteins and nucleic acids degradation). Procedures for tumor sample handling and shipping are described in the Pathology Manual.

5.4.4.2 Fcγ Receptor-Polymorphism

Whole blood samples (3.0 mL per sample) for assessment of Fcγ-receptor polymorphism will be collected once at baseline and will be fully used by a central laboratory exclusively for this purpose.

All technical details for the collection and management of the blood samples are specified in the Study Sample Handling and Logistics Manual.

5.4.4.3 Exploratory Biomarker Analysis

Any left-over from tumor samples (only tumor blocks, no slides) used by the central laboratory for central confirmation of HER2 status at screening (see section 5.4.1,) or for assessment of HER2 status at surgery (see section 5.4.4.1) will be kept in the Roche Clinical Repository (RCR) for exploratory biomarker analysis.

These research specimens will be used only for research purposes to identify dynamic biomarkers that may be predictive of response to trastuzumab treatment (in terms of efficacy, dose, safety and tolerability) and will help to better understand the pathogenesis, course and outcome of breast cancer and related diseases. Specimens for dynamic biomarker discovery will be coded in order to protect the privacy of the patient.

All RCR specimens will be destroyed no later than 15 years after the final freeze of the respective clinical database unless regulatory authorities require that specimens be maintained for a longer period. The implementation and use of the RCR specimens is governed by the Roche Clinical Repository policy to ensure the appropriate use of the RCR specimens.

5.5 Pharmacokinetic (PK) Assessments

This study is driven by the co-primary endpoint C_{trough} therefore adherence to the PK assessment schedule is of utmost importance. Surgery will be performed after 8 cycles of chemotherapy without interruption of the trastuzumab treatment schedule. For each day of a scheduled PK assessment, the exact time of trastuzumab administration and blood collection for PK assessments will need to be recorded in the eCRF.

Blood samples for PK analysis will be collected as described in Table 10 and Table 11 below. On each day of IV dosing the PK blood samples need to be drawn from the arm not receiving the trastuzumab infusion. Blood samples drawn prior to drug administration will give information on serum trough levels of trastuzumab.

The time of sampling at the end of infusion (IV arm) and on non-dosing days (SC and IV arms) is at the discretion of the investigator with the requirement to record the exact date and time of sampling. The total volume blood loss for pharmacokinetic assessments will be approximately 2 ml per blood draw, amounting to a total of 50 ml for a patient in the SC arm and approximately 74 ml for a patient in the IV arm.

Additional 4 ml blood in each study arm will need to be drawn for determination of serum trastuzumab concentration at month 3 and 6 in the treatment-free follow up which will be needed for the evaluation of the anti-trastuzumab antibody analysis (see section 5.4.3).

The procedures for the collection, handling and shipping of PK samples are specified in the Study Sample Handling and Logistics Manual.

Table 10 Schedule of PK Sampling (SC arm)

Sample Number	Cycle	Day of Cycle	Timepoint
1-1	1	1	Pre-dose
1-2		2	Post-dose
1-3		15	Post-dose
1-4	2	1	Pre-dose
1-5	3	1	Pre-dose
1-6	4	1	Pre-dose
1-7	5	1	Pre-dose
1-8	6	1	Pre-dose
1-9	7	1	Pre-dose
1-10		2	Post-dose
1-11		4	Post-dose
1-12		8	Post-dose
1-13		15	Post-dose
1-14	8	1	Pre-dose
1-15	9	1	Pre-dose
1-16		2	Post-dose
1-17		15	Post-dose
1-18	10	1	Pre-dose
1-19	11	1	Pre-dose
1-20	12	1	Pre-dose
1-21		2	Post-dose
1-22		4	Post-dose
1-23		8	Post-dose
1-24		15	Post-dose
1-25	13	1	Pre-Dose

* within a window of 0 and +4 hours

Table 11 Schedule of PK sampling (IV arm)

Sample Number	Cycle	Day of Cycle	Timepoint
2-1	1	1	Pre-dose
2-2		1	At the end of infusion
2-3		2	Post-dose
2-4		15	Post-dose
2-5	2	1	Pre-dose
2-6		1	At the end of infusion
2-7	3	1	Pre-dose
2-8		1	At the end of infusion
2-9	4	1	Pre-dose
2-10		1	At the end of infusion
2-11	5	1	Pre-dose
2-12		1	At the end of infusion
2-13	6	1	Pre-dose
2-14		1	At the end of infusion
2-15	7	1	Pre-dose
2-16		1	At the end of infusion
2-17		2	Post-dose
2-18		4	Post-dose
2-19		8	Post-dose
2-20		15	Post-dose
2-21	8	1	Pre-dose
2-22		1	At the end of infusion
2-23	9	1	Pre-dose
2-24		1	At the end of infusion
2-25		2	Post-dose
2-26		15	Post-dose
2-27	10	1	Pre-dose
2-28		1	At the end of infusion
2-29	11	1	Pre-dose
2-30		1	At the end of infusion
2-31	12	1	Pre-dose
2-32		1	At the end of infusion
2-33		2	Post-dose
2-34		4	Post-dose
2-35		8	Post-dose
2-36		15	Post-dose
2-37	13	1	Pre-dose

6. INVESTIGATIONAL MEDICINAL PRODUCT

The study treatment is defined as neoadjuvant (pre-surgery) and adjuvant (post-surgery) treatment. Throughout the study, the investigational medicinal product is SC trastuzumab. The comparator is IV trastuzumab (Herceptin®) which is also considered investigational medicinal product in this trial.

5-Fluorouracil, epirubicin, cyclophosphamide and docetaxel are administered in accordance with their SmPCs and not subject of investigation in this study. Therefore they are not regarded as Investigational Medicinal Products.

6.1 Dose and Schedule of Investigational Drug and Comparator

SC and IV trastuzumab will be administered every 3 weeks for 18 cycles unless intolerable toxicity or investigator-assessed disease progression occurs.

SC and IV trastuzumab will be administered before starting the infusion of chemotherapy.

6.1.1 SC Trastuzumab

A fixed dose of 600 mg SC trastuzumab irrespective of the patient's weight will be administered throughout the treatment phase. All doses of SC trastuzumab will be administered as a subcutaneous injection into the thigh over about 5 minutes by a trained health care professional. Injection-related symptoms must have resolved before chemotherapy is started or the patient is discharged unless deemed clinically not significant by the investigator. Patients who experience injection-related symptoms may be pre-medicated with paracetamol and antihistamines for subsequent injections. Dose reductions for toxicity are not permitted.

6.1.2 IV Trastuzumab

IV trastuzumab will be administered as described in the Herceptin® Investigator Brochure. Weight should be recorded at screening and at every scheduled visit for all patients.

IV trastuzumab will be administered on day 1 of cycle 1 at the required loading dose of 8 mg/kg. On Day 22 (three weeks after the first dose, and every three weeks thereafter), IV trastuzumab will be administered at a maintenance dose of 6 mg/kg. The first infusion is given over 90 minutes. If the first dose is well tolerated, subsequent doses may be given over 30 minutes. It is recommended to observe patients from start of the infusion for fever, chills and other infusion-related symptoms. Interruption or slowing of the infusion may help control such symptoms and may be resumed when symptoms abate. All infusion-related symptoms must have resolved before chemotherapy is given or the patient is discharged unless deemed clinically not significant by the investigator. Patients who experience infusion-related symptoms may be pre-medicated with paracetamol and antihistamines for subsequent infusions. Dose reductions for toxicity are not permitted.

6.2 Dose and Schedule of Chemotherapy

Doses will be calculated for all drugs according to the patient's body surface area (BSA). In calculating BSA, actual weights should be used. No downward adjustments to "ideal body weight" are allowed. The principle applies to all individuals whose calculated BSA is ≤ 2.2 m^2. In the few patients who should have a larger calculated BSA, the limit of 2.2 m^2 should be used.

Docetaxel

Docetaxel will be administered at 75 mg/m^2 as an IV infusion over 60 ($\pm$ 10) minutes after completion of trastuzumab administration. Premedication consisting of an oral corticosteroid, such as dexamethasone 16 mg per day in divided doses for 3 days starting 1 day prior to docetaxel administration, unless contra-indicated, should be used.

Patients will be closely observed from the start of the infusion for hypersensitivity reactions which may occur within minutes. Severe hypotension, bronchospasm or generalized rash/erythema requires immediate discontinuation of docetaxel and appropriate treatment. The infusion may be slowed for minor symptoms like flushing or local cutaneous reactions. Patients experiencing severe hypersensitivity reactions should be discontinued from the study. Similarly, prophylactic G-CSF may be used to mitigate the risk of hematological toxicities. Dose delays and reductions are permitted and prophylactic G-CSF may be given to maintain the dosing schedule (see Section 6.3). Docetaxel will be administered every three weeks for four cycles (cycles 1 to 4).

5-Fluorouracil

5-Fluorouracil will be administered at 500 mg/m2 on day 1 of cycles 5 to 8. It may be given as an IV bolus over 3 - 5 minutes or as an infusion over 30 minutes. Dose delays and reduction for toxicity are permitted (see Section 6.3).

Epirubicin

Epirubicin will be administered at 75 mg/m^2 on day 1 of cycles 5 to 8. It may be given as an IV bolus over 3 - 5 minutes or as an infusion over 30 minutes. Dose delays and reduction for toxicity are permitted (see Section 6.3).

Cyclophosphamide

Cyclophosphamide will be administered at 500 mg/m2 on day 1 of cycles 5 - 8. It should be given as an IV bolus over 3 - 5 minutes, in accordance with local policy. Patients with body surface area > 2 m^2 should be dose capped at 1200 mg. Dose delays and reduction for toxicity are permitted (see Section 6.3).

6.3 Dose Modifications and Delays

Chemotherapy and trastuzumab are to be given on the same day in a three-weekly regimen. Administration of chemotherapy or trastuzumab may need to deviate from the planned schedule e.g. due to adverse events. If the delay in dosing for either drug is foreseen to be 7 days or less, then chemotherapy and trastuzumab should be given together on the same day. In case the dosing delay is more than 7 days, chemotherapy and trastuzumab administration may be uncoupled and given on different days as clinically indicated. However, chemotherapy and trastuzumab should be brought back to a common administration schedule as soon as possible.

Trastuzumab

Administration of trastuzumab may be delayed to assess or treat adverse events, such as cardiac adverse events or myelosuppression as shown in Table 12. Where this impacts PK sampling the accurate recording of dosing dates and times and PK sampling times is paramount to ensure the data are usable.

If IV trastuzumab administration is delayed more than 7 days from schedule a re-loading dose of 8 mg/kg needs to be administered before continuing again with the maintenance dose of 6 mg/kg.

No dose adjustment is needed in case of delayed administration of SC trastuzumab as a fixed dose of SC trastuzumab is given in this study.

Table 12 Actions to be Taken in Case of Trastuzumab-Related Toxicity

Toxicity related to study treatment	Action
1. Non-hematological, grade 1 or 2 (excluding cardiac) toxicity	Continue with study treatment (all medication in the cycle)
2. Non-hematological, grade 3 or 4 (excluding cardiac) toxicity	Hold study treatment (all medication in the cycle) until recovery to grade ≤ 2. Toxicity resolved to grade ≤ 1 within a maximum of 5 weeks calculated from last administration: Resume study treatment. Toxicity did NOT resolve to grade ≤ 2 within a maximum of 5 weeks calculated from last administration: Discontinue trastuzumab permanently. Take patient off study. Continue treatment as deemed suitable by local investigator.
3. Recurrence of non-hematological, grade 3 or 4 (excluding cardiac) toxicity upon re-challenge	Discontinue trastuzumab permanently. Take patient off study. Continue treatment as deemed suitable by local investigator.
4. Cardiac toxicity (significant asymptomatic drop in LVEF ($\geq$ 10 percentage points from baseline to a LVEF < 50%)	Study treatment (all medication in the cycle) to be held, continued or resumed according to the algorithm depicted in Appendix 4.
5. Cardiac toxicity (symptomatic congestive heart failure)	Trastuzumab to be discontinued permanently (patient to be taken off study)
6. Cardiac toxicity (other than significant asymptomatic LVEF drop or CHF)	Actions must follow rules 1. to 3. for non-hematological toxicities
7. Hematological toxicity – Neutropenia < 1.5×10^9/L	Hold study treatment (all medication in the cycle) until neutrophils $\geq 1.5 \times 10^9$/L.

Docetaxel, 5-Fluorouracil, epirubicin and cyclophosphamide

Dose delays will be allowed for myelosuppression, hepatic dysfunction, and other dose limiting toxicities. Dose reductions should be managed as indicated in the individual SmPCs and managed as per local practice.

6.4 Preparation and Administration of Study Drugs

6.4.1 IV Trastuzumab

Trastuzumab for IV administration is supplied for use as a freeze-dried preparation in two vial sizes (150 mg per vial and 440 mg per vial) for parenteral administration. Each vial of trastuzumab is reconstituted with sterile water for injection dependent on the vial size, as follows:

- Trastuzumab 440 mg vial is mixed with 20.0 mL of sterile water for injection (not supplied)
- Trastuzumab 150 mg vial is mixed with 7.2 mL of sterile water for injection (not supplied)

Use of other reconstitution solvents is not allowed. The reconstituted solution contains 21 mg/mL trastuzumab. None of the above formulations contain a preservative. The product is not intended to be stored after reconstitution and dilution unless this has taken place under aseptic conditions. Once the infusion is prepared it should be administered immediately and for single use only. The formulation must be infused within 8 hours after reconstitution.

The trastuzumab 440 and 150 mg vials provided for this study are both to be used as a SINGLE DOSE VIAL ONLY. Each vial should not be used for more than one administration of trastuzumab or for more than one patient at a time.

DO NOT FREEZE TRASTUZUMAB THAT HAS BEEN RECONSTITUTED.

Appropriate aseptic technique should be used. Each vial of trastuzumab is reconstituted with Sterile Water for Injection (SWI) as described above. Trastuzumab should be carefully handled during reconstitution. Causing excessive foaming during reconstitution or shaking the reconstituted trastuzumab may result in problems with the amount of trastuzumab that can be withdrawn from the vial.

The following instructions have to be followed:

1. Using a sterile syringe, slowly inject the Sterile Water for Injections in the vial containing the lyophilised trastuzumab, directing the stream into the lyophilised cake.
2. Swirl vial gently to aid reconstitution. DO NOT SHAKE.

Slight foaming of the product upon reconstitution is not unusual. Allow the vial to stand undisturbed for approximately 5 minutes. The reconstituted trastuzumab results in a colourless to pale yellow transparent solution and should be essentially free of visible particulates.

Trastuzumab should not be mixed or diluted with other drugs.

Determine the volume of the solution required based on a loading dose of 8 mg trastuzumab/kg body weight, or a subsequent 3 weekly dose of 6 mg trastuzumab/kg body weight:

$$\text{Volume (mL)} = \frac{\text{Body weight (kg)} \times \text{dose (8 mg/kg for loading or 6 mg/kg for maintenance)}}{\text{21 (mg/mL, concentration of reconstituted solution)}}$$

The appropriate amount of solution should be withdrawn from the vial and added to an infusion bag containing 250 ml of 0.9% sodium chloride solution. Do not use with glucose-containing solutions since it causes aggregation of the protein. The bag should be gently inverted to mix the solution in order to avoid foaming. Parenteral solutions should be inspected visually for particulates and discoloration prior to administration**.** The product is not to be stored after reconstitution and dilution unless this has taken place under aseptic conditions. Once the infusion is prepared it should be administered immediately. If diluted aseptically the infusion may be stored for a maximum of 24 hours (at 2-8 °C).

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed.

6.4.2 SC Trastuzumab

SC trastuzumab is supplied as a ready to use solution with a nominal content of 120 mg/mL drug substance and MUST NOT BE DILUTED prior to administration.

6.4.3 5-Fluorouracil, Epirubicin, Cyclophosphamide and Docetaxel

Preparation and administration will be performed by the site as governed by local guidelines and regulations.

6.5 Formulation, Packaging and Labeling

Packaging of IV and SC trastuzumab will be overseen by the Roche Clinical Trial Supplies department and bearing a label with the identification required by local law, the protocol number, drug identification and dosage.

The packaging and labeling of IV and SC trastuzumab will be in accordance with Roche standard and local regulations.

Upon arrival of investigational products at the site, site personnel should check them for damage and verify proper identity, quantity, integrity of vials and temperature conditions, and report any deviations or product complaints to the monitor upon discovery.

6.5.1 IV Trastuzumab

Trastuzumab IV drug product will be provided in 150 mg (Ro 45-2317/V03) and 440 mg (Ro 45-2317/V01) vials as freeze-dried lyophilisate formulated in histindine/histidine HCl, α,α-trehalose dehydrate and polysorbate 20. Batch-specific details and information on shelf-life are given in the packaging label. The recommended storage conditions are 2-8°C, the vials are not to be shaken. For reconstitution of the lysophilisate, storage and handling instructions, please see section 6.4.1.

6.5.2 SC Trastuzumab

Trastuzumab for SC administration (Ro 045-2317/F04) is supplied as a ready to use liquid formulation with a nominal content of 120 mg/mL drug substance and MUST NOT BE DILUTED prior to administration. The drug product contains 2,000 U/mL rHuPH20 (manufactured in a CHO cell line) acting as a permeation enhancer, histidine/histidine-HCl (buffer), alpha,alpha-trehalose dihydrate (bulking agent), methionine (stabilizer), and polysorbate 20 (stabilizer/emulsifier) in water for injection (WFI) at a pH of 5.5 ± 0.5. The drug product is a sterile, colorless to yellowish, clear to opalescent liquid in colorless 10-mL vials (8.0 mL fill).

The recommended storage conditions are 2°C - 8°C, protected from light. From a microbiological point of view the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C-8°C. Batch-specific details and information on shelf-life are given in the packaging label.

6.5.3 5-Fluorouracil, Epirubicin, Cyclophosphamide and Docetaxel

5-FU, Epirubicin, cyclophosphamide and docetaxel will not be provided by Roche. They will be sourced locally by the investigational site.

6.6 Blinding and Unblinding

Not applicable as the study is open label.

6.7 Assessment of Compliance

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing" and return records.

Accurate records must be kept for each study drug provided by the sponsor. These records must contain the following information:

- documentation of drug shipments received from the sponsor (date received and quantity)
- disposition of unused study drug not dispensed to patient

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the patient to whom the study medication was dispensed
- the date(s), quantity of the study medication dispensed to the patient

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and copies of the dispensing & inventory logs, must be returned to the Roche Monitor at the end of the study, unless alternate destruction has been authorized by Roche, or required by local or institutional regulations.

6.8 Destruction of Study Drug

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons e.g. cytotoxicity. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed

on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity (batch numbers or subject numbers) of investigational product(s) destroyed
- Quantity of investigational product(s) destroyed
- Date of destruction (date discarded in designated hazardous container for destruction)
- Method of destruction (the site must provide the sponsor with documentation of their institutional policy and procedures for handling and disposing of hazardous drugs)
- Name and signature of responsible person who discarded the investigational product in a hazardous container for destruction

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 Adverse Events and Laboratory Abnormalities

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

7.1.1.1 Intensity

Intensity of all adverse events will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 on a five-point scale (Grade 1 to 5) (Appendix 6) and reported in detail on the eCRF. As the sole exception, symptomatic congestive heart failure should in addition be graded according to NYHA criteria (Appendix 5).

Adverse events not listed on the CTCAE should be graded as follows:

CTC Grade	Equivalent To:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the patient
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the patient at direct risk.
Grade 4	Life threatening/disabling	An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.
Grade 5	Death	AE resulting in death

7.1.1.2 Drug – Adverse Event Relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as Yes.

The following criteria should be considered in order to assess the relationship as Yes:

- Reasonable temporal association with drug administration
- It may or may not have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as No:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- It does not follow a known pattern of response to the suspected drug
- It does not reappear or worsen when the drug is readministered

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in death; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

The study will comply with all local regulatory requirements and adhere to the full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 (see Appendix 1).

7.1.1.4 Progression of Underlying Malignancy

Progression of breast cancer is not to be reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria (applicable before surgery), or other criteria as specified in section 5.3.2.3 (applicable after surgery).

Progression of the primary tumor as defined by clinical increase of primary tumor or evidence of metastasis, in the pre-operative setting (cycles 1 - 8) requires immediate discontinuation from the study, and local practice should be followed such as: second-line cytotoxic regimen, surgery and or radiotherapy.

Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of disease, or does not fit the expected pattern of progression of disease.

If there is any uncertainty about an adverse event being due only to breast cancer progression, it should be reported as an AE or SAE, as applicable.

7.1.2 Treatment and Follow-up of AEs

After the 28 day period following the last dose of study drug in an individual patient, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure.

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure.

Unrelated Grade 1 or Grade 2 AEs: Follow until one of the following occurs:

- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure.

The final outcome of each adverse event must be recorded on the eCRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will be recorded on the laboratory results eForms of the eCRF.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the eCRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the eCRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed until they have returned to the normal range, baseline value and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the eCRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Adverse Events

All AEs occurring from the start of screening procedures until the start of study treatment (in case the patient is randomized) will need to be collected and be recorded in the sites' source documents. For patients who are randomized, screening AEs will be captured on the medical history page unless they are serious in which case an AE page and an SAE report needs to be completed. For patients who fail screening, AE information will not be reported to Roche but will stay with the patient's medical records.

The terminal half-life of trastuzumab, derived from population PK analysis, is approximately 28 days. Therefore, trastuzumab may be present in the circulation for up to 24 weeks after the last administration.

Until 28 days after the last administration of trastuzumab, all AEs must be reported irrespective of type of disorder and drug event relationship. Between 28 days after last study drug until the end of the study only related AEs and cardiac AEs should be reported.

Table 13 Reporting Time Windows for Adverse Events and Serious Adverse Events

	during treatment and up to 28 days after stop of trastuzumab treatment	28 days from stop of trastuzumab treatment until 6 months from stop of trastuzumab treatment	6 months from stop of trastuzumab treatment until end of the study
AEs	all	related, cardiac	related, cardiac
SAEs	all	related, unrelated, cardiac	related, cardiac

7.2.2 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* (as defined in section 7.1.1.3 above) and which occurs during the course of the study, regardless of the treatment arm, must be reported to Roche *immediately (i.e., no more than 24 hours after* the investigator becoming aware of the event.

All SAEs occurring from the start of screening procedures until the start of study treatment (in case the patient is randomized) will need to be collected.

Related Serious Adverse Events *MUST* be collected and reported regardless of the time elapsed from the last study drug administration, until the end of the study.

Unrelated Serious Adverse Events must be collected and reported during the study treatment phase and for up to 6 months after the last dose of trastuzumab unless the patient went off study and started a new anticancer treatment.

The definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to. Complete information can be found in Appendix 1.

7.2.3 Reporting of Non-serious events of Special Interest

7.2.3.1 Left Ventricular Systolic Dysfunction

Symptomatic left ventricular systolic dysfunction should be reported with the event term "Congestive heart failure" and not as individual signs and symptoms thereof. Signs and symptoms should however be entered in the comments part of the AE page in the eCRF Congestive heart failure should be graded according to NCI-CTCAE v3.0 and in addition according to the NYHA classification (Appendix 5).

Symptomatic congestive heart failure must be reported irrespective of causal relationship during the full course of the study.

Asymptomatic declines in LVEF should not be reported as adverse events since LVEF data are collected separately in the eCRF. Exceptions to this rule are as follows:

- Any asymptomatic decline in LVEF requiring treatment or leading to withholding/discontinuation of trastuzumab must be reported as an adverse event. In this case the event should be reported with the event term "left ventricular systolic dysfunction" and graded according to NCI-CTCAE v3.0 (Appendix 6).

The following table summarizes the reporting conventions for left ventricular systolic dysfunction:

Table 14 Reporting Conventions for Left Ventricular Systolic Dysfunction

Observation	How to report	Term to be reported	Grading
Asymptomatic decline in LVEF	no AE		
Asymptomatic decline in LVEF requiring treatment or leading to withholding/discontinuation of trastuzumab	AE	Left ventricular systolic dysfunction	NCI CTCAE for "left ventricular systolic dysfunction"
Symptomatic left ventricular systolic dysfunction	AE SAE report if applicable	Congestive heart failure	NCI CTCAE for "left ventricular systolic dysfunction" and NYHA

A significant drop in LVEF identified by MUGA scan or echocardiography requires a follow-up LVEF assessment for confirmation approximately three weeks later. This applies throughout the full course of the study (treatment phase and follow-up phase).

A significant LVEF drop is defined as an absolute decrease of at least 10 ejection fraction (EF) points below baseline and to below 50%.

In addition, please refer to the algorithm in Appendix 4 to assist the decision to continue or discontinue study medication based on LVEF assessment in asymptomatic patients.

7.2.3.2 Administration Associated Reactions

Special attention will be given to trastuzumab administration-associated reactions. Timing and type of adverse drug reactions may be different between IV and SC trastuzumab. The currently available PK data from the BP22023 study suggest that maximal serum concentration of SC trastuzumab is only reached after 108-156 hours from injection due to slower absorption. IV trastuzumab reaches maximal serum concentrations much sooner. The maximal serum concentration *of SC trastuzumab* is lower compared to IV trastuzumab. IV trastuzumab-associated reactions (e.g. chills, fever) tend to occur at the first administration, but infrequently thereafter. Preliminary safety data from the BP22023 study show that SC trastuzumab seems to be well tolerated (see section 1.1.2.4.1).

Patients should be informed about the potential occurrence of administration-associated adverse reactions and be provided with instructions how to contact the site in case of need. All patients will be closely followed as they will attend the site for further PK sampling during the first cycle of IV or SC administration (see Table 6). At all scheduled PK sampling visits potential adverse events should be investigated and recorded in the eCRF. In case an adverse event is considered to be an administration-associated reaction not only the date, but also the the time of onset will be collected in the eCRF.

7.2.4 Pregnancy

In patients of childbearing potential and women < 1 year after the onset of menopause, appropriate contraceptive measures are mandatory during study treatment (examples: barrier method (condoms, diaphragm); intrauterine devices; surgical methods, or abstinence. Based on pharmacokinetic considerations, contraceptive measures are recommended for at least 6 months following the last dose of trastuzumab.

A female patient must be instructed to immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor. The investigator should counsel the patient, discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Pregnancies occurring up to 6 months after the completion of trastuzumab treatment must also be reported to the investigator.

7.3 Warnings and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate for IV trastuzumab, other than those noted in the Herceptin ® Investigators' Brochure.

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate for 5-fluorouracil, epirubicin, cyclophosphamide or docetaxel, other than those noted in the respective SmPCs.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

8.1 Primary and Secondary Study Variables

8.1.1 Primary and Secondary Study Variables for the PK-Analysis

The comparability of the SC and IV dose of trastuzumab will be assessed by a one-sided testing procedure. The primary variable for the pharmacokinetic analysis will be the observed trough concentration C_{trough} of trastuzumab at cycle 7 (ie. the measured pre-dose concentration value at cycle 8). The C_{trough}SC/ C_{trough}IV mean ratio of the SC dose of trastuzumab relative to the IV dose will be estimated together with the 90% confidence interval based on the log-transformed trough concentration values. Bio-non-inferiority will be concluded if the lower bound of the confidence interval is equal or greater than 0.8.

Observed C_{trough} is a PK *co*-primary endpoint. Predicted C_{trough} will be considered as supportive endpoint of the measured C_{trough}. The aim to use predicted C_{trough} is to take into account possible deviations from the protocol (i.e. sampling schedule or dosing interval) and extra-noise (i.e. precision of analytical measurement). Any potential differences between the outcomes of the statistical test performed on observed and predicted C_{trough} will be discussed in the final study report.

The same analysis will be performed as a secondary objective for the post-surgery period on the C_{trough} level observed at cycle 12 (i.e the measured pre-dose concentration value at cycle 13).

The number and percentage of patients exceeding C_{trough} concentrations of 20 μg/mL will be determined as a secondary analysis. Other pharmacokinetic variables will be regarded as secondary.

Modelling of the PK data will be performed using a population approach based on a model previously developed on a combined SC and IV dataset from the BP22023 study. The structural and statistical model will be defined using non-linear mixed effect model (NONMEM). The model will be parametrized in terms of clearances and volumes and the individual measures of exposure i.e. C^{p}_{trough} and $AUC^{p}0\text{-}\tau$ will be predicted from the final pharmacokinetic model.

All pharmacokinetic variables will be presented by listings and descriptive summary statistics including arithmetic mean, geometric mean, median, range, standard deviation and coefficient of variation and 95%-confidence limits.

The PK model will be used to assess whether the SC trastuzumab starting dose results in the expected range of concentrations on the first 8 patients in each arm. In case the variability in PK is too high to allow a conclusion, samples from additional patients (up to 12 per arm) will be included into this analysis.

8.1.2 *Co*-primary efficacy variable - Pathological Complete Response

The *co*-primary efficacy variable is **pathological Complete Response (pCR).** This is defined as the absence of invasive neoplastic cells of the primary tumor in the breast after surgery. Pathological complete response *in situ* (pCRis) which is defined as carcinoma in situ found in the primary tumor remnants does not constitute a pCR .

8.1.3 Secondary efficacy Variables

Total pathological Complete Response (tpCR): defined as the absence of invasive neoplastic cells in the primary tumor remnants and in the axillary lymph nodes

Overall Response Rate (ORR): defined as clinical complete or partial tumor response

Event Free Survival (EFS): This is defined as the time from the date of randomization to the date where an event occurs. An event is disease recurrence or progression(local, regional, distant or contralateral) or death due to any cause.

Overall survival: This is defined as the time from the date of randomization to the date of death, regardless of the cause of death. Subjects who were alive at the time of the analysis will be censored at the date of the last follow up assessment. Subjects without follow up assessment will be censored at the day of last dose and subjects with no post baseline information will be censored at the time of randomization.

Time to response: For subjects with a best overall response of CR or PR this is defined as the time from the first drug administration to the date of first CR or PR. which will be the date the response is first documented by objective evidence. Time to response will be summarized for all subjects with documented response by descriptive statistics (mean, SD and median). The response rate at preplanned assessment time points will be summarized in addition.

Pathological Complete Response *in situ* (pCRis): defined as carcinoma *in situ* found in the primary tumor remnants at surgery

8.1.4 Safety

The safety and tolerability will be assessed as a secondary objective. This will include adverse events (including symptomatic cardiac dysfunction), laboratory parameters and LVEF assessments.

8.2 Statistical and Analytical Methods

8.2.1 Statistical Model

8.2.1.1 Primary Variables

8.2.1.1.1 Trastuzumab Serum Trough Concentration

The ANOVA model

$$y_{ij} = \mu' + \tau_i + \varepsilon_{ij} \qquad (i = SC, IV,\ j = 1,..., n_i)$$

will be applied to the logarithmically transformed primary variable $\log(C_{trough})$, and the log-transformed secondary variables $\log(C^p_{trough})$, $\log(AUC^p 0\text{-}\tau)$. C_{max} will not be considered for PK bridging. The reason is that comparable C_{max} is not likely because

trastuzumab diffusion from the interstitial space is expected to be slower than an IV infusion directly into the central compartment μ' denotes the general mean of the transformed variable, τ_i the effect of the treatment i and ε_{ij} the random deviation of the observation y_{ij} and n_i the number of subjects in treatment i included in the analysis. The random deviations ε_{ij} are assumed to be independently and identically normally distributed with mean zero and variance σ_ε^2. Under this model $\mu_{SC} / \mu_{IV} = \exp(\tau_{SC} - \tau_{IV})$ holds, where μ_i (i = SC, IV), denotes the population mean of treatment i for the untransformed variable.

The contrast $\tau_{SC} - \tau_{IV}$, its 90% confidence limits, and the intra-subject variance σ_ε^2 will be estimated from the ANOVA. An estimate of the treatment effects ratio μ_{SC} / μ_{IV} and the corresponding 90% confidence limits for the untransformed variables will be calculated by exponentiation of the estimate of contrast $\tau_{SC} - \tau_{IV}$ and the 90% confidence limits. The intra–subject coefficient of variation for the untransformed primary variables will be estimated using the relationship $CV_\varepsilon = \sqrt{\exp(\sigma_\varepsilon^2) - 1}$.

8.2.1.1.2 Pathological Complete Response (pCR)

The Per Protocol Set (PPS) will be the main analysis set. Sensitivity analysis will be provided on the ITT population.

Non-inferiority in pCR rate will be established if the lower limit of the one-sided 97.5% confidence interval for the difference in pCR rate using the continuity correction of Anderson and Hauck (1986) is above -12.5% (absolute percentage points, see Appendix 8). The difference in pCR rates is defined as pCR rate in the SC trastuzumab arm minus the pCR rate in the IV trastuzumab arm.

pCR rates and 95%-confidence limits according to Pearson-Clopper will also be calculated for single treatment groups. Logistic regression including stratification factors will be applied in an exploratory manner.

8.2.1.2 Secondary Variables

All analyses will be provided on the PPS. Sensitivity analysis will be provided on the ITT population.

95% confidence interval for the difference in tpCR and ORR rate using the continuity correction of Anderson and Hauck (1986) will be provided for the PPS and ITT population.

TpCR and ORR rates and 95%-confidence limits according to Pearson-Clopper will also be calculated for the individual study arms. pCRis will be evaluated in an exploratory manner.

For time to event variables, Kaplan-Meier curves will be calculated and displayed. Median survival times and corresponding 95% confidence interval will be provided. The estimated hazard ratio and corresponding confidence intervals will be obtained from the

Cox regression model. Expanded analyses on subgroups including stratification factors will be applied in an exploratory manner.

8.2.2 Hypothesis Testing

8.2.2.1 Trastuzumab Serum Trough Concentration

For the PK co-primary variable "observed C_{trough}", the following hypotheses will be tested:

H_0: The SC-dose is inferior to the IV-dose, i.e. the $C_{trough}SC/ C_{trough}IV$ mean ratio of the SC dose of trastuzumab relative to the IV dose is not greater than 0.8
vs.
H_1: The SC-dose is non-inferior to the IV-dose, i.e. the $C_{trough}SC/ C_{trough}IV$ mean ratio of the SC dose of trastuzumab relative to the IV dose is equal or greater than 0.8

The null-hypothesis will be rejected if the lower bound of the 90%-confidence interval for the mean ratio is equal or greater than 0.8.

This test will be performed for the observed serum trough concentrations at cycle 7 (i.e. cycle 8 pre-dose trastuzumab serum trough concentration) as primary analysis. A similar test will be conducted for the same cycle as supportive analysis on the predicted trastuzumab serum trough concentration. Furthermore, this test will also be conducted as secondary analysis on the serum trough concentrations at cycle 12 (i.e. cycle 13 pre-dose trastuzumab serum trough concentration) on both observed and predicted trastuzumab serum trough concentration and for the secondary variable AUC0-tau.

8.2.2.2 Pathological Complete Response.

For the efficacy co-primary variable pathological Complete Response, the following hypotheses will be tested:

$$H_0: \Pi_{SC} \leq \Pi_{IV} - \delta \quad \text{vs.} \quad H_1: \Pi_{SC} > \Pi_{IV} - \delta$$

Where ΠSC and ΠIV are the pathological Complete responses in the SC trastuzumab arm and the IV trastuzumab arm respectively, and δ is the non-inferiority margin (12.5%).

The null-hypothesis will be rejected if the lower limit of the one-sided 97.5 % confidence interval for the difference in pCR rate using the continuity correction of Anderson and Hauck (1986) is above -12.5% (absolute percentage points).

8.2.3 Types of Analyses

8.2.3.1 Efficacy Analysis

Efficacy analysis will be primarily based on the per-protocol population consisting of all randomized patients without any major protocol violation (defined in section 8.2.3.2 .). In addition, an Intent-to-Treat analysis will be reported consisting of all patients who had at least one efficacy assessment after first drug administration.

8.2.3.2 Exclusion of Data from Analysis

Per-protocol population:

The per-protocol population will be defined as the subset of the intent-to-treat population defined by the following exclusions:

- Less than 8 cycles of Herceptin/chemotherapy treatment
- Metastatic breast cancer before entering the study
- Previous anti-cancer therapy
- HER2 negative patients
- major violation of inclusion/exclusion criteria

Patients will be assigned to treatment groups as treated.

Intent to treat population:

All patients having at least one efficacy assessment after first study drug administration will be included in the intent to treat population (Patients will be assigned to treatment groups as randomized for analysis purposes)

Safety population:

All patients who received at least one dose of study medication will be included in the safety population. (Patients will be assigned to treatment groups as treated.)

8.2.3.3 Safety Interim Analysis

Safety summaries and listings will be provided to the DSMB for safety monitoring.

8.2.3.4 Main and follow-up analyses

The main analysis on the two co-primary variables will take place after all patients in the study have undergone surgery and at least 100 patients of each study arm have completed treatment. All analyses on secondary variables will also be run at this time, although the number of events for the time to events analyses is expected to be limited. *F*ollow-up analysis will be run once all patients in the study have completed at least 24 months of treatment-free follow-up *and after end of study (60 months of treatment–free follow-up).*

8.2.4 Safety Data Analysis

The safety analysis population will include all subjects who receive at least one dose/infusion of SC or IV trastuzumab. All safety parameters will be summarized and presented in tables based on this safety population.

Adverse event data will be presented in frequency tables (overall and by intensity) by body system. For adverse events the most extreme intensity will be used for reporting. Administration-associated reactions will be summarized.

Laboratory data will be presented as summary statistics for each sampling time point using both shift and frequency tables.

All adverse events, including symptomatic cardiac dysfunction, and abnormal laboratory variables will be assessed according to the NCICTC v3.0 grading system.

Information on study drug will be summarized by duration, starting dose and cumulative dose using descriptive statistics.

Treatment exposure will be summarized as the number of cycles received by each subject, and as the percentage of the planned dose of each agent given at each cycle.

LVEF will be summarized by presenting frequencies over time for each treatment group of the following categories:

- Decrease of ≥15 points from baseline
- Decrease of ≥ 10 points from baseline to a value < 50%
- Decrease of ≥ 15 points from baseline to a value < 50%

Symptomatic left ventricular dysfunction will be summarized by NYHA classification.

8.2.5 Other Analyses

8.2.5.1 Pharmacokinetic Analysis

A per–protocol analysis including all subjects randomized and adherent to the protocol will be performed.

Subjects will be excluded from the pharmacokinetic analysis if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol or if data are unavailable or incomplete which may influence the pharmacokinetic analysis. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure.

8.2.5.2 Pharmacodynamic Analysis

The relationship between tumor response and the serum concentrations of trastuzumab may be explored.

8.2.5.3 Quality of Life Analysis

NA

8.2.5.4 Pharmacoeconomic Analysis

NA

8.3 Sample Size

Assuming pCR response rates of at least 40% in both arms 552 patients are necessary to conclude non-inferiority in pathological complete response rate with a power of 80% using a one-sided 97.5% confidence limit for the difference of the response rates and a non-inferiority margin of 12.5%. This number of patients accounts for a 10% drop-out rate in the patient population.

The chemotherapeutic regimen used in this trial has been derived from the regimen used by Buzdar et al 2005 which yielded consistently pCR rates above 50% (see section 3.1.2). The replacement of weekly paclitaxel by three-weekly docetaxel and the different patient

population (stage IIIB, IIIC including inflammatory breast cancer) may impact on the pCR rate. Data from the NOAH trial, however, achieving a pCR rate of 43% in patients with locally advanced disease including inflammatory breast cancer with a combination regimen including anthracyclines and taxanes suggest that a pCR rate of 40% seems a reasonable assumption for this trial [27].

PK sample size calculations are based on the CV% for the trough concentrations of trastuzumab from previous studies in MBC and EBC patients after q3w treatment. Since the situation prior to surgery is comparable to the MBC setting, an inter-patient coefficient of variation of 60% is assumed. Hence, 130 patients per arm (i.e., a total of 260 patients) is needed to demonstrate C_{trough} comparability with a power of 80% if the true means of the two formulations do not differ by more than 5%.

A population PK approach has been adopted for the analysis which allows to restrict the number of blood samples to be taken from each patient. However, the draw-back to this approach is that a relatively large number of patients needs to be included from which the data need to be collected. In the PK loading study (MO16982) only 37 of the 72 patients gave a useable amount of PK data for non-compartmental analysis. The number of patients with useable PK-data increased to 70 when the data were evaluated by model-dependent (population PK) methods [62].

9. DATA COLLECTION, MANAGEMENT AND QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the electronic Case Report Form against the investigator's records by the study monitor (source document verification), and the maintenance of a drug–dispensing log by the investigator.

Data for this study will be recorded via an Electronic Data Capture (EDC) system using electronic Case Report Forms. It will be transcribed by the site from the paper source documents onto the eCRF. (In no case is the eCRF to be considered as source data for this trial.)

A comprehensive validation check program utilizing front-end checks in the eCRF and back-end checks in the Roche data base will verify the data and discrepancies will be generated accordingly and transferred electronically to the eCRF at the site for resolution by the investigator.

Throughout the study the Study Management Team will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the eCRF.

10. STUDY COMMITTEES

Data Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) will be responsible for the assessment of risks and benefits reviewing key safety data accruing in the study. The DSMB will meet in regular intervals and report the conclusion of their deliberations to the Sponsor with recommendations or not for modification of trial conduct. A separate DSMB Charter will outline the committee's composition, the members' roles and responsibilities and the frequency of meetings.

11. REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P: Global Cancer Statistics 2002, CA Cancer J Clin, Mar 2005; 55: 74 – 108.

2. Yarden Y, Sliwkowski M. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127-37.

3. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer 2004; 4:361-70.

4. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, Science 1987;235:177-82.

5. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707-12.

6. Fisher B, Bryant J, Wolmark N et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer , JCO 1998 16:2672-2685.

7. Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: A meta analysis. J Natl Cancer Inst 2005; 97:188-194.

8. Kaufmann M, Hortobagyi GN, Goldhirsch A et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update, JCO 2006 Apr 20;24(12):1940-9.

9. Bear HD, Anderson S, Smith RE et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27, JCO 2006 May 1;24(13):2019-27.

10. Herceptin (trastuzumab) population pharmacokinetics and covariates analysis using data from Herceptin phase II/III studies BO15935, WO16229, BO15899 and M77004. part I. Report No 1018264. 31 March 2005.

11. Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, G; HERA study team. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 2007;369:29-3.

12. Perez EA, Romond EH, Suman VJ et al. Updated results of the combined analysis of NCCTG N9831 and NSABP B-31. Adjuvant Chemotherapy with or without trastuzumab (H) in patients with HER2-positive breast cancer. ASCO 2007, abstract 512).

13. Slamon D, Eiermann W, Robert N et al., BCIRG 006 : 2nd interim analysis phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC T) with doxorubicin and cyclophosphamide followed by doxetaxel and trastuzumab (AC TH) with docetaxel, carboplatin and trastuzumab (TCH) in Her2neu positive early breast cancer patients, Abstract # 52 SABCS 2006.

14. Suter TM, Procter M, van Veldhuisen DJ, Trastuzumab-associated cardiac adverse effects in the herceptin adjuvant trial, J Clin Oncol. 2007 Sep 1;25(25):3859-65. Epub 2007 Jul 23.

15. Rastogi P, Jeong J, Geyer CE, Five year update of cardiac dysfunction on NSABP B-31, a randomized trial of sequential doxorubicin/cyclophosphamide (AC)→paclitaxel (T) vs. AC→T with trastuzumab(H), Journal of Clinical Oncology, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: LBA513.

16. Perez EA, Suman VJ, Davidson NE et al., Cardiac Safety Analysis of Doxorubicin and Cyclophosphamide Followed by Paclitaxel With or Without Trastuzumab in the North Central Cancer Treatment Group N9831 Adjuvant Breast Cancer Trial, JCO 26,8 2008.

17. Sachelarie I, Grossbard ML, Chadha M, Feldman S, Ghesani M, Blum RH. Primary systemic therapy of breast cancer. Oncologist. 2006; 11(6): 574-89.

18. Lazaridis G, Pentheroudakis G, Pavlidis N. Crit Rev Oncol Hematol. Integrating trastuzumab in the neoadjuvant treatment of primary breast cancer: accumulating evidence of efficacy, synergy and safety. Crit Rev Oncol Hema 2008; 66(1): 31-41.

19. Buzdar AU, Ibrahim NK, Francis D et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer, JCO 2005 ; 23 : 3676-3685.

20. Gianni L, Semiglazov V, Manikhas GM et al. Neoadjuvant trastuzumab in locally advanced breast cancer (NOAH): Antitumor and safety analysis, Journal of Clinical Oncology, 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S (June 20 Supplement), 2007: 532.

21. Buzdar A, Valero V, Ibrahim N et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2 – positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 2007; 13(1) 228-233.

22. Pernas S., Urruticoechea A., Falo A. ET AL. High pathologic complete response rate effectively predicted by proliferation index in HER2-positive localized breast cancer treated with weekly paclitaxel followed by FEC with concurrent trastuzumab. Poster Session V: treatment Neoadjuvant Therapy: SABCS, Dec. 15, 2007.

23. Dawood S, Gonzalez-Angulo AM, Peintinger F et al. Efficacy and Safety of neoadjuvant trastuzumab combined with paclitaxel and epirubicin. Cancer 2007;110:1195-200.

24. Coudert BP., Arnould L., Moreau L. et al. Pre-operative systemic (neo-adjuvant) therapy with trastuzumab and docetaxel for HER2-overexpressing stage II or III breast cancer: results of a multicenter phase II trial. Annals of Oncology 17: 409-414, 2006.

25. Limentani SA., Brufsky AM., Erban JK. et al. Phase II Study of Neoadjuvant Docetaxel, Vinorelbine, and Trastuzumab followed by Surgery and Adjuvant Doxorubicin Plus Cyclophosphamide in Women with Human Epidermal Growth Factor Recpetor 2- Overexpressing Locally Advanced Breast Cancer. J Clin Oncol. Vol 25:10: 2007.

26. Lybaert W., Wildiers H., Neven P. et al. Docetaxel (T) + Capecitabine (X) with or without Trastuzumab (H) neoadjuvant therapy for locally advanced breast cancer (BC): Phase II study. J Clin Oncol, 2007 ASCO Annual Meetings Proceedings Part I. Vol 25: 18S, 2007.

27. Coudert BP., Largillier R., Arnould L. et al. Multicenter Phase II Trial of Neoadjuvant Therapy with Trastuzumab, Docetaxel and Carboplatin for human Epidermal Growth Factor receptor-2-Overexpressing Stage II or III Breast Cancer: Results of the GETN (A)-1 Trial. J Clin Oncol Vol 25: no. 19. July 1, 2007.

28. Untch M, Stoeckl D, Konecny G et al. A multicenter phase II study of preoperative epirubicin, cyclophosphamide (EC) followed by paclitaxel (P) plus trastuzumab (T) in HER2 positive primary breast cancer. SABCS 2005, abstract 871.

29. Untch M, Rezai M, Loibl S et al. Neoadjuvant treatment of HER2 overexpressing primary breast cancer with trastuzumab given concomitantly to epirubicinscyclophosphamide followed by docetaxel ± capecitabine. First analysis of efficacy and safety of the GBG/AGO multicenter intergroup study, European Journal of Cancer Supplements Volume 6, No. 7, April 2008, page 47.

30. Harris L., Burstein J., Gelman R. et al. Preoperative trastuzumab and vinorelbine (HN) is a highly active, well-tolerated regimen for HER2 3+/FISH+ Stage II/III breast cancer. Proc Am Soc Clin Oncol 22:2003 (abstr 86).

31. Tripathy D., Moisa C. Gluck S. Neoadjuvant capecitabine plus docetaxel +/- trastuzumab therapy for recently diagnosed breast câncer: phase II results. SABCS, Dec.15, 2007.

32. Hurley J., Doliny P., Reis I. et al. Docetaxel, Cisplatin and Trastuzumab As Primary Systemic Therapy for Human Epidermal Growth Factor Receptor 2 – Positive Locally Advanced Breast Cancer. J Clin Oncol. Vol 24: 12: 2006.

33. Carey LA., Dees EC., Sawyer L. et al. Response to trastuzumab (Herceptin®) given with paclitaxel (Taxol® T) immediately following 4AC as initial therapy for primary breast cancer (BrCa). SABCS 2002 (abstract 424).

34. Burstein HJ., Harris L., Gelman R. et al. Preoperative Therapy with trastuzumab and Paclitaxel Followed by Sequential Adjuvant Doxorubicin/Cyclophosamide for HER2 Overexpressing Stage II or III Breast Cancer: A Pilot Study. Clin Oncol Vol 2 Nr 1 2003.

35. Torrisi R., Magni E., Dellapasqua S. et al. Trastuzumab and oral vinorelbine (+/- endocrine therapy) as primary therapy in patients (pts) with HER-2/neu positive breast cancer. SABCS, Dec. 15, 2007

36. Bines J., Murad S., Lago B., Ferrari J. et al. Multicenter brazilian study of weekly docetaxel and trastuzumab as primary therapy in stage III, HER-2 overexpressing breast cancer. Proc. AM Soc Clin Oncol 22: 2003 (abstr 268).

37. Baselga J, Semiglazov V, Manikhas GM et al. Efficacy of neoadjuvant trastuzumab in patients with inflammatory breast cancer (IBC): data from the NOAH (Neoadjuvant Herceptin) Phase III trial. ECCO 2007, abstract O#2030.

38. Gianni L, Eiermann W, Semiglazov V et al. Neoadjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer: primary efficacy analysis of the NOAH trial. SABCS 2008, abstract 31.

39. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N Engl J Med 2001;344:783-92

40. Hortobagyi GN, Yap HY, Kau SW, et al: A comparative study of doxorubicin and epirubicin in patients with metastatic breast cancer. Am J Clin Oncol 12:57-62, 1989.

41. Riccardi A, Tinelli C, Brugnatelli S, et al: Doubling of the epirubicin dosage within the 5-fluorouracil, epirubicin and cyclophosphamide regimen: a prospective, randomized, multicentric study on antitumor effect and quality of life in advanced breast cancer. Int J Oncol 16:769-776, 2000.

42. Robert J: Epirubicin. Clinical pharmacology and dose-effect relationship. Drugs 45:20-30, 1993 (suppl 2).

43. Clinical Study Report – M77003 Main Analysis - Cardiac safety of recombinant humanized anti-p185HER2 monoclonal antibody trastuzumab (Herceptin®) in combination with epirubicin/cyclophosphamide as first line therapy in anthracycline-naïve patients with HER2 overexpressing metastatic breast cancer. Report No. 1029798; October, 2008.

44. Lundin J et al. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). Blood 2002; 100(3):768-773.

45. Hylenex: Full Prescribing Information; Administrative Review of NDA 21-859 http://www.fda.gov/cder/foi/label/2005/021859lbl.pdf (accessed 20080418).

46. Frost GI. Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration. Expert Opin. Drug Deliv. (2007) 4(4):427-440.

47. 6.8749 Harris J. Clinical Review of NDA 21-640. FDA Report: December 1 2003:1-18. http://www.fda.gov/cder/foi/nda/2004/21-640_Vitrase_Medr.pdf (accessed 20051210).

48. Smith WB et al. Pharmacokinetics and pharmacodynamics of epoetin delta in two studies in healthy volunteers and two studies in patients with chronic kidney disease. Clin Ther. 2007; 29(7): 1368-80.

49. Hale G et al. Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration. Blood. 2004 Aug 15; 104 (4):948-55.

50. Srinivas NR, et al. Assessment of dose proportionality, absolute bioavailability, and immunogenicity response of CTLA4Ig (BMS-188667), a novel immunosuppressive agent. Pharma Research 1997;14(7)911-6.

51. Sparano JA, Wang M, Martino S et al. Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med 2008; 358(16):1663-71.

52. Griggs JJ, Schiffhauer LM, Sahasrabudhe K et al. Safety and Effectiveness of primary systemic therapy with docetaxel and trastuzumab in HER2-positive breast cancer. JCO 2005; 23(16S):793.

53. Harvey V, Mouridsen H, Semiglazov V et al. Phase III trial comparing three doses of docetaxel for second-line treatment of advanced breast cancer. JCO 2006; 24(31):4963-4969.

54. Bono P, Kellokumpu-Lehtinen PL, Alanko T et al. Docetaxel 100 versus 80 mg/m2 as adjuvant treatments of early breast cancer: an exploratory analysis of a randomised trial. Ann Oncol advanced access, January 19, 2009.

55. Clinical Study Report – A phase I/II study of a loading regimen (6 mg/kg weekly for 3 weeks) followed by a maintenance regimen (6 mg/kg every 3 weeks), of Herceptin monotherapy in women with HER2 positive metastatic breast cancer. Protocol MO16982/Report 1026709. April 2008.

56. Mittendorf EA, Esteva FJ, Wu Y et al., Determination of HER2 status in patients achieving less than a pathologic complete response following neoadjuvant therapy with combination chemotherapy plus trastuzumab, ASCO 2008, abstract # 150.

57. Varga Z, Caduff R,·Pestalozzi B. Stability of the HER2 gene after primary chemotherapy in advanced breast cancer, Virchows Arch., 2005 Feb;446(2):136-41. Epub 2005 Jan 14.

58. Neubauer H, Gall C, Vogel U, et al., Changes in tumor biological markers during primary systemic chemotherapy (PST). Anticancer Res. 2008 May-Jun; 28(3B):1797-804.

59. Arens N, Bleyl U, Hildenbrand R. HER2/neu, p53, Ki67, and hormone receptors do not change during neoadjuvant chemotherapy in breast cancer, Virchows Arch. 2005 May;446(5):489-96. Epub 2005 Apr 19.

60. Foster C, Harris K, Ostland M, et al. FCGR3A V/F polymorphism does not predict response to Herceptin (H) monotherapy. Proc Am Soc Clin Oncol 2002; 21: Abstract #227.

61. Musolino A., Naldi N, Bortesi B. et al., Immunoglobulin G Fragment C Receptor Polymorphisms and Clinical Efficacy of Trastuzumab-Based Therapy in Patients With HER-2/neu–Positive Metastatic Breast Cancer. J Clin Oncol. 2008 Apr 10;26(11):1789-96. Epub 2008 Mar 17.

62. Therasse P, Arbuck SG, Eisenhauer EA et al, New Guidelines to evaluate the response to treatment in solid tumors, Journal of the National Cancer Institute. Page 205, Vol 92, No. 3, Feb 2000.

63. Clinical Study Report – HERA (BO16348) Interim Analysis: A randomized multi-center comparison of 1 year of Herceptin treatment versus observation only in women with HER2-positive primary breast cancer who have completed adjuvant therapy. Research Report 1019820 / January 2006.

64. Gianni L, Eiermann W, Semiglazov V et al. Neoadjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer: a randomized phase III trial with a parallel HER2-negative cohort (the NOAH trial). 2009 (submitted).

65. Khatcheressian JL, Wolff AC, Smith TJ et al., American Society of Clinical Oncology 2006 Update of the Breast Cancer Follow-up and Management Guidelines in the Adjuvant Setting, J Clin Oncol. 2006 Nov 1;24(31):5091-7. Epub 2006 Oct 10.

66. Guideline on the choice of Non-Inferiotrity Margin. Committee for Medicinal Products for human use (CHMP), European Medicines Agency – Pre-authorisation Evaluation of Medicines for human Use. London, 27 July 2005, Doc. Ref. EMEA/CPMP/EWP/2158/99.

67. ICH Harmonised Tripartite Guideline. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. M3 (R2). Current step 2 Version, dated 15th July 2008.

68. Wynne C, Harvey V, Schwabe C et al. Comparison of subcutaneous and intravenous administration of trastuzumab: a phase I/Ib trial in health male volunteers and patients with HER2-positive breast cancer. J Clin Pharm. 2012 Feb 22 [Epub ahead of print].

69. Ismael G, Hegg R, Muehlbauer S et al. Subcutaneous versus intravenous administration of (neo)adjuvant trastuzumab in patients with HER2-positive, clinical stage I-III breast cancer (HannaH study): a phase 3, open-label, multicentre, randomised trial. Lancet Oncol. 2012 Sep;13(9):869-78. Epub 2012 Aug 9.

PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

12. ETHICAL ASPECTS

12.1 Local Regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC].

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

12.2 Informed Consent

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The electronic Case Report Forms [eCRFs] for this study contain a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

12.3 Independent Ethics Committees

This protocol and any accompanying material provided to the subject [such as subject information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

13. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

14. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

15. STUDY DOCUMENTATION, eCRFS AND RECORD KEEPING

15.1 Investigator's Files / Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] subject clinical source documents.

The Investigator's Study File will contain the protocol/amendments, , Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc. In addition, at the end of the study, the investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data, query resolution correspondence and reasons for changes, in a human readable format on CD which also has to be kept with the Investigator's Study file.

Subject clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the eCRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and randomization logs. The Investigator must keep these two categories of documents (including the archival

CD) on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator can not guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

15.2 Source Documents and Background Data

The investigator shall supply the sponsor on request with any required background data from the study documentation or clinic records. This is particularly important when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

15.3 Audits and Inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the electronic Case Report Form data must be by direct inspection of source documents.

15.4 Electronic Case Report Forms

Data for this study will be captured via an Electronic Data Capture (EDC) system. An audit trail will maintain a record of initial entries and changes made, reasons for change, time and date of entry and user name of person authorizing entry or change.

For each patient randomized, an eCRF must be completed and electronically signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study (this does not apply to patients who fail to be randomized). If a patient withdraws from the study, the reason must be noted on the eCRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

The investigator should ensure the accuracy, completeness and timeliness of the data reported to the sponsor in the eCRFs and in all required reports.

16. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [eCRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the eCRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the eCRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

17. CONFIDENTIALITY OF TRIAL DOCUMENTS AND SUBJECT RECORDS

The investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. On eCRFs or other documents submitted to the sponsor, patients should not be identified by their names, but by an identification code. The investigator should keep a patient randomization log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., patients' written consent forms, in strict confidence.

18. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Details on the publication of study results will be outlined in a separate publication policy document.

Appendix 1 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event]
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe]
- requires in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

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

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event are indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 1 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 (Cont.)

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs eForm of the eCRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor see attached list: 'Administrative and contact information'.

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science see attached list: 'Administrative and contact information'.

Appendix 2 Guideline for assessment of pathological response

The size of the tumor or the size of the gross area presumed to be tumor must be measured and all gross alterations within the size of the primary must be described.

NOTE: For any type of surgical specimen it is advisable to assess whether the margins are grossly tumor free, particularly when surgical resection is very close to the tumor

Primary tumor histology

The definition of WHO histologic type and grade (Elston & Ellis) will be made following the usual terminology.

Also non-invasive components must be reported and specified separately.

Report of microscopic findings after primary chemotherapy

For each individual patient the pattern(s) of residual tumor will be described.

For tumor remnants, the following mutually exclusive categories are suggested:

- Absent (no neoplastic cells) with or without fibrosis;
- Single neoplastic cells;
- Microscopic evidence of a single focus (diameter in mm to be specified);
- Microscopic evidence of multiple foci (largest diameter in mm to be specified);
- Macroscopic evidence of tumor (measures to be specified).

All the above information will be reported in the appropriate eCRF section.

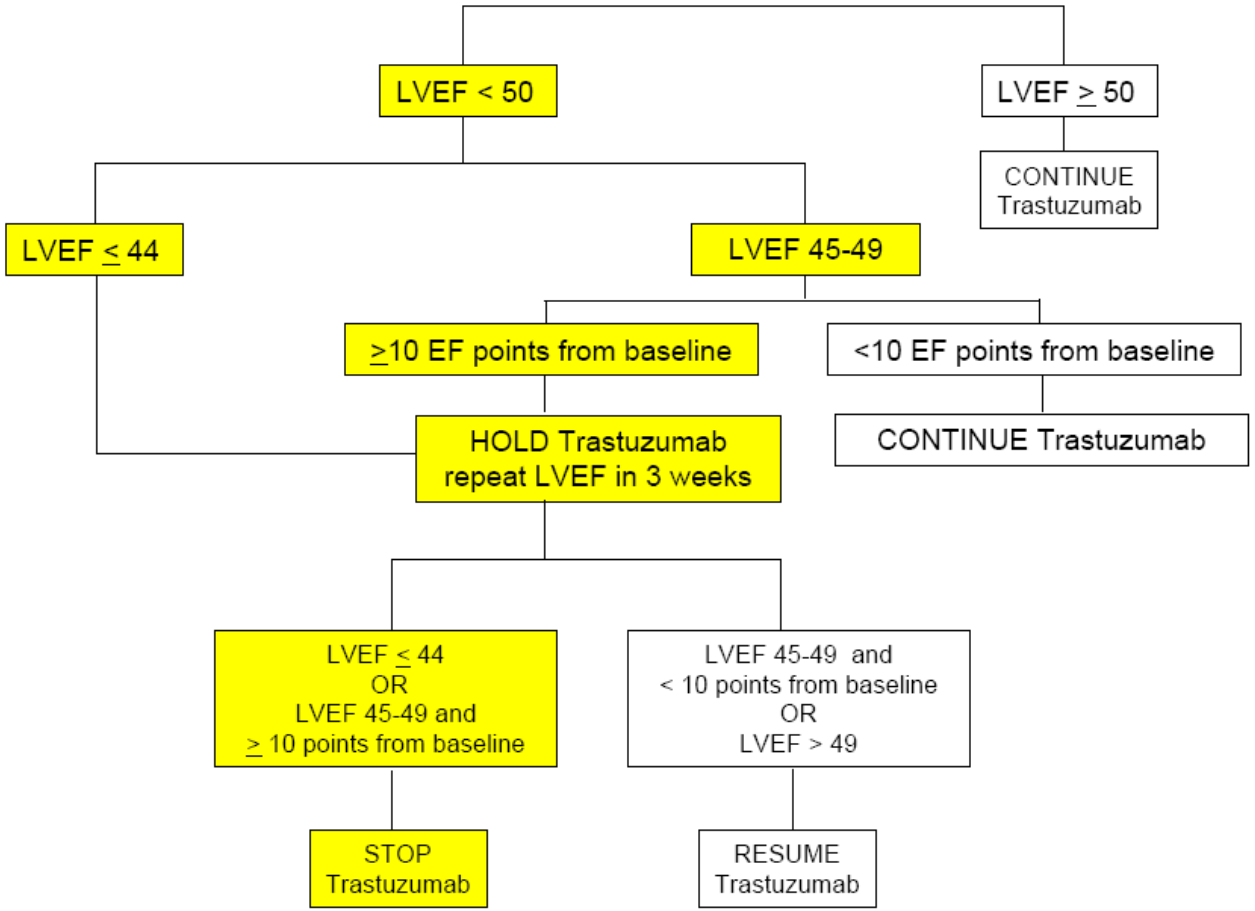
Appendix 3 ECOG performance status scale

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Reference:

Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 4 Algorithm for Continuation and Discontinuation of trastuzumab SC or IV based on LVEF Assessment in Asymptomatic Patients



Appendix 5 New York Heart Association (NYHA) functional classification system for heart failure

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

Appendix 6 Common Terminology Criteria for Adverse Events v3.0

The CTCAE v3.0 manual can be found at the following URL

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

Appendix 7 Breast Cancer Type by cTNM Classification

operable	locally advanced inoperable	inflammatory
T1bN0M0	T1bN2M0	T4dN0M0
T1bN1M0	T1bN3M0	T4dN1M0
T1cN0M0	T1cN2M0	T4dN2M0
T1cN1M0	T1cN3M0	T4dN3M0
T2N0M0	T2N2M0	
T2N1M0	T2N3M0	
T3N0M0	T3N2M0	
T3N1M0	T3N3M0	
	T4aN0M0	
	T4bN0M0	
	T4cN0M0	
	T4aN1M0	
	T4bN1M0	
	T4cN1M0	
	T4aN2M0	
	T4bN2M0	
	T4cN2M0	
	T4aN3M0	
	T4bN3M0	
	T4cN3M0	

Appendix 8 Selection of the Non-inferiority Margin in the non-Inferiority Analysis of pCR

Based on the EMEA guideline on The Choice of the non-Inferiority Margin [66] the non-inferiority margin for pCR in this study was selected as the lower bound of the 95% CI [pCR_{SC}-pCR_{IV}] that would ensure that the lower bound of the indirect 95% CI [pCR_{SC}-$pCR_{chemo\ alone}$] will be above zero.

Using a conservative approach the lower bound of the 95% CI [pCR_{SC}-$pCR_{chemo\ alone}$] can be derived by adding the lower bound of the CI [pCR_{SC}-pCR_{IV}] (i.e. pre-defined non-inferiority margin) and the lower bound of the CI [pCR_{IV}-$pCR_{chemo\ alone}$] (derived from metaanalysis, i.e. 12.76%). Thus in order to observe a lower bound of the indirect CI [pCR_{SC}-$pCR_{chemo\ alone}$] which is greater than 0, the lower bound of the CI [pCR_{SC}-pCR_{IV}] must be higher than 12.5%.

The meta-analysis on pCR rates and their confidence intervals which was performed on the two randomised studies investigating the efficacy of IV trastuzumab in the neo-adjuvant setting [19] [64] demonstrated that a non-inferiority margin of 12.5% for pCR indeed met this requirement:

Treatment effect [pCR_{IV}-$pCR_{chemo\ alone}$] 23.7%

95% two-sided CI [pCRIV-$pCR_{chemo\ alone}$] : [12.76%; 34.56%]

Treatment effect [pCR_{IV}-$pCR_{chemo\ alone}$] 23.7%

90% two-sided CI [pCRIV-$pCR_{chemo\ alone}$] : [14.51% ; 32.81%]

Calculation details for lower bound of confidence intervals:

Weighted analysis with the reciprocal of the variances as weights:

$w_i=1/(p_{1i}*(1-p_{1i})/n_{1i}+p_{2i}*(1-p_{2i})/n_{2i})$.
$d_i=p_{1i}-p_{2i}$

$effect=sum(w_i*d_i)/sum(w_i)$

lower confidence limit (95% two-sided CI)= effect - 1.96 $sqrt(1/sum(w_i))$
lower confidence limit (90% two-sided CI)= effect - 1.645 $sqrt(1/sum(w_i))$

where :

- w_i is the weight for study i,
- p_{1i} and n_{1i} are the pCR rate and number of patients in the Herceptin IV arm in study i
- p_{2i} and n_{2i} are the pCR rate and number of patients in the control arm in study i.
- d_i is the treatment effect in study i

.