Investigating Denosumab as an add-on to neoadjuvant chemotherapy in RANK/L-positive or RANK/L-negative primary breast cancer and two different nab-Paclitaxel schedules in a 2x2 factorial design (GeparX)

GBG 88

EudraCT No.: 2015-001755-72

Protocol Amendment 3 (Version 11.04.2019)

1. ADDRESSES, RESPONSIBILITIES AND SIGNATURES

Sponsor

GBG Forschungs GmbH

D-63263 Neu-Isenburg, Martin-Behaim-Straße 12

Phone: +49 (0) 6102 / 7480-0

Fax: +49 (0) 6102 / 7480-440

Email: GeparX@GermanBreastGroup.de

Co-ordinating Investigator (according to the German drug law AMG)

Prof. Dr. med. Jens-Uwe Blohmer

Study Co-Chair

Prof. Dr. med. Sibylle Loibl

GBG Subboard "Neoadjuvant"

Prof. Dr. J. U. Blohmer, Berlin

Prof. Dr. C. Denkert, Berlin (Pathology)

Prof. Dr. P.A. Fasching, Erlangen, L.A. / USA

Dr. C. Hanusch, München

Prof. Dr. J. Huober, Ulm

Prof. Dr. C. Jackisch, Offenbach

Dr. Theresa Link, Dresden

Prof. Dr. S. Loibl, Neu-Isenburg/FFM

PD Dr. Kerstin Rhiem, Köln

Prof. Dr. A. Schneeweiss, Heidelberg

Prof. Dr. C. Solbach, Frankfurt

Prof. Dr. M. Untch, Berlin

Protocol Board AGO-B

Prof. Dr. P.A. Fasching, Erlangen, L.A. / USA

Prof. Dr. T. Fehm, Düsseldorf

Prof. Dr. C. Jackisch, Offenbach

Prof. Dr. W. Janni, Ulm

Prof. Dr. T. Kühn, Esslingen

Prof. Dr. H.-J. Lück, Hannover

Prof. Dr. V. Möbus, Frankfurt

Prof. Dr. V. Müller, Hamburg

PD Dr. B. Rack, München

Prof. Dr. M. Schmidt, Mainz

Prof. Dr. A. Schneeweiss, Heidelberg

Prof. Dr. E. Stickeler, Aachen

Prof. Dr. C. Thomssen, Halle / Saale

Prof. Dr. M. Untch, Berlin

Project Management, Registration and Safety/SAE Reporting

Konstantin Reißmüller

GBG Forschungs GmbH, 63263 Neu-Isenburg, Martin-Behaim-Straße 12

Phone: +49 (0) 6102 / 7480-438

Fax: +49 (0) 6102 / 7480-440

Email: GeparX@GBG.de

Statistics

Dr. Valentina Nekljudova

GBG Forschungs GmbH, 63263 Neu-Isenburg, Martin-Behaim-Straße 12

Phone: +49 (0) 6102 / 7480-467

Fax: +49 (0) 6102 / 7480-440

Email: Valentina.Nekljudova@GBG.de

Monitoring Coordination

Udo Pfeil

GBG Forschungs GmbH, 63263 Neu-Isenburg, Martin-Behaim-Straße 12

Phone: +49 (0) 6102 / 7480-421

Fax: +49 (0) 6102 / 7480-440

Email: Udo.Pfeil@GBG.de

2. APPROVAL SIGNATURES

Study Title:

Investigating Denosumab as an add-on to neoadjuvant chemotherapy in RANK/L-positive or RANK/L-negative primary breast cancer and two different nab-Paclitaxel schedules in a 2x2 factorial design (GeparX)

Study Number:

GBG 88

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the study.

Signature: Date:

11.04.2019

_____ _____

Prof. Dr. Sibylle Loibl (GBG Forschungs GmbH)

Representing the Sponsor

11.04.2019

_____ _____

Prof. Dr. Jens-Uwe Blohmer

Coordinating Investigator

11.04.2019

_____ _____

Dr. V. Nekljudova (GBG Forschungs GmbH)

Study Biostatistician

3. PROTOCOL SYNOPSES

3.1 Protocol Synopsis (English)

Study Title	**Investigating Denosumab as an add-on to neoadjuvant chemotherapy in RANK/L-positive or RANK/L-negative primary breast cancer and two different nab-Paclitaxel schedules in a 2x2 factorial design (GeparX)**
Study Code	GBG 88
EudraCT Number	2015-001755-72
Sponsor	GBG Forschungs GmbH, Neu-Isenburg
Development Phase	Randomized phase IIb (including non-randomized cohort study for HER2+ primary breast cancer)
Rationale	RANK ligand (RANKL), a key factor for bone remodeling and metastasis, is crucial for the development of mouse mammary glands during pregnancy. RANKL functions as a major paracrine effector of the mitogenic action of progesterone in mouse and human mammary epithelium via its receptor RANK and has a role in ovarian hormone-dependent expansion and regenerative potential of mammary stem cells. Pharmacologic inhibition of RANKL attenuates the development of mammary carcinoma and inhibits metastatic progression in multiple mouse models.[1] In a retrospective analysis of 601 patients treated in the GeparTrio study with chemotherapy (TAC) we could demonstrate that elevated expression of RANK (immunohistochemical score > 8.5 using the N-1H8 antibody by Amgen) was found in 14.5% of patients overall.[2] The ABCSG-18 study showed that adjuvant denosumab reduces clinical fractures, improves bone health, and can be administered without added toxicity.[3] Moreover denosumab showed a trend in improvement of disease-free survival in postmenopausal woman with hormone receptor positive breast cancer.[4] It appears therefore reasonable to test denosumab, a clinically available antibody against RANKL in patients with primary breast cancer as an adjunct to neoadjuvant chemotherapy for its ability to

	increase pCR rate and improve outcome overall and in relation to the expression of RANK/L. The backbone chemotherapy consists of nab-Paclitaxel because the pCR rate in the GeparSepto study could be increased by using nab-Paclitaxel instead of sb paclitaxel. Two different nab-Paclitaxel regimen will be compared.
Rationale of the Investigation of Trastuzumab (ABP 980) in combination with pertuzumab (HER2+ Substudy)	Monoclonal Antibodies are complex proteins with high molecular weight (MW). Biosimilars have the potential to significantly improve access to expensive agents. Biosimilarity is defined as follows: The biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components. There are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency. ABP 980, a candidate as a biosimilar to trastuzumab, has been shown to be as effective as the reference product Herceptin®, in terms of pCR achievement - in early breast cancer (Lilac, NCT 01901146). This was the first time this was demonstrated for a biosimilar for trastuzumab in the neoadjuvant setting. ABP 980 has similar binding to $Fc_{\gamma}RIIIa$ as Herceptin®. In vivo and in vitro data confirmed similar function. In a neoadjuvant study randomizing 725 patients, ABP 980 was compared to Herceptin as part of a standard neoadjuvant EC-Paclitaxel regimen and showed comparable pCR rates. Patients receiving Herceptin were randomized to continue after surgery with Herceptin or transitioned to ABP 980. All other short and long term parameters assessed were also not significantly different. Currently the dual blockade of trastuzumab and pertuzumab in combination with chemotherapy is indicated as neoadjuvant therapy in HER2+ primary breast cancer. So GeparX will also evaluate the safety and efficacy of ABP 980 in combination with pertuzumab as neoadjuvant therapy in the treatment of HER 2+ primary breast cancer. All patients with HER2+ disease will receive Pertuzumab in addition to Trastuzumab (ABP 980) throughout the trial.
Co-Primary	• A: To compare the pathological complete response (pCR= ypT0 ypN0) rates of neoadjuvant treatment with or

Objectives	without denosumab in addition to backbone treatment consisting of nPac 125mg/m² weekly (Cb)→EC or nPac 125mg/m² day 1,8 q22 (Cb) →EC plus anti-HER2 treatment (i. e. trastuzumab/pertuzumab in case of positive HER2-status) in patients with early breast cancer. • B: To compare the pathological complete response (pCR= ypT0 ypN0) rates of nPac 125mg/m² weekly(Cb)→EC or nPac 125mg/m² day 1,8 q22 (Cb)→EC plus anti-HER2 treatment (i. e. trastuzumab/pertuzumab in case of positive HER2-status) in patients with early breast cancer.
Secondary Objectives	• To test for interaction of denosumab treatment with RANK expression. The cutoff for the RANK expression high vs low will be defined in the SAP. • To assess the pCR rates per arm in subgroups according to stratification (minimization) factors. • To assess the pCR rates per arm for patients with RANK high and RANK low prospectively and centrally by IHC. • To determine the rates of ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0 for both randomizations. • To determine the response rates of the breast tumor and axillary nodes based on physical examination and imaging tests (sonography, mammography, or MRI) after treatment in both arms for each randomization. • To determine the breast conservation rate after each treatment. • To assess the toxicity and compliance, including time to onset of peripheral sensory neuropathy grade 2-4 and resolution of peripheral sensory neuropathy grade 2-4 to grade 1. • To determine loco-regional invasive recurrence free survival (LRRFS), distant-disease-free survival (DDFS), invasive disease-free survival (IDFS), EFS (event free survival) and overall survival (OS) for all treatment arms and according to stratified subpopulations. • To compare RANK/L expression from baseline to surgery. • To compare Ki67 from baseline to surgery. • To correlate response (complete vs. partial vs. no change) measured by best appropriate imaging method after the first two cycles of treatment with pCR. • To assess mammographic density–changes induced by denosumab.

	• To assess quality of life with a focus on persisting peripheral sensory neuropathy using the FACT-Taxane (Version 4) questionnaire.
Correlative Science Objectives	• To assess, characterize, and correlate disseminated tumor cells with the treatment effect (DTC Substudy). To correlate Single Nucleotide Polymorphisms (SNPs) of genes with the associated toxicity and histologically assessed treatment effect (Pharmacogenetic substudy). • To examine and compare the impact on the pCR of the pre-specified molecular markers such as TILs, RANK/L and others on core biopsies as well as clinical characteristics (e.g. age). • To assess molecular markers at baseline and surgery. • Detection of microRNA and correlation with pCR (Substudy on urinary miRNA sampling (UMS))**.**
Primary Objectives of the HER2+ Substudy	• To assess the pathological complete response (pCR= ypT0 ypN0) rate of neoadjuvant treatment with ABP 980 and pertuzumab in the overall HER2+ cohort and compare with the results obtained in GeparSepto study. • To compare the pathological complete response (pCR= ypT0 ypN0) rate of nPac 125mg/m² weekly→EC or nPac 125mg/m² day 1,8 q22 →EC plus anti-HER2 treatment (i. e. ABP 980 / pertuzumab in case of positive HER2-status) in patients with early breast cancer.
Study Design and Treatment	This is a multicenter, prospective, 2x2 randomized, open-label phase IIb study to compare neoadjuvant treatment with and without denosumab in patients with untreated breast cancer and two different nab-paclitaxel schedules. Patients will be first randomized (using Pocock minimization) to one of the following two treatments in addition to neoadjuvant therapy: • Denosumab (120 mg s.c. q4w) • No denosumab Stratification (minimization) factors for the randomization will be: • LPBC (negative (defined as ≤50% stromal tumour infiltrating lymphocytes) / present (defined as >50% stromal tumour infiltrating lymphocytes)) • Subtype (HER2-/HR+ vs TNBC vs. HER2+)

	• EC every 2 vs EC every 3 weeks Secondarily patients will be randomized (using Pocock minimization) to: • nPac 125mg/m² weekly (Cb)→EC • nPac 125mg/m² day 1,8 q22 (Cb)→EC The first randomization (denosumab) will be an additional minimization factor for the second randomization (chemotherapy regimen). The HER2+ substudy is a cohort study investigating open label non-randomized use of ABP 980 in combination with pertuzumab. In all study arms, treatment will be given until surgery, disease progression, unacceptable toxicity, withdrawal of consent of the patient, or termination by the Sponsor.
Inclusion Criteria	Patients will be eligible for study participation only if they comply with the following criteria: • Written informed consent according to local regulatory requirements prior to beginning specific protocol procedures. • Complete baseline documentation must be submitted via MedCODES to GBG Forschungs GmbH. • Unilateral or bilateral primary carcinoma of the breast, confirmed histologically by core biopsy. Fine-needle aspiration from the breast lesion alone is not sufficient. Incisional biopsy or axillary clearance is not allowed. In case of bilateral cancer, the investigator has to decide prospectively which side will be evaluated for the primary endpoint. • Tumor lesion in the breast with a palpable size of $\geq$ 2 cm or a sonographical size of $\geq$ 1 cm in maximum diameter. The lesion has to be measurable in two dimensions, preferably by sonography. In case tumor isn't measurable by sonography, then MRI or mammography is sufficient. In case of inflammatory disease, the extent of inflammation can be used as measurable lesion. • Patients must be in the following stages of disease: - cT2 - cT4a-d or - cT1c and cN+ or - cT1c and pN_{SLN}+ or - cT1c and ER-neg and PR-neg or - cT1c and Ki67>20% or

	- cT1c and HER2-pos • In patients with multifocal or multicentric breast cancer, the largest lesion should be measured. • Centrally confirmed ER-, PR- and HER2-status. Central pathology includes also assessment of Ki-67, TIL and RANK/L status on core biopsy. TNBC is defined as ER<1% and PR<10% stained cells and HER2-negative; and HER2-positive is defined as IHC 3+ or in-situ hybridization (ISH) and according to ASCO-CAP guidelines as of 2013. LPBC (lymphocyte predominant breast cancer) is defined as more than 50% stromal tumour infiltrating lymphocytes. Formalin-fixed, paraffin-embedded (FFPE) breast tissue from core biopsy has therefore to be sent to the GBG central pathology laboratory prior to randomization. Patients will be eligible for the HER2+ substudy if they have a centrally confirmed HER2+ tumor. • Age ≥ 18 years. • Karnofsky Performance status index ≥ 90%. • Confirmed normal cardiac function by ECG and cardiac ultrasound (LVEF or shortening fraction) within 3 months prior to randomization. Results must be above the normal limit of the institution. For patients with HER2-positive tumors LVEF must be above 55%. • Laboratory requirements: Hematology - Absolute neutrophil count (ANC) ≥ 2.0 x 10^9 / L and - Platelets ≥ 100 x 10^9 / L and - Hemoglobin ≥ 10 g/dL (≥ 6.2 mmol/L) Hepatic function - Total bilirubin ≤ 1.5x UNL and - ASAT (SGOT) and ALAT (SGPT) ≤ 1.5x UNL and - Alkaline phosphatase ≤ 2.5x UNL. • Serum calcium or albumin-adjusted serum calcium ≥2.0 mmol/L (8.0 mg/dL) and ≤2.9 mmol/L (11.5 mg/dL). Hypocalcemia has to be corrected before study entry by supplementation of calcium and vitamine D. • Negative serum pregnancy test within 14 days prior to randomization for all women of childbearing potential with the result available before dosing. • Complete staging work-up within 3 months prior to randomization. All patients must have bilateral mammography, breast ultrasound (≤ 21 days), breast MRI (optional). Chest X-ray

	(PA and lateral), abdominal ultrasound or CT scan or MRI, and bone scan in case of high risk for primary metastasis. In case of a positive bone scan, bone X-ray (or CT or MRI) is mandatory. Other tests may be performed as clinically indicated. • Patients must agree with central pathology testing of core biopsy specimen and final pathology specimen and be available and compliant for treatment and follow-up.
Exclusion Criteria	• Pure lobular carcinomas (lobular histology and G1/G2 and HR+/HER2-) • Patients with stages cT1a, cT1b, or any M1. • Prior chemotherapy for any malignancy. • Prior radiation therapy for breast cancer. • History of disease with influence on bone metabolism, such as osteoporosis, Paget's disease of bone, primary hyperparathyreoidism requiring treatment at the time of randomization or considered likely to become necessary within the subsequent six months. • Use of bisphosphonates or denosumab within the past 1 year. • Significant dental/oral disease, including prior history or current evidence of osteonecrosis/ osteomyelitis of the jaw, active dental or jaw condition which requires oral surgery, non-healed dental/oral surgery, planned invasive dental procedure for the course of the study. • Last visit at dentist > ½ year ago. • Pregnant or lactating patients. Patients of childbearing potential must agree to use highly effective non-hormonal contraceptive measures during study treatment and 7 months following the last dose of mAbs. • Inadequate general condition (not fit for anthracycline-taxane-targeted agents-based chemotherapy). • Previous malignant disease being disease-free for less than 5 years (except CIS of the cervix and non-melanomatous skin cancer). • Known or suspected congestive heart failure (>NYHA I) and / or coronary heart disease, angina pectoris requiring antianginal medication, previous history of myocardial infarction, evidence of transmural infarction on ECG, uncontrolled or poorly controlled arterial hypertension (e.g. BP >140 / 90 mm Hg under treatment with two antihypertensive drugs), controlled arterial hypertension under treatment with three or more antihypertensive drugs, rhythm abnormalities requiring

	permanent treatment, clinically significant valvular heart disease. • History of significant neurological or psychiatric disorders including psychotic disorders, dementia or seizures that would prohibit the understanding and giving of informed consent. • Pre-existing motor or sensory neuropathy of a severity ≥ grade 2 by NCI-CTC criteria v 4.0. • Currently active infection. • Incomplete wound healing. • Definite contraindications for the use of corticosteroids. • Known hypersensitivity reaction to one of the compounds or incorporated substances used in this protocol inclusive calcium and vitamine D. Known hereditary fructose intolerance. • Concurrent treatment with: - chronic corticosteroids unless initiated > 6 months prior to study entry and at low dose (10mg or less methylprednisolone or equivalent). - sex hormones. Prior treatment must be stopped before study entry. - other experimental drugs or any other anti-cancer therapy. • Participation in another clinical trial with any investigational, not marketed drug within 30 days prior to study entry.
Investigational products and formulation	Denosumab 120mg s.c. every 4 weeks for 6 cycles. nab-Paclitaxel 125mg/m² weekly for 12 weeks (days 1, 8, 15 every 3 weeks for 4 cycles) or nab-Paclitaxel 125mg/m² day 1, 8 q22 for 4 cycles (12 weeks). For patients with HER2-positive disease: ABP 980 Loading dose: 8mg/kg, thereafter 6 mg/kg, every 3 weeks simultaneously to all chemotherapy cycles. After surgery all patients will change to either the reference product Herceptin or to another approved biosimilar trastuzumab per investigator`s decision/local standard. The protocol will provide procedures for specific adverse events requesting dose modifications or delays.
Non-investigational product and formulation	For all patients: Epirubicin 90mg/m² and Cyclophosphamide 600mg/m² every 2 or 3 weeks (Investigator's decision before randomization) after nab-Paclitaxel (Cb). For patients with triple-negative disease:

	• Carboplatin AUC 2 weekly in parallel to the cycles of nab-paclitaxel. For patients with HER2-positive disease: • Pertuzumab 840mg loading dose i.v. followed by 420mg i.v. every 3 weeks simultaneously to chemotherapy for at least 4 applications (according to label). These agents are used according to marketed formulation via normal procedures at each site and applied according to recommendations of the manufacturers.
Supportive treatment	Supplementation of at least daily 500mg calcium and 400 IU vitamine D is required in all patients receiving denosumab, unless hypercalcaemia is present. If hypocalcemia occurs, short-term augmentation of calcium supplementation to 1000mg/daily may be necessary. Good oral hygiene practices should be maintained during treatment with denosumab. Avoid invasive dental procedures during treatment with denosumab. For patients in whom invasive dental procedures cannot be avoided, the clinical judgment of the treating physician should guide the management plan (postpone dental treatment vs interruption of denosumab) of each patient based on individual benefit/risk assessment. Other supportive treatments are recommended during chemotherapy according to AGO, ESMO, or ASCO guidelines (e.g. www.asco.org/guidelines/antiemetics).
Primary endpoint	**Primary efficacy endpoint:** **Pathological complete response of breast and lymph nodes (ypT0 ypN0; primary endpoint)** No microscopic evidence of residual invasive or non-invasive viable tumor cells in all resected specimens of the breast and axilla. Pathological response will be assessed considering all removed breast and lymphatic tissues from all surgeries. Patients with negative sentinel node biopsy prior to treatment start and no axilla surgery after neoadjuvant chemotherapy will be counted as pCR if no invasive and non-invasive residual tumor is detected in the removed breast tissue. Patients with histologically/cytologically positive nodes prior to treatment start and no axilla surgery after chemotherapy will be

	counted as no pCR (preferably axillary dissection instead of sentinel node biopsy is strongly recommended in this situation). Patients with positive sentinel node biopsy prior to treatment start and no invasive and non-invasive residual tumor detected in the removed breast tissue and lymph nodes after chemotherapy will be counted as pCR.
Secondary endpoints	**Secondary short-time efficacy endpoints** ypT0/Tis ypN0 is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast and axilla; in case of sentinel node biopsy prior to treatment start, the axillary lymph nodes will be evaluated as described for the primary endpoint. ypT0 ypN0/+ is defined as no microscopic evidence of residual invasive or non-invasieve viable tumor cells in all resected specimens of the breast; ypT0/Tis ypN0/+ is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast; patients with a sentinel node biopsy prior to treatment start will be evaluated for $ypT_{(any)}$ ypN0 similarly to the description given for the primary endpoint. Clinical (c) and imaging (i) response will be assessed every 2nd cycle and before surgery by physical examination and imaging tests. Sonography is the preferred examination, however, if sonography appears not to provide valid results or is not performed, MRI, mammography or palpation will be considered with decreasing priority. The same imaging method should be considered for the measurement before, during and after treatment. For defined categories of efficacy (complete, partial, stable, or progression), the proportion of patients with success will be determined and appropriate confidence intervals will be calculated. The response categories of the breast are: • **Complete response (CR):** complete disappearance of all tumor signs in the breast as assessed by all available imaging test and palpation. The response of the axillary nodes is not to be considered. • **Partial response (PR):** reduction in the product of the two largest perpendicular diameters of the primary tumor size by 50% or more assessed by imaging test or palpation. In patients with multifocal or multicentric disease, the lesion with the

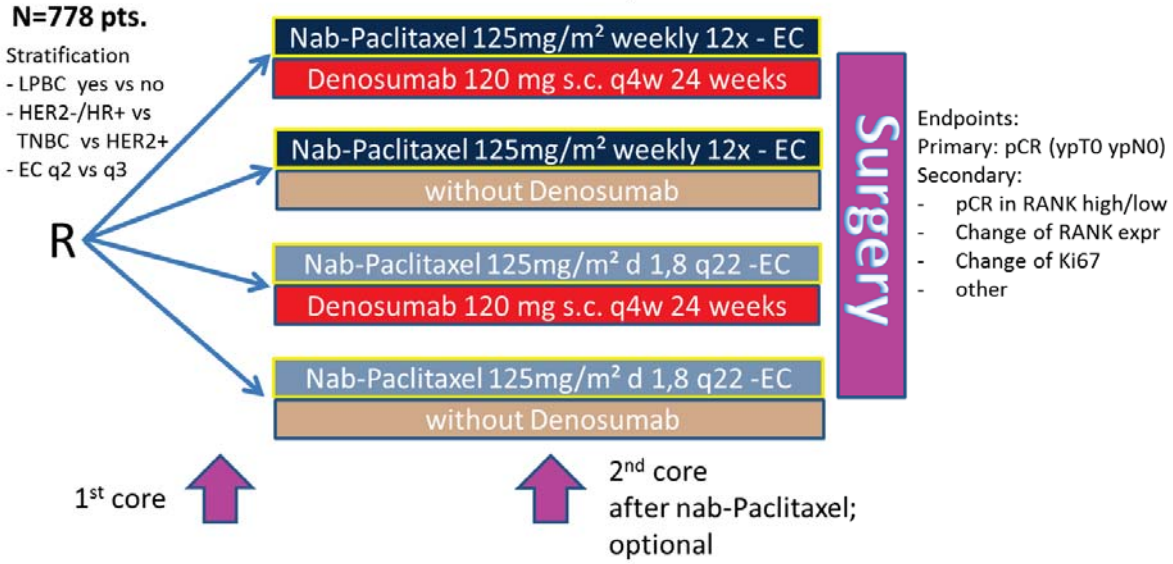
	largest diameters should be chosen for follow-up. The response of the axillary nodes is not to be considered. • **Stable disease (NC):** no significant change in tumor size during treatment which means an estimated reduction of the tumor area by less than 50%, or an estimated increase in the size of the tumor area lesions of less than 25%. • **Progressive disease (PD):** development of new, previously undetected lesions, or an estimated increase in the size of pre-existing lesions by 25% or more after at least two cycles of therapy. Breast conservation is defined as tumorectomy, segmentectomy or quadrantectomy as a most radical surgery. Patients in whom success cannot be determined (e.g. patients in whom histology is not evaluable) will be included in the denominator, i.e. these patients will affect the success rate in the same way as treatment failures. **LRRFS, DDFS, IDFS, EFS and OS** are defined as the time period between randomisation and first event and will be analyzed after the end of the study by referring to data from GBG patient's registry. Progressions during neoadjuvant treatment are not considered as events unless the patient is not amenable for surgery. **Tolerability and Safety:** Descriptive statistics for the 4 treatments (+/- anti-HER2-treatment) will be given on the number of patients whose treatment had to be reduced, delayed or permanently stopped. The reason for termination includes aspects of efficacy (e.g. termination due to tumor progression), safety (e.g. termination due to adverse events) and compliance (e.g. termination due to patient's withdrawal of consent). Reasons for premature termination will be categorized according to the main reason and will be presented in frequency tables. Safety by toxicity grades are defined by the NCI-CTCAE version 4.0. **Correlative science research:** Exploratory analyses will be performed to identify possible relationships between biomarkers and drug activity. The aim is to identify potential prognostic/predictive biomarkers of short and long term outcome parameters (pCR, EFS, and OS). Mammographic density of the pre-treatment and pre-surgical mammogram will be assessed centrally. Missing data on response evaluation will be set to no response.

Statistical Methods	An 'intent-to-treat' (ITT) analysis will be conducted for all patients randomized in the study. In addition, a 'per-protocol' analysis will be conducted; the detailed definition of the per-protocol analysis set will be given in the statistical analysis plan. All HER2+ patients will be analysed for subgroups and multivariate analyses of the main study irrespective of the anti-HER2 treatment according to the general ITT principles.
Statistical Methods Primary endpoint	Primary objectives A and B will be tested according to the improved Bonferroni procedure: the smaller of the two p-values will be compared with $\alpha = 0.1$ and the larger p-value will be compared with $\alpha = 0.2$ to keep the overall significance level of the study of $\alpha = 0.2$.[5] The primary endpoint will be summarized as pathological complete response rate for each treatment group for both randomizations. Two-sided 90% confidence intervals will be calculated according to Pearson and Clopper.[6] The difference in the rates of pathological complete response will be evaluated as rate difference (for primary objective A denosumab arm minus no-denosumab arm; for primary objective B nPac 125w (Cb)→ EC minus nPac day 1,8 q22 (Cb)→ EC arm) with 90% confidence interval. Additionally, an odds ratio with the 90% confidence interval will be reported. The significance will be tested with the two-sided continuity corrected χ^2-test according to the improved Bonferroni procedure. The null hypothesis is that there is no difference in pCR rates between treatment arms; the alternative hypothesis is that there is a difference for both randomizations. The significance level for all other tests is set to 2-sided $\alpha = 0.05$. There will be no adjustment for multiple comparisons in the analyses for the stratified subpopulations. A secondary logistic regression analysis correcting for the minimization factors will be conducted for the primary endpoint. Uni- and multivariate logistic regression will be performed for pCR to adjust for the known factors (treatment group for both randomizations, minimization factors, age, tumor size, nodal status, grade, histological type), based on the ITT population. Additionally, a multivariate logistic regression including all factors above and interaction between denosumab and chemotherapy arms will be performed.

	Primary and secondary objectives for the HER2+ substudy will be assessed in all patients who have received at least one dose of ABP 980. The pCR rates with a 95% CI will be reported and compared between chemotherapy treatment arms using the continuity corrected χ^2-test.
Statistical Methods Sample size	The sample size calculation is based on the following assumptions for the primary endpoint: • Improvement of the pCR rate by denosumab in all patients from 35% to 46% (OR=1.58) • Improvement of the pCR rate by different schedules of chemotherapy (nPac 125mg day 1,8 q22 (Cb) → EC arm to nPac 125mg w (Cb) → EC) will be 36% to 45% (OR=1.45) With 778 recruited patients, the primary continuity corrected χ^2-test of pCR rates between denosumab and no denosumab arms will have 92% power to the 2-sided significance level α=0.10. The continuity corrected χ^2-test of pCR rates between nPac 125mg w (Cb)→ EC) to nPac 125mg day 1,8 q22 (Cb) → EC arms will have 80% power to the 2-sided significance level α=0.10. Sample size for the continuity corrected χ^2-test was computed using nQuery Advisor 6.02. It is planned to recruit 778 subjects into this study. The sample size calculation for the HER2+ substudy is based on the primary endpoint of the main study: All patients with HER2+ disease enrolled into the study will receive ABP 980 in addition to pertuzumab and backbone chemotherapy. It is planned to recruit approximately 150 subjects into this substudy.
Statistical Methods Secondary Endpoints	**Secondary short-time efficacy endpoints** (ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0, response by physical examination, imaging response, breast conservation) will also be summarized as rates in each treatment group, two-sided 95% confidence intervals will be calculated according to Pearson and Clopper, and the continuity corrected Pearson χ^2 test will be performed to evaluate the difference of rates in treatment arms; these tests are considered explorative. The significance level for all tests is set to 2-sided $\alpha = 0.05$. Subgroup and multivariate analyses

	will be performed for ypT0/Tis ypN0 in the same way as for the primary endpoint. A Breslow-Day test for interaction will be performed to asses difference of treatment effect between high RANK and low RANK subgroups (the cutpoint will be defined in SAP) with 2-sided $\alpha = 0.1$. The null hypothesis is that the odds ratios of pCR in denosumab arm to no denosumab arm are equal in the RANK+ and RANK- subgroups, the alternative hypothesis is that odds ratios are not equal. For **LRRFS, DDFS, IDFS, EFS and OS** curves will be estimated using the Kaplan-Meier method, based on the ITT population. 3 year and 5 year survival (and 95%CIs) will be estimated. Univariate and multivariate Cox-proportional hazards model will be used to adjust hazard ratios for minimization factors and the above defined covariates. Time to the first occurrence of grade 2-4 peripheral neuropathy and time to improvement of peripheral neuropathy will be analyzed using Kaplan-Meier curves and log-rank test. Safety and compliance for HER2+ substudy will be reported descriptively in treatment arms. More details will be in the SAP and follow the general safety assessment of the main study.
Number of sites	It is planned to conduct the study within approximately 60 sites.
Enrollment Period	Approximately 24 months (Q-I 2017 – Q-IV 2018).
Study duration	Approximately 32 months (24 months recruitment + 6 months treatment duration + maximum 2 months time to surgery).
Follow-up Period	As no study specific treatment or investigation is planned after 90 days after surgery, follow up is not part of this study. However, information on subsequent cancer specific treatments and the health status of the patients is collected either based on yearly chart reviews at the sites or based on information deriving from the GBG registry of previous study participants. Information on date and site of recurrences, date and cause of deaths as well as secondary malignancies and important long-term side effects will be collected.

GeparX: Investigating Denosumab as an add-on neoadjuvant treatment for RANK-positive or RANK-negative primary breast cancer and two different nab-Paclitaxel schedules ; 2x2 factorial design



Assumptions for primary endpoint (α=0.2, β=0.2):
Denosumab: 35% to 46% OR 1.58
nab-Pac: 36% to 45%; OR 1.45 ;

Treatment backbone:
HER2+:+ Trastuzumab (ABP 980) + Pertuzumab
TNBC: + Carboplatin weekly AUC 2

3.2 Protocol Synopsis (German) / Deutsche Zusammenfassung

Studientitel	Denosumab als Ergänzung zur neoadjuvanten Therapie beim RANK/L-positiven oder RANK/L-negativen primären Mammakarzinom und zwei verschiedenen nab-Paclitaxel Therapie-Schemata in einem 2x2 faktoriellen Design
Studiencode	GBG 88
EudraCT-Nummer	2015-001755-72
Sponsor	GBG Forschungs GmbH, Neu-Isenburg
Phase	Randomisierte Phase IIb (mit einer nicht-randomisierten Kohorten-Studie für HER2-positiven primären Brustkrebs)
Rationale	RANK-Ligand (RANKL), ein Schlüsselfaktor für den Knochenumbau und die Metastase, ist entscheidend für die Entwicklung von Brustdrüsen der Maus während der Schwangerschaft. RANKL funktioniert als übergeordneter parakriner Effektor der mitogenen Wirkung von Progesteron im Brustepithel der Maus und des Menschen über seinen Rezeptor RANK und spielt bei der ovarialen hormonabhängigen Ausdehnung und dem regenerativen Potential von Mamma-Stammzellen eine Rolle. Eine pharmakologische Hemmung von RANKL schwächt die Entwicklung des Brustkarzinoms ab und inhibiert den metastatischen Progress bei multiplen Mausarten.[1] In einer retrospektiven Analyse von 601 in der GeparTrio-Studie mit Chemotherapie (TAC) behandelten Patienten konnte gezeigt werden, dass eine erhöhte Expression von RANK (immunhistochemischer Score > 8,5 unter Verwendung des N-1H8-Antikörpers von Amgen) bei 14,5% der Patienten insgesamt nachgewiesen wurde. [2] Die Primäranalyse zur ABCSG-18 (ASCO 2015) zeigte, dass der adjuvante Einsatz von Denosumab klinische Frakturen reduziert, die Knochengesundheit verbessert und ohne zusätzliche Toxizitäten verabreicht werden kann.[3] Daher kann Denosumab adjuvant positive Auswirkungen auf das Ansprechen bei

	postmenopausalen Brustkrebspatienten haben.[4] Es erscheint daher sinnvoll, bei Patienten mit primärem Brustkrebs Denosumab als Ergänzung zu neoadjuvanter Chemotherapie im Hinblick auf die Möglichkeit einer Erhöhung der pCR-Rate und eine Verbesserung des Outcomes insgesamt und in Relation zur Ausprägung von RANK/L zu testen. Die Chemotherapie besteht aus nab-Paclitaxel, da die pCR-Rate in der GeparSepto-Studie unter Verwendung nab-Paclitaxel statt sb Paclitaxel erhöht werden konnte. Zwei verschiedene nab-Paclitaxel-Regime werden verglichen.
Rationale der Untersuchung von Trastuzumab (ABP 980) in Kombination mit Pertuzumab (HER2+ Substudie)	Monoklonale Antikörper sind komplexe Proteine mit hohem Molekulargewicht (MW). Biosimilars haben das Potenzial, den Zugang zu teuren Wirkstoffen deutlich zu verbessern. Biosimilarität ist wie folgt definiert: Das biologische Produkt ist dem Referenzprodukt trotz kleiner Unterschiede in klinisch inaktiven Komponenten sehr ähnlich. Es gibt keine klinisch bedeutsamen Unterschiede zwischen dem biologischen Produkt und dem Referenzprodukt hinsichtlich der Sicherheit, Reinheit und Potenz. ABP 980, ein Kandidat als Biosimilar zu Trastuzumab, hat sich in Bezug auf die pCR beim primären Brustkreb als genauso effektiv wie das Referenzprodukt Herceptin® erwiesen (Lilac, NCT 01901146). Dies war das erste Mal, dass dies für ein Biosimilar für Trastuzumab auf neoadjuvantem Gebiet gezeigt wurde. ABP 980 hat eine ähnliche Bindung an $Fc_{\gamma}RIIIa$ wie Herceptin®. In vivo- und in vitro-Daten bestätigten ähnliche Funktionen. In einer neoadjuvanten Studie mit 725 randomisierten Patienten wurde ABP 980 mit Herceptin® als Teil eines standardmäßigen neoadjuvanten EC-Paclitaxel-Regimes verglichen und zeigte vergleichbare pCR-Werte. Patienten, die Herceptin® erhielten, wurden randomisiert, nach der Operation mit Herceptin® fortzufahren oder auf ABP 980 umzustellen. Alle anderen Kurz- und Langzeitparameter waren ebenfalls nicht signifikant verschieden. Derzeit ist die doppelte Blockade von Trastuzumab und Pertuzumab in Kombination mit Chemotherapie als neoadjuvante Therapie bei HER2+ primärem Brustkrebs indiziert. So wird GeparX auch die Sicherheit und Wirksamkeit von ABP 980 in Kombination mit Pertuzumab als neoadjuvante Therapie bei der

	Behandlung von HER 2+ primärem Brustkrebs evaluieren. Alle Patienten mit HER2-positiver Erkrankung erhalten Pertuzumab in Ergänzung zu Trastuzumab (ABP 980) während der gesamten Studie.
Co-Primäres Zielkriterium	• A: Vergleich der pathologischen Komplettremissions-Rate (pCR= ypT0 ypN0) von neoadjuvanter Behandlung mit oder ohne Denosumab als Ergänzung zur Behandlung mit nPac 125mg/m² wöchentlich (Cb)→EC oder nPac 125mg/m² an den Tagen 1,8 q22 (Cb)→EC plus anti-HER2-Behandlung (i. e. Trastuzumab/Pertuzumab bei positivem HER2-Status) bei Patienten mit Brustkrebs im Frühstadium. • B: Vergleich der pathologischen Komplettremissions-Rate (pCR= ypT0 ypN0) von nPac 125mg/m² wöchentlich (Cb)→EC oder nPac 125mg/m² an den Tagen 1,8 q22 (Cb)→EC plus anti-HER2-Behandlung (i. e. Trastuzumab/Pertuzumab bei positivem HER2-Status) bei Patienten mit Brustkrebs im Frühstadium.
Sekundäre Zielkriterien	• Test der Interaktion von Denosumab-Behandlung und RANK-Expression. Der Cutoff für die RANK-Expression hoch vs. niedrig wird im SAP definiert. • Bestimmung der pCR-Raten pro Arm in Subgruppen entsprechend Stratifikations- (Minimierungs-) Faktoren. • Bestimmung der pCR-Rate pro Arm bei Patienten mit hoher und niedriger RANK-Expression prospektiv und zentral durch IHC. • Bestimmung der Raten von ypT0/is ypN0; ypT0 ypN0/+; ypT0/is ypN0/+; $ypT_{(jedes\ Stadium)}$ ypN0 in beiden Armen. • Bestimmung der Ansprechrate des Brusttumors und der axillären Lymphknoten anhand körperlicher Untersuchung und bildgebender Verfahren (Sonographie, Mammographie oder MRT) nach Behandlung in beiden Armen für jede Randomisation. • Bestimmung der Rate der brusterhaltenden Therapie nach jeder Behandlung. • Bestimmung von Toxizität und Compliance einschließlich der Dauer bis zum Auftreten einer peripheralen sensorischen Neuropathie von Grad 2-4 und des

	Rückgangs einer peripheren sensorischen Neuropathie von Grad 2-4 auf Grad 1. • Bestimmung des loko-regionären invasiv rezidivfreien Überlebens (LRRFS), des Fernmetastasen-freien Überlebens (DDFS), des invasiv krankheitsfreien Überlebens (IDFS), des Ereignis-freien Überlebens (EFS) und des Gesamtüberlebens (OS) in allen Behandlungsarmen und entsprechend den stratifizierten Subgruppen. • Vergleich der RANK/L-Expression der prätherapeutischen Core-Biopsie und dem Operationsresektat. • Vergleich der Ki-67-Expression der prätherapeutischen Core-Biopsie und dem Operationsresektat. • Ansprechrate (komplett vs. partiell vs. unverändert) anhand der Messungen durch das jeweils am besten geeignete bildgebende Verfahren nach den beiden ersten Behandlungszyklen in Korrelation mit der pCR. • Veränderung der Mammographie-Dichte mit und ohne Denosumab. • Bestimmung der Lebensqualität besonders zu andauernder peripherer sensorischer Neuropathie unter Verwendung des Fragebogens FACT-Taxan (Version 4).
Ziele der Substudien	• Untersuchung, Charakterisierung und Korrelation disseminierter Tumorzellen im Knochenmark mit den Therapieeffekten (DTC Substudie). • Evaluation genomweiter Single Nucleotide Polymorphismen (SNPs) zur Entdeckung von Genen, die mit der beobachteten Toxizität und der histologisch festgestellten Therapieeffizienz in Zusammenhang gebracht werden können (Pharmakogenetische Substudie). • Untersuchung und Vergleich von vorab definierten molekularen Markern wie z.B. TILs (für LPBC), RANK/L und andere wie auch klinische Charakteristika (z.B. Alter) an der prätherapeutischen Core-Biopsie und am Operationsresektat. • Untersuchung molekularer Marker bei Baseline und Operation.

	• Nachweis von microRNA und Korrelation mit dem klinischen Ansprechen (Substudie zur Untersuchung von Urin auf miRNA (UMS)).
Primäre Zielkriterien der HER2+ Substudie	• Vergleich der pathologischen Komplettremissions-Rate (pCR= ypT0 ypN0) von neoadjuvanter Behandlung mit ABP 980 und Pertuzumab in der HER2+ Gesamtkohorte und Vergleich mit den in der GeparSepto-Studie erzielten Resultaten. • Vergleich der pathologischen Komplettremissions-Rate (pCR= ypT0 ypN0) von nPac 125mg/m² wöchentlich →EC oder nPac 125mg/m² an den Tagen 1,8 q22 →EC plus anti-HER2-Behandlung (i. e. ABP 980/Pertuzumab bei positivem HER2-Status) bei Patienten mit Brustkrebs im Frühstadium.
Studiendesign und Behandlung	Dies ist eine multizentrische, prospektive, 2x2 randomisierte, offene Phase IIb-Studie zum Vergleich einer neoadjuvanten Chemotherapie mit und ohne Denosumab bei Patientinnen mit unbehandeltem Brustkrebs. Patienten werden zuerst zu einer der beiden folgenden Behandlungen zusätzlich zur neoadjuvanten Therapie randomisiert: • Denosumab (120 mg s.c. q4w) • Kein Denosumab Stratifikations- (Minimierungs-) Faktoren für die Randomisation sind: • LPBC (negativ (definiert als ≤50% stromaler Tumor-infiltrierender Lymphozyten) / vorhanden (definiert als >50% stromaler Tumor-infiltrierender Lymphozyten) • Subtyp (HER2-/HR+ vs. TNBC vs. HER2+) • EC alle 2 vs. alle 3 Wochen Zweitens werden die Patienten (unter Verwendung der Pocock-Minimierung) randomisiert zu: • nPac 125 mg/m² wöchentlich (Cb) → EC • nPac 125 mg/m² an den Tagen 1,8, q22 (Cb) → EC Die erste Randomisation (Denosumab) wird ein zusätzlicher Minimierungsfaktor für die zweite Randomisation (Chemotherapie-Regime).

	Die HER2+ Substudie ist eine Kohorten-Studie mit Untersuchung des offenen, nicht-randomisierten Gebrauchs von ABP 980 in Kombination mit Pertuzumab. In allen Studienarmen wird die Behandlung bis zur Operation, Auftreten eines Progresses, inakzeptabler Toxizität, Rücknahme der Einwilligung des Patienten oder der Beendigung seitens des Sponsors durchgeführt.
Einschlußkriterien	Patienten können nur bei Erfüllung folgender Ein- und Ausschlußkriterien an der Studie teilnehmen: • Eine schriftliche Einwilligungserklärung liegt für alle protokollspezifischen Maßnahmen gemäß den lokalen gesetzlichen Bestimmungen vor Beginn der Untersuchungen vor. • Die Baseline-Dokumentation muss der GBG Forschungs GmbH via MedCODES vollständig vorliegen. • Histologisch durch Stanzbiopsie gesichertes, unilaterales oder bilaterales primäres Mammakarzinom der Brust. Eine Feinnadelaspiration ist nicht ausreichend. Eine Inzisionsbiopsie ist nicht erlaubt. Bei bilateralem Mammakarzinom wird vom Prüfarzt die für die Auswertung des primären Endpunkts relevante Seite prospektiv festgelegt. • Brustläsion mit einer palpablen Größe von ≥ 2 cm oder einer sonographischen Größe von ≥ 1 cm im größten Durchmesser. Die Läsion muss in zwei Dimensionen, vorzugsweise im Ultraschall, messbar sein. Wenn der Tumor sonographisch nicht meßbar ist, sind MRT oder Mammographie ausreichend. Im Falle eines inflammatorischen Karzinoms kann das Ausmaß der Rötung als messbare Läsion verwendet werden. • Patienten müssen folgende Erkrankungsstadien aufweisen: - cT2 - cT4a-d oder - cT1c und cN+ oder - cT1c und pNSLN+ oder - cT1c und ER-neg. und PR-neg. oder - cT1c und Ki67 >20% - cT1c und HER2-pos. • Bei Patienten mit multifokalem oder multizentrischem Brustkrebs sollte die größte Lesion gemessen werden.

	• Zentral bestätigter ER-, PR- und HER2- Status. Die Zentralpathologie beinhaltet auch die Bestimmung von HER2-, Ki-67-, TIL- und RANK/L-Status der Stanzbiopsie. TNBC ist definiert als ER < 1% und PR< 10% gefärbter Zellen und HER2-negativ; HER2-positiv als IHC 3+ oder in-situ-Hybridisierung (ISH) nach den Richtlinien der ASCO-CAP-Richtlinien von 2013. LPBC (Lymphozyten-prädominanter Brustkrebs) ist als mehr als 50% stromaler Tumor-infiltrierender Lymphozyten definiert. Formalinfixiertes, in Paraffin eingebettetes Gewebe (FFPE) der Stanzbiopsie muß daher vor Randomisation zur GBG-Zentralpathologie gesendet werden. Für die HER2+ Substudie einschlussfähig sind Patienten mit zentral bestätigtem HER2-positivem Tumor. • Alter $\geq$ 18 Jahre. • Karnofsky Performance-Status-Index $\geq$ 90%. • Normale Herzfunktion muss durch EKG und Herz-Ultraschall (LVEF oder Shortening Fraction) innerhalb von 3 Monaten vor Randomisation bestätigt werden (LVEF > 55%). • Laboruntersuchungen: <u>Hämatologie</u> - Neutrophile (ANC) $\geq$ 2,0 x 10^9/l und - Thrombozyten $\geq$ 100 x 10^9/l und - Hämoglobin $\geq$ 10 g/dL ($\geq$ 6,2 mmol/l). <u>Leberfunktion</u> - Gesamt-Bilirubin $\leq$ 1,5x oberer Normalwert und - ASAT (SGOT) und ALAT (SGPT) $\leq$ 1,5x oberer Normalwert und - Alkalische Phosphatase $\leq$ 2,5x oberer Normalwert. • Serum Calcium oder Albumin-adjustiertes Serum Calcium ≥2.0 mmol/L (8.0 mg/dL) und ≤2.9 mmol/L (11.5 mg/dL). Eine Hypocalcämie muss vor Studieneinschluss durch eine Calcium und Vitamin D Substitution korrigiert werden. • Negativer Schwangerschaftstest (Serum) innerhalb von 14 Tagen vor Randomisation bei allen Frauen im gebärfähigen Alter mit verfügbarem Resultat vor Therapiestart. • Komplette Staging-Untersuchungen innerhalb von drei Monaten vor Randomisation. Für alle Patientinnen müssen bilaterale Mammographie, Brustultraschall ($\leq$ 21 Tage),

	Brust-MRT (optional), Röntgen-Thorax (PA und lateral), Ultraschall oder CT oder MRT des Abdomens sowie ein Knochenszintigramm durchgeführt werden. Im Falle eines positiven Knochenszintigramms ist eine Röntgenuntersuchung (oder CT oder MRT) der Knochen durchzuführen. Falls klinisch indiziert, können weitere Untersuchungen durchgeführt werden. • Die Patienten müssen der Befundung der ersten Stanzbiopsie und des OP-Gewebes durch die Zentralpathologie zustimmen und für Behandlung und Nachbeobachtung geeignet sein und zur Verfügung stehen.
Ausschlußkriterien	• Rein lobuläre Karzinome (lobuläre Histologie und G1/G2 und HR+/HER2-). • Erkrankungsstadium cT1a, cT1b oder jeglicher M1. • Vorangegangene Chemotherapie für jedwede Erkrankung. • Vorangegangene Strahlentherapie für Brustkrebs. • Vorausgegangene Erkrankungen mit Beeinflussung des Knochenstoffwechsels wie z. B. Osteoporose, Morbus Paget des Knochens, primärer Hyperparathyreoidismus, welche zum Zeitpunkt der Studienaufnahme oder absehbar während der Studienteilnahme behandlungsbedürftig sind/sein werden. • Anwendung von Bisphosphonaten oder von Denosumab innerhalb 1 Jahr vor Studienteilnahme. • Signifikante Zahn-/Mundhöhlenerkrankungen, z.B. anamnestische oder aktuelle Kieferosteonekrose/-myelitis, aktive Zahn- oder Kiefererkrankung, welche einen oralchirurgischen Eingriff notwendig macht, nicht abgeheilte Zahn/Mundhöhlenoperation, geplante invasive Zahneingriffe während der Zeitdauer der Studienteilnahme. • Letzter Zahnarztbesuch liegt länger als ½ Jahr zurück. • Schwangere oder stillende Patienten. Patienten im gebärfähigen Alter müssen zustimmen, während der Studienbehandlung und 7 Monate nach der letzten Einnahme der monoklonalen Antikörper (mAbs) hochwirksame nicht-hormonelle Verhütungsmethoden anzuwenden. • Unzureichender Allgemeinzustand (nicht geeignet für eine Anthracyclin-Taxan-basierte zielgerichtete Chemotherapie).

	• Vorangegangene maligne Erkrankung mit einem krankheitsfreien Intervall von weniger als 5 Jahren (außer CIS der Cervix und nicht-melanomatösem Hautkrebs). • Bekannte oder vermutete Herzinsuffizienz (> NYHA I) und / oder koronare Herzkrankheit, Angina pectoris mit erforderlichen antianginalen Medikamenten, Vorgeschichte mit Herzinfarkt, Nachweis von transmuralem Infarkt im EKG, unkontrollierte oder schlecht eingestellte arterielle Hypertonie (z.B. BP > 140 / 90 mm Hg unter Behandlung mit zwei blutdrucksenkenden Medikamenten), kontrollierte arterielle Hypertonie unter Behandlung mit drei oder mehr blutdrucksenkenden Medikamenten, Rhythmusstörungen mit erforderlicher dauerhafter Behandlung, klinisch signifikante Herzklappenerkrankung. • Vorgeschichte signifikanter neurologischer oder psychiatrischer Erkrankungen wie psychotischer Störungen, Demenz oder Anfälle, die Verständnis und Erklärung der Einwilligung ausschließen würden. • Vorbestehende motorische oder sensorische Neuropathie Schweregrad $\geq$ 2 gemäß NCI-CTC-Kriterien v 4.0. • Akute Infektion. • Unvollständige Wundheilung. • Eindeutige Kontraindikationen für Anwendung von Corticosteroiden. • Bekannte Überempfindlichkeitsreaktion auf eine der in diesem Studienprotokoll verwendeten Verbindungen oder Substanzen inklusive Calcium und Vitamin D. Bekannte erbliche Fruktoseintoleranz. • Momentane Behandlung mit: o Dauerbehandlung mit Kortikosteroiden, die $\leq$ 6 Monate vor Studienbeginn begonnen wurde und mit mehr als 10 mg Methylprednisolon (oder Äquivalent) dosiert ist. o antihormoneller Therapie. Die Einnahme muss vor Studieneintritt beendet werden. o anderen experimentellen Substanzen oder eine andere Krebstherapie. • Teilnahme an einer anderen klinischen Studie mit nicht zugelassener Prüfsubstanz innerhalb von 30 Tagen vor Einschluss in die Studie.

Prüfsubstanzen	Denosumab 120 mg s.c. alle 4 Wochen für 6 Zyklen parallel zur neoadjuvanten Chemotherapie. nPac 125 mg/m² wöchentlich über 12 Wochen oder nPac 125 mg/m² an Tag 1, 8 q22 für 4 Zyklen (12 Wochen). Für Patientinnen mit HER2-positiver Erkrankung: ABP 980 8 mg/kg i.v. Initialdosis, danach 6 mg/kg i.v. alle 3 Wochen simultan zu allen Chemotherapie-Zyklen. Nach der Operation wechseln alle Patienten entweder zum Referenzprodukt Herceptin oder einem anderen zugelassenen Trastuzumab Biosimilar gemäß der Entscheidung des Arztes / lokalem Standard. Das Protokoll beinhaltet die Vorgehensweise bei bestimmten schwerwiegenden Ereignissen, die Dosisanpassungen oder -verschiebungen erfordern.
Andere Substanzen	Für alle Patienten: • Epirubicin 90mg/m² + Cyclophosphamid (600mg/m²) (EC) i.v. alle 2 oder 3 Wochen (Entscheidung des Prüfarztes vor Randomisation) für 4 Zyklen nach nab-Paclitaxel. Für Patientinnen mit triple-negativer Erkrankung: • Carboplatin AUC 2 wöchentlich während der Zyklen mit nab-Paclitaxel. Für Patientinnen mit HER2-positiver Erkrankung: • Pertuzumab 840 mg i.v. Initialdosis, danach 420 mg i.v. alle 3 Wochen simultan zur Chemotherapie für mindestens 4 Zyklen (gemäß Zulassung). Diese Medikamente werden gemäß den Anwendungshinweisen und Empfehlungen der Hersteller appliziert.
Begleitmedikation	Supplementierung von mindestens täglich 500 mg Calcium und 400 IU Vitamin D ist bei allen Patientinnen, die Denosumab erhalten, notwendig, es sei denn es besteht eine Hypercalcämie. Eine gute Mundhygienepraxis sollte während der Behandlung mit Denosumab eingehalten werden. Invasive Zahnbehandlungen sollten während der Behandlung mit Denosumab vermieden werden. Für Patientinnen, bei denen invasive Zahnbehandlungen nicht vermieden werden können, soll die klinische Einschätzung des behandelnden Arztes den individuellen Behandlungsplan (Verschiebung des Zahneingriffes vs. Unterbrechung von Denosumab) leiten. Andere supportive Therapien sollen während der Chemotherapie

	entsprechend den Richtlinien der AGO, EMSO oder ASCO verabreicht werden (e.g. www.asco.org/guidelines/antiemetics).
Primärer Endpunkt	**Primärer Effektivitäts-Endpunkt:** **Pathologische Komplettremission von Brust und Lymphknoten (ypT0 ypN0; primärer Endpunkt)** Kein miskroskopischer Nachweis von verbliebenen nicht-invasiven oder invasiv vitalen Tumorzellen in sämtlichen resezierten Gewebeproben von Brust und Axilla. Das pathologische Ansprechen wird anhand des entnommenen Brustgewebes und der Lymphknoten aus allen Operationen bewertet. Patienten mit negativer Sentinel-Lymphknoten-Biopsie vor Behandlungsbeginn und keiner Axilla-OP nach der Chemotherapie werden als pCR gezählt, wenn kein invasiver und kein nicht-invasiver Resttumor im entnommenen Brustgewebe nachgewiesen wird. Patienten mit positiver Sentinel-Lymphknoten-Biopsie vor Behandlungsbeginn und keiner Axilla-OP nach der Chemotherapie werden als nicht pCR gezählt (vorzugsweise Axilladissektion anstelle von Sentinel-Lymphknoten-Biopsie wird in dieser Situation besonders empfohlen). Patienten mit positiver Sentinel-Lymphknoten-Biopsie vor Behandlungsbeginn und ohne nachweisbaren invasiven und nicht-invasiven Tumorrest im entnommenen Brustgewebe und den Lymphknoten nach der Chemotherapie werden als pCR gezählt.
Sekundäre Endpunkte	Sekundäre kurzzeitige Effektivitätsendpunkte ypT0/is ypN0 wird definiert als kein mikroskopischer Nachweis von verbliebenen invasiv lebensfähigen Tumorzellen in allen resezierten Proben von Brust und Axilla; im Falle einer Sentinelnode-Biopsie vor Behandlungsbeginn werden die axillären Lymphknoten wie für den primären Endpunkt beschrieben ausgewertet. ypT0 ypN0 /+ wird definiert als kein mikroskopischer Nachweis von verbliebenen invasiv oder nicht-invasiv lebensfähigen Tumorzellen in allen resezierten Proben der Brust; ypT0 / is ypN0 /+ wird definiert als kein mikroskopischer Nachweis von verbliebenen invasiv lebensfähigen Tumorzellen in allen resezierten Proben der Brust; Patienten mit einer Sentinelnode-

	Biopsie vor Behandlungsbeginn werden für ypT ausgewertet (jedes Stadium) ypN0 wie in der Beschreibung für den primären Endpunkt angegeben. Klinisches und bildliches Ansprechen werden jeden 2. Zyklus und vor der Operation durch körperliche Untersuchung und bildgebende Verfahren gemessen. Bevorzugte Untersuchungsmethode ist die Sonographie, wenn sie jedoch keine validen Resultate zu liefern scheint oder nicht durchgeführt wird, sind MRT, Mammographie oder Palpation mit abnehmender Priorität zu berücksichtigen. Für die Messung vor, während und nach der Behandlung sollte dasselbe bildgebende Verfahren angewendet werden. Für definierte Kategorien der Effektivität (vollständig, teilweise, unverändert oder Progress) wird der Anteil der Patienten mit Behandlungserfolg festgelegt und entsprechende Konfidenzintervalle berechnet.Die Kategorien für das Ansprechen sind: • **Komplettremission (CR)**: Vollständiges Verschwinden aller Anzeichen des Tumors in der Brust, festgestellt anhand aller verfügbaren Bildgebungen und Palpation. Das Ansprechen der axillären Lymphknoten wird nicht berücksichtigt. • **Teil-Ansprechen (pCR)**: Reduktion des Produkts der beiden größten senkrechten Durchmesser der Primärtumorgröße um 50% oder mehr anhand von Bildgebung oder Palpation. Bei Patienten mit multifokaler oder multizentrischer Erkrankung sollte die Läsion mit dem größten Durchmesser für die Nachverfolgung gewählt werden. Das Ansprechen der axillären Lymphknoten wird nicht berücksichtigt. • **Stabile Erkrankung (NC)**: Keine signifikante Veränderung in der Größe des Tumors während der Behandlung, was eine geschätzte Reduktion des Tumorareals um weniger als 50% oder eine geschätzte Zunahme der Läsionen im Tumorareal von weniger als 25% bedeutet. • **Progressive Krankheit (PD)**: Entwicklung neuer, zuvor unentdeckter Läsionen oder eine geschätzte Zunahme der Größe der bereits vorhandenen Läsionen um 25% oder mehr nach mindestens zwei Zyklen der Therapie. Brusterhaltung wird definiert als Tumorektomie, Segmentektomie oder Quadrantektomie als radikalste Operation.

	Patienten, bei denen kein Behandlungserfolg bestimmt werden kann (z. B. Patienten, bei denen die Histologie nicht auswertbar ist), sind im Nenner enthalten, d.h., diese Patienten werden die Erfolgsrate in der gleichen Weise wie das Therapieversagen beeinflussen. LRRFS, DDFS, IDFS, EFS und OS sind als Zeitraum zwischen Registrierung und dem ersten Ereignis definiert und werden nach Studienende mit Bezug zu den Daten der Patientenregistrierung analysiert. Progresse während der neoadjuvanten Behandlung werden nicht als Ereignisse angesehen, außer die Patientin kann nicht operiert werden. **Verträglichkeit und Sicherheit**: Beschreibende Statistiken für die 4 Behandlungsarme (+/- Anti-HER2-Therapie) wird für die Anzahl von Patienten bereitgestellt, deren Behandlung reduziert, verschoben oder dauerhaft gestoppt werden mußte. Der Grund für die Beendigung beinhaltet Aspekte der Wirksamkeit (z.B. Beendigung wegen Progress), Sicherheit (z.B. Beendigung aufgrund schwerwiegender Ereignisse) und Eignung (z.B. Beendigung wegen Widerruf der Einwilligung des Patienten). Gründe für die vorzeitige Beendigung werden nach dem Hauptgrund kategorisiert und in Häufigkeitstabellen dargestellt. Die Sicherheit nach Toxizitätsgraden wird nach der NCI-CTCAE Version 4.0 definiert. Korrelative wissenschaftliche Forschung: Explorative Analysen werden durchgeführt, um mögliche Zusammenhänge zwischen Biomarkern und Wirkstoffaktivität zu identifizieren. Ziel ist die Identifizierung möglicher prognostischer/prädiktiver Biomarker zu kurz- und langfristigen Parametern (pCR, EFS und OS). Fehlende Daten zur Evaluierung des Ansprechens werden dem Nichtansprechen zugerechnet.
Statistische Methoden	Eine "intent-to-treat" (ITT)-Analyse wird für alle Patienten durchgeführt, die randomisiert wurden. Darüber hinaus wird eine "per-protocol"-Analyse durchgeführt; die detaillierte Definition des per-protocol Analyse-Sets ist im statistischen Analyseplan aufgeführt. Alle HER2-positiven Patienten werden für die Subgruppen und multivariate Analysen der Hauptstudie unabhängig von der Anti-HER2-Behandlung nach den allgemeinen ITT-Grundsätzen analysiert.

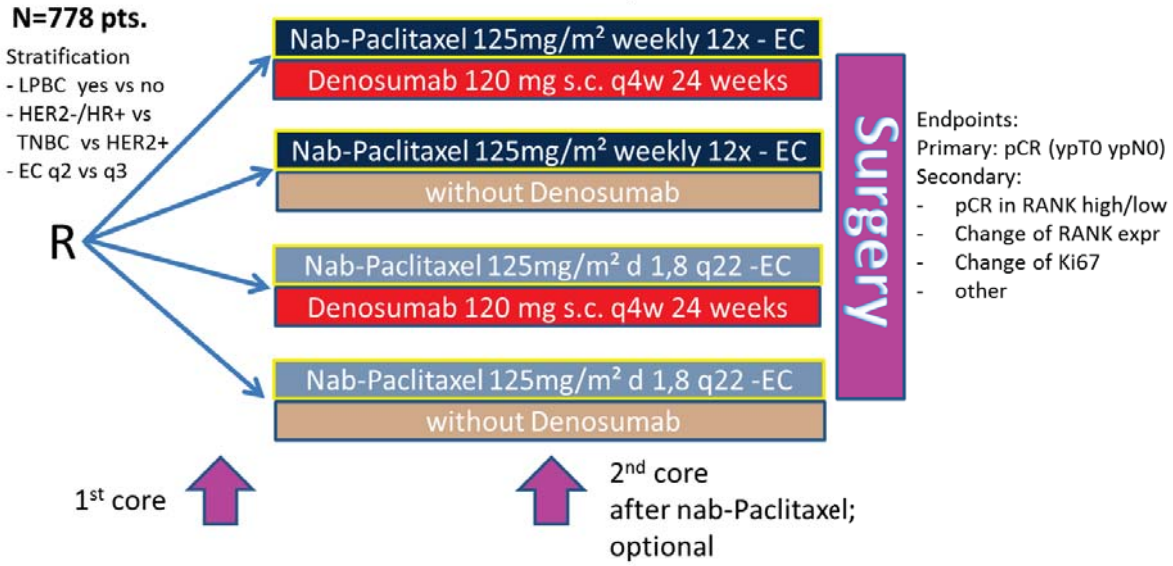
Statistische Methoden Primärer Endpunkt	Die primären Zielkriterien A und B werden getestet nach dem verbesserten Bonferroni Verfahren: der kleinere von 2 p-Werten wird verglichen mit α = 0,1 und der größere mit α = 0,2, um den Gesamtsignifikanzlevel der Studie auf α = 0,2 zu halten.[5] Der primäre Endpunkt wird als pathologische Komplettremissionsrate für jede Behandlungsgruppe für beide Randomisierungen zusammengefasst. 2-seitige 90% Konfidenzintervalle werden nach Pearson und Clopper berechnet.[6] Die Differenz bei den Raten der pathologischen Komplettremissionen wird als Differenzrate ausgewertet (für das primäre Zielkriterium A Denosumab-Arm minus kein-Denosumab-Arm); für das primäre Zielkriterium B nPac 125 mg/m² wöchentlich (Cb) → EG minus nPac Tag 1,8 q22 (Cb) → EC- Arm) mit 90% Konfidenzintervall. Zusätzlich wird eine odds ratio mit 90% Konfidenzintervall angegeben. Die Signifikanz wird mit dem zweiseitigen Kontinuitäts-korrigierten χ2-Test entsprechend dem verbesserten Bonferroni-Verfahren getestet. Die Nullhypothese ist, dass es keinen Unterschied in den pCR-Raten zwischen den Behandlungsarmen gibt, die alternative Hypothese, dass es einen Unterschied zwischen den beiden Randomisierungen gibt. Das Signifikanzniveau für alle anderen Tests wird auf 2-seitig α = 0,05 festgelegt. Es gibt keine Anpassung für Mehrfachvergleiche in den Analysen für die stratifizierten Subpopulationen. Eine sekundäre logistische Regressionsanalyse zur Korrektur für die Minimierungsfaktoren wird für den primären Endpunkt durchgeführt. Uni- und multivariate logistische Regression wird für die pCR zur Anpassung an die auf der ITT-Population basierenden üblichen Faktoren durchgeführt (Behandlungsgruppe für beide Randomisierungen, Minimierungsfaktoren, Alter, Tumorgröße, Nodalstatus, Grad, histologischer Typ). Zusätzlich wird eine multivariate logistische Regression mit allen

	oben genannten Faktoren und der Interaktion zwischen Denosumab und den Chemotherapie-Armen durchgeführt. Primäre und sekundäre Zielkriterien für die HER2+ Substudie werden bei allen Patienten, die mindestens eine Dosis ABP 980 erhalten haben, bestimmt. Die pCR-Raten mit einem 95% CI werden berichtet und zwischen den Behandlungsarmen der Chemotherapie unter Verwendung des Kontinuitätskorrigierten χ2-Tests verglichen.
Statistische Methoden Patientenzahl	Die Kalkulation der Fallzahl basiert auf folgenden Annahmen für den primären Endpunkt: • Verbesserung der pCR-Rate durch Denosumab bei allen Patienten von 35% auf 46% (OR=1.58) • Verbesserung der pCR-Rate durch unterschiedliche Chemotherapien (nPac 125mg/m² an Tag 1,8 q22 (Cb) → EC verglichen mit nPac 125mg/m² wöchentlich (Cb) → EC wird 36% gegenüber 45% (OR=1.45) sein. Mit 778 rekrutierten Patienten hat der primäre Kontinuitäts-korrigierte χ^2-Test der pCR-Raten zwischen Denosumab und nicht Denosumab eine 92%ige Power für den 2-seitigen Signifikanzlevel von α=0.10. Der Kontinuitäts-korrigierte χ^2-Test der pCR-Raten zwischen nPac 125mg/m² wöchentlich (Cb)→ EC und nPac 125mg/m² an Tag 1,8 q22 (Cb) → EC hat eine 80%ige Power für den 2-seitigen Signifikanzlevel von α=0.10. Die Berechnung für den Kontinuitäts-korrigierten χ2-Test wird mit nQuery Advisor 6.02 durchgeführt. Es ist geplant, 778 Patienten in die Studie zu rekrutieren. Die Kalkulation der Fallzahl für die HER2+ Substudie basiert auf dem primären Endpunkt der Hauptstudie: Alle in die Studie eingebrachten Patienten mit HER2-positiver Erkrankung erhalten ABP 980 zusätzlich zu Pertuzumab und der Chemotherapie. Es ist geplant, ungefähr 150 Patienten in diese Substudie einzubringen.
Statistische Methoden	Sekundäre kurzzeitige Wirksamkeitsendpunkte (ypT0 / is ypN0;

Sekundäre Endpunkte	ypT0 ypN0 /+; ypT0 / is ypN0 /+; ypT ($_{\text{jedes Stadium}}$) ypN0, Ansprechen anhand körperlicher Untersuchung, Bildgebung, Brusterhaltungsrate) wird auch als Ansprechrate in jedem Behandlungsarm zusammengefasst, 2-seitige 95%ige Konfidenzintervalle werden nach Pearson und Clopper kalkuliert und der Kontinuitäts-korrigierte Pearson χ^2-Test wird durchgeführt, um die Differenz der Ansprechraten in den Behandlungsarmen zu evaluieren; diese Tests werden als explorativ angesehen. Das Signifikanzniveau wird für alle Tests auf 2-seitig α = 0.05 festgelegt. Subgruppen- und multivariate Analysen werden für ypT0 /is ypN0 in der gleichen Weise wie für den primären Endpunkt durchgeführt. Ein Breslow-Day Test für die Interaktion wird durchgeführt, um den Unterschied des Behandlungseffekts zwischen den Subgruppen mit hohem und niedrigem RANK (der Schnittpunkt wird im SAP definiert) mit 2-seitigem α = 0.1 zu bewerten. Die Nullhypothese ist, dass die odds ratios der pCR im Denosumab- Arm und im Arm ohne Denosumab in den Subgruppen RANK+ und Rank- gleich sind, die alternative Hypothese, dass die odds ratios nicht gleich sind. Für **LRRFS, DDFS, IDFS EFS** und **OS** werden Kurven unter Verwendung des Kaplan-Meier-Verfahrens, basierend auf der ITT-Population, angelegt. 3 Jahre und 5 Jahre Überlebensrate (und 95% CIs) werden geschätzt. Univariate und multivariate Cox-proportionale Hazard-Modelle werden verwendet, um die Hazard Ratios für Minimierungsfaktoren und die oben definierten Kovariaten anzupassen. Die Zeit bis zum ersten Auftreten einer peripheren Neuropathie Grad 2-4 und die Zeit bis zu einer Besserung der peripheren Neuropathie wird mittels Kaplan-Meier-Kurven und einem Log-Rank-Test analysiert. Sicherheit und Compliance für die HER2+ Substudie werden beschreibend in den Behandlungsarmen berichtet. Weitere Details finden sich im SAP und folgen der allgemeinen Sicherheitsbewertung der Hauptstudie.
Zentrenanzahl	Die Studie soll in ca. 60 Zentren in Deutschland durchgeführt werden.
Rekrutierungszeitraum	Ca. 24 Monate (Q-I 2017 – Q-IV 2018).

Studiendauer	Ca. 32 Monate (24 Monate Rekrutierung + 6 Monate Behandlungsdauer + max. 2 Monate bis OP).
Follow-up-Periode	Da keine studienspezifische Behandlung oder Untersuchung nach 90 Tagen nach der Operation geplant ist, ist das Follow-up kein Teil dieser Studie. Allerdings werden Informationen über den Gesundheitszustand der Patienten entweder anhand der jährlichen Krankenaktenauswertung an den Zentren oder mittels GBG-Patientenregister eingeholt. Gesammelt werden Informationen zum Zeitpunkt und Ort der Rezidive, Todesdatum und -ursache sowie Zweiterkrankungen und wichtige Langzeitnebenwirkungen.

GeparX: Investigating Denosumab as an add-on neoadjuvant treatment for RANK-positive or RANK-negative primary breast cancer and two different nab-Paclitaxel schedules ; 2x2 factorial design



Assumptions for primary endpoint (α=0.2, β=0.2):
Denosumab: 35% to 46% OR 1.58
nab-Pac: 36% to 45%; OR 1.45 ;

Treatment backbone:
HER2+:+ Trastuzumab (ABP 980) + Pertuzumab
TNBC: + Carboplatin weekly AUC 2

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5. INTRODUCTION

5.1 Current Status of Neoadjuvant Chemotherapy in Primary Breast Cancer

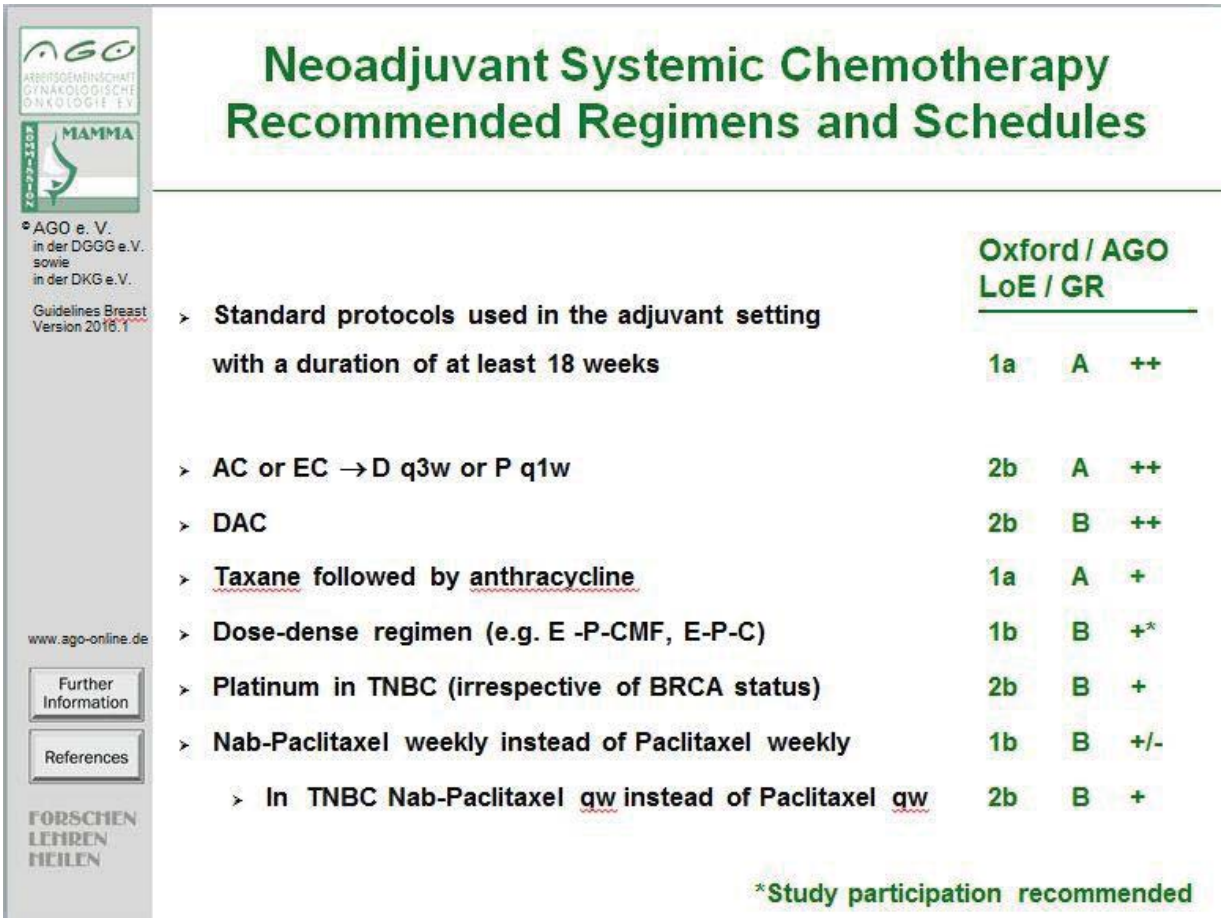
It has been widely accepted that breast cancer is a systemic disease with a local component and needs to be treated by systemic treatment as well as local procedures (surgery and radiotherapy). The main goal of neoadjuvant chemotherapy (NACT) before surgery for patients with large, inoperable or inflammatory disease is to convert inoperable to operable primary tumors. For patients with operable disease, requiring mastectomy, NACT may allow some to have breast conservation after NACT.[7] Today, it appears even realistic, that a group of patients can be identified, in which the chance for a pathological complete response (pCR) is that high, that surgery might be avoided.

NACT in primary operable disease is a clinical option to improve surgical options and to gain information on response. NACT and adjuvant chemotherapy are accepted as equally effective. [8, 9, 10, 11] Therefore, patients who are candidates for adjuvant chemotherapy should be offered NACT as an additional option. In reverse, NACT should be avoided in patients for whom the need and the type of adjuvant chemotherapy cannot be defined a priori. In this subset of patients NACT might be an over-treatment if the individual risk is overestimated. On the other hand, patients with triple-negative (TNBC) or HER2-positive cancer can be relieved from an unfavorable prognosis, if a pCR can be achieved, as those patients show only a very low relapse rate (in opposite to the high recurrence rate in patients without a pCR). [12, 13, 14]

Outside of clinical trials, the AGO guidelines for neoadjuvant treatment recommend the use of either a sequential anthracycline-based regimen for 4 cycles followed by a taxane-based regimen for 4 cycles or 6 cycles of an anthracycline-taxane-combination (Figure 1).[15]

Data on the dosage and administration of weekly paclitaxel demonstrated very good tolerability combined with high efficacy, which is therefore accepted as the optimal schedule to deliver paclitaxel. [16, 17]

Figure 1: Current recommendation of regimen and schedules for neoadjuvant chemotherapy by the AGO Breast Commission (AGO guidelines version 2016.1).



5.2 Use of nab-Paclitaxel as part of neoadjuvant treatment for early breast cancer

The GeparSepto trial randomly assigned 1206 women with histologically proven operable and inoperable breast cancer to 12 weeks of weekly nab-paclitaxel at 150 or 125 mg/m^2 or to 12 weeks of weekly solvent-based paclitaxel at 80 mg/m^2. In both study arms after the taxane 4 cycles of epirubicin 90 mg/ m^2 in combination with cyclophospahmide 600 mg/ m^2 were given at 3 weekly intervals. All patients with HER2-positive tumors received concurrent to neoadjuvant chemotherapy a combination of the two monoclonal antibodies trastuzumab at 6 mg/ kg together with pertuzumab 840 mg every three weeks.

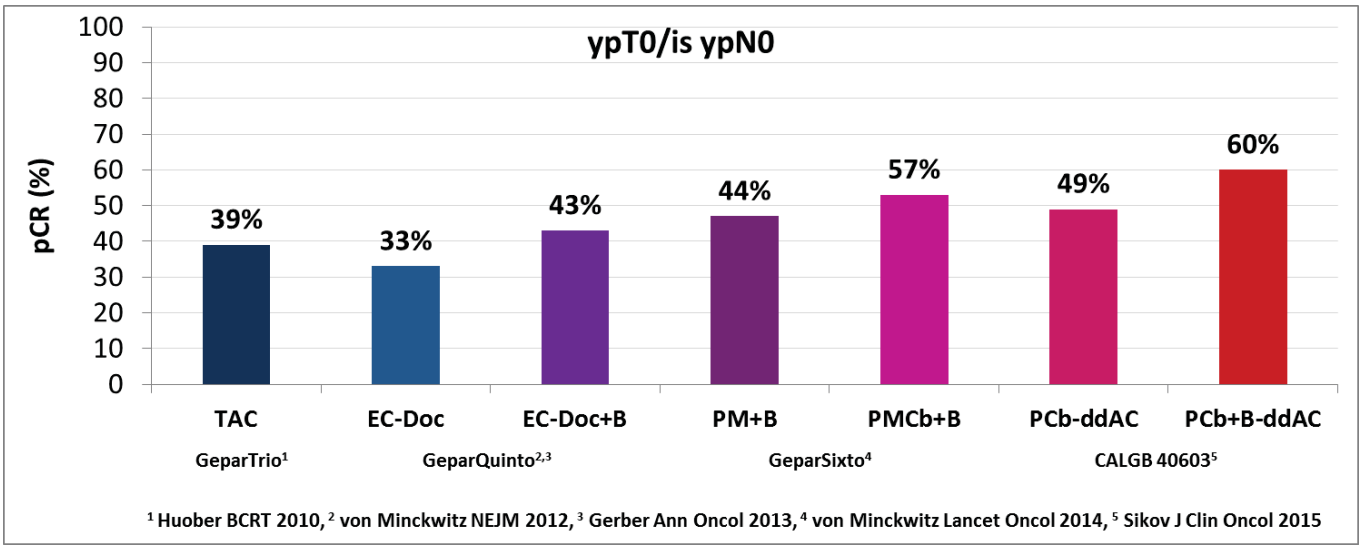
The pathologic complete response rate (ypT0, ypN0) was significantly higher in the nab-paclitaxel arm (38%), compared to the paclitaxel arm (29%), p<0.001. This result was consistent in all stratified subgroups (biological subgroups, Ki-67, SPARC, HER2) with an odds ratio of 1.53 and was of greatest magnitude in patients with triple-negative tumors with an odds ratio of 2.61. Grade 3 to 4 toxicities for paclitaxel and nab-paclitaxel respectively, were anemia (0.7% vs 2.1%, p=0.048), neutropenia (61.9%, 60.9%, p=0.72), febrile neutropenia (4.0%, 4.6% p=0.67), sensory neuropathy (2.7%, 10.4%, p< 0.001), diarrhea (2.8%, 3.3%, p=0.74), skin rash (0.7%, 1.2%, p=0.55), hand foot syndrome (1.0%, 2.1%, p =0.16). Moreover

the risk-benefit ratio of nab-paclitaxel 125 mg/m² was improved over nab-paclitaxel 150 mg/m² with better drug adherence and relative-total dose intensity, lower frequency of peripheral sensory neuropathy grade 3/4 (15% vs 8%) and higher pCR rate (32% vs 41%).[18]
This trial supports the substitution of solvent-based paclitaxel with nab-paclitaxel in clinical trials.

5.3 Use of carboplatin as part of neoadjuvant treatment of triple-negative breast cancer.

Recently, results from GeparSixto study showed that adding carboplatin to paclitaxel plus non-pegylated liposomal doxorubicin given as a neoadjuvant weekly regimen for 18 weeks in patients with TNBC leads to an absolute increase of more than 20% in pCR rate.[19] In this trial, 315 TNBC patients were randomized to receive 18 weeks of weekly paclitaxel 80mg/m² and weekly non-pegylated-liposomal doxorubicin 20mg/m² (PM) plus bevacizumab 15mg/kg q2w ± weekly carboplatin (Cb) AUC 1.5. The pCR rates (ypT0 ypN0) were 36.9% with PM and 53.2% with PMCb (p=0.005). Overall, the addition of carboplatin was associated a higher rate of dose discontinuations due to hematological toxicity, nausea, diarrhea and anorexia.
The CALGB 40603 (Alliance), a 2 × 2 factorial, open-label, randomized phase II trial, evaluated the impact of adding carboplatin and/or bevacizumab in 443 patients with stage II to III TNBC which received paclitaxel 80 mg/m^2 once per week (wP) for 12 weeks, followed by doxorubicin plus cyclophosphamide once every 2 weeks (ddAC) for four cycles, and were randomly assigned to concurrent carboplatin (area under curve, AUC 6) once every 3 weeks for four cycles and/or bevacizumab 10 mg/kg once every 2 weeks for nine cycles. Effects of adding these agents on pCR breast (ypT0/Tis), pCR breast/axilla (ypT0/TisN0), treatment delivery, and toxicities were analyzed. Patients assigned to either carboplatin or bevacizumab were less likely to complete wP and ddAC without skipped doses, dose modification, or early discontinuation resulting from toxicity. Grade ≥ 3 neutropenia and thrombocytopenia were more common with carboplatin, as were hypertension, infection, thromboembolic events, bleeding, and postoperative complications with bevacizumab. Employing one-sided p values, addition of either carboplatin (60% v 44%; p=0.0018) or bevacizumab (59% v 48%; p=0.0089) significantly increased pCR breast, whereas only carboplatin (54% v 41%; p=0.0029) significantly raised pCR breast/axilla. More-than-additive interactions between the two agents could not be demonstrated.[20] Moreover the ADAPT TN trial suggested high efficacy and favorable toxixity of 12 weeks therapy with nab-paclitaxel and carboplatin compared to nab-paclitaxel and gemcitabine in TNBC.[21]
The optimal regimen for neoadjuvant treatment of patients with TNBC could be therefore considered as a weekly application of at least 18 weeks of an adequately dosed anthracycline-taxane- based regimen combined with carboplatin with or without bevacizumab (Figure 2).

Figure 2: Summary of the pCR rate (ypT0/is ypN0) among TNBC breast cancer patients enrolled in the GeparTrio, GeparQuinto, and GeparSixto trial.



5.4 Use of trastuzumab und pertuzumab as part of neoadjvuant treatment of patients with HER2-positive breast cancer

Several trials have investigated the use of trastuzumab in the adjuvant therapy of breast cancer. All of them demonstrated a tremendous effect of adding trastuzumab to the standard treatment in any of the settings. [22, 23, 24]

The first data of 42 patients with HER2-positive disease with operable breast cancer who were randomly assigned to either 4 cycles of paclitaxel followed by 4 cycles of 5-fluorouracil, epirubicin, and cyclophosphamide or to the same chemotherapy with simultaneous weekly trastuzumab for 24 weeks show a pCR rate of 25% for the chemotherapy group and 66.7% (p=0.02) for the chemotherapy plus trastuzumab group.[25]

Up to now there are several well conducted larger scale neoadjuvant clinical trials in HER2-positive patients receiving trastuzumab in combination with chemotherapy as preoperative therapy. The TECHNO trial [26] applied trastuzumab concurrently with paclitaxel over 12 weeks whereas within the GeparQuattro [27] trial as well as the NOAH study [28] patients received trastuzumab concurrently with the anthracycline as well as the taxane over 24 and 36 weeks, respectively.

Trastuzumab has been given in parallel with epirubicin in the GeparQuattro study (450 patients) and in parallel with doxorubicin in the NOAH trial (118 patients). None of the trials suggested that this combination is not safe enough. The incidence of decreased left ventricular ejection fraction (LVEF) in the TECHNO study (2.8%) was lower than in the NOAH study, in which a reduction in LVEF by more than 10 percentage points was observed in 9.5% of patients in the trastuzumab-treated group after 14 months of follow-up. In addition,

congestive heart failure (CHF) was diagnosed in 2.2% of patients in the NOAH study, but not in the TECHNO study. The GeparQuattro study has shown an even lower incidence of cardiac dysfunction as only 4 of 445 HER2-positive patients showed a decrease in LVEF of more than 10 percentage points and 1 CHF. The results have been confirmed by the GeparQuinto study having treated additional 320 patients with the same chemotherapy-trastuzumab regimen. [29] In addition, data from the metastatic setting in the HERCULES study combining epirubicin with trastuzumab indicated that this is feasible.[30]

The NeoALTTO [31] and the NeoSphere [32] studies recently showed that a dual blockage of the HER2 receptor can further increase the pCR rate in HER2 positive breast cancer. With trastuzumab alone pCR (ypT0/is ypN0) rates were 27.6% after 4 cycles weekly paclitaxel and a 6 weeks upfront window of trastuzumab alone and 21.5% after 4 cycles docetaxel. When lapatinib or pertuzumab were added, the corresponding pCR rates were 46.9% and 39.3%. These pCR rates are comparable with the 45% pCR rate of the GeparQuinto study with a 24 week EC-Docetaxel regimen in combination with trastuzumab given during all cycles.[29]

In particular, in the NeoSphere trial, [32] eligible patients with newly-diagnosed HER2-positive breast cancer were randomized to receive either A) trastuzumab/docetaxel (reference treatment) or B) trastuzumab/docetaxel and pertuzumab (primary comparison arm) or C) trastuzumab/pertuzumab without chemotherapy or D) pertuzumab/docetaxel. After four cycles of study treatment, patients then underwent surgery, the primary endpoint of the study being pCR rate at surgery. Following surgery, patients completed chemotherapy and treatment to one year with trastuzumab so that all patients received overall standard therapy as a minimum. The main therapeutic result showed a very high rate of tumor eradication in the breast when pertuzumab was added to conventional trastuzumab and docetaxel treatment: 45.8 % for the triplet regimen (arm B) and 29% for the comparator arm (arm A). The pCR rate accounted for 16.8% in women receiving the chemotherapy-free regimen (arm C) and 24% in arm D when pertuzumab and docetaxel were used together.

Data from German neoadjuvant breast cancer trials suggest that an increasing number of chemotherapy cycles is associated with an augmented pCR rate, so that treatment duration might be relevant also in this specific setting. This is supported by recent results from the Tryphaena study[33] that assessed the addition of pertuzumab to standard preoperative treatment in patients with early HER2-positive breast cancer. In this study, patients were randomly assigned to received A) 5-fluorouracil, epirubicin, cyclophosphamide (FEC) followed by docetaxel (T), with trastuzumab (H) and pertuzumab (P) given concurrently throughout (FEC+H+P ×3→T+H+P ×3); B) FEC followed by THP (FEC ×3→T+H+P ×3); or C) T, carboplatin (Carb), H with P (TCarbH+P ×6). Following neoadjuvant therapy, patients underwent surgery and continued trastuzumab to complete 1 year of treatment. The observed pCR rates were exceeding the 60% threshold and are so far the highest pCR results presented in a large multicenter study.

We postulate that an optimal neoadjuvant regimen for HER2-positive breast cancer should last at least 18 weeks, using anthracyclines and taxanes and preferably includes two anti-HER2 agents to increase the pCR rate further.

Efficacy and tolerability of pertuzumab in HER2-positive breast cancer

The BO17929 study was a two-stage, phase II study of 66 patients with HER2-positive, metastatic breast cancer whose disease had progressed on trastuzumab therapy.[34] Patients received a combination of trastuzumab (either weekly or three weekly dosing) and pertuzumab with a loading dose of 840 mg and maintenance dose of 420 mg every three weeks (q3w). The objective response rate (ORR) was 24.2%, and the clinical benefit rate (CBR) was 50%. Five patients (7.6%) experienced a complete response, 11 patients (16.7%) experienced a partial response, and 17 patients (25.8%) experienced stable disease of ≥6 months. A further cohort of 29 patients [35] was added to the BO17929 study, in which patients received only pertuzumab, but were allowed to have trastuzumab re-introduced if the tumor did not respond to pertuzumab alone, or responded and then relapsed. All 29 patients enrolled for pertuzumab monotherapy experienced disease progression. During pertuzumab monotherapy, the ORR was 3.4% and CBR was 10.3%. With the addition of trastuzumab, the ORR and CBR were 17.6% and 41.2%, respectively. Treatment was well tolerated with minimal cardiac dysfunction. In the CLEOPATRA study 808 patients with HER2-positive metastatic breast cancer were randomly assigned to receive placebo plus trastuzumab plus docetaxel (control group) or pertuzumab plus trastuzumab plus docetaxel (pertuzumab group) as first-line treatment until the time of disease progression or unacceptable toxicity. The median overall survival was 56.5 months in the group receiving the pertuzumab combination, as compared with 40.8 months in the group receiving the placebo combination. Median progression-free survival as assessed by investigators improved by 6.3 months in the pertuzumab group Pertuzumab extended the median duration of response by 7.7 months, as independently assessed. [36] Pertuzumab in combination with trastuzumab has been generally well tolerated. [37] Overall, 407 patients received at least one dose of pertuzumab in combination with trastuzumab and docetaxel. The most common adverse drug reactions (ADRS) (> 50%) were diarrhea, alopecia and neutropenia. The most common NCI-CTCAE (version 3) grade 3-4 ADRS (> 10%) were neutropenia, febrile neutropenia and leucopenia, and the most common serious adverse events (SAE) were febrile neutropenia, neutropenia and diarrhea. Deaths from other causes than disease progression occurred in 2% of patients in the pertuzumab-treated group and 2.5% in the placebo-treated group; mainly due to infection. After 1 year of additional follow-up, left ventricular systolic dysfunction (any grade) was reported more frequently in the control group than in the pertuzumab group (8.3% vs. 4.4%). Left ventricular systolic dysfunction of grade 3 or higher was reported in 2.8% of the patients in the control group and in 1.2% of the patients in the pertuzumab group. Among patients in whom the left ventricular ejection fraction was assessed after the baseline assessment, 6.6% in the control group and 3.8% in the pertuzumab group had declines of 10 percentage points or more from baseline that resulted in a left ventricular ejection fraction of less than 50%. After discontinuation of docetaxel, all ADRs in the pertuzumab and trastuzumab treated group occurred in < 10% of patients with the exception of diarrhea (19.1%), upper respiratory tract infection (12.8%), rash (11.7%), headache (11.4%) and fatigue (11.1%). Also in the BO17929 study[34] the combination of pertuzumab and

trastuzumab in metastatic setting was well tolerated and adverse events were mild to moderate. Cardiac dysfunction was minimal, and no patients withdrew as a result of cardiac-related adverse events.
In the NeoSphere study,[32] 108 patients received trastuzumab and pertuzumab without chemotherapy. Diarrhea (28%), headache (14%), nausea (12%), and fatigue (11%) were the most frequest AEs in this treatment arm. Moreover, only 4 patients experienced SAEs during neoadjuvant treatment; 1 out of 4 patients experienced CHF.
In the Tryphaena study,[33] patients received concomitantly trastuzumab and pertuzumab in all three treatment arms (FEC (+HP)→Docetaxel (+HP); FEC→Docetaxel (+HP); TCH (+HP)). Diarrhea, alopecia, and nausea all grades were reported in >50% of patients across all treatment arms, during the neoadjuvant treatment period. Neutropenia, febrile neutropenia, and leucopenia were the most frequently reported grade ≥3 adverse events. Only 1 out of 223 patients (0.4%) who received trastuzumab and pertuzumab in combination with standard chemotherapy developed symptomatic left ventricular systolic dysfunction during the treatment. In Tryphaena patients received up to 6 cycles of pertuzumab/trastuzumab preoperatively. Whereas in the GeparSepto trial patients received up to eight cycles of pertuzumab/trastuzumab in parallel to a standard chemotherapy regimen with (nab)Paclitaxel and EC.
In September 2013, based on the results of the NeoSphere and Tryphaena trials, the Food and Drug Administration (FDA) granted accelerated approval to pertuzumab as part of a complete treatment regimen for patients with HER2-positive early stage breast cancer in neoadjuvant setting. In Europe EMA granted approval in August 2015.
A large confirmatory trial testing pertuzumab plus trastuzumab and chemotherapy as adjuvant therapy for HER2-positive breast cancer has recently completed accrual of 4810 patients (Aphinity trial, NCT01358877) and is now in follow-up. Patients were randomized after surgery to either pertuzumab or placebo in addition to chemotherapy and trastuzumab to test whether the triple combination improves invasive disease-free survival.

Cardiac toxicity of HER2 targeted agents

Since pertuzumab targets HER2, such as trastuzumab, there might be a potential risk of cardiac side effects, particularly in patients who have received prior anthracycline treatment. All patients enrolled in pertuzumab studies undergo routine cardiac monitoring by echocardiography or multiple-gated acquisition (MUGA) scan. Overall, the data so far show that pertuzumab as a single agent, or combined with other therapies (trastuzumab or cytotoxic chemotherapy) has an acceptable cardiac safety profile.

The cardiac side-effect profile appears to be similar to that of trastuzumab, and combination of the two antibodies has not increased the rate of cardiac events in carefully selected patients studied so far.
The incidence of cardiac dysfunction has been assessed using the following criteria: an absolute decrease from baseline of ≥10 percentage points in LVEF to a value of <50% at any post-baseline LVEF assessment. Using this definition, the majorities of patients with cardiac

dysfunction were asymptomatic and have shown improvement or return to baseline function on follow-up, in line with the experience with trastuzumab.
The rate of asymptomatic declines, as assessed using these criteria in pooled studies was:
• 5.2% (20/386) in the single-agent pertuzumab population.
• 3.0% (6/202) for pertuzumab in combination with trastuzumab (based on patients receiving this regimen in studies BO17929, NEOSPHERE and TOC3478s).
• 5.8% (43/738) for pertuzumab in combination with any cytotoxic chemotherapy and trastuzumab (includes events occurring during the neoadjuvant, adjuvant and treatment-free follow-up periods in NEOSPHERE and TRYPHAENA), of which incidence was 3.8% (13/408) for patients receiving Ptz+T+D (events in the treatment period only) in the pivotal study CLEOPATRA. At median follow-up of 50 months there was no evidence of cumulative or late toxicity. The long-term cardiac safety profile was maintained.[36] To minimize the risk of cardiac toxicity, only patients with HER2-positive tumors who have adequate cardiac function at baseline (LVEF ≥55%) has been enrolledinto the study. In addition, patients who have particular cardiac risk factors has been excluded.

Infusion-associated reactions

An infusion reaction was defined in the CLEOPATRA trial[36] as any event described as hypersensitivity, anaphylactic reaction, acute infusion reaction or cytokine release syndrome occurring during an infusion or on the same day as the infusion. In this trial, the initial dose of pertuzumab was given the day before trastuzumab and docetaxel to allow for the examination of pertuzumab-associated reactions. During this window, the overall frequency of infusion reactions was 9.8% in the placebo-treated group and 13.0% in the pertuzumab-treated group, with the majority of infusion reactions being mild or moderate. The most common infusion reactions (> 1.0%) in the pertuzumab-treated group were pyrexia, chills, fatigue, headache, asthenia, hypersensitivity and vomiting.
During the second cycle when all medicinal products were administered on the same day, the most common infusion reactions in the pertuzumab-treated group (> 1.0%) were fatigue, dysgeusia, hypersensitivity, myalgia and vomiting.
Overall, the frequency of hypersensitivity/anaphylaxis events (not including acute infusion reactions/cytokine release syndrome) during the entire treatment period was 9.1% in the placebo-treated group and 10.8% in the pertuzumab-treated group (Pertuzumab IB September 2015).

5.5 Role of Denosumab in the treatment of breast cancer

5.5.1 Denosumab and the RANK/RANKL pathway

Denosumab is a fully human monoclonal immunoglobulin type 2 (IgG2) antibody that binds with high affinity (dissociation equilibrium constant [Kd] 3 x 10^{-12} M) and specificity to RANK ligand (Receptor Activator of NF-κB Ligand; RANKL) and neutralizes the activity of human RANKL, similar to the action of endogenous osteoprotegerin (OPG). Denosumab binding

prevents the activation of RANK and inhibits the formation, activation, and survival of osteoclasts. As a consequence, bone resorption and cancer-induced bone destruction are reduced.

The pathophysiology of bone metastasis is thought to depend on a vicious cycle, where growth factors and cytokines, released from cancer cells and bone, activate osteoclasts to induce bone resorption.[38] RANKL-activated osteoclasts resorbing bone in turn release growth factors from the bone that promote survival and proliferation of tumor cells in bone, creating an environment conducive for the establishment of metastatic deposits. Denosumab inhibits osteoclast formation, activation, and survival. By this action, denosumab has the potential to interrupt the vicious cycle on interactions between cancer cells and the bone microenvironment, and thereby delaying the establishment and progression of bone metastases.[39]

The hypothesis that increased bone turnover can promote skeletal tumor growth or bone metastasis has been addressed experimentally in several model systems.[40, 41, 42] Experimental inhibition of osteoclasts by inhibition of RANKL (using the soluble receptor OPG-Fc) in rodent models of breast cancer bone metastasis not only prevented tumor-induced osteolysis and decreased progression of established skeletal tumors,[43] but also significantly delayed de novo formation of bone metastases.[44] Similarly, nonclinical studies suggest that RANKL inhibition suppresses the establishment and progression of osteoblastic prostate tumors in the bone.[45]

Stimulation of the RANKL-RANK system has been identified as a main driver for increased osteoclastic activity.[46] Thus, it is possible that inhibition of RANKL and osteoclastic bone resorption by denosumab will prevent or delay bone metastasis in patients with breast cancer. Given that the bone microenvironment is a reservoir of micrometastatic tumor cells,[47] denosumab may additionally reduce tumor cell spread to soft tissue sites.[48, 49, 50]

5.5.1.1 Direct role of RANK/RANKL in breast cancer

Recent studies also suggest the potential for a direct role for RANK/RANKL in breast cancer: RANK is expressed in some human breast tumors, and can be functional on cancer cells (eg, certain epithelial [breast and prostate] cancer) and melanoma cell lines.[51, 52, 53] Transgenic mice in which RANKL is overexpressed within mammary epithelium exhibit precocious ductal side-branching and alveolar budding with clear evidence of mammary hyperplasia.[54] Studies in mice indicate that RANKL not only contributes to mammary epithelial proliferation but also the expansion and regenerative capacity of mammary stem cells[55, 56] which altogether may mediate increased risk of mammary cancer. In support of this hypothesis, mice overexpressing RANK exhibit increased mammary tumorigenesis relative to wild type mice in both carcinogen induced and spontaneous models of mammary tumorigenesis. RANK expression is associated with an increased incidence of more extensive ductular hyperplasia and mammary intraepithelial neoplasia, an increased incidence of adenocarcinoma, as well as a shorter latency to tumor formation and increased numbers of tumors per gland and per

mouse.[57] In addition, genetic ablation of RANK in mammary-gland epithelial cells prevented hormone-induced epithelial proliferation, impaired the expansion of the mammary stem cell population, and sensitized mammary epithelial cells to irradiation-induced cell death.[58] Inhibition of RANKL reduced mammary tumorigenesis induced by hormone, carcinogen and in the MMTV-neu oncogene model, and, in addition, reduced pulmonary metastasis was observed in the MMTV-neu model upon RANKL inhibition.[57]

Primary breast carcinoma samples from the neoadjuvant GeparTrio study were analyzed to correlate the expression of human RANK and RANKL with pCR, DFS, and OS.[59] Pre-treatment formalin-fixed, paraffin-embedded (FFPE) core biopsies (n=601) were analyzed for percentage and intensity of immunohistochemical RANK and RANKL expression. Antibodies against human RANK (N-1H8; Amgen) and human RANKL (M366; Amgen) were used. RANK protein was expressed in 160 (27.0%) patients. Increased RANK expression was observed in 14.5% of patients and correlated with high tumor grade (p=0.023) and negative HR status (p=0.001). Patients with high RANK expression showed a higher pCR rate (23.0 % vs. 12.6%, p=0.010), shorter DFS (p=0.038), and OS (p=0.011). However, prognostic and predictive information was not an independent parameter. Only 6.0% of samples expressed RANKL, which was not correlated with any clinical features. Higher RANK expression in the primary tumor is associated with a higher sensitivity to chemotherapy, but also a higher risk of relapse and death.

These observations support the hypothesis that inhibition of RANKL with denosumab may, by both osteoclast dependent and independent mechanisms, delay the development of clinical metastasis and disease recurrence in breast cancer. RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes.

5.5.2 Nonclinical experience with denosumab

The biologic effects of denosumab have been asessed in numerous in vitro and in vivo models and nonclinical pharmacodynamic, pharmacokinetic, and toxicology studies conducted in mice, rats, and cynomolgus monkeys. Since the biological activity of denosumab in animals is specific to nonhuman primates, evaluation of genetically engineered (knockout) mice or use of other biological inhibitors of the RANK/RANKL pathway, such as OPG linked to an immunoglobulin crystallizable fragment (OPG-Fc) and RANK linked to an immunoglobulin crystallizable fragment (RANK-Fc), were used to evaluate the pharmacodynamic properties of denosumab in rodent models. Nonclinical experience in models of bone metastasis is limited to rodent-based experiments using RANKL inhibitors such as OPG-Fc or RANK-Fc, with mechanisms of action that are considered similar to that of denosumab. In mouse bone metastasis models of estrogen receptor positive and negative human breast cancer,[60, 43] prostate cancer, and non small cell lung cancer, OPG-Fc reduced osteolytic, osteoblastic, and osteolytic/osteoblastic lesions, delayed formation of de novo bone metastases, and reduced skeletal tumor growth. When OPG-Fc was combined with hormonal therapy (tamoxifen) or

chemotherapy (docetaxel) in these models, there was additive inhibition of skeletal tumor growth in breast, and prostate, or lung cancer respectively. In a mouse model of mammary tumor induction, RANK-Fc delayed tumor formation.

These studies show that denosumab is a potent inhibitor of bone resorption via inhibition of RANKL. The toxicological effects observed in animal studies were attributable to the pharmacological activity of denosumab.

Genetic ablation of RANKL in knockout mice, and lifelong inhibition of RANKL by OPG in transgenic rats, was associated with deleterious changes in long bone growth, geometry and/or strength.[61, 62, 63] RANKL knockout mice, but not OPG transgenic rats, also exhibited failure of tooth eruption.[61,63] Preclinical pharmacology studies in neonatal rats administered RANKL inhibitors also resulted in reduced bone growth, altered growth plates, and impaired tooth eruption. All of these changes were partially reversible when dosing of RANKL inhibitors was discontinued. Studies in mice suggest absence of RANKL during pregnancy may interfere with maturation of the mammary gland leading to impaired lactation post-partum. However, this was not seen in pregnant cynomolgus monkeys treated with monthly doses of 50 mg/kg denosumab throughout pregnancy.[64]

In utero denosumab exposure in cynomolgus monkeys resulted in increased fetal loss, stillbirths, and postnatal mortality, along with evidence of absent peripheral lymph nodes, abnormal bone growth, and decreased neonatal growth.

5.5.3 Nonclinical pharmacokinetics and drug metabolism of denosumab

The single-dose pharmacokinetics and multiple-dose toxicokinetics (TK) of denosumab following IV or SC administration were evaluated in mice, rats, and cynomolgus monkeys. In addition, tissue distribution (by solid scintillation counting) and quantitative whole body autoradiography studies were conducted in cynomolgus monkeys following SC administration of denosumab. In mice and rats, species in which denosumab does not bind RANKL, the IV pharmacokinetics of denosumab were linear over the dose range of approximately 0.1 to 10 mg/kg, with low clearance (CL) and a volume of distribution at steady-state (Vss) that indicated a lack of extensive extravascular distribution. Approximately 6- and 15-fold higher CL was observed in knock-in mice that express a chimeric form of RANKL to which denosumab binds and knock-out mice lacking expression of the Fc neonatal receptor (FcRn), respectively, indicating important roles of RANKL and FcRn in denosumab disposition. In cynomolgus monkeys, the IV pharmacokinetics of denosumab were nonlinear over the dose range of 0.0016 to 1 mg/kg (with approximately 16-fold lower CL at the highest relative to lowest dose) but were approximately dose-linear for doses ≥1 mg/kg. At all doses, the Vss indicated a lack of extensive extravascular distribution. The SC pharmacokinetics of denosumab were also nonlinear in monkeys over the dose range of 0.0016 to 1 mg/kg, but were approximately dose-linear between 1 and 3 mg/kg.

In tissue distribution and quantitative whole-body autoradiography studies in cynomolgus monkeys, radioactivity following SC administration of 125 I-labeled denosumab was widely distributed. In general, systemic (serum) radioactivity was largely (> 85%) acid-precipitable, indicating that the majority of circulating radioactivity was most likely the intact antibody and iodinated peptide fragments. Concentrations of radioactivity in bone (eg, femur or lumbar vertebrae) and bone marrow were much less than (generally < 10%) those in serum and declined in parallel, indicating no specific uptake or sequestration in bone.

The multiple-dose TK of denosumab was evaluated for up to 16 months in cynomolgus monkeys for once weekly or monthly SC doses ranging from 1 to 50 mg/kg. Exposure following the first dose increased approximately dose-proportionally, indicating linear pharmacokinetics over this dose range. No sex difference in denosumab TK was observed. The development of antidrug antibodies led to decreased exposure relative to exposure after the first dose and to animals that were antibody negative. In antibody-negative animals, no evidence of time-dependent changes in denosumab TK was observed.[65]

5.5.4 Clinical experience with denosumab

In the United States (US), European Union (EU), and a number of other regions, XGEVA® (denosumab, 120 mg every 4 weeks [Q4W]) is currently approved for the prevention of skeletal related events (SREs) in patients with bone metastases from solid tumors, as well as for the treatment of adults and skeletally mature adolescents with giant cell tumour of bone that is unresectable or where surgical resection is likely to result in severe morbidity. Detailed information on the nonclinical effects of denosumab and its clinical effects in this patient population is provided in the current country-specific prescribing information for denosumab. The EU Summary of Product Characteristics (SmPC) provide detailed product information for investigators and is summarized in Table 1[65]

The clinical program evaluating denosumab in the prevention of bone metastases in subjects with advanced malignancies includes 2 studies. Study 20050147 was a phase 3, randomized, double-blind, placebo-controlled study in men with hormone-refractory CRPC. The primary objective of this study was to compare the treatment effect of denosumab with placebo on prolonging bone metastasis-free survival in men with hormone-refractory CRPC who had no bone metastasis at baseline. The secondary objectives were to compare the treatment effect of denosumab with placebo on the time to first bone metastasis (excluding deaths) and overall survival, and to assess the safety and tolerability of denosumab compared with placebo.[66]

Study 20060359 [NCT01077154]is an ongoing, phase 3, randomized, double-blind, placebo-controlled study in women with stage II or III breast cancer who are at high risk of disease recurrence to evaluate the prevention of bone metastasis and extraosseous disease recurrence. As this study is currently ongoing and blinded, results are not yet available.

In study 20050147[66], denosumab significantly prolonged bone metastasis-free survival; subjects receiving denosumab had a 15% relative risk reduction for bone metastasis or death compared with subjects receiving placebo (hazard ratio [95% confidence interval (CI)]: 0.85 [0.73, 0.98]; p-value = 0.0284). Kaplan-Meier curves for the 2 treatment groups diverged at 3 months of treatment and continued to separate, indicating that the treatment effect was sustained over time. Three hundred thirty-five subjects (46.8%) receiving denosumab and 370 subjects (51.7%) receiving placebo developed a bone metastasis or died during the primary blinded treatment period. The median bone metastasis-free survival time was 4.2 months longer for subjects who received denosumab compared with subjects who received placebo (29.5 months and 25.2 months, respectively). Denosumab also significantly reduced the risk of developing a first bone metastasis by 16% (difference in median time to first bone metastasis = 3.7 months) compared with placebo (hazard ratio of 0.84 (95% CI: 0.71, 0.98); p-value = 0.0317). Treatment with denosumab did not improve overall survival (including deaths on study and follow-up; a secondary efficacy endpoint) relative to placebo (hazard ratio of 1.01 [95% CI: 0.85, 1.20]; p-value= 0.9125). The study design required discontinuation of investigational product following development of bone metastases so that subjects could receive treatment for prevention of SREs. Systemic cancer treatments also could have been initiated. Given that most deaths (approximately 80%) included in the overall survival endpoint occurred in subjects who had discontinued treatment, the period from development of bone metastasis to death was long (median= 19 months), and multiple agents could have been used during this period to prolong survival (information on use of these agents was not collected), the potential to measure any impact of study treatment on subsequent survival was limited.

Time to overall prostate cancer disease progression (hazard ratio of 0.90 [95% CI: 0.78, 1.03]; p = 0.1287) and progression-free survival (hazard ratio of 0.89 [95% CI: 0.78, 1.02]; p = 0.0931) (exploratory efficacy endpoints) were directionally favorable in the denosumab group, although the differences between groups did not reach statistical significance. Prostate-specific antigen increased over time similarly in both treatment groups.

Table 1: Effect of denosumab on the occurrence of skeletal related events (SRE) in various malignant diseases

	Study 1 breast cancer		Study 2 other solid tumours** or multiple myeloma		Study 3 prostate cancer		Combined advanced cancer	
	XGEVA	zoledronic acid	XGEVA	zoledronic acid	XGEVA	zoledronic acid	XGEVA	zoledronic acid
N	1026	1020	886	890	950	951	2862	2861
First SRE								
Median time (months)	NR	26.4	20.6	16.3	20.7	17.1	27.6	19.4
Difference in median time (months)	NA		4.2		3.5		8.2	
HR (95% CI) / RRR (%)	0.82 (0.71, 0.95) / 18		0.84 (0.71, 0.98) / 16		0.82 (0.71, 0.95) / 18		0.83 (0.76, 0.90) / 17	
Non-inferiority / Superiority p-values	< 0.0001† / 0.0101†		0.0007† / 0.0619†		0.0002† / 0.0085†		< 0.0001 / < 0.0001	
Proportion of subjects (%)	30.7	36.5	31.4	36.3	35.9	40.6	32.6	37.8
First and subsequent SRE*								
Mean number/patient	0.46	0.60	0.44	0.49	0.52	0.61	0.48	0.57
Rate ratio (95% CI) / RRR (%)	0.77 (0.66, 0.89) / 23		0.90 (0.77, 1.04) / 10		0.82 (0.71, 0.94) / 18		0.82 (0.75, 0.89) / 18	
Superiority p-value	0.0012†		0.1447†		0.0085†		< 0.0001	
SMR per Year	0.45	0.58	0.86	1.04	0.79	0.83	0.69	0.81
First SRE or HCM								
Median time (months)	NR	25.2	19.0	14.4	20.3	17.1	26.6	19.4
HR (95% CI) / RRR (%)	0.82 (0.70, 0.95) / 18		0.83 (0.71, 0.97) / 17		0.83 (0.72, 0.96) / 17		0.83 (0.76, 0.90) / 17	
Superiority p-value	0.0074		0.0215		0.0134		< 0.0001	
First radiation to bone								
Median time (months)	NR	NR	NR	NR	NR	28.6	NR	33.2
HR (95% CI) / RRR (%)	0.74 (0.59, 0.94) / 26		0.78 (0.63, 0.97) / 22		0.78 (0.66, 0.94) / 22		0.77 (0.69, 0.87) / 23	
Superiority p-value	0.0121		0.0256		0.0071		< 0.0001	

NR = not reached; NA = not available; HCM = hypercalcaemia of malignancy; SMR = skeletal morbidity rate; HR = Hazard Ratio; RRR = Relative Risk Reduction †Adjusted p-values are presented for Studies 1, 2 and 3 (first SRE and first and subsequent SRE endpoints); *Accounts for all skeletal events over time; only events occurring ≥ 21 days after the previous event are counted.

** Including NSCLC, renal cell cancer, colorectal cancer, small cell lung cancer, bladder cancer, head and neck cancer, GI/genitourinary cancer and others, excluding breast and prostate cancer

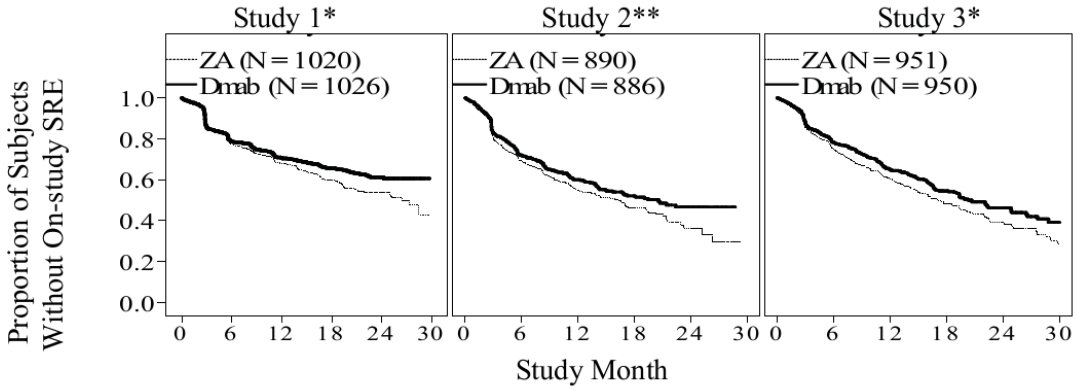
References: Stopeck et al. J Clin oncol 2010; Henry et al. J Clin oncol 2011; Fizazi et al. Lancet 2011; Lipton EJC 2012; SmPC XGEVA®.

The ABCSG 18 is a randomised, double-blind, placebo-controlled, phase 3 Study that aims to determine the treatment effect of denosumab (60 mg q6 months) in postmenopausal patients with early breast cancer receiving aromatase inhibitors. Denosumab led to a significantly delayed time to first clinical fracture, increased bone mineral density from baseline at all timepoints and measurement site and reduced the incidence of new or worsening vertebral fractures at 36 months.[3] Moreover at a median follow-up of 4 years denosumab impoves disease-free survival (HR=0.82, p=0.051).[4] The sensitivity analysis confirmed the estimate of DFS difference observed in the intention-to-treat population analysis. A subgroup analysis showed a benefit in DFS in particular for patients with hormone-receptor positive breast cancer (HR=0.75) and with tumors greater than 2cm (HR=0.66) (interaction test was not statistically significant).

5.5.4.1 Effect on disease progression and overall survival

Disease progression was similar between denosumab and zoledronic acid in all three studies and in the pre-specified analysis of all three-studies combined. In all three studies overall survival was balanced between denosumab and zoledronic acid in patients with advanced malignancies involving bone: patients with breast cancer (hazard ratio and 95% CI was 0.95 [0.81, 1.11])[67], patients with prostate cancer (hazard ratio and 95% CI was 1.03 [0.91, 1.17])[68], and patients with other solid tumours or multiple myeloma (hazard ratio and 95% CI was 0.95 [0.83, 1.08])[69]. A post-hoc analysis in study 2 (patients with other solid tumours or multiple myeloma) examined overall survival for the 3 tumour types used for stratification (non-small cell lung cancer, multiple myeloma, and other). Overall survival was longer for denosumab in non-small cell lung cancer (hazard ratio [95% CI] of 0.79 [0.65, 0.95]; n = 702) and longer for zoledronic acid in multiple myeloma (hazard ratio [95% CI] of 2.26 [1.13, 4.50]; n = 180) and similar between denosumab and zoledronic acid in other tumour types (hazard ratio [95% CI] of 1.08 (0.90, 1.30); n = 894). This study did not control for prognostic factors and anti-neoplastic treatments. In a combined pre-specified analysis from studies 1, 2 and 3, overall survival was similar between denosumab and zoledronic acid (hazard ratio and 95% CI 0.99 [0.91, 1.07])[70].Moreover long-term results on denosumab therapy deriving from the open label extension phase of two phase 3 studies in patients with metastatic breast and prostate cancer show no new safety signals. Hypocalcemia rates were similar in the blinded treatment and open-label phases. ONJ rates increased with increasing exposure to antiresorptives. During the blinded treatment phase, the combined incidence, adjusted for years of patient follow-up, of positively adjudicated ONJ for both trials was 1.9 % in the denosumab group and 1.2 % in the zoledronic acid group. The patient incidence of ONJ during the open-label extension phase, not adjusted for years of patient follow-up, was 6.9 % for patients continuing denosumab and 5.5 % for patients swithching to denosumab.[71, 65]

Table 2: Kaplan-Meier plots of time to first on-study SRE with zoledronic acid compared to denosumab.



N = Number of subjects randomised
*= Statistically significant for superiority; **= Statistically significant for non-inferiority

5.5.4.2 Effect on pain

SREs are associated with increased pain and analgesic use in patients with bone metastases. Therefore treatments that prevent SREs may decrease pain and the need for opioid analgesics and reduce the impact of pain on daily functioning.[72]

The time to pain improvement (i.e., ≥ 2 point decrease from baseline in BPI-SF worst pain score) was similar for denosumab and zoledronic acid in each study and the integrated analyses. In a post-hoc analysis of the combined dataset, the median time to worsening pain (> 4-point worst pain score) in patients with mild or no pain at baseline was delayed for XGEVA compared to zoledronic acid (198 versus 143 days) (p = 0.0002).[70]

5.5.4.3 Denosumab for the prevention of bone metastasis and disease recurrence in early breast cancer

Denosumab is also being evaluated in women with breast cancer as a potential treatment for the prevention of bone metastasis and disease recurrence. This study (D-CARE) is an ongoing, phase 3, randomized, double-blind, placebo-controlled study in women with early stage breast cancer at high risk of recurrence.

Denosumab has also gained marketing approval in the US, EU, and a number of other regions under the proprietary name Prolia® (60 mg every 6 months [Q6M]) for the treatment of postmenopausal women and men with osteoporosis and for the treatment of bone loss due to hormone ablation therapy in patients with breast or prostate cancer.[73]

5.5.4.4 Special populations

No overall differences in safety or efficacy were observed between geriatric patients and younger patients. Controlled clinical studies of denosumab in patients with advanced malignancies involving bone over age 65 revealed similar efficacy and safety in older and younger adult patients. No dose adjustment is required in elderly patients.

In a study of 55 patients without advanced cancer but with varying degrees of renal function, including patients on dialysis, the degree of renal impairment had no effect on the pharmacokinetics of denosumab. There is no need for renal monitoring when receiving denosumab.

No specific study in patients with hepatic impairment was performed. In general, monoclonal antibodies are not eliminated via hepatic metabolic mechanisms. The pharmacokinetics of denosumab is not expected to be affected by hepatic impairment.

The pharmacokinetic profile in paediatric populations has not been assessed.[65]

5.5.5 Toxicology

5.5.5.1 Preclinical studies

Preclinical studies assessing the safety of denosumab included in vitro tissue cross-reactivity studies, assessment of acute effects on the cardiovascular and respiratory system in cynomolgus monkeys, repeated-dose toxicity studies in cynomolgus monkeys up to 1 year in duration, and reproductive and embryo-fetal toxicity studies in cynomolgus monkeys. No toxicologically significant effects were observed with administration of denosumab once monthly for 1 year at doses of 1, 10, and 50 mg/kg; however, abnormal growth plates were observed, which is consistent with the pharmacological action of denosumab. Denosumab had no effect on male or female fertility, and was not an embryo-fetal or a maternal toxicant when administered to cynomolgus monkeys during the first trimester of pregnancy (lymph nodes were not examined in this study).

In another study, pregnant cynomolgus monkeys were given monthly SC injections of 0 (control) or 50 mg/kg/dose denosumab from approximately gestation day 20 until parturition (up to 6 doses). Administration of denosumab resulted in effects on the pregnant females and their offspring. There were increased stillbirths and postnatal mortality; abnormal bone growth resulting in reduced bone strength, reduced hematopoiesis, and tooth malalignment (with no effect on tooth eruption); absence of peripheral lymph nodes; and decreased neonatal growth. There was no evidence of maternal harm prior to labor; adverse maternal effects occurred infrequently during labor. Following a recovery period from birth out to 6 months of age, the effects on bone strength and quality returned to normal; some denosumab-related effects persisted (absent/decreased size of lymph nodes, extramedullary hematopoiesis, and dental dysplasia). There were no adverse effects on tooth eruption and minimal to moderate mineralization in multiple tissues was seen in one recovery animal.

Maternal mammary gland development was normal. Very low concentrations of denosumab were present in the maternal milk of cynomologus monkeys up to 1 month after the last dose of denosumab (≤ 0.5% milk:serum ratio). In general, the effects observed in mothers and infants were consistent with the pharmacological action of denosumab as a monoclonal antibody against RANKL and an inhibitor of osteoclastic bone resorption.[65 above]

5.5.5.2 Adverse events in humans

Regulatory authorization for the commercial use of denosumab (XGEVA®) for the prevention SREs in patients with bone metastases from solid tumors and for the treatment of adults and skeletally mature adolescents with giant cell tumour of bone is based on an extensive clinical database. This clinical database, which also includes studies conducted to support regulatory authorization of denosumab for bone loss indications (Prolia, 60 mg every 6 months [Q6M]), consists of 58 clinical studies (44 completed and 14 ongoing) in healthy adults and patients with osteoporosis (approximately 15,200 subjects), bone loss associated with hormone-ablation therapy (approximately 5,800 subjects), rheumatoid arthritis (approximately 200 subjects), advanced cancer (multiple myeloma and advanced malignancies involving bone [approximately 13,500 subjects]) and giant cell tumor of the bone (approximately 540 subjects) collected between June 2001 and September 2014.

Denosumab at a dose of 120 mg SC Q4W had an acceptable safety profile in a phase III trial of subjects with CRPC without bone metastases.

Overall, the subject incidences of adverse events, serious adverse events, fatal adverse events, and grade 3 to 5 adverse events were generally similar between treatment groups. As expected given the subjects' underlying cancer and the length of study participation (on average, approximately 20 months for denosumab, 19 months for placebo), most subjects in both treatment groups (94% denosumab, 93% placebo) had at least 1 adverse event. In most cases, adverse events did not lead to withdrawal of investigational product or withdrawal from the study. Also consistent with subjects' disease status, 46% of subjects in each treatment group had a serious adverse event, 53% and 50% of the subjects in the denosumab and placebo groups, respectively, had grade 3 or higher adverse events, and 10% of subjects in each treatment group had fatal adverse events. Adverse events were considered related to investigational product for 26% and 23% of subjects in the denosumab and placebo groups, respectively. The overall incidence of fatal adverse events was similar between denosumab (10.1%) and placebo (9.5%) and was consistent with the underlying disease state of elderly subjects with CRPC. Fatal adverse events were generally associated with disease progression or age-related comorbities.

The most common adverse events in either treatment group of the phase III Study 20050147 were backpain (23.3% denosumab, 22.1% placebo), constipation (17.6%, 16.9%), and arthralgia (17.1%, 15.9%). Serious adverse events were generally similar between treatment groups, were generally reflective of underlying disease, and led to investigational product or study discontinuation in < 6% of subjects in each treatment group. The most common serious

adverse events in either treatment group by preferred term in Study 20050147 were urinary retention (7.5% denosumab, 4.4% placebo), hematuria (4.9%, 3.4%), and anemia (3.1%, 1.7%). Subjects with events of ONJ leading to withdrawal from investigational product in the denosumab group accounted for most of the overall difference between treatment groups in withdrawal rates. The overall subject incidence of ONJ positively adjudicated by the external, blinded ONJ committee was 4.6% (33 subjects) and 0.0% in the denosumab and placebo groups, respectively. Similar to what was observed in the studies for the prevention of SREs, ONJ was infrequent during the first year of exposure to denosumab. The incidence of ONJ was higher with longer exposure, with the annual incremental risk being approximately constant after 2 years.

The safety of denosumab was evaluated in 5,931 patients with advanced malignancies involving bone and is derived from active-controlled, clinical trials examining the efficacy and safety of denosumab versus zoledronic acid in preventing the occurrence of skeletal related events. The adverse reactions are presented in Table 3.

Table 3: Adverse reactions reported in three phase III and one phase II active-controlled clinical studies in patients with advanced malignancies involving bone

MedDRA system organ class	**Frequency category**	**Adverse reactions**
Infections and infestations	Uncommon	Cellulitis[1]
Immune system disorder	Uncommon	Drug hypersensitivity
Metabolism and nutrition disorders	Common	Hypocalcaemia[1]
	Common	Hypophosphataemia
Respiratory, thoracic and mediastinal disorders	Very common	Dyspnoea
Gastrointestinal disorders	Very common	Diarrhoea
	Common	Tooth extraction
Skin and subcutaneous tissues disorders	Common	Hyperhidrosis
Musculoskeletal and connective tissue disorders	Common	Osteonecrosis of the jaw[1]

[1] See section Description of selected adverse reactions

The following convention has been used for the classification of the adverse reactions reported in three phase III and one phase II clinical studies (see table 1): very common (≥ 1/10), common 5 (≥ 1/100 to < 1/10), uncommon (≥ 1/1,000 to < 1/100), rare (≥ 1/10,000 to < 1/1,000) and very rare (< 1/10,000). Within each frequency grouping and system organ class, adverse reactions are presented in order of decreasing seriousness.

Please refer always to the last updated SmPC for the last available information on denosumab.[65]

5.6 Rationale of the Study

RANK ligand (RANKL), a key factor for bone remodeling and metastasis, is crucial for the development of mouse mammary glands during pregnancy. RANKL functions as a major paracrine effector of the mitogenic action of progesterone in mouse and human mammary epithelium via its receptor RANK and has a role in ovarian hormone-dependent expansion and regenerative potential of mammary stem cells. Pharmacologic inhibition of RANKL attenuates the development of mammary carcinoma and inhibits metastatic progression in multiple mouse models.[1]

In a retrospective analysis of 601 patients treated in the GeparTrio study with chemotherapy (TAC) we could demonstrate that elevated expression of RANK (immunohistochemical score > 8.5 using the N-1H8 antibody by Amgen) was found in 14.5% of patients overall. Patients with high RANK expression showed a higher pCR rate (23.0 % vs. 12.6 %, p = 0.010), shorter DFS (p = 0.038), and OS (p = 0.011)[2] The ABCSG-18 study (denosumab 60 mg s.c. q6m) showed that adjuvant denosumab reduces risk of fractures, improves bone health, and can be administered without added toxicity.[3] Moreover denosumab improves disease-free survival in postmenopausal woman with hormone receptor positive (ER- and/or PR-positive) breast cancer.[4]

It appears therefore reasonable to investigate denosumab, a clinically available antibody against RANKL in patients with primary breast cancer as an adjunct to neoadjuvant chemotherapy for its ability to increase pCR rate and improve outcome overall and in relation to the expression of RANK/L.

The backbone chemotherapy consists of nab-Paclitaxel because the pCR rate in the GeparSepto study could be increased by using nab-Paclitaxel instead of sb paclitaxel. Two different nab-Paclitaxel regimens will be compared.

For rationale for the HER2+ Substudy see chapter 16.4.

5.7 Discussion of the study design

5.7.1 Control treatments

The chemotherapy backbone used in this study is in high concordance with current recommendations by the AGO for neoadjuvant treatment of early or locally advanced breast cancer. The EC followed by weekly paclitaxel regime received the highest degree (double plus) of recommendation as this regimen has also been extensively investigated as adjuvant treatment and has shown highly favorable survival results. A similar recommendation is given for trastuzumab for patients with HER2-positive tumors. Pertuzumab received conditional approval by FDA for neoadjuvant use in combination with trastuzumab, however, the AGO

gave only a “one plus” recommendation as no data on DFS an OS are available and the label was so far not extended for neoadjuvant use by the EMA. Nab-paclitaxel as well as carboplatin (for patients with TNBC) are recommended as neoadjuvant treatment also with “one plus”, as also no survival data are available for patients with early breast cancer.

5.7.2 Drug-drug interactions of denosumab

No formal drug-drug interaction studies have been conducted with denosumab.

In clinical trials, denosumab has been administered in combination with standard anti-cancer treatment and in subjects previously receiving bisphosphonates.[71] The pharmacokinetics and pharmacodynamics of denosumab were not altered by concomitant chemotherapy and/or hormone therapy, nor by previous intravenous bisphosphonate exposure.[65 above]

5.8 Selection of study population

The main eligibility criteria of the GeparX study are comparable to those used in previous GBG neoadjuvant studies, e.g. GeparSixto[74] (only TNBC and HER2+ disease), GeparOcto, GeparSepto, and GeparQuinto.[29 above,75,76]

5.8.1 Removal of patients from therapy or assessment

Patients with progressive disease, medical or subjective intolerability of toxicity, or lack of compliance will be removed from the trial treatment. However, the investigators are asked to continue medical and/or local surgical treatment and radiotherapy as closely as possible to the guidelines given by the protocol. These patients will be followed up for efficacy at surgery and toxicity as long as the patient does not withdraw her consent.

5.8.2 Blinding

This randomized phase II study is not blinded. Pathologic complete response is considered as the objective endpoint, the pathologist in general is not informed about the study treatment and the histology reports will also be centrally reviewed in a blinded fashion.

5.8.3 Treatment compliance

All chemotherapy compounds are administered intravenously; denosumab will be administered subcutaneously. No laboratory tests for compliance will be used. The doses that were administered will be documented in the e-data capturing system (e-CRF).

5.8.4 Appropriateness of the primary efficacy variable

Pathologic complete remission (pCR) is considered to be a surrogacy endpoint and recently FDA as well as EMA approved for studies on neoadjuvant chemotherapy with high risk breast

cancer. Especially in triple-negative and HER2-positive breast cancer pCR is highly correlated with favorable survival.
A recent pooled-analysis of 11,955 patients showed that patients who attain pathological complete response (most of them receiving adequately doses and cycles of anthracyclines and taxanes with or without trastuzumab), defined as ypT0 ypN0 or ypT0/Tis ypN0, have improved survival.[99] Patients with pathological complete response (ypT0/Tis ypN0) had improved EFS (HR= 0.48, 95% CI 0.43-0.54) and OS (HR=0.36, 95% CI 0.31-0.42) compared to patients with residual tumor. This relationship was strongest in patients with TNBC (EFS HR=0.24, 95% CI 0.18-0.33; OS HR=0.16, 95% CI 0.11-0.25), hormone-receptor-negative/HER2-positive tumors (EFS HR=0.25, 95% CI 0.18-0.34; OS HR= 0.19, 95% CI 0.12-0.31), and grade 3 hormone-receptor-positive tumors (EFS HR=0.27, 95% CI: 0.14-0.50; OS HR= 0.29, 95% CI: 0.13-0.65). Accordingly, the "FDA Guidance for Industry" supported the use of pCR as a surrogate endpoint to predict clinical benefit.
To assure homogeneity regarding histologic assessment, a central review of histology reports at surgery will be performed. Central blinded pathological assessment of pCR is not planned in this large-scale study as a standardized preparation of the surgical tissue by the local pathologist would be a prerequisite (which is not feasible).
Hemoglobin levels are considered as a standard surrogate marker for symptomatic anemia. Levels above 11g/dl are usually not associated with symptoms of anemia.

5.8.5 Risk-benefit Analysis for the Participants

All study participants will receive an up-to-date neoadjuvant treatment, consisting of antra-taxanes based chemotherapy associated with douple anti-HER2 blockage, if HER2 positive. Trial participation allows also patients to receive nab-paclitaxel (not yet imbursed for the primary BC setting) and patients with HER2-positive tumors to receive pertuzumab in addition to trastuzumab. Denosumab has been widely used for treatment for patients with bone metastasis and has shown a highly favorable toxicity profile. No interaction with other cancer treatments has been reported until now. By participating in the study patients might have a higher chance for a pCR when randomized to the denosumab arm.
Participating patients will have an additional burden due to investigations required for study participation. However, due to the severity of the underlying disease and the high risk of relapse and death due to the biological subtype of disease, this burden of disease appears to be less relevant compared to the potential higher efficacy and cure rate.

5.8.6 Interpretation of potential study results

Three scenarios of results can be envisaged which will lead to the following conclusions:

- Denosumab leads to a significant (p<0.1) increase in pCR rate in all patients. The risk-benefit analysis will then be positive as it is unlikely that new safety signals will be observed in this study. Such a positive result will lead – assuming also positive data from currently ongoing adjuvant trials - to the conclusion that denosumab can be used for neoadjuvant treatment outside of clinical trials. This trial is not powered to detect an improvement of DFS (or any of the long-term outcome parameters), but two adjuvant studies (ABCSG 18 [NCT00556374] and D-Care [NCT01077154]) are addressing this question and are expected to report in the near future.
- Denosumab leads to a significant (p<0.1) increase in pCR rate only in patients with RANK-overexpressing tumors. This data will help to better target a population where denosumab has an anti-tumor and not only bone-protective effects . Results have to be validated e.g. using the above mentioned adjuvant studies or by conducting a confirmative neoadjuvant phase III study.
- Denosumab shows no different or even a lower pCR rate in the total population as well as in in the predefined subgroups. In this case, a direct and relevant anti-tumor effect of denosumab becomes quite unlikely.
- nab Paclitaxel 125mg/m² weekly has the same toxicity profile as in day 1,8 q22 but is more efficacious than the day 1,8 q22 regimen. This would be the standard dosing in primary breast cancer
- In case there is equal or less toxicity for day 1,8 q22 regimen but equal efficacy with nab-Paclitaxel 125mg/m² weekly this would be the preferred regimen.

6. STUDY OBJECTIVES

6.1 Co-Primary Objectives

- A: To compare the pathological complete response (pCR= ypT0 ypN0) rates of neoadjuvant treatment with or without denosumab in addition to backbone treatment consisting of nPac 125mg/m² weekly (Cb)→EC or nPac 125mg/m² day 1,8 q22 (Cb) →EC plus anti-HER2 treatment (i. e. trastuzumab/pertuzumab in case of positive HER2-status) in patients with early breast cancer.

- B: To compare the pathological complete response (pCR= ypT0 ypN0) rates of nPac 125mg/m² weekly(Cb)→EC or nPac 125mg/m² day 1,8 q22 (Cb)→EC plus anti-HER2 treatment (i. e. trastuzumab/pertuzumab in case of positive HER2-status) in patients with early breast cancer.

6.2 Secondary Objectives

- To test for interaction of denosumab treatment with RANK expression. The cutoff for the RANK expression high vs low will be defined in the SAP.
- To assess the pCR rates per arm in subgroups according to stratification (minimization) factors.
- To assess the pCR rates per arm for patients with RANK high and RANK low prospectively and centrally by IHC.
- To determine the rates of ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0 for both randomizations.
- To determine the response rates of the breast tumor and axillary nodes based on physical examination and imaging tests (sonography, mammography, or MRI) after treatment in both arms for each randomization.
- To determine the breast conservation rate after each treatment.
- To assess the toxicity and compliance, including time to onset of peripheral sensory neuropathy grade 2-4 and resolution of peripheral sensory neuropathy grade 2-4 to grade 1.
- To determine loco-regional invasive recurrence free survival (LRRFS), distant-disease-free survival (DDFS), invasive disease-free survival (IDFS), EFS (event free survival) and overall survival (OS) for all treatment arms and according to stratified subpopulations.
- To compare RANK/L expression from baseline to surgery.
- To compare Ki67 from baseline to surgery.
- To correlate response (complete vs. partial vs. no change) measured by best appropriate imaging method after the first two cycles of treatment with pCR.
- To assess mammographic density–changes induced by denosumab.
- To assess quality of life with a focus on persisting peripheral sensory neuropathy using the FACT-Taxane (Version 4) questionnaire.

6.3 Correlative Science Objectives

- To assess, characterize, and correlate disseminated tumor cells with the treatment effect (DTC Substudy).
- To correlate Single Nucleotide Polymorphisms (SNPs) of genes with the associated toxicity and histologically assessed treatment effect (Pharmacogenetic substudy).
- To examine and compare the impact on the pCR of the pre-specified molecular markers such as TILs, RANK/L and others on core biopsies as well as clinical characteristics (e.g. age).
- To assess molecular markers at baseline and surgery.

- Detection of microRNA and correlation with pCR (Substudy on urinary miRNA sampling (UMS)).

Primary objectives of the HER2+ Substudy:

- To assess the pathological complete response (pCR= ypT0 ypN0) rate of neoadjuvant treatment with ABP 980 and pertuzumab in the overall HER2+ cohort and compare with the results obtained in GeparSepto study.
- To compare the pathological complete response (pCR= ypT0 ypN0) rate of nPac 125mg/m² weekly→EC or nPac 125mg/m² day 1,8 q22 →EC plus anti-HER2 treatment (i. e. ABP 980 / pertuzumab in case of positive HER2-status) in patients with early breast cancer.

For secondary objectives of the HER2+ Substudy see chapter 16.4.

7. STUDY DESIGN

This is a multicenter, prospective, 2x2 randomized, open-label phase IIb study to compare neoadjuvant treatment with and without denosumab in patients with untreated breast cancer and two different nab-paclitaxel schedules.

Patients will be first randomized (using Pocock minimization) to one of the following two treatments in addition to neoadjuvant therapy:

- Denosumab (120 mg s.c. q4w)
- No denosumab

Stratification (minimization) factors for the randomization will be:

- LPBC (negative (defined as ≤50% stromal tumour infiltrating lymphocytes) / present (defined as >50% stromal tumour infiltrating lymphocytes))
- Subtype (HER2-/HR+ vs TNBC vs. HER2+)
- EC every 2 vs EC every 3 weeks

Secondarily patients will be randomized (using Pocock minimization) to:

- nPac 125mg/m² weekly (Cb)→EC
- nPac 125mg/m² day 1,8 q22 (Cb)→EC

The first randomization (denosumab) will be an additional minimization factor for the second randomization (chemotherapy regimen).

The HER2+ substudy is a cohort study investigating open label non- randomized use of ABP 980 in combination with pertuzumab.

In all study arms, treatment will be given until surgery, disease progression, unacceptable toxicity, withdrawal of consent of the patient, or termination by the Sponsor.

8. STUDY POPULATION

8.1 Number of patients

It is planned to recruit 778 subjects into this study.

8.2 Inclusion Criteria

Patients will be eligible for study participation only if they comply with the following criteria:

- Written informed consent according to local regulatory requirements prior to beginning specific protocol procedures.
- Complete baseline documentation must be submitted via MedCODES to GBG Forschungs GmbH.
- Unilateral or bilateral primary carcinoma of the breast, confirmed histologically by core biopsy. Fine-needle aspiration from the breast lesion alone is not sufficient. Incisional biopsy or axillary clearance is not allowed. In case of bilateral cancer, the investigator has to decide prospectively which side will be evaluated for the primary endpoint.
- Tumor lesion in the breast with a palpable size of $\geq$ 2 cm or a sonographical size of $\geq$ 1 cm in maximum diameter. The lesion has to be measurable in two dimensions, preferably by sonography. In case tumor isn't measurable by sonography, then MRI or mammography is sufficient. In case of inflammatory disease, the extent of inflammation can be used as measurable lesion.
- Patients must be in the following stages of disease:
  - cT2 - cT4a-d or
  - cT1c and cN+ or
  - cT1c and pNSLN+ or
  - cT1c and ER-neg and PR-neg or
  - cT1c and Ki67>20% or
  - cT1c and HER2-pos

  In patients with multifocal or multicentric breast cancer, the largest lesion should be measured.
- Centrally confirmed ER-, PR- and HER2-status. Central pathology includes also assessment of Ki-67, TIL and RANK/L status on core biopsy. TNBC is defined as ER<1% and PR<10% stained cells and HER2-negative; and HER2-positive is defined as IHC 3+ or in-situ hybridization (ISH) and according to ASCO-CAP guidelines as of 2013. LPBC (lymphocyte predominant breast cancer) is defined as more than 50% stromal tumour

infiltrating lymphocytes. Formalin-fixed, paraffin-embedded (FFPE) breast tissue from core biopsy has therefore to be sent to the GBG central pathology laboratory prior to randomization.

- Patients will be eligible for the HER2+ substudy if they have a centrally confirmed HER2+ tumor.
- Age ≥ 18 years.
- Karnofsky Performance status index ≥ 90%.
- Confirmed normal cardiac function by ECG and cardiac ultrasound (LVEF or shortening fraction) within 3 months prior to randomization. Results must be above the normal limit of the institution. For patients with HER2-positive tumors LVEF must be above 55%.
- Laboratory requirements:
- Hematology
- - Absolute neutrophil count (ANC) ≥ 2.0 x 109 / L and
  - Platelets ≥ 100 x 109 / L and
  - Hemoglobin ≥ 10 g/dL (≥ 6.2 mmol/L)
- Hepatic function
- - Total bilirubin ≤ 1.5x UNL and
  - ASAT (SGOT) and ALAT (SGPT) ≤ 1.5x UNL and
  - Alkaline phosphatase ≤ 2.5x UNL.
- Serum calcium or albumin-adjusted serum calcium ≥2.0 mmol/L (8.0 mg/dL) and ≤2.9 mmol/L (11.5 mg/dL). Hypocalcemia has to be corrected before study entry by supplementation of calcium and vitamine D.

  Negative serum pregnancy test within 14 days prior to randomization for all women of childbearing potential with the result available before dosing.

  Complete staging work-up within 3 months prior to randomization. All patients must have bilateral mammography, breast ultrasound (≤ 21 days), breast MRI (optional). Chest X-ray (PA and lateral), abdominal ultrasound or CT scan or MRI, and bone scan in case of high risk for primary metastasis. In case of a positive bone scan, bone X-ray (or CT or MRI) is mandatory. Other tests may be performed as clinically indicated.
- Patients must agree with central pathology testing of core biopsy specimen and final pathology specimen and be available and compliant for treatment and follow-up.

8.3 Exclusion Criteria

- Pure lobular carcinomas (lobular histology and G1/G2 and HR+/HER2-)
- Patients with stages cT1a, cT1b, or any M1.
- Prior chemotherapy for any malignancy.
- Prior radiation therapy for breast cancer.
- History of disease with influence on bone metabolism, such as osteoporosis, Paget's disease of bone, primary hyperparathyreoidism requiring treatment at the time of

randomization or considered likely to become necessary within the subsequent six months.

- Use of bisphosphonates or denosumab within the past 1 year.
- Significant dental/oral disease, including prior history or current evidence of osteonecrosis/ osteomyelitis of the jaw, active dental or jaw condition which requires oral surgery, non-healed dental/oral surgery, planned invasive dental procedure for the course of the study.
- Last visit at dentist > ½ year ago.
- Pregnant or lactating patients. Patients of childbearing potential must agree to use highly effective non-hormonal contraceptive measures during study treatment and 7 months following the last dose of mAbs.
- Inadequate general condition (not fit for anthracycline-taxane-targeted agents-based chemotherapy).
- Previous malignant disease being disease-free for less than 5 years (except CIS of the cervix and non-melanomatous skin cancer).
- Known or suspected congestive heart failure (>NYHA I) and / or coronary heart disease, angina pectoris requiring antianginal medication, previous history of myocardial infarction, evidence of transmural infarction on ECG, uncontrolled or poorly controlled arterial hypertension (e.g. BP >140 / 90 mm Hg under treatment with two antihypertensive drugs), controlled arterial hypertension under treatment with three or more antihypertensive drugs, rhythm abnormalities requiring permanent treatment, clinically significant valvular heart disease.
- History of significant neurological or psychiatric disorders including psychotic disorders, dementia or seizures that would prohibit the understanding and giving of informed consent.
- Pre-existing motor or sensory neuropathy of a severity ≥ grade 2 by NCI-CTC criteria v 4.0.
- Currently active infection.
- Incomplete wound healing.
- Definite contraindications for the use of corticosteroids.
- Known hypersensitivity reaction to one of the compounds or incorporated substances used in this protocol inclusive calcium and vitamine D. Known hereditary fructose intolerance.
- Concurrent treatment with:
- chronic corticosteroids unless initiated > 6 months prior to study entry and at low dose (10mg or less methylprednisolone or equivalent).
- sex hormones. Prior treatment must be stopped before study entry.
- other experimental drugs or any other anti-cancer therapy.
- Participation in another clinical trial with any investigational, not marketed drug within 30 days prior to study entry.

8.4 Removal of Patients from Study

8.4.1 Drop-outs

This study is conducted according to the principle of intent-to-treat.

Drop-outs are defined as those patients who have been randomized in the study but withdrew their consent or are withdrawn by the investigator from the study immediately thereafter but prior to first application of study medication. The reasons are collected and reported in the Consort Statement. Such patients are not included in the safety population but are included in the ITT analysis and will not be replaced.

8.4.2 Premature Treatment Discontinuation

Patients who have been randomized and have received study medication and, for whatever reason, did not participate throughout the entire study are classified as premature treatment discontinuation and will be counted as no pCR in case of missing surgery information; if patient discontinues treatment and proceeds to surgery, she is counted for pCR according to the histological report of the surgery.

Patients may discontinue study treatment at any time. Reasons for discontinuation must be documented in the case report form (CRF) and in the patient's medical records. Investigators must attempt to contact patients who fail to attend scheduled visits by telephone, letter, visit, etc., to exclude the possibility of an adverse event being the cause. Should this be the case, the adverse event must be documented, reported and followed-up as described in Section 14. The GBG project manager and/or monitor should be informed immediately of each discontinuation and the reason for it. A final examination should be performed if possible on each discontinuation. Patients should receive off study treatment as close as possible to the protocol. Treatment and outcome should be documented on the CRFs. Only in case a patient withdraws her consent for future data collection further CRFs have to be completed until date of withdrawal.

9. STUDY TREATMENT

- nab-Paclitaxel 125mg/m² weekly (days 1, 8, 15 every 3 weeks for 4 cycles) for 12 weeks or nab-Paclitaxel 125mg/m² day 1,8 q22 for 4 cycles (12 weeks)
- with or without Denosumab 120 mg s.c. on day 1 every 4 weeks for 6 cycles.

For patients with HER2-positive disease:

- ABP 980 Loading dose: 8mg/kg, thereafter 6 mg/kg, every 3 weeks simultaneously to all chemotherapy cycles. After surgery all patients will change to standard Herceptin®.

For all patients:

- Epirubicin 90mg/m² and Cyclophosphamide 600mg/m² every 2 or 3 weeks (Investigator's decision before randomization) after nab-Paclitaxel (Cb).

For patients with triple-negative disease:

- Carboplatin AUC 2 weekly in parallel to the cycles of nab-paclitaxel.

For patients with HER2-positive disease:

- Pertuzumab 840 mg loading dose i.v. followed by 420 mg i.v. every 3 weeks simultaneously to chemotherapy for at least 4 applications (according to label).

Study design EC two-weekly
week
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Arm A
R
Arm B
12 weeks
8 weeks
Core biopsy*
(before study entry)
Surgery
if HER2 positive:
trastuzumab
acc. to AGO Guideline
Core biopsy
optional
*Centrally assessed:
- Subtypes HER2/HR
- Ki67
- TILs
- RANK
If HER2 positive:
Trastuzumab (ABP 980) 8 mg/kg
(loading dose) followed by 6 mg/kg
Pertuzumab (absolute dose per
application) 840 mg (loading dose)
followed by 420 mg
nab-Paclitaxel
125 mg/m²
according to
randomization
weekly or 2 of 3
weeks
Denosumab 120 mg s.c. every 4
weeks for 6 cycles
Triple-neg.:
Carboplatin AUC 2.0 q1w
during nab-Paclitaxel
max. dose 300 mg
Epirubicin 90 mg/m²
Cyclophosphamide 600 mg/m²

Study design EC three-weekly
Core biopsy*
(before study entry)
R
Arm A
Arm B
week
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
12 weeks
9 weeks
Surgery
Core biopsy optional
*Centrally assessed:
- Subtypes HER2/HR
- Ki67
- TILs
- RANK
nab-Paclitaxel 125 mg/m² according to randomization weekly or 2 of 3 weeks
Denosumab 120 mg s.c. every 4 weeks for 6 cycles
Triple-neg.:
Carboplatin AUC 2.0 q1w during nab-Paclitaxel
max. dose 300 mg
If HER2 positive:
Trastuzumab (ABP 980) 8 mg/kg (loading dose) followed by 6 mg/kg
Pertuzumab (absolute dose per application) 840 mg (loading dose) followed by 420 mg
Epirubicin 90 mg/m²
Cyclophosphamide 600 mg/m²
If HER2 positive:
trastuzumab
acc. to AGO Guidelines

9.1 Investigational Products

Name: Denosumab (XGEVA®)

Dosage Form: 120 mg s.c. into the thigh, abdomen or upper arm.

Supplementation of at least 500 mg calcium and 400 IU vitamine D is required in all patients, unless hypercalcaemia is present If hypocalcemia occurs, short-term augmentation of calcium

Schedule: day 1 (+/- 3 days) every 4 weeks for 6 cycles. But the first injection should be given on day 2 after the administration of anti-HER2 treatments.

Denosumab will be supplied as a sterile, clear, colorless to slightly yellow, preservative-free liquid, in single-use 3.0 mL glass vials containing a deliverable dose of 1.7 mL.

Denosumab is a human monoclonal IgG2 antibody produced in a mammalian cell line (CHO) by recombinant DNA technology.

Upon receipt, and to ensure stability of denosumab it must be stored under the conditions specified below.

Denosumab is shipped by courier maintained at 2°C to 8°C. Denosumab vials will arrive in a secondary packaging container and should be immediately placed in a refrigerator maintained at 2°C to 8°C in a secured location until planned use. The set point is a single temperature for the refrigerator and should remain constant as shown in the table below.

Refrigerator Set Point	Acceptable Parameters:	Acceptable Range:
5°C	± 3°C	2°C to 8°C

Denosumab should be stored protected from light in a secure refrigerator. Denosumab is stable if maintained in accordance with the guidelines described and the provided expiration date. Actual storage conditions during the period of the study must be recorded. The Sponsor must be notified if any vial undergoes temperature excursions i.e. exposed to temperatures outside the requirements or if vials become cracked or broken. The denosumab supply may need to be returned for destruction and replaced with a new denosumab shipment.

Do not:

- Freeze the vial
- Shake denosumab in vials vigorously
- Deviate from the storage times and temperatures above
- Do not directly expose denosumab to CO_2 or dry ice, heat

Failure to follow the instruction above may lead to denaturation and inactivation of denosumab. If foreign particulate matter or discoloration is observed then contact the Sponsor for further instructions.

Prior to administration, remove the vial from the refrigerator and bring to room temperature in the original container. This generally takes 15 to 30 minutes. Do not warm the vial in any other way. Once removed from the refrigerator, the vial must not be exposed to temperatures outside the requirements as defined above and **must be used within 24 hours**. If not used within this time duration, the vial must be discarded. Do not freeze the vial. Protect the vial from light and heat. Gently swirl vial to homogenize the contents, swirling may result in the formation of bubbles, which is normal. Avoid vigorous shaking. Preparation of the clinical supplies should be performed using aseptic techniques and under sterile conditions.

Administration of Subcutaneous Dose

It is recommended to use a 25-to-27-gauge ½-inch to 5/8-inch in length needle to withdraw and inject the entire contents of the vial using an appropriate size syringe. Do not re-enter the vial. Discard the vial after single use or entry.

A 30-gauge needle should not be used, as this may affect the quality of the product.

All IP must be administered by a qualified health care professional.

Manufacturer: Amgen Europe B.V.
Minervum 7061
NL-4817 ZK Breda

Amgen is interested in hearing about any concern or irregularity at any stage of the study. Should any such concerns or irregularities occur please do not use the IP or other Amgen provided protocol required drug until Amgen confirms that it is permissible to use.
The following could be considered potential product complaints that need to be reported to Amgen:

- Packaging: for example, broken container or cracked container
- Devices: issues with delivery of IP by device
- Usage: for example, subject or healthcare provider cannot appropriately use the product
- Labeling: for example, missing labels, illegible labels, incorrect labels, and/or suspect labels
- Change in IP appearance: for example color change or presence of foreign material
- Unexpected quantity in bottle: for example number of tablets or amount of fluid
- Evidence of tampering or stolen material

If possible, please have the IP available for examination when making the call for a product complaint. Maintain IP at appropriate storage conditions described in this manual until further instructions are received from Amgen. You will also be asked for the following, site location and name of institution/investigator, protocol number, product name, lot number (from label), date problem was noticed, a full description of the problem and whether or not a subject was dosed with the impacted product/device.

Name:	nab-paclitaxel (Abraxane®)
Dosage Form:	i.v. over 30 min
Schedule:	125 mg/m², given days 1, 8, 15 every 3 weeks for 4 cycles or days 1, 8 every 3 weeks for 4 cycles
Manufacturer:	Celgene Europe Ltd. 1 Longwalk Road Stockley Park Uxbridge UB11 1DB United Kingdom

Nab-paclitaxel (ABI-007, nab-paclitaxel, nanoparticle albumin-bound paclitaxel) is a Cremophor® EL-free, albumin-bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Each 50-mL single-use vial contains 100 mg of paclitaxel, and approximately 900 mg of human albumin. Nab-paclitaxel is supplied as a white to off-white sterile lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection USP.

At the study site, nab-paclitaxel has to be stored in a locked, safe area under the responsibility of the (hospital) pharmacist, or the investigator, or other personnel allowed storing and dispensing study drug to prevent unauthorized access, according to national regulations.

Unopend vials must be kept in the outer carton in order to protect them from light.

The investigator is responsible to ensure that the investigational drug is used only in accordance with the protocol and under no circumstances will be supplied to third parties.

If any study drug is lost or damaged, its disposition should be documented in the source documents.

The investigator or a pharmacist or other appropriate individual who is assigned by the local principal investigator should maintain records of the inventory for at the site, the use for

each subject, and the delivery, storage and destruction of study drug in accordance with the national regulations.

Name:	Trastuzumab (ABP 980) (for patients with HER2-positive tumors only)
Dosage Form:	i.v.
Schedule:	Loading dose: 8 mg/kg body weight at the first. Infusion over 90 min; monitor patient for 4.5 h afterwards. Maintenance dose: 6 mg/kg body weight over 30-90 min; monitor patient for 30 min afterwards.
Application	The first injection will be given the day before the application of nabP and denosumab. The following injections on day 1 q day 22 for 8 cycles (8 infusions) together with nabP-EC
Manufacturer:	Amgen Europe B.V. Minervum 7061 NL-4817 ZK Breda

Intravenous administration of trastuzumab (ABP 980) as pertuzumab should be performed in a setting with emergency equipment and staff who are trained to monitor medical situations and respond to medical emergencies. Patients should be monitored during and following completion of each infusion for any adverse effects. Since there is the potential for delayed onset infusion-associated reactions, patients should be warned of this possibility and instructed to contact the treating physician with any concerns. Unless otherwise specified in the protocol, the initial dose should be administered over 60 minutes (± 10 minutes). If prior infusions were well tolerated, subsequent doses may be administered over 30 minutes (± 10 minutes). Patients should be observed for fever, chills, and other infusion-associated symptoms for at least 60 minutes after the first infusion and for 30 minutes after subsequent infusions. If symptoms occur, the infusion should be slowed, interrupted, or discontinued. When the patient's symptoms have completely resolved, the infusion may be continued at 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full dose during the next cycle. Patients who experience infusion-associated symptoms may be premedicated for subsequent infusions.

Trastuzumab (ABP 980) drug product is provided as a single use formulation containing 30 mg/mL trastuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20. Each 20 mL vial contains 420 mg of trastuzumab (14.0 mL/vial). Upon receipt, trastuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Trastuzumab vials should not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and

should not be frozen. The solution of trastuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at a temperature range of 2°C–25°C. However, since diluted trastuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

9.2 Non-investigational Products

9.2.1 Description, Formulation, and Handling of the non-investigational products

Name:	Epirubicin
Dosage Form:	i.v. over at least 30 min an implanted port system or via catheter to the subclavian vein
Schedule:	90 mg/m², given on day 1 every 2 or 3 weeks for 4 cycles
Manufacturers:	various

Name:	Cyclophosphamide
Dosage Form:	i.v. over 60 min.
Schedule:	600 mg/m², given on day 1 every 2 or 3 weeks for 4 cycles. Cyclophosphamide should be given on the same days as epirubicin after the end of the epirubicin infusion.
Manufacturers:	various

Name:	Carboplatin (for patients with TNBC only)
Dosage Form:	i.v. infusion over 15 – 60 minutes
Schedule:	AUC 2.0, given on days 1, 8, 15 every 3 weeks for 4 cycles Carboplatin should be given on the same days as nab-paclitaxel and after the end of nab-Paclitaxel infusion.
Manufacturers:	various

Carboplatin dose is calculated in mg, using a modified Calvert formula as follows:

Total dose (mg) = target AUC (mg/mL/min) × (creatinine clearance (mL/min) + 25)

IMPORTANT NOTE:

The creatinine clearence used in the Calvert formula to calculate AUC-dosing should not exceed 125mL/min.

The maximum carboplatin dose should not exceed target AUC (mg/mL/min) x 150 mL/min. For this study, the maximum dose of carboplatin at AUC 2 is 300 mg, at AUC 1.5 is 225 mg and at AUC 1.1 is 165 mg.

No 'correction factor' should be applied for calculating the dose of carboplatin.

Creatinine clearance can be estimated using the Cockroft-Gault formula, as follows:

Female Creatinine Clearance (mL/min) = 0.85 x (140 – age in years) × (weight in kg) divided by 72 × serum creatinine in mg/dL.

For the dosing of carboplatin using the Calvert formula, creatinine will be determined every 3 weeks. Carboplatin dose should be re-calculated every 3 weeks using actual weight and actual creatinine value.

For calculating the AUC and creatinine clearance the following link can be used:

http://www.cato.eu/gfr-cockcroft-gault-2.html

Name:	Pertuzumab (for patients with HER2-positive tumors only)
Dosage Form:	i.v.
Schedule:	840 mg the day before the first nab-Paclitaxel cycle and denosumab administration, thereafter 420 mg on day 1 q day 22 for a minimum of 4 cycles (acording to label)
Manufacturer:	Roche Pharma AG Emil-Barell-Str.1 79639 Grenzach-Wyhlen

Trastuzumab followed by pertuzumab are given directly before application of cytotoxic treatment at day 1 of all chemotherapy cycles until surgery.

Do not administer trastuzumab and pertuzumab as an i.v. push or bolus. On very rare occasions, patients experienced the onset of infusion symptoms or pulmonary symptoms more than six hours after the start of the trastuzumab infusion. Patients should be warned of the possibility of such a late onset and should be instructed to contact their physician if these symptoms occur.

There is no upper or lower limit on the amount that can be administered. The amount to be administered should be recalculated at each cycle according to the patient's weight. If the

patient's body weight has changed by more than 5% from the last calculation the amount to be administered should be recalculated.

9.3 General Principles of Study Treatment Administration

Each patient should be scheduled to receive all cytotoxic agents at a dose calculated according to body surface area (BSA). An adaptation of the total dose of the consecutive cycles should be performed in case BSA has increased/decreased for more than 5% due to weight changes.

No dose adjustment is recommended in case of BSA is calculated above 2.0 m^2.

9.4 Supportive Treatment

Supplementation of at least daily 500 mg calcium and 400 IU vitamine D is required in all patients receiving denosumab, unless hypercalcaemia is present. If hypocalcemia occurs, short-term augmentation of calcium supplementation to 1000 mg/daily may be necessary

Good oral hygiene practices should be maintained during treatment with denosumab.

Avoid invasive dental procedures during treatment with denosumab. For patients in whom invasive dental procedures cannot be avoided, the clinical judgment of the treating physician should guide the management plan (postpone dental treatment vs interruption of denosumab) of each patient based on individual benefit/risk assessment.

Other supportive treatments are recommended during chemotherapy according to AGO, ESMO, or ASCO guidelines (e.g. www.asco.org/guidelines/antiemetics).

The following supportive treatments are recommended for patients receiving:

- the **EC** regimen:

- dexamethasone: 8 mg i.v. before infusion.
- dexamethasone: 4 mg p.o. bid days 2-3, 4 mg p.o. on day 4
- NK1-antagonist (e.g. aprepitant, 125mg on day 1, 80mg on day 2-3), 5-HT3-antagonists, dopaminantagonists or metoclopramide according to local practice.
- The oral combination of netupitant (NK1) and palonosetron (5HT3) (fixed-dose oral combination agent NEPA) plus dexamethasone is an additional treatment option in this setting. Moreover olanzapine (olanzapine 5 mg os d1-4) may be considered for highly emetogenic regimens with a 5-HT3 receptor antagonist plus dexamethasone, particularly when nausea is an issue.
- Pegfilgrastim s.c. as primary prophylaxis on day 2 (only for patients receiving EC every 2 ws).
- Ciprofloxacin 500mg 2x1 tablet/day 5-12 as primary prophylaxis (only for patients receiving EC every 2 ws)

- the **nab-Paclitaxel** regimen:

- No specific supportive treatment is recommended (when given without carboplatin).
- A single antiemetic agent, such as dexamethasone, a 5-HT3 receptor antagonist, or a dopamine receptor antagonist, such as metoclopramide, may be considered.

- the **nab-Paclitaxel/Carboplatin** regimen:

- Antiemetic prophylaxis according to high emetogenic risk regimen*:*
    - 5-HT_3 receptor antagonist (palonosetron 0.25mg) + dexamethasone 8 mg + 30 min before administration of therapy but not at the days thereafter. Patients with a tendency to constipation might be considered not to receive palonosetron.
    - Carboplatin could be highly emetogenic in some patients. Therefore a triple antiemetic combination is an option (dexamethasone, NK1-antagonist, 5-HT3 receptor antagonist). Moreover olanzapine (olanzapine 5 mg os d1-4) may be considered for highly emetogenic regimens with a 5-HT3 receptor antagonist plus dexamethasone, particularly when nausea is an issue.
    - Clemastine 2 mg.
    - G-CSF as secondary prophylaxis on day 2-4 if indicated.

No additional supportive treatment is necessary for **trastuzumab (ABP 980), pertuzumab or denosumab.**

9.5 Treatment Discontinuation due to Interruptions or Early Progression

If treatment is interrupted for more than 6 weeks in a row due to any reason whatsoever, patient should stop study treatment and will be treated according to investigator's decision. Treatment interruption for more than 10 weeks altogether is not recommended. Results of surgery should still be documented. In case of a shorter interruption the full number of cycles should remain unchanged.

If a patient shows progressive disease (increase in tumor area by 25% or detection of new lesion) or inacceptable toxicity occurs during nabP(Cb), it is recommended to stop this part of the treatment and continue with EC.

If tumor progression or intolerable toxicity occurs during EC systemic treatment should be discontinued and patients should undergo immediate local treatment.

If a patients wishes to discontinue neoadjuvant treatment or the investigator decides that this is for the best benefit of the patient, immediate surgery (in the case of given operability), radiotherapy (in the case of inoperability) is recommended.

It is recommended to the investigator to follow as closely as possible attached current guidelines for surgical or radiation treatment as well as for postsurgical systemic treatment as the patient still remains a study participant and will be included in the intent-to-treat analysis.

The reason and date of treatment discontinuation for all patients will be documented on the Case Report Form (e.g. progressive disease, death, adverse event, withdrawal of consent, lost to follow-up, etc.).

The investigator will attempt to complete at the time of discontinuation of systemic treatment all study procedures that are being asked to be performed before and at surgery. The procedures and the surgery data have to be documented in the CRF.

9.6 Post-study Treatment

After completion of neoadjuvant therapy, and assessment of response, all patients should undergo surgery according to current treatment guidelines.

Definitive surgery should be performed 1-14 days after completion (after day 21) of the last chemotherapy cycle. In any case full hematologic recovery to normal limit is recommended

Surgery should be performed according to the current guidelines summarized in appendix 18.3. Pseudonymized surgical reports will be collected and analyzed centrally. Sentinel node assessment after neoadjuvant therapy is preferred to optimally assess pCR.

International guidelines do not recommend performing SNB prior to start of neoadjuvant therapy, in order to properly assess the nodal response to therapy.

The excised breast tissue should be examined by the pathologist according to the general guidelines summarized in appendix 18.4. Pseudonymized histology reports will be sent to GBG and analyzed centrally.

Radiotherapy should be applied according to the guidelines given in appendix 5. Pseudonymized radiotherapy reports will be sent to GBG and analyzed centrally.

Further post-operative systemic treatment:

No post-surgical chemotherapy is generally recommended (unless patients discontinued planned neoadjuvant chemotherapy). Denosumab will end prior to surgery as will the chemotherapy. No data support further treatment with Denosumab postsurgically. Individual decisions can be made for chemotherapy or other agents based on the postsurgical results.

Within three weeks after surgery, treatment should be (re)started if any is considered.

For tumor stages ypT1-4 and/or ypN1-3 and in case of g*BRCA1/2* mutation: Participation within the Olympia trial will be possible.

Pseudomized surgical reports will be collected and analyzed centrally. Administration of systemic adjuvant therapy is possible within a clinical trial if allowed by the study protocol (e.g. Olympia study see above).

The excised breast tissue should be examined by the pathologist according to the general guidelines summarized in appendix 18.4. Pseudomized histology reports will be sent to GBG and analyzed centrally.

Radiotherapy should be applied according to the guidelines given in appendix 18.5. Pseudomized radiotherapy reports will be sent to GBG and analyzed centrally.

Further post-operative systemic treatment is recommended for patients with:

HER2-positive disease: patients should complete anti-HER2-treatment with either the reference product Herceptin or with another approved biosimilar trastuzumab according to current standard recommendations (www.ago-online.org), including three-monthly cardiac ultrasound examination with measurement of the LVEF. Participation in a postneoadjuvant study is allowed.

Within three weeks after surgery, treatment should be restarted.

No post-surgical chemotherapy is recommended (unless patients discontinued planned neoadjuvant chemotherapy).

For tumor stages ypT1-4 and/or ypN1-3: Participation within postsurgical trials will only be possible after positive decision by the Protocol Board.

Adjuvant bisphosphonates are allowed according to current treatment guidelines (www.ago-online.org).

9.7 Concomitant Treatment

9.7.1 Concomitant Treatment and Supportive Care Guidelines

9.7.1.1 Permitted Medications

All patients will be asked to provide a complete list of prescription and over-the-counter medications that have been taken within the previous 4 weeks prior to screening. The investigator must be informed as soon as possible about any new medication(s) taken from the time of screening until the completion of the post-treatment follow-up visit.

Relevant concomitant medications taken during the study will be recorded in the case report form (CRF) with indication, dose information, and dates of administration.

Patients should receive full and appropriate supportive care during the study, including transfusion of blood and blood products, treatment with antibiotics, analgesics, iron supplementation or erythropoietin according to guidelines.

Antiemetics (as recommended and indicated) should be administered prophylactically in the event of nausea.

Permitted concomitant treatments are:

- Antiemetics
- Antiallergic measures
- Iron supplementation
- G-CSF (pegylated or non-pegylated)
- i.v. antibiotics in case of febrile neutropenia or documented infection
- Lipid lowering drugs in addition to dietary advice to patients, as HMG-CoA reductase inhibitor such as atorvastatin, pravastatin or fluvastatin

Ancillary treatments will be given as medically indicated. They must be specified in the CRF.

9.7.1.2 Prohibited Medications

Not permitted concomitant treatments are:

- Patients must not receive any other non-licensed, investigational drug or anticancer treatment until end of study treatment
- Sex hormones are not allowed. Prior treatment should be stopped before study entry
- LHRH agonists are allowed for fertility preservation as all patients have a HR- tumour, but a careful risk benefit assessment is required.
  Preoperative use of bisphophosphonates: postoperatively, bisphosphonates are not recommended but may be used for osteoporosis according to current treatment guidelines of the AGO (http://www.ago-online.de)
- Systemic corticosteroids are not allowed, except as premedication, or if started > 6 months before randomization and given at daily doses ≤ 10mg Methylprednisolone or equivalent
- Concomitant treatment with amifostine (Ethyol®) or cardioprotectors (e.g. Savene®) will not be allowed during the course of study treatment

9.7.2 Immunogenicity

In clinical studies, neutralizing antibodies have not been observed for denosumab. Using a sensitive immunoassay, < 1% of patients treated with denosumab tested positive for non-neutralizing binding antibodies, with no evidence of altered pharmacokinetics, pharmacodynamic response or toxicity

9.7.3 Treatment of Investigational Product Overdose

There is no experience with overdosage of denosumab in human clinical trials. Denosumab has been administered in clinical studies using doses up to 180 mg every 4 weeks and 120 mg weekly for 3 weeks. There is no known antidote for overdose.[65]

In the event of a suspected overdose, which has to be reported as an SAE, the patient should be closely monitored.

Treatment should be directed at the major anticipated toxicities which are hypocalcemia for denosumab and bone marrow suppression, mucositis and peripheral sensory neuropathy for the cytotoxic agents.

9.8 Dose delay and modifications due to adverse events

There will be no dose-modifications of denosumab for any reason are foreseen in this study. Denosumab will be given every 28 days (+/- 3 days). In case of missing first dose denosumab (logistic delay) denosumab may be administered at day 8 (first cycle only).

Denosumab should be withheld 30 days prior to any elective invasive oral/ dental procedure and until documented evidence of complete mucosal healing.

Dose reductions or treatment discontinuations of chemotherapy are mandatory in case of severe hematological and/or non-hematological toxicities (please refer to section 9.9). Recommendations have to be chosen according to the system organic class (SOC) showing the greatest degree of toxicity. If a patient experiences several toxicities at the same degree and there are conflicting recommendations, please follow the most conservative dose adjustment recommended.

Note that the doses which have been reduced for toxicity ***must not*** be re-escalated (except for liver function tests if improved to within ranges given).

Toxicities are graded using the NCI common toxicity criteria (NCI-CTC version 4.0; see appendix 2).

Table 4: Dose reductions

(mg, mg/m² or AUC)	Level 0	Level -1	Level -2
Denosumab	120	-	-
Epirubicin mg/m²	90	75	60
Cyclophosphamide mg/m²	600	500	stop
nab-paclitaxel mg/m²	125	100	80
Carboplatin (AUC)	2.0	1.5	1.1

Trastuzumab / Pertuzumab	No dose reduction is recommended. In case of severe toxicity probably related to this compound, treatment should be discontinued. If toxicity is recovered within 3 weeks to grade 1, a restart of treatment should be considered.

No further dose-reductions are recommended. In the case of prolonged toxicity, patient should stop the agent(s) that is/are most likely to be related to the observed toxicity. It is up to the investigator to decide when to discontinue all systemic treatment and to perform surgery immediately.

9.8.1 Recurrence of Disease or Breast Cancer Related Death

Recurrence of/or death due to breast cancer should not be reported as an adverse event or serious adverse event. Findings that are *clearly* consistent with the expected progression of the underlying cancer should not be reported as an adverse event. However, if there is any uncertainty about a finding being due solely to progression of breast cancer, the finding should be reported as an adverse event or serious adverse event as appropriate.

9.9 Toxicity-specific Recommendations for Treatments

9.9.1 Hematological Adverse Events

9.9.1.1 Anaemia

Occurrence of anaemia strongly correlates with fatigue symptoms and reduced quality of life. Moreover, severe anemia increases the incidence of surgical complications and prolongs recovery from surgery.[77, 78]

Adverse Event	Grade			
	1	2	3	4
Anaemia (hemoglobin)	< LLN - 10.0 g/dL < LLN - 6.2 mmol/L < LLN - 100 g/L	< 10.0 - 8.0 g/dL < 6.2 - 4.9 mmol/L < 100 - 80 g/L	< 8.0 g/dL < 4.9 mmol/L < 80 g/L. Transfusion indicated.	Life-threating consequences, urgent intervention indicated.

Adverse event	Action to be taken for subsequent cycles
Hemoglobin 8 - 10 g/dL (6.2 - 4.9 mmol/L)	subjects should be screened for (functional) iron deficiency and iron replacement therapy should be introduced

Hemoglobin < 8 g/dL (<4.9 mmol/L)or clinical signs of anemia	Blood transfusions until hemoglobin rises above 9 g/dl (5.5 mmol/L).

For blood transfusions, patients will receive leukocyte-reduced and filtered concentrates of erythrocytes from single donors. The reason, number, and frequency of erythrocyte transfusion must be documented.[79]

Cytotoxic treatment should be stopped as long as hemoglobin levels are below 8.0 g/dL (< 4.9 mmol/L) and the patient is symptomatic. If hemoglobin has not recovered to ≤ grade 1 within 3 weeks and symptoms of anaemia are still present study treatment should be discontinued. If the patient is asymptomatic the decision of stopping the treatment is up to the investigator balancing the risk/benefit ratio of the single patient.

9.9.1.2 Neutropenia and Febrile Neutropenia

Neutropenia and febrile neutropenia should be graded using the NCI grading system:

Toxicity Grade - Neutropenia/Febrile Neutropenia:

Adverse event	**Grade**			
	1	**2**	**3**	**4**
Neutrophils / Granulocytes (ANC / AGC)	< LLN - 1500/mm^3 < LLN - 1.5 x 10^9/L	< 1500 - 1000/mm^3 < 1.5 - 1.0 x 10^9/L	< 1000 - 500/mm^3 < 1.0 - 0.5 x 10^9/L	< 500/mm^3 < 0.5 x 10^9/L
Febrile neutropenia			ANC < 1000/mm^3 with a single temperature of > 38.3°C or a sustained tempe-rature of ≥ 38°C for more than one hour.	Life-threatening consequences (e.g. septic shock, hypotension, acidosis,necrosis), urgent intervention indicated.

Severe neutropenia is defined as:

- Neutrophils < 0.5 x 10^9/L longer than 7 days.
- Neutrophils < 0.1 x 10^9/L longer than 3 days.
- Every grade 3 neutropenia concomitant with fever (3 oral temperature determinations > 38°C during a 24-hour period or a single elevation above 38.5°C).

Blood counts prior to application of the next course of chemotherapy:

Neutrophils (x 10^9/L)	**Action to be taken**

ANC ≥ 1.5	• Treat on time
ANC 1.0 – <1.5	• Treat on time at full dose with secondary G-CSF prophylaxis. Use non-long acting G-CSF eg. on days 2-4 during weekly schedules
ANC < 1.0	• Postpone next treatment application until ANC ≥ 1.0. • Start of G-CSF. Complete blood count should be repeated every other day. Proceed with next treatment at full-dose study treatment as soon as ANC ≥ 1.0 in addition with G-CSF and give G-CSF as secondary prophylaxis for all subsequent cycles; • If there is no recovery (ANC < 1.0) within 3 weeks after the last treatment application despite use of G-CSF, the patient will go off study treatment. • If there is a second episode of prolonged recovery of neutrophils (>8 days) despite use of G-CSF, dosage of all cytotoxic agents will be reduced by 1 dose level for the subsequent cycles. No further dose reductions are planned thereafter.

General rules as summarized by ASCO [80] "Antibacterial and antifungal prophylaxis are only recommended for patients expected to have 100 neutrophils/L for 7 days, unless other factors increase risks for complications or mortality to similar levels. Inpatient treatment is standard to manage febrile neutropenic episodes, although carefully selected patients may be managed as outpatients after systematic assessment beginning with a validated risk index (eg, Multinational Association for Supportive Care in Cancer [MASCC] score or Talcott's rules) according to ASCO guidelines.[80] Patients with MASCC scores 21 or in Talcott group 4, and without other risk factors, can be managed safely as outpatients. Febrile neutropenic patients should receive initial doses of empirical antibacterial therapy within an hour of triage and should either be monitored for at least 4 hours to determine suitability for outpatient management or be admitted to the hospital. An oral fluoroquinolone plus amoxicillin/clavulanate (or plus clindamycin if penicillin allergic) is recommended as empiric therapy, unless fluoroquinolone prophylaxis was used before fever developed."

Febrile Neutropenia:

Febrile neutropenia (FN) is defined as an absolute neutrophil count (ANC) of <0.5 x 10^9/L, or <1.0 x 10^9/L predicted to fall below 0.5 x 10^9/L within 48h, with fever or clinical signs of sepsis but without any evidence of infection.

Adverse event	**Action to be taken**
Febrile neutropenia	• discontinuation of all study treatments • hospital admission or consider out-patient treatment in case patient is at low risk (≥21 points) according to the MASCC Scoring System or below to Talcott´s group 4 (please check under www.asco.org/guidelines/outpatientfn [80] • pre-antibiotic collection of specimens for bacteriology in case of fever or an infection • full blood count with differential count and blood culture should be performed every other day until recovery of ANC ≥ 1.0 x 10^9/L or temperature < 38.1°C. • If ANC 0.1 - 0.5 x 10^9/L and low risk start of an empirical antibiotic therapy with oral therapy with a fluoroquinolone (ciprofloxacin or levofloxacin) plus amoxicillin/clavulanate (e.g. ciprofloxacin (2-3 x 750 mg/d) plus ampicillin / clavulanic acid (2x 875 / 125 mg/d). G-CSF should be given according to the recommendation of the guidelines.[80] • In high-risk situations (ANC < 0.1 x 10^9/L or uncontrolled infection, pneumonia, hypotension, multi-organ failure, mucositis grade 4 with diarrhea, invasive fungal infection age > 65 years, or lymphopenia, with or without fever), i.v. antibiotic and antifungal treatment is recommended. • Beware that in case of severe neutropenia, despite an infection, fever can be missing!

In case of ANC < 0.5 x 10^9/L for >3 days, or grade 3 infection, a therapeutic intervention should proceed immediately including all actions to be taken according to the following table:

Adverse event	Action to be taken
Documented grade 3 infection **or** **ANC < 0.5 x 10^9/L for >3 days**	• The first episode of documented infection or ANC < 500 x 10^9/L for > 3 days will result in the addition of G-CSF days 2-5 according to manufacturer's recommendation for the remaining subsequent cycles • If there is a second episode while on G-CSF, dosage of cytotoxic agents will be reduced by 1 dose level during the subsequent cycles. No further dose reductions are planned thereafter.

Severe neutropenia and febrile neutropenia (as defined above) have to be reported as SAE, all other neutropenia (or leucopenia) will only be documented as AE.

9.9.1.3 Thrombocytopenia

Adverse event	Grade			
	1	2	3	4
Platelet count decreased	< LLN - 75000/mm^3 < LLN - 75.0 x 10^9/L	< 75000 - 50000/mm^3 < 75.0 - 50.0 x 10^9/L	< 50000 - 25000/mm^3< 50.0 - 25.0 x 10^9/L	< 25000/mm^3 < 25.0 x 10^9/L

Transfusions of platelets are indicated if platelets drop below 20000/µl or (petechial) bleeding is observed. The number and type (pooled or single donor products) should be documented.

All study medications should be stopped in case of grade 3 or 4 thrombocytopenia.

Platelets have to recover to ≥ 100 x 10^9/L before the start of the next chemotherapy cycle. If this results in a delay of the next treatment application, a blood count has to be repeated every second day, to restart treatment as soon as possible. If platelets have not recoveredwithin 3 weeks, treatment should be discontinued.

After one episode of thrombocytopenia grade 4 (< 25000/µl) or a second episode of prolonged recovery, the chemotherapeutics implicated in the event should be dose-reduced by 1 dose level thereafter, or if the dose was already reduced, the ongoing chemotherapy regimen should be permanently stopped.

9.9.2 Non-haematological Adverse Events

In the event of NCI-CTCAE grade 3 or 4 non-haematological AE(s) that the investigator considers to be due to suspected disease progression, re-evaluation of tumor status is indicated irrespective of scheduled clinic visits.

If any of the following conditions occur, administration of cytotoxic agents may be interrupted for a maximum of 14 days to allow the AE to resolve or decrease in severity:

- NCI-CTCAE grade 3 or 4 or unacceptable adverse events, e.g. cosmetic effect of grade 2 rash;
- No consideration and/or corroborative evidence that the AE is due to progressive disease;
- The AE is consistent with previously described adverse events.

At a minimum, reassessment of adverse events should be done weekly and more frequently if clinically indicated. When the AE decreases in severity to NCI-CTCAE Grade 1, the patient may continue to take the assigned dose.

9.9.2.1 Cellulitis

In three phase III active-controlled clinical trials in patients with advanced malignancies involving bone, skin infections leading to hospitalisation (predominantly cellulitis) were reported more frequently in patients receiving XGEVA® 120mg every 4 weeks (0.9%) compared with zoledronic acid 4mg every 3 weeks (0.7%). In postmenopausal women with osteoporosis, skin infections leading to hospitalisation were reported for 0.4% women receiving Prolia® (denosumab 60 mg every 6 months) and for 0.1% women receiving placebo.

Patients should be advised to seek prompt medical attention if they develop signs or symptoms of cellulitis (skin infection).

9.9.2.2 Hand-foot syndrome

Chemotherapy related adverse event

Adverse event	**Grade**			
	1	**2**	**3**	**4**
Palmar-plantar erythrodys-estheesia syndrome	Minimal skin changes or dermatitis (e.g. erythema, edema or hyperkeratosis) without pain.	Skin changes (e.g. peeling, blisters, bleeding, edema or hyperkeratosis) with pain; limiting instrumental ADL.	Severe skin changes (e.g. peeling, blisters, bleeding, edema or hyperkeratosis) with pain; limiting self care ADL.	-

Grade 0-1:

No dose modification is recommended.

Prevention:

- Avoidance of mechanical stress
- Removal of sweat/sudor (lukewarm water)
- Cooling gel pads can be used starting 15 min before until 15 min after chemotherapy infusion to prevent more severe hand-foot syndromes.

Treatment:

- Hyperkeratosis: Acid. Salicyl. 5-10 % (e.g. in Vas. alba);
- Urea pura 10-20 % (e.g. in Ungt. molle)

In case of inflammation:

Topical glucocortikosteroids (e.g. Mometasonfuroat Ecural®, Clobetasolproprionat Dermoxin®)

Astringend hand- / foot – baths (e.g. potassiumpermanganate, Tannolact®)

Grade 2-3:

Therapy at investigator´s discretion (please consult a dermatologist). Discontinue treatment until resolution to grade ≤ 1 and treat symptomatically. Use of vitamine B6 pyridoxine (50 to 150 mg BID) has been reported to be of possible benefit [81] and is permitted for symptomatic or secondary prophylactic treatment of hand-foot skin reaction. Delay all chemotherapy application for a maximum of two weeks until ≤ grade 1. Then reduce dose of all cytotoxic agents by one dose level according to table Table 4. If no recovery to ≤ grade 1 within two weeks delay, patient will go off chemotherapy

Grade 4: Patient will go off chemotherapy.

9.9.2.2.1 Skin Rash

Adverse event	Grade			
	1	2	3	4
Rash acneiform	Papules and/or pustules covering < 10% BSA, which may or may not be associated with symptoms of pruritus or tenderness.	Papules and/or pustules covering 10 - 30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; associated with psychosocial impact; limiting instrumental ADL.	Papules and/or pustules covering > 30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; limiting self care ADL; associated with local superinfection with oral antibiotics indicated.	Papules and/or pustules covering any % BSA, which may or may not be associated with symptoms of pruritus or tenderness and are associated with extensive superinfection with IV antibiotics indicated; life-threatining consequences.

Adverse event	Grade			
	1	2	3	4
Rash maculo-papular	Macules/papules covering > 10% BSA with or without symptoms (e.g. pruritus, burning, tightness).	Macules/papules covering 10 - 30 % BSA with or without symptoms (e.g. pruritus, burning, tightness); limiting instrumental ADL.	Macules/papules covering > 30% BSA with or without associated symptoms; limiting self care ADL.	-

A proactive and early approach to management of rash is crucial. Rash can be managed by a variety of treatment options to relieve symptoms and to reduce the rash.

The recommendations for management of skin rash are as follows (please consult a dermatologist in case of grade ≥ 2):

Rash grade 1

Mild rash may not need treatment. However, if treatment is considered necessary, topical treatments can be used.

antiseptic topical therapy			anti-inflammatory topical treatment			antibiotic topical treatment	
2x daily (BID)	octenidine (e.g. Octenisept®-solution)		glucocorticoids -corticosteroids, 1-3x daily	mometasone furoate cream (e.g. Ecural®)		2x daily (BID)	fusidic acid (e.g. Fucidine®, Fusicutan®)
	polyhexanide (e.g. Lavanid®-solution, Lavasorb®)			prednicarbate (e.g. Dermatop®)			metronidazol (z.B. Metrocreme®, Rozex®)
	povidone iodine (e.g. Betaisodona®-solution or ointment)			clobetasol 17α-propionate (e.g. Dermoxin®)			Nadifloxacin Creme (Nadixa®)
				Calcineurin-Inhibitors, 1-2x täglich			
				tacrolimus (Protopic 0,1% Salbe®)			

Rash grade 2

Relief from major symptoms caused by CTCAE grade 2 skin-related adverse events should be achieved by a combination of local and in addition systemic therapies including:

Topical treatment see above (e.g. hydrocortisone 2.5% cream, mometasone furoate cream, clindamycin 1% gel, tacrolimus 0.1% cream, etc.)

Systemic antibiotics (after antibiogram , doxycycline or minocycline 100 mg 1-0-1 (BID) for 2 weeks or metronidazol 400 mg 1-0-1 (BID) for 2 weeks, etc.),

Systemic oral corticosteroid (low dose and short term, i.e. < 10 days treatment-prednisolone 1 mg/ kg initial, followed by a 20mg daily dose-reduction) may be added at the investigator´s discretion –

Before study treatment is discontinued the dermatologist should be consulted. Systemic retinoids may be administered and monitored by a dermatologist (Isotretinoin 0,3-0,4 mg/ kg (Aknenormin®))

Systemic and topical treatment should be initiated at the start of CTCAE grade 2 rash and continued until improvement or resolution to CTCAE grade ≤ 1.
If grade 2 rash persists for ≥ 7 days despite treatment and is poorly tolerated by the patient, the investigator may choose to pause treatment for up to 14 days followed by a reduction in the dose of study treatment by 1 dose level.

Rash grade 3

May be treated in a manner similar to CTCAE grade 2 rash. In the event of CTCAE grade ≥ 3 rash, study treatment should be paused until recovery to CTCAE grade ≤ 1. Treatment should be resumed at a reduced dose by -1 dose level.
If CTCAE grade ≥ 3 rash does not resolve to CTCAE grade ≤ 1 within 14 days of delay and despite optimal supportive care, the patient will go off study treatment.

9.9.2.2.2 Paronychia and Rhagades

At the investigator´s discretion:

Mild forms: Topical treatment with Antiseptics, Antiobioticsas well asantifungal actions (e.g. Fusidic Acid ointment Fucidine®+ Ciclopirox olaminelacquer like Batrafen®)

Severe forms: Systemic antibiotics after resistogram and -as the case may be- antifungal systemic administration (please consult a dermatologist)

Granuloma pyogenicum	Topical treatment with silver nitrate or surgical decontamination
Rhagades	Tissue glue/adhesive (e.g. Cyanacrylat Dermabond®)

9.9.2.2.3 Pruritus (itching)

At the investigator´s discretion:

lipid replenishing cream (e.g. Excipial U Lipolotio®)

Polidocanol containing agent (Optiderm® Lotio)

Antihistamines: Cetirizin®, Tavegil®

Aprepitant 40 mg up to 80 mg tablets. (Emend®)

9.9.2.2.4 Interdigital folds ulceration

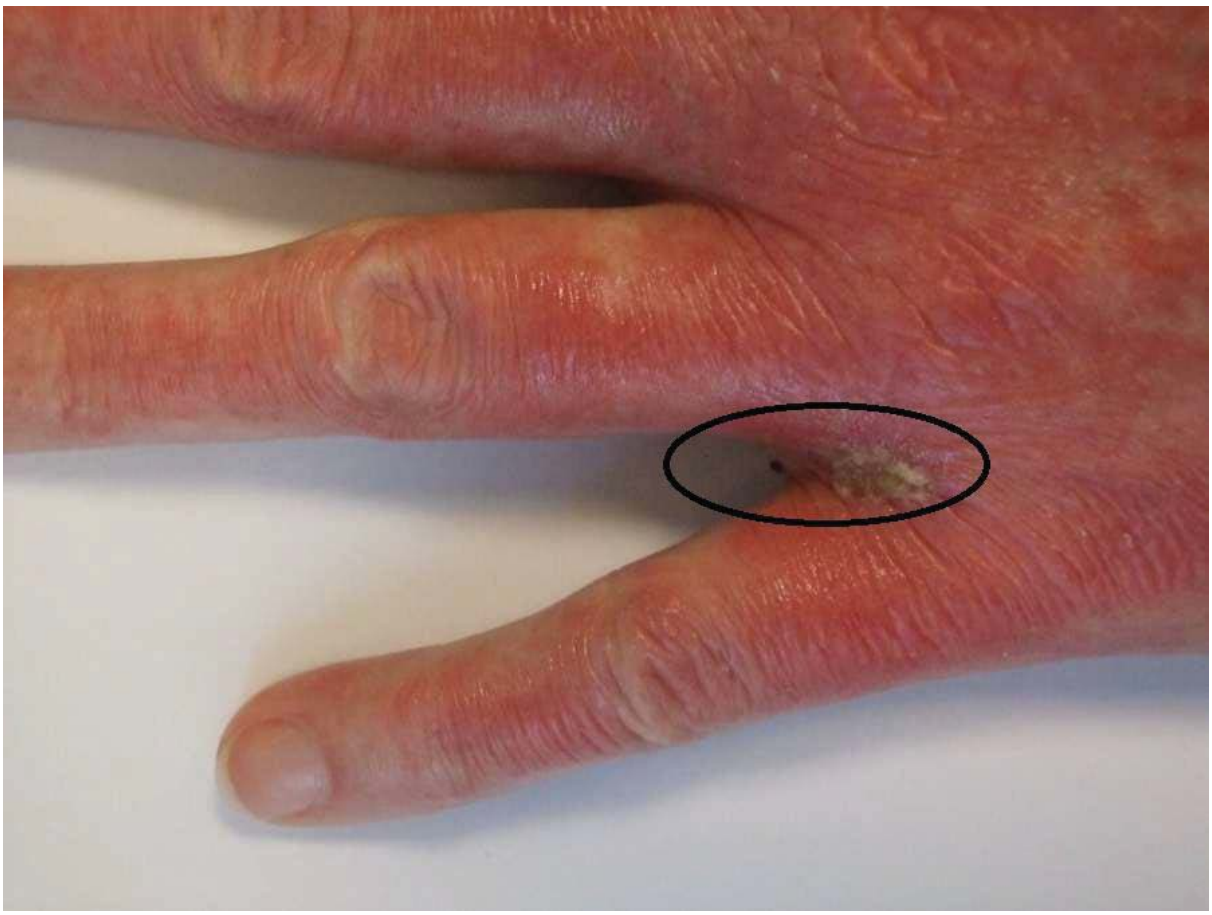
-Therapy proposal at investigator´s discretion (please consult a dermatologist):

- Octenisept®-solution
- FuciCort®-ointment. (twice daily)
- (+) Eosin-disodium solution(2%)
    - Candida –as the case may be-

    + Clotrimazoletopical (e.g. Canesten®)

    + linen wound dressing (Leinenläppchen)

Image: Example and location of interdigital folds ulceration



9.9.2.3 Nausea, vomiting, or both

In case of significant emesis premedications should be optimized according to current guidelines (www.asco.org/guidelines/emetics).

9.9.2.4 Mucositis

Adverse event	Grade			
	1	2	3	4
Mucositis oral	Asymptomatic or mild symptoms; intervention not indicated.	Moderate pain; not interfering with oral intake; modified diet indicated.	Severe pain; interfering with oral intake.	Life-threatening consequences; urgent intervention indicated.
Mucositis rectal	Asymptomatic or mild symptoms; intervention not indicated.	Symptomatic; medical intervention indicated; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	Life-threatening consequences; urgent operative intervention indicated.

Development of mucositis ≥ grade 2: Discontinue treatment until resolution to grade 0-1, reduce dose of nab-paclitaxel, or epirubicine/cyclophosphamide by 1 dose level for the remaining cycles.

If despite dose reduction, mucositis grade ≥ 3 will re-occur, the patient will go off all study treatments as per investigator discretion.

9.9.2.5 Diarrhea

Adverse event	Grade			
	1	2	3	4
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4-6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of ≥ 7 stools per day over baseline; incontinence; hospitalisation indicated; severe increase in ostomy output compared to baseline; limiting self care with ADL.	Life -threatening consequences; urgent intervention indicated.

* ADL, activities of daily living.

Diarrhea can be debilitating and potentially life-threatening on rare occasions. Guidelines developed by an ASCO panel for treating chemotherapy-induced diarrhea are abstracted below.[82]

No prophylactic treatment for diarrhea is recommended, however, patient should be educated, that their involvement in the management of diarrhoea is crucial as well as about dietary modifications.

Dietary modifications which are essential in the management of diarrhoea include the following recommendations (American Cancer Society; National Cancer Institute):

a. Stop all lactose containing products and eat small meals

b. Avoid spicy, fried and fatty foods, raw vegetables and other foods high in fiber

- Eat foods low in fiber (i.e., lean meat, rice, skinless chicken or turkey, fish, eggs, canned or cooked skinless fruits, cooked/pureed vegetables)

c. Avoid caffeine and alcohol as they can irritate the bowel and increase motility

d. Hydration:Drink 8-10 large glasses of clear liquids a day (e.g., water, electrolyte drink).

- Avoid acidic drinks such as tomato juice and fizzy soft drinks.

e. Supplement diet to include foods rich in potassium (e.g., bananas, potatoes)

In case of grade 1 to 2 diarrhea without impairment of quality of life:

- Continue study treatment
- start with the first loose stool supportive carewith loperamide administered as an initial 4-mg dose followed by 2-mg doses every four hours. Limit the time on

loperamide to 3 days before switching to 2nd line agents if grade 2 diarrhea persists.

In case of prolonged (> 3 days) grade 2 diarrhoea and grade 2 diarrhoea with impairment of quality of life:

- Reduce dose of cytotoxic agent by 1 dose level
- Continue with loperamide

In case of grade 3 or 4 diarrhoea apart from the above mentioned approaches the following approaches are recommended:

- Hold cytotoxic treatment immediately
- Re-evaluate diarrhea every 2nd day
- If diarrhoea grade 3/4 persist for > 2 weeks, stop cytotoxic treatment permanently
- if diarrhoea grade 2 persists with impaired quality of life for > 2 weeks, stop cytotoxic treatment permanently. In case of no improvement after 1 week, dose of cytotoxic treatments should be also reduced by 1 dose level.
- if diarrhoea resolves to grade ≤ 2 without impairment of quality of life, reduce dose of cytotoxic agent by 1 dose-level and start supportive care with loperamide (as above).

Other therapeutic options for extensive diarrhoea are:

- Opioids, clonidin, nonsteroidal anti-inflammatory drugs, and the serotonine antagonist cryoheptadine have been shown to be effective in controlling diarrhea associated with inflammation of the bowel.
- The synthetic octapeptide octreotide has been shown to be effective in the control of diarrhea induced by fluoropyrimidine-based chemotherapy regimens when administered as an escalating dose by continuous infusion or subcutaneous injection. Octreotide can be administered at doses ranging from 100 micrograms twice daily to 500 micrograms three times daily, with a maximum tolerated dose of 2000 micrograms three times daily in a 5-day regimen.

9.9.2.6 Hypocalcemia

In three phase III active-controlled clinical trials in patients with advanced malignancies involving bone, hypocalcemia was reported in 9.6% of patients treated with denosumab 120mg every 4 weeks and 5.0% of patients treated with zoledronic acid. A grade 3 decrease in serum calcium levels was experienced in 2.5% of patients treated with denosumab and 1.2% of patients treated with zoledronic acid. A grade 4 decrease in serum calcium levels was

experienced in 0.6% of patients treated with denosumab and 0.2% of patients treated with zoledronic acid.[70] During the open-label extension phase of two phase 3 trials, the incidences of hypocalcemia were 4.3 and 3.1%, in patients continuing and switching to denosumab, respectively.[71]

Monitoring of calcium levels is recommended during treatment, especially in the first weeks of initiating therapy. If hypocalcemia occurs, short-term augmentation of calcium supplementation to 1000 mg/daily may be necessary. Calcium level needs to be normalized before the administration of denosumab.[65]

9.9.2.7 Osteonecrosis of the Jaw (ONJ)

ONJ has occurred in patients treated with denosumab (XGEVA®). In three phase 3 active-controlled clinical trials in patients with advanced malignancies involving bone, ONJ was confirmed in 1.8% of patients in the denosumab group (median exposure of 12.0 months; range 0.1 – 40.5) and 1.3% of patients in the zoledronic acid group. The trials in patients with breast or prostate cancer included denosumab extension treatment phase (median overall exposure of 14.9 months; range 0.1 – 67.2). The patient-year adjusted incidence of confirmed ONJ was 1.1% during the first year of treatment and 4.1% thereafter. The median time to ONJ was 20.6 months (range: 4 to 53).

Poor oral hygiene, invasive dental procedures (eg, tooth extraction), treatment with anti-angiogenic medication, local gum or oral infection were risk factors for ONJ in patients receiving denosumab in clinical trials.

Patients who are suspected of having or who develop ONJ while on Denosumab should receive care by a dentist or an oral surgeon. In patients who develop ONJ during treatment with denosumab, a temporary interruption of treatment should be considered based on individual risk/benefit assessment until the condition resolves.[65 above]

9.9.2.8 Atypical femoral fractures

Atypical femoral fracture has been reported with denosumab (XGEVA®). Atypical femoral fractures may occur with little or no trauma in the subtrochanteric and diaphyseal regions of the femur and may be bilateral. Specific radiographic findings characterize these events. Atypical femoral fractures have also been reported in patients with certain comorbid conditions (e.g. vitamine D deficiency, rheumatoid arthiritis, hypophosphatasia) and with use of certain pharmaceutical agents (e.g. bisphosphonates, glucocorticoids, proton pump inhibitors). These events have also occurred without antiresorptive therapy. During denosumab treatment, patients should be advised to report new or unusual thigh, hip, or groin pain. Patients presenting with such symptoms should be evaluated for an incomplete femoral fracture, and the contralateral femur should also be examined. If an atypical femoral fracture occurs, denosumab should be permanently discontinued.[65 above]

9.9.2.9 Bilirubin and Impaired Liver Function Tests

In cases with abnormal liver function ≥ grade 3, liver imaging has to be performed to rule out the eventuality of occurrence of metastatic disease.

When a separate LFT panel is tested, it should include the following: ALT, AST, alkaline phosphatase, GGT, and total bilirubin. A direct bilirubin level should be obtained if the total bilirubin level is ≥ 2.0x UNL. Liver chemistry threshold stopping criteria and dose modification guidelines have been designed to assure subject safety.

Grade 1 abnormal bilirubin and/or ALAT: re-test LFTs every week, continue study treatment.

Grade 2 abnormal bilirubin and/or ALAT: hold chemotherapy, re-test LFTs every week until improvement to Grade 1. Re-start chemotherapy at a lower dose level CHECK LFT weekly. Grade 3 or 4: stop chemotherapy permanently.

In the event of abnormal ASAT and / or ALAT and / or alkaline phosphatase levels in the absence of relapse, the following dose modifications for the cytotoxic agents should apply.

ASAT / ALAT and alkaline phosphatase

ASAT and / or ALAT values	Alkaline phosphatase value	Dose modification
≤ 1.5x UNL	≤ 5x UNL	no dose modification
> 1.5x UNL to ≤ 2.5x UNL	≤ 2.5x UNL	no dose modification
> 2.5x UNL to ≤ 5x UNL	≤ 2.5x UNL	Reduce all cytotoxic agents by one dose level.
> 1.5x UNL to ≤ 5x UNL	> 2.5x UNL to ≤ 5x UNL	Reduce all cytotoxic agents by one dose level.
> 5x UNL	> 5x UNL	Dose delay all cytotoxic agents by a maximum of 2 weeks. If then no recovery to the above figures, patient should go off study treatment.

Once the dose is reduced due to impaired liver function, no further dose reduction is recommended if again an increase of the liver values is observed. In this case, all study treatments should be discontinued.

9.9.2.10 Renal Impairment

Adverse event	Grade			
	1	2	3	4
Creatinine	> ULN - 1.5x ULN	> 1.5 - 3.0x ULN	> 3.0 - 6.0x ULN	> 6.0x ULN

As no renal clearance characterized denosumab elimination, no dose adjustment of denosumab is necessary in patients with renal impairment.

In clinical studies of subjects without advanced cancer with varying degrees of renal function (including patients with severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), there was a greater risk of developing hypocalcemia with increasing degree of renal impairment and in the absence of calcium supplementation. Monitoring calcium levels and adequate intake of calcium and vitamine D is important in patients with severe renal impairment or receiving dialysis.

In case of grade 3 abnormal creatinine or if creatinine clearance is below 30 mL/min, all study treatments should be discontinued permanently.

9.9.2.11 Cardiac Toxicity

Anti-HER2 monoclonal antibody related adverse event:

Adverse event	Grade			
	1	2	3	4
Left ventricular systolic dysfunction	-	-	Symptomatic due to drop in ejection fraction responsive to intervention	Refractory or poorly controlled heart failure due to drop in ejection fraction; intervention such as ventricular assist device, intravenous vasopressor support, or heart transplant indicated

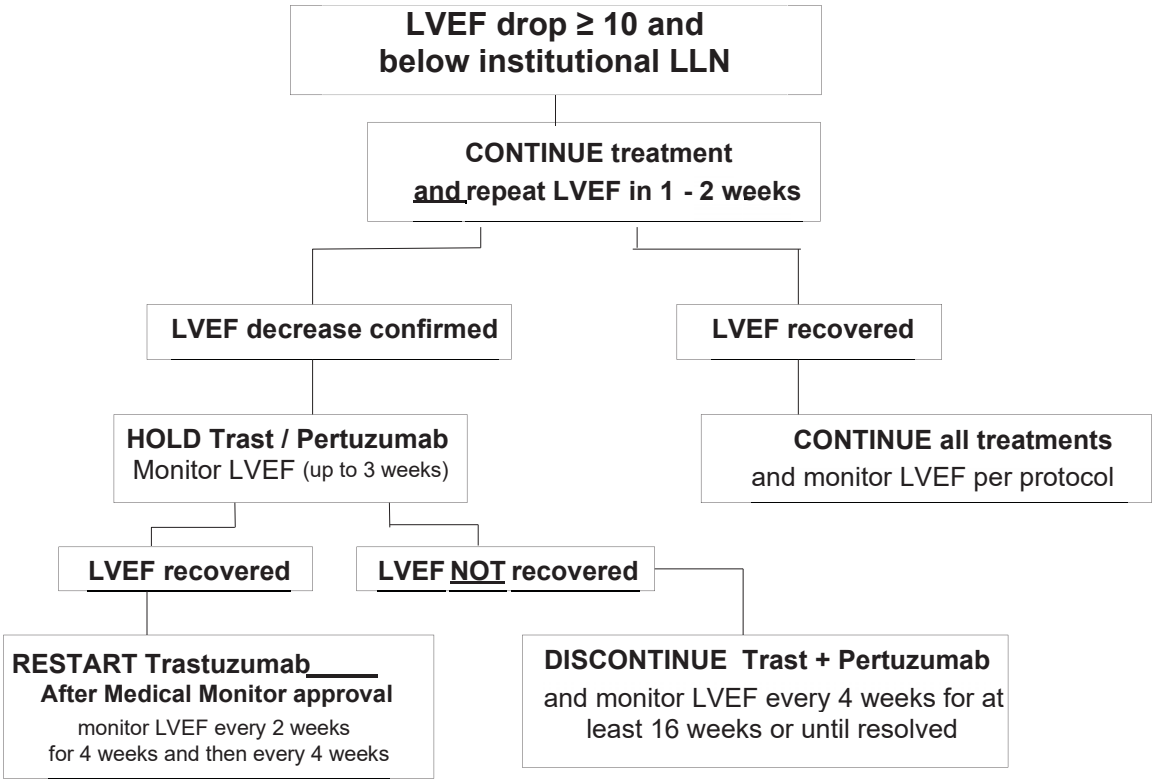
NYHA	Class I	Class II	Class III	Class IV
Cardiac	Patients with no limitation of activities; they suffer no symptoms from ordinary activities.	Patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.	Patients with marked limitation of activity; they are comfortable only at rest.	Patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

Cardiovascular events have been seen in patients taking compounds that inhibit ErbB2. Such an event is considered as AE's of special interest (see chapter 14.5)

- Patients who have a ≥10% decrease in left ventricular cardiac ejection fraction (LVEF) from baseline, and the LVEF is below the institution's lower limit of normal, should have a repeat evaluation of ejection fraction 1-2 weeks later while still receiving trastuzumab and pertuzumab.
- If the repeat ejection fraction evaluation confirms a ≥10% decrease in LVEF, and the ejection fraction is below the institution's lower limit of normal, then trastuzumab and pertuzumab should be temporarily discontinued.
- If the LVEF recovers during the next three weeks, after consultation and approval of the co-ordinating investigator, the patient may be restarted on trastuzumab, but not on pertuzumab. For such patients, monitoring of LVEF will then be performed two weeks and four weeks after re-challenge, and then every four weeks thereafter.
- If the repeat ejection fraction evaluation still shows a decrease ≥10% in LVEF from baseline and the value is below the institution's lower limit of normal, then the patient should be withdrawn from trastuzumaband pertuzumab therapy. Ejection fraction should continue to be monitored every four weeks for at least 16 weeks or until resolution.

Patients with NCI CTCAE Grade 3 or 4 left ventricular systolic dysfunction must be withdrawn from any study treatment immediately.

Details on the workflow can be found in the following figure.



9.9.2.12 Anaphylactic Type Reactions and Hypersensitivity Reactions

Nab-paclitaxel, trastuzumab or pertuzumab:

- In the event of a hypersensitivity reaction occurring despite premedication, it will very likely occur *within a few minutes of the start of the first or of the second infusion of nab-paclitaxel or trastuzumab or pertuzumab*. Therefore, the first and the second infusion must be given drop by drop for the first 5 minutes, and a careful evaluation of general sense of well-being and, whenever possible, blood pressure and heart rate monitoring will be performed so that immediate intervention is provided in response to symptoms of an untoward reaction.

Denosumab:

- In the post-marketing setting, events of hypersensitivity, including rare events of anaphylactic reactions, have been reported in patients receiving XGEVA®.

Facilities and equipment for resuscitation must be immediately available: antihistamine, corticosteroids, aminophylline, epinephrine.

If a reaction occurs, the specific treatment that is medically indicated for a given symptom (e.g. epinephrine in case of anaphylactic shock, aminophylline in case of bronchospasm, etc.) will be instituted. In addition, it is recommended to take the measures listed below:

Mild symptoms / grade 1 Localized cutaneous reaction, such as: pruritus, flushing, rash	Consider decreasing the rate of infusion until recovery of symptoms, stay at bedside. Then, complete study drug infusion at the initially planned rate. At subsequent cycle, use the same premedication outlined in chapter 5.2.
Moderate symptoms / grade 2 Any symptom not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic blood pressure (BP) > 80 mm Hg	Stop study drug infusion. Give i.v. dexamethasone 10 mg (or equivalent) and i.v. diphenhydramine 50 mg (or equivalent). Resume study drug infusion after recovery of symptoms. At subsequent cycle, give i.v. dexamethasone 10 mg (or equivalent) and i.v. diphenhydramine 50 mg (or equivalent) one hour before infusion, in addition to the premedication planned in chapter 5.2. If < Grade 3 trastuzumab/pertuzumab-associated reaction (cytokine release syndrome/acute infusion reaction or allergic reaction/hypersensitivity such as fever, rash, urticaria) occurs, pre-medication should be given with the next dose, but the infusion time may not be decreased for the subsequent infusion. If the next dose is well tolerated with pre-medication, the subsequent infusion time may be decreased by 30±10 minutes as long as pre-medication continues to be used. If infusion-related AEs occur with the 60-minute infusion, all subsequent doses should be given over 90 ±15 minutes (with pre-medication). If infusion-related AEs occur with the 30-minute infusion, all subsequent doses should be given over 60 ±10 minutes (with pre-medication).

Severe symptoms / grade 3 Such as bronchospasm, generalized urticaria, hypotension with systolic BP $\leq$ 80 mm Hg, angioedema	Stop study drug infusion. Give i.v. dexamethasone 10 mg (or equivalent) and i.v. diphenhydramine 50 mg (or equivalent), add epinephrine as needed. Whenever possible resume study drug infusion within three hours after recovery or reinfuse the patient within 72 hours using i.v. dexamethasone 20 mg (or equivalent) and i.v. diphenhydramine 50 mg (or equivalent) one hour prior to resumption of infusion. At subsequent cycle, dexamethasone (or equivalent) will be given at 20 mg orally the evening before taxane chemotherapy, the morning of chemotherapy and one hour before taxane infusion. Additionally diphenhydramine (or equivalent) will be given at 50 mg i.v. 1 hour before taxene infusion. If the reaction is caused by trastuzumab or pertuzumab, this medication should be discontinued permanently. If a severe reaction recurs, patient will go off study treatment.
Anaphylaxis (grade 4)	No further study drug therapy!

9.9.2.13 Peripheral neuropathy

Chemotherapy related adverse event

Adverse event	Grade			
	1	2	3	4
Peripheral sensory neuropathy	Asymptomatic; loss of deep tendon reflexes or paresthesia.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	Life-threatening consequences; urgent intervention indicated.

In case of symptoms or signs experienced by the patient, dose modification should be performed as follows:

Grade 0-1: No change.

Grade 2: Retreat nab-paclitaxel at dose level -1 (no further dose reduction is planned). The next application of taxane is cancelled without substitution until resolved to grade 1. Therapy of the subsequent applications is continued with reduced dose in a 3 of 4 schedule, i.e. 3 applications, the next is cancelled, etc. If symptoms are not resolved to grade 1 within 3 weeks, taxane treatment should be stopped definitively.

Grade 3/4: Patient will go off nab-paclitaxel.

All other drugs might be continued without dose reduction.

9.9.2.14 Other Adverse Events

For any other NCI-CTCAE v 4.0 grade 3 or 4 adverse event or any clinically significant, lower-grade adverse event, cytotoxic treatments should be interrupted for a maximum of 14 days until the patient recovers completely or the adverse event reverts to NCI-CTCAE v4.0 grade 1 or to baseline grade.

If recurrence of adverse event after drug holiday / interruptions is observed a dose reduction by 1 dose level for all agents (except trastuzumab and pertuzumab) is recommended. Dose reduction should only be implemented when all supportive care measures have been exhausted without an improvement of patient.

9.9.3 Pregnancy and Lactation

The safety and efficacy of denosumab in pregnant women has not been established.

Denosumab is not recommended for use in pregnant women. Women should be advised not to become pregnant during and for at least 5 months after treatment with denosumab as single agent. At AUC exposures up to 16-fold higher than the human exposure (120mg s.c. every 4 weeks), denosumab showed no evidence of impaired fertility in female cynomolgus monkeys. In a study of cynomolgus monkeys dosed with denosumab during the period equivalent to the first trimester at AUC exposures up to 10-fold higher than the human dose (120mg s.c. every 4 weeks), there was no evidence of maternal or fetal harm. In this study, fetal lymph nodes were not examined.

In another study of cynomolgus monkeys dosed with denosumab throughout pregnancy at AUC exposures 12-fold higher than the human dose (120mg s.c. every 4 weeks), there were increased stillbirths and postnatal mortality; abnormal bone growth resulting in reduced bone strength, reduced hematopoiesis, and tooth malalignment; absence of peripheral lymph nodes; and decreased neonatal growth. There was no evidence of maternal harm prior to labor; adverse maternal effects occurred infrequently during labor. Maternal mammary gland development was normal.

Studies in mice suggest absence of RANKL during pregnancy may interfere with maturation of the mammary gland leading to impaired lactation post-partum. It is not known if denosumab is excreted in human milk. Because denosumab has the potential to cause adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or discontinue the drug.[65]

All other cytotoxic medication must not be administered to pregnant women, or to women who are breastfeeding. Treatment has to immediately been stopped as soon as a pregnancy is diagnosed.

A negative pregnancy test is required prior to study entry. Highly effective non-hormonal contraceptive methods that result in a low failure rate should be applied, i.e. some IUDs (including progestogen-containing intrauterine devices), sexual abstinence or vasectomised partner (CPMP/ICH/286/95). The use of hormonal contraceptives is not allowed during the entire duration of the study. Patients should be advised not to get pregnant or breast feeding during the first 7 months after therapy.

In case a patient becomes pregnant during therapy, this has to be documented as an SAE immediately.

In addition, the pregnancy and its outcome, of the patient or his partner, during therapy or 7 months thereafter, has to be reported on a separate form provided by the Sponsor.

If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking IP should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

10. STUDY PROCEDURE - ASSESSMENTS AND SCHEDULE

10.1 Study Procedures at Screening

	Investigations	Timing (within days / months prior to randomisation)
Patient informed consent	Obtained	Prior to protocol procedures
History and physical exam	History - including: diagnosis of breast cancer, menopausal status, family history of cancer, general medical history including cardiac history and allergy,	< 21 days

	concurrent illness, pre-treatment sentinel node biopsy Concomitant medications, and their indication, used within one month prior to study entry Physical exam - including: height and weight, Karnofsky index for performance status / vital signs, heart rate (pulse), blood pressure Palpation of breasts, axillary, supra- and infraclavicular region Dental examination with appropriate preventive dentistry < 6 months Preexisting signs and symptoms Check FSH and E2 in patients aged <50 and history of hysterectomy.	
Hematology *	Hemoglobin White blood cells (WBC) and absolute neutrophil count (ANC) Platelet count	< 21 days
Biochemistry *	Serum Calcium Alkaline phosphatase ASAT (SGOT), ALAT (SGPT) Total bilirubin Serum creatinine (if ≤ 175 µmol/L [2 mg/dL]: creatinine clearance should be calculated)	< 21 days (Liver function tests are to be repeated within 3 days if abnormal results.)
Pregnancy test	Serum (if applicable) Agreement must be obtained to use highly effective non-hormonal contraceptive measures. Provision of highly effective non-hormonal contraceptive methods, i.e. some IUDs (including progestogen-containing intrauterine devices), sexual abstinence or vasectomized partner.	< 14 days (if applicable)

Imaging tests **	Mandatory for all patients: Bilateral mammography (A copy of the mammogram before study entry should be pseudomized and sent to GBG for central mammographic density assessment) Bilateral breast ultrasound In case of high risk for primary metastasis: Chest-X-Ray (PA and lateral) Abdominal ultrasound and / or CT scan and / or MRI Bone scan and bone X-ray (or CT scan or MRI) in case of hot spots in bone scan Other imaging tests as indicated	< 3 months (either breast ultrasound and/or mammography has to be ≤ 21 days)
Cardiac Monitoring	ECG, cardiac ultrasound (LVEF)	< 3 months
Biomaterials	Formalin-fixed paraffin-embedded (FFPE) tumor tissue from diagnostic core to be sent to central pathology (mandatory) RNAlater™ conserved tissue (optional) Fresh frozen tissue (optional) 10 mL whole blood for serum (mandatory) 20 mL whole blood for plasma (mandatory) 10 mL whole blood for SNP analysis (mandatory; can also be taken any time after randomisation) 10 ml urine (optional) according to subprotocol 2x 10 ml bone aspirate (optional) according to subprotocol	prior to randomization
Other Investigations	as clinically indicated	< 3 months
QoL	QoL Questionnaire FACT-Taxane	< 21 days

*Laboratory assessments will be performed whenever possible by the same laboratory throughout the study.

**To ensure comparability, the repeated breast imaging methods should be performed using identical techniques.

10.2 Evaluation during Chemotherapy up to 90 days after Surgery

	Investigations	Timing
History and physical exam since previous infusion	Clinical history and concomitant medication (use of cytotoxic drugs) Physical exam - including: weight, Karnofsky index for performance status, heart rate (pulse), blood pressure Palpation of breasts, axillary supra- and infraclavicular region Symptoms and toxicities	every cycle (day 1 or day -1 of each chemotherapy cycle) and before surgery Serious Adverse Events must be reported within 24 hours.
Imaging	Breast ultrasound including tumor measurement of the longest diameter and, if appropriate, mammography and/or MRI A copy of the presurgical mammogram should be pseudomized and sent to GBG	every 6 weeks and before breast surgery before surgery
Cardiac monitoring	Cardiac ultrasound (LVEF)	according to guidelines for anti-HER2 treatment and anthracyline therapy (e.g. after taxane and prior to surgery)
Hematology	Hemoglobin White blood cells (WBC) and absolute neutrophil count (ANC) Platelet count	every week and before surgery
Biochemistry	Serum Calcium Alkaline phosphatase ASAT (SGOT), ALAT (SGPT) Total bilirubin Serum creatinine	every cycle (within 3 days prior to chemotherapy) and before surgery

Biomaterials	10 mL whole blood for serum (mandatory) 20 mL whole blood for plasma (mandatory) 10 ml urine (optional) according to subprotocol	after 6 weeks of chemotherapy
	Formalin-fixed paraffin-embedded (FFPE) tumor tissue from core biopsy (optional) Fresh frozen tissue (optional) 10 ml urine (optional) according to subprotocol	after end of taxane treatment
	FFPE from breast tumor tissue and lymph node (if involved) (mandatory) Fresh-frozen from breast and lymph node (optional) 10 mL whole blood for serum (mandatory) 20 mL whole blood for plasma (mandatory) 2x 10 ml bone aspirate (optional) according to subprotocol	at surgery
Reports	Collect pseudonymized surgery, radiotherapy and histopathology report	Any time after surgery
Other Investigations		as clinically indicated within 90 days
QoL	QoL Questionnaire FACT-Taxane	After nab-Pac, after EOT (prior to surgery) and 90 days after surgery
Laboratory assessments will be performed whenever possible by the same laboratory throughout the study. Toxicities will be recorded according to NCI-CTC criteria with the maximum grade per cycle. Every effort will be made to use the same instrumental examination from baseline until end of study treatment.		

10.3 Collection of Biomaterials

For marker analysis

- Collection of formalin-fixed paraffin-embedded (FFPE) tumor tissue from diagnostic core before start of chemotherapy and at surgery (breast tumor tissue and involved lymph node) is mandatory. Optionally FFPE tumor tissue from core after end of taxane treatment can be collected. For central review at least 3 diagnostic cores should be sent to the central pathology, in concordance with the German S3 guidelines. Material will be stored centrally in a biomaterial bank based at the GBG pathological biomaterial repository.
- Optionally, an additional sample of the core biopsy before start of chemotherapy will be immediately transferred to RNAlater™ samples, sent and stored at GBG liquid and frozen biomaterial repository.
- Optionally, fresh frozen tissue can be collected before start of study treatment, after end of taxane treatment and at surgery (breast tumor tissue and lymph node).
- One serum sample (out of 10 mL whole blood) should be collected before start of study treatment, after 6 weeks of chemotherapy and before surgery (mandatory). Serum preparation should be performed as follows: collect 10 mL of peripheral blood in an S-Monovette (Sarstedt; monovette contains granula-bound coagulation agent), invert the tube to mix blood with coagulation agent and leave the sample 30 min at room temperature. After 30 min, the clotting of the blood is completed. Please centrifuge the sample for 15 min at 1500g and transfer the clear or yellow supernatant (upper phase/layer = serum) into the 5 fresh tubes supplied. For tube labeling use adhesive labels provided with the lab kit. Please write the center´s number, the patient number and the date of blood sampling on the labels. The serum samples must be frozen immediately at -20°C to -80°C. Samples will be picked up by a courier service. Please contact the GBG (trafo@gbg.de) to arrange pick up dates or for further information.
- For plasma isolation 20 mL of peripheral blood should collected before start of study treatment, after 6 weeks of chemotherapy and before surgery (mandatory). The plasma samples should be processed within two hours, Peripheral blood will be sampled in two EDTA-Monovette (Sarstedt)and inverted for several times (5x). After centrifugation for 15 min at 1500g, the supernatant can be transferred carefully into two fresh sample tubes. The supernatant should be centrifuged for a second time for 15 min at 1500 g. The plasma can be collected from the supernatant and transferred carfully into 10 fresh tubes. The samples must be frozen immediately at -20^{o}C to -80^{o}C and stored at the center until pick up by a courier service. For pick up arrangement please contact the GBG (trafo@gbg.de).
- One whole blood sample (10 mL) of each patient will be collected preferably before start of study treatment (but can be also collected later) for SNP analysis (mandatory).

The sample will be shipped at room temperature and stored at the GBG liquid and frozen biomaterial repository.

All technical and transportation devices (material for blood and tissue sampling and packaging) will be provided by the GBG.

10.4 End of Treatment (EOT)

The regular end of treatment is defined as 4 weeks after the last infusion of nabP(Cb) or Denosumab or EC whichever comes last.

10.5 Treatment Discontinuation of Individual Patient

If a patient shows one of the following reasons the study treatment has to be discontinued:

- Increase of breast tumor or axillary nodes in maximum diameter by 25% (see response categories in chapter 12.4.2 according to WHO assessment [83]),
- Detection of a new lesion,
- Unacceptable toxicity,
- Patients request or non-compliance.

The reason and date of discontinuation for all patients will be documented on the Case Report Form (e.g. progressive disease, death, adverse event, withdrawal of consent, lost to follow-up, etc.).

Treatment with single agents can be discontinued despite the continuation of the others.

The investigator will attempt to complete all discharge procedures at the time of discontinuation of systemic treatment. The procedures have to be documented in the CRF.

10.6 End of Study (EOS)

10.6.1 Regular End of Study

The end of this study is defined as 90 days after surgery. Planned end of study is Q IV 2019.

10.6.2 Premature Termination of Study

The study may be terminated prematurely for safety reasons, slow accrual, or upcoming new data impairing the relevance of the study objective by the GBG Forschungs GmbH as the sponsor or the protocol board. The Independent Data Monitoring Committee will provide advice. The sponsor is allowed to close the trial for any reason at any time. A decision to prematurely terminate the study is binding to all investigators of all study sites. Responsible

ethics committees and regulatory authorities will be informed about the reason(s) and time of termination according to the applicable laws and regulations.

If the study is terminated prematurely, all investigators have to inform their patients and take care of appropriate follow-up and further treatment of the patients.

10.6.3 Premature Termination of Study at a Particular Study Site

The GBG Forschungs GmbH as the sponsor reserves the right to discontinue the study at a particular study site at any time. The reasons will be discussed with the investigator.

The GBG Forschungs GmbH may terminate this study in one particular study site for one of the following reasons:

- Non-compliance with the protocol, GCP and/or regulatory requirements.
- Insufficient number of recruited patients.
- False documentation in the CRF due to carelessness or deliberately.
- Inadequate co-operation with GBG Forschungs GmbH or its representatives.
- The Investigator request to close of his/her study site.

If the study is prematurely terminated in a study site, the responsible investigators have to inform their patients and take care of appropriate follow-up and further treatment of the patients. The responsible ethics committee and regulatory authorities will be informed about the reason and time of termination according to the applicable laws and regulations.

10.7 Follow-up Period

As no study specific treatment or investigation is planned after 90 days after surgery, follow up is not part of this study. However, information on subsequent cancer specific treatments and the health status of the patients is collected either based on yearly chart reviews at the sites or based on information deriving from the GBG registry of previous study participants.

Information on date and site of recurrences, date and cause of deaths as well as secondary malignancies and long-term side effects will be collected.

11. DATA QUALITY ASSURANCE

11.1 Data Management and Documentation

Data management will be carried out by the GBG Forschungs GmbH using the proprietary GBG Forschungs web-based EDC system, "GBG MedCODES®- Medical CRF Online Documentation & Evaluation System". Data management activities include CRF design, database creation, MedCODES® application hosting, Data Entry and Data Validation.

11.1.1 Data Entry and Queries

All CRF data will be entered into the trial database using the MedCODES® application, which will perform automated plausibility and value range checks before accepting the data into the database. All CRF data will be reviewed by a data entry clerk, who will create queries for data fields that do not match the trial guidelines. These queries are stored and forwarded (within MedCODES®) to the center for resolution. The resolved queries will be checked again by a data entry clerk and either closed or re-queried.

11.1.2 Data Validation

Visual and computerized methods of data validation are applied in order to ensure accurate, consistent and reliable data.

11.1.3 Database Close and Lock

At the end of recruitment , new patient randomisation or registration functionality is stopped. New data entry is not permitted and all patients' data is set to "Final Status" and no data changes permitted. The database is locked to any kind of manipulation and handed over to the Statistics Department.

11.1.4 Privacy Protection and Data Safety

11.1.4.1 **Data Transfer and Network Access**

All Communication between the MedCODES® server and the client computers is conducted via 256 Bit encrypted HTTPS (Secure HTTP) connections.

11.1.4.2 **Pseudonymisation**

In order to protect patient data confidentiality and for safeguarding the privileged doctor patient relationship, each participating patient is assigned a unique GBG reference number. Instead of the true patient identity the pseudonym is used in all communication between the trial site and the GBG Forschungs GmbH.

11.1.4.3 **User Access Control**

Every user is provided with a personal username and password which defines their access rights as well. Access control is based on the Users Role in MedCODES. Therefore users can only access and amend those datasets necessary for them to fulfill their tasks.

11.1.5 Record retention

Copies of all pertinent on-site information (investigator's file and source data) are retained by the investigator for a period of at least 15 years from the end of the trial. The Trial Master File and Trial Databases (representing the original Case Report Forms) are kept at the GBG Forschungs GmbH for the same period of time.

11.2 Monitoring and Source Data Verification

All source data verification (SDV) is conducted according to GBG monitoring standard operations procedures (SOP).

The investigator must permit the monitor, the sponsor's internal auditors and representatives from the regulatory authorities to inspect all study-related documents and pertinent hospital or medical records for confirmation of data contained within the CRFs. Source data verification is then performed by consulting the patient file. In case of discrepancies the monitor creates queries which must be resolved by the center.

11.3 Definition Protocol Violations

Major protocol violations according to protocol are:

- Prior chemotherapy treatment
- Absence of documentation of protocol specified tumor
- No surgery unless due to progression or death

11.4 Computer Systems

All data are collected and stored using the MedCODES® application. The MedCODES® application is based on an Apache 2.2 / PHP 5.2 application server and a MySQL 5 database backend.

Due to the nature of the MedCODES® application, the trial centers must be equipped with computer terminals with online access and current versions of Microsoft Internet Explorer, Mozilla Firefox or Apple Safari. JavaScript execution must be enabled with the web browser.

12. STATISTICS

The statistical analysis of the present study is performed in accordance with the principles stated in the Consensus Guideline E9 (Statistical Principles for Clinical Trials) of the International Conference on Harmonization (ICH).

12.1 Analysis Sets

12.1.1 Intent-To-Treat Set

The intent-to-treat analysis set consists of all patients that are randomized. Patients who consented to participation and fulfilled all study criteria but did not receive any study medication after randomization are included in the intent-to-treat analysis but are excluded from the safety set and are listed separately together with their reason (if known) for not starting study treatment.

12.1.2 Per-Protocol Set

Patients who fulfilled all study criteria at the time of randomization, started assigned treatment and in whom no major protocol violation (which will be defined in SAP) occurred in the course of the study will be included into the per-protocol analysis.

12.1.3 Safety Set

Patients of the intent-to-treat population who received at least one dose of the study medication are included into the safety analysis. If a patient has accidentally received the wrong treatment, this patient is analyzed according to the actual treatment.

12.2 Sample Size Determination

The sample size calculation is based on the following assumptions for the primary endpoint:

- Improvement of the pCR rate by denosumab in all patients from 35% to 46% (OR=1.58)
- Improvement of the pCR rate by different schedules of chemotherapy (nPac 125mg day 1,8 q22 (Cb) → EC arm to nPac 125mg w (Cb) → EC) will be 36% to 45% (OR=1.45)

With 778 recruited patients, the primary continuity corrected $\chi 2$-test of pCR rates between

denosumab and no denosumab arms will have 92% power to the 2-sided significance level α=0.10. The continuity corrected χ2-test of pCR rates between nPac 125mg w (Cb)→ EC) to nPac 125mg day 1,8 q22 (Cb) → EC arms will have 80% power to the 2-sided significance level α=0.10.

Sample size for the continuity corrected χ2-test was computed using nQuery Advisor 6.02.

It is planned to recruit 778 subjects into this study.

The sample size calculation for the HER2+ substudy is based on the primary endpoint of the main study:

All patients with HER2+ disease enrolled into the study will receive ABP 980 in addition to pertuzumab and backbone chemotherapy.

It is planned to recruit approximately 150 subjects into this substudy.

12.3 Treatment Stratification

Stratification (minimization) factors for the randomization will be:

- LPBC (negative (defined as ≤50% stromal tumour infiltrating lymphocytes) / present (defined as >50% stromal tumour infiltrating lymphocytes))
- Subtype (HER2-/HR+ vs TNBC vs. HER2+)
- EC every 2 vs EC every 3 weeks

The first randomization will be a minimization factor for the second randomization.

12.4 Statistical Analyses

12.4.1 Evaluation of Primary Endpoints

Primary efficacy endpoint:

Pathological complete response of breast and lymph nodes (ypT0 ypN0; primary endpoint)

No microscopic evidence of residual invasive or non-invasive viable tumor cells in all resected specimens of the breast and axilla.

Pathological response will be assessed considering all removed breast and lymphatic tissues from all surgeries.

Patients with negative sentinel node biopsy prior to treatment start and no axilla surgery after neoadjuvant chemotherapy will be counted as pCR if no invasive and non-invasive residual tumor is detected in the removed breast tissue.

Patients with histologically/cytologically positive nodes prior to treatment start and no axilla surgery after chemotherapy will be counted as no pCR (preferably axillary dissection instead of sentinel node biopsy is strongly recommended in this situation).

Patients with positive sentinel node biopsy prior to treatment start and no invasive and non-invasive residual tumor detected in the removed breast tissue and lymph nodes after chemotherapy will be counted as pCR.

An 'intent-to-treat' (ITT) analysis will be conducted for all patients randomized in the study. In addition, a 'per-protocol' analysis will be conducted; the detailed definition of the per-protocol analysis set will be given in the statistical analysis plan. All HER2+ patients will be analysed for subgroups and multivariate analyses of the main study irrespective of the anti-HER2 treatment according to the general ITT principles.

Primary objectives A and B will be tested according to the improved Bonferroni procedure: the smaller of the two p-values will be compared with $\alpha = 0.1$ and the larger p-value will be compared with $\alpha = 0.2$ to keep the overall significance level of the study of $\alpha = 0.2$.

The primary endpoint will be summarized as pathological complete response rate for each treatment group for both randomizations. Two-sided 90% confidence intervals will be calculated according to Pearson and Clopper.

The difference in the rates of pathological complete response will be evaluated as rate difference (for primary objective A denosumab arm minus no-denosumab arm; for primary objective B nPac 125w(Cb)→ EC minus nPac day 1,8 q22 (Cb)→ EC arm) with 90% confidence interval. Additionally, an odds ratio with the 90% confidence interval will be reported. The significance will be tested with the two-sided continuity corrected χ^2-test according to the improved Bonferroni procedure.

The null hypothesis is that there is no difference in pCR rates between treatment arms; the alternative hypothesis is that there is a difference for both randomization.

The significance level for all other tests is set to 2-sided $\alpha = 0.05$. There will be no adjustment for multiple comparisons in the analyses for the stratified subpopulations. A secondary logistic regression analysis correcting for the stratification factors will be conducted for the primary endpoint.

Uni- and multivariate logistic regression will be performed for pCR to adjust for the known factors (treatment group for both randomizations, stratification factors LPBC and HER2, age, tumor size, nodal status, grade, histological type), based on the ITT population.

Additionally, a multivariate logistic regression including all factors above and interaction between denosumab and chemotherapy arms will be performed.

Primary and secondary objectives for the HER2+ substudy will be assessed in all patients who have received at least one dose of ABP 980. The pCR rates with a 95% CI will be reported and compared between chemotherapy treatment arms using the continuity corrected χ^2-test.

12.4.2 Evaluation of secondary efficacy endpoints

Secondary short-time efficacy endpoints

ypT0/Tis ypN0 is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast and axilla; in case of sentinel node biopsy prior to treatment start, the axillary lymph nodes will be evaluated as described for the primary endpoint.

ypT0 ypN0/+ is defined as no microscopic evidence of residual invasive or non-invasieve viable tumor cells in all resected specimens of the breast; ypT0/Tis ypN0/+ is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast; patients with a sentinel node biopsy prior to treatment start will be evaluated for $ypT_{(any)}$ ypN0 similarly to the description given for the primary endpoint.

Clinical (c) and imaging (i) response will be assessed every 2nd cycle and before surgery by physical examination and imaging tests. Sonography is the preferred examination, however, if sonography appears not to provide valid results or is not performed, MRI, mammography or palpation will be considered with decreasing priority. The same imaging method should be considered for the measurement before, during and after treatment.

For defined categories of efficacy (complete, partial, stable, or progression), the proportion of patients with success will be determined and appropriate confidence intervals will be calculated.

The response categories of the breast are:

- **Complete response (CR):** complete disappearance of all tumor signs in the breast as assessed by all available imaging test and palpation. The response of the axillary nodes is not to be considered.
- **Partial response (PR):** reduction in the product of the two largest perpendicular diameters of the primary tumor size by 50% or more assessed by imaging test or palpation. In patients with multifocal or multicentric disease, the lesion with the largest diameters should be chosen for follow-up. The response of the axillary nodes is not to be considered.

- **Stable disease (NC):** no significant change in tumor size during treatment which means an estimated reduction of the tumor area by less than 50%, or an estimated increase in the size of the tumor area lesions of less than 25%.
- **Progressive disease (PD):** development of new, previously undetected lesions, or an estimated increase in the size of pre-existing lesions by 25% or more after at least two cycles of therapy.

Breast conservation is defined as tumorectomy, segmentectomy or quadrantectomy as a most radical surgery.

Patients in whom success cannot be determined (e.g. patients in whom histology is not evaluable) will be included in the denominator, i.e. these patients will affect the success rate in the same way as treatment failures.

LRRFS, DDFS, IDFS, EFS and OS are defined as the time period between randomisation and first event and will be analyzed after the end of the study by referring to data from GBG patient's registry. Progressions during neoadjuvant treatment are not considered as events unless the patient is not amenable for surgery.

Tolerability and Safety: Descriptive statistics for the 4 treatments (+/- anti-HER2-treatment) will be given on the number of patients whose treatment had to be reduced, delayed or permanently stopped. The reason for termination includes aspects of efficacy (e.g. termination due to tumor progression), safety (e.g. termination due to adverse events) and compliance (e.g. termination due to patient's withdrawal of consent). Reasons for premature termination will be categorized according to the main reason and will be presented in frequency tables. Safety by toxicity grades are defined by the NCI-CTCAE version 4.0.

Correlative science research: Exploratory analyses will be performed to identify possible relationships between biomarkers and drug activity. The aim is to identify potential prognostic/predictive biomarkers of short and long term outcome parameters (pCR, EFS, and OS). Mammographic density of the pre-treatment and pre-surgical mammogram will be assessed centrally. Missing data on response evaluation will be set to no response.

Secondary short-time efficacy endpoints (ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0, response by physical examination, imaging response, breast conservation) will also be summarized as rates in each treatment group, two-sided 95% confidence intervals will be calculated according to Pearson and Clopper, and the continuity corrected Pearson χ^2 test will be performed to evaluate the difference of rates in treatment arms; these tests are considered explorative. The significance level for all tests is set to 2-sided $\alpha = 0.05$. Subgroup and multivariate analyses will be performed for ypT0/Tis ypN0 in the same way as for the primary endpoint.

A Breslow-Day test for interaction will be performed to asses difference of treatment effect between high RANK and low RANK subgroups (the cutpoint will be defined in SAP) with 2-

sided α = 0.1. The null hypothesis is that the odds ratios of pCR in denosumab arm to no denosumab arm are equal in the RANK+ and RANK- subgroups, the alternative hypothesis is that odds ratios are not equal.

For **LRRFS, DDFS, IDFS, EFS and OS** curves will be estimated using the Kaplan-Meier method, based on the ITT population. 3 year and 5 year survival (and 95%CIs) will be estimated. Univariate and multivariate Cox-proportional hazards model will be used to adjust hazard ratios for stratification factor and the above defined covariates.

Time to the first occurrence of grade 2-4 peripheral neuropathy and time to improvement of peripheral neuropathy will be analyzed using Kaplan-Meier curves and log-rank test.

Safety and compliance for HER2+ substudy will be reported descriptively in treatment arms. More details will be in the SAP and follow the general safety assessment of the main study.

12.4.3 Interim Analysis for Safety

One interim safety analysis will be performed after the first 200 patients have completed the nab-Paclitaxel treatment. The analysis will be presented to the IDMC and the protocol board for further decision making.

12.4.4 Interim Analysis for Efficacy

No interim efficacy analysis will be performed.

12.5 Further Analysis after the End of the Study

Time-to-event endpoints will be analyzed at a later time point. LRRFS, DDFS, IDFS and OS are defined as the time period between registration and first event and will be analyzed after the end of the study by referring to data from GBG patient's registry. Progression during neoadjuvant treatment is not considered as an event. Curves will be estimated using the Kaplan-Meier method, based on the ITT population, and compared using log-rank test. The 3 year and 5 year survival (and 95% CIs) will be estimated. Univariate and multivariate Cox-proportional hazards model will be used to adjust hazard ratios for stratification factor and the above defined covariates.

The following definitions will be used (based on Hudis)[84]:

Figure 3: Definitions of long term efficacy endpoints

End Point	Invasive Ipsilateral Breast Tumor Recurrence	Local/Regional Invasive Recurrence	Distant Recurrence*	Death From Breast Cancer	Death From Nonbreast Cancer	Death From Unknown Cause	Invasive Contralateral Breast Cancer	Ipsilateral DCIS	Contralateral DCIS	Second Primary Invasive Cancer (nonbreast)

OS				X	X	X				
IDFS	X	X	X	X	X	X	X			X
DDFS			X	X	X	X				X
LRRFS	X	X					X	X		
LRFS	X							X		
RRFS		X								

Abbreviations: DCIS, ductal carcinoma in situ; OS, overall survival; IDFS, invasive disease-free survival-invasive; DDFS, distant disease-free survival; LRRFS, loco-regional recurrence-free survival; (LRFS, local recurrence-free survival; RRFS regional recurrence-free survival – not relevant for this study).

The EFS (event-free survival) is defined [85] as time in months from randomization until disease progression under neo-adjuvant therapy resulting in inoperability, any invasive loco-regional (ipsilateral breast, local/regional lymph nodes) recurrence of disease after neoadjuvant therapy, any invasive contralateral breast cancer, any distant recurrence of disease or death due to any cause, whichever occurs first.

The multivariate Cox proportional hazards model will be used to assess the disease-free and overall survival in order to adjust for the major prognostic factors.

13. INDEPENDENT DATA MONITORING COMMITTEE (IDMC)

13.1 IDMC Members and Mission

In addition to the Protocol Board, the Independent Data Monitoring Committee (IDMC) of the GBG reviews and monitors the conduct of the trial. The IDMC consists of five members, three medical oncologists, one biometrician and a patients advocate. The members are independent of the trial and familiar with the methodology of oncology trials. They are aware of the dangers of conclusions based on immature data and have agreed with the design and the goals of this protocol. IDMC meetings are held every six months. The mission of the IDMC is to ensure the ethical conduct of the trial and to protect patients' safety interests in this study.

13.2 Documentation Provided to the IDMC

Before any meeting of the IDMC, the trial statistician should provide the IDMC with at least the following key documents:

- Patient baseline characteristics,
- Disease recurrence rates,

- Serious Adverse Events Listing with outcome,
- SAE summary table,
- Recruitment summary,
- Narratives of deaths,
- Study protocol.

All data will be broken down by treatment arm, participating institution and patient (whenever necessary).

13.3 Recommendations of the IDMC

After each meeting, the IDMC will provide the Protocol Board with a written recommendation to either modify the trial (with reasons), or discontinue the trial (with reasons), or continue the trial unchanged. The final decision to amend the protocol or to discontinue the trial will be taken only by the Protocol Board.

13.4 Early Termination of the Trial

Early termination of the trial will be considered by the Protocol Board based on the suggestion of the IDMC if less than 50 patients are recruited within 12 months.

14. ADVERSE EVENTS

All subjects will be monitored for AEs during the study by the study phycisian.

Patients will be instructed by the investigator to report the occurrence of any adverse event.

14.1 Adverse Event

The International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) E6 (R1) defines an AE as:

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 product, whether or not considered related to the medicinal product.

An AE includes but is not limited to any clinically significant worsening of a subject's pre existing condition. An abnormal laboratory finding (including ECG finding) that requires an action or intervention by the investigator, or a finding judged by the investigator to represent a change beyond the range of normal physiologic fluctuation, should be reported as an AE.

Adverse events may be treatment emergent (ie, occurring after initial receipt of investigational product) or nontreatment emergent. A nontreatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the subject has received investigational product.

Elective treatment or surgery or preplanned treatment or surgery (that was scheduled prior to the subject being enrolled into the study) for a documented pre-existing condition, that did not worsen from baseline, is not considered an AE (serious or nonserious). An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or SAE.

The term AE is used to include both serious and non-serious AEs.

All AEs will be recorded by the Investigator after the first administration of study treatment to at least 30 days after the last dose of IP or until the last study visit, whichever period is longer. AEs and serious adverse events (SAEs) will be recorded on the AE page of the CRF and in the subject's source documents. All SAEs must be reported according to section 14.7.

Adverse events will be graded according to NCI-CTCAE version 4.0.

14.2 Adverse Reaction

Adverse reactions are all untoward and unintended responses to a medicinal product related to any dose administered.

All expected Adverse Reactions are listed in the Investigator's Brochure (IB) for an unapproved investigational medicinal product or in the Summary of Product Characteristics (SmPC) for an authorized product. If the nature or the severity of an adverse reaction is not consistent with the applicable product information, the adverse reaction is defined as unexpected. The base for the decision is the current version of the corresponding reference document that has been submitted and approved by the competent authority and the ethics committees.

Documentation and Reporting of Adverse Events related to concomitant medication including supportive treatment: Suspected Adverse Drug Reactions with concomitant medication fall under the reporting requirements according to the "Berufsordnung für Ärzte" (Professional Code for Physicians in Germany) and must be handled by the treating physician accordingly. For this protocol all suspected adverse drug reactions serious and non-serious for concomitant medication must be documented in the CRF and/or SAE form.

14.3 Serious Adverse Event/Serious Adverse Reaction

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires or prolongs hospitalization, results in persistent or significant disability or incapacity, a congenital anomaly or birth defect, or

an important medical event after start of first administration of medication. Important medical events are those which may not be immediately life-threatening, but are clearly of major clinical significance.

Progression of a patient's underlying condition leading to one of the above should not be reported as a serious adverse event.

Severe neutropenia and febrile neutropenia (as defined above) have to be reported as SAE, all other neutropenia (or leucopenia) will only be documented as AE.

Pregnancy (Pregnancy reporting form) and AEs of special interest (see Section 14.5) must also be documented as a serious adverse event.

Exceptions:

- Hospitalization which is due solely to a planned study visit and without prolongation does not constitute a Serious Adverse Event.
- An overnight stay in the hospital that is only due to transportation, organisation or accommodation problems and without medical background does not need to be handled/documented as a Serious Adverse Event.
- Leucopenia and (non-febrile) neutropenia of any grade without hospitalisation which do not meet the criteria in Section 9.9.1.2

14.4 Suspected Unexpected Serious Adverse Reactions

All unexpected serious adverse events judged by either the investigator or the GBG Forschungs GmbH as the sponsor to have a reasonable suspected causal relationship to an investigational medicinal product qualify as suspected unexpected serious adverse reactions (SUSAR).

All Suspected Serious Adverse Reactions (SAR), which might be unexpected, must be reported to the GBG Forschungs GmbH as the sponsor immediately, regardless of the time which has elapsed during the clinical trial (treatment and follow-up phase).

14.5 Adverse Events of Special Interest

The Adverse Events (AE) of special interest must be reported on the SAE report form.

The following events are defined as AEs of Special Interest:

For patients on denosumab:

- Hypocalcemia grade ≥3
- Osteonecrosis of the jaw
- Atypical fractures of the femur

For patients on nab-paclitaxel:

- any adverse event effecting cranial nerves
- Anaphylaxis
- Macular edema

For patients on trastuzumab (ABP 980):

- Cardiac failure
- Infusion reactions
- Pulmonary toxicity
- Hypersensitivity
- infections and infestations

14.6 Death on Study

Any death occurring during the study must be reported to the GBG Forschungs GmbH regardless of the relation to study drug(s) on the death report form section of the CRF. The cause of death should be documented (tumor-related, treatment-related, tumor- and treatment-unrelated). Autopsy reports should be collected whenever possible and sent to the GBG Forschungs GmbH.

Deaths that do not occur due to tumor progression during the treatment phase or within 30 days following the last treatment of study have to be reported as serious adverse events within one working day to the GBG Forschungs GmbH. Deaths after the end of treatment which are considered to be related to study treatment also have to be reported as SAEs on the same eCRF form.

14.7 SAE Reporting

All serious adverse events occurring during the study treatment period or within 30 days following the last administration must be reported according to the procedure described below. Any late SAE (occurring after this 30-day period) possibly or probably related to the study medication should follow the same reporting procedure.

Progression or relapse of the tumour and their related symptoms must not be reported as SAE but must be documented elsewhere on the CRF.

SAEs must be reported to GBG Forschungs GmbH within 24 hours of the Investigator's knowledge of the event by MedCODES or facsimile, using the SAE Report Form.

Address for reports on serious adverse events:

GBG Forschungs GmbH Martin-Behaim-Straße 12 63263 Neu-Isenburg Germany	Phone: +49 (0) 6102 / 7480-0	Via MedCODES or alternatively via Fax: + 49 (0) 6102 / 7480-440

The GBG will report all SAEs immediately to the Co-ordinating Investigator and to the pharmaceutical manufacturer.

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to IP (causality), action taken regarding IP, and outcome.

All SAEs will be followed-up by the investigator until satisfactory resolution. Annually all SAEs will be reported as a Development Safety Update Report (DSUR) by the GBG to the competent authoritiesand the leading ethics committee, including all SUSARs.

Withdrawal from the study and further treatment shall be at the discretion of the investigator.

14.8 SUSAR Reporting

Expected serious adverse reactions are listed in the Investigator's Brochure of Denosumab and the Summary of Product Characteristics (SmPC) of nab-Paclitaxel, Pertuzumab, Trastuzumab, Carboplatin, Epirubicin and Cyclophosphamide. All serious unexpected adverse events judged by either the investigator or the GBG Forschungs GmbH as the sponsor to have a reasonable suspected causal relationship to an investigational or an accompanying medicinal product are qualified as suspected unexpected serious adverse reactions (SUSAR). SUSARs have to be reported to the competent authorities and the corresponding ethics committees by the GBG within 15 days, and in case of fatal or life-threatening events as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will be subsequently be submitted. SUSAR reporting can be delegated to an adequately qualified person and organisation. In this case the responsibility and commitment still lies with the sponsor of the study.

15. ADMINISTRATIVE EXECUTION

15.1 Monitoring

On-site visits will be made before the study begins and at regular intervals during the study. Other forms of communication (e.g. by telephone, mail, fax etc.) may be used as needed to supplement visits.

The monitor has the responsibility of reviewing the ongoing study with the investigator to verify adherence to the protocol and to deal with any problems if and when they arise.

Special items monitored are: patient enrollment, completeness, exactness and plausibility of data entered on the CRFs, verification against source data and occurrence of Adverse Events. At all times, the confidentiality of study documents is maintained. Monitoring is performed by GBG Forschungs GmbH.

It is the responsibility of the investigator to ensure that, the dispensing and return/destruction of study medication on the drug accountabilityforms provided is documentedcorrectly .The investigator should ensure that the investigational product use is documented in such a way as to ensure correct dosage. This documentation should confirm that each subject did receive the product dispensed for him or her and state the identity, including the dosage, of the product received.Drug reconciliation will be verified by a second responsible personat the close-out visit to the site by the study monitor. All discrepancies are accounted for and documented.

The investigator agrees to allow the monitor access to all study materials needed for the monitor to properly review the study progress. The investigator (or deputy) agrees to assist the monitor in resolving any problem that may be detected during the monitoring visit.

15.2 Sponsor's Responsibilities

The GBG Forschungs GmbH as the sponsor

- agrees to provide the investigator with sufficient material and support to permit the investigator to conduct the study according to the agreed protocol.
- reserves the right to request the withdrawal of a patient due to protocol violations, administrative or other reasons.
- reserves the right to terminate the study prematurely due to persistent protocol violations, administrative or other reasons. Should this be necessary, the procedures will be arranged after review and after consultation by both parties to ensure protection of the patients' interests.

15.3 Investigator's Responsibilities

The investigator agrees to conduct the study in accordance with the procedures and requirements laid out in this protocol. In particular, the investigator agrees to conduct the study in accordance with strict ethical principles. Any modification to the agreed protocol must be approved in writing by both GBG Forschungs GmbH as the sponsor and, if appropriate, the ethics committee(s) approving the original protocol before any modifications are put into effect.

On receipt of study medication, the investigator (or deputy) will conduct an inventory of the supplies and complete a supplies receipt. The investigator will retain a copy of this receipt at the site and return the original receipt to the study monitor.

It is the responsibility of the investigator to complete the CRFs for each patient in the study, and when a patient completes the study, the investigator must (electronically) sign all CRFs.

In addition to the CRFs, the investigator will maintain adequate records that fully document the progress of the study. The investigator has to state that the patient has taken part in a study and record the study number in the patient's medical records. The exact dates of the beginning and the end of treatment should be given as well.

Copies of these study records (and all study-related documents) shall be kept by the investigator for the maximum period of time permitted by the hospital, institution or private practice. All documentation and materials provided by GBG Forschungs GmbH for this study are to be retained in a secure place and treated as confidential material.

The investigator has the right to request termination of the study for administrative or other reasons. Should this be necessary and agreed upon, the procedures will be arranged after review and after consultation by both parties, to ensure protection of the patients' interests.

By signing this document the investigator indicates that he/she has read the protocol, fully understands the requirements and agrees to abide by all protocol requirements.

Further obligations of the investigator are agreed on in the investigator's contract with the GBG Forschungs GmbH as the sponsor.

15.4 Patient Informed Consent

Prior to the beginning of specific protocol procedures, the patient is informed about the nature of the study drug and is given pertinent information as to the intended purpose, possible benefits, and possible adverse experiences. The procedures and possible hazards to which the patient will be exposed are explained. Patient insurance for the compensation of patients for possible study-related injury is provided by the GBG Forschungs GmbH as the sponsor according to local law.

An approved informed consent statement will then be read and signed by the patient, and, if required, a witness, and the investigator. The patient will be provided with a copy of the signed informed consent statement. The patient may withdraw from the study at any time without prejudicing future medical treatment. Verification of a signed informed consent statement will be noted on the patient's study Case Report Form.

Patients are informed that pseudonymised data from their case may be stored electronically and that such data will not be revealed to any unauthorised third party. Data will be reviewed by the monitor, an independent auditor and possibly by representatives of regulatory authorities and/or ethics committees. The terms of the local data protection legislation will be applied as appropriate.

The patient information sheet and a sample informed consent form are provided in Appendix 2.

15.5 Confidential Follow-up

The investigator will be responsible for retaining sufficient information about each patient (e.g. informed consent form, name, address, phone number, and identity in the study) so that regulatory agencies or the GBG Forschungs GmbH as the sponsor may access this information should the need to do so arise. These records should be retained in a confidential manner for as long as legally mandated according to local requirements.

15.6 Ethics and Regulatory Considerations

The study described in this protocol is conducted in compliance with the ICH guideline for Good Clinical Practice and applicable regulations in all aspects of preparation, monitoring, reporting, auditing, and archiving.

The final approved protocol and the informed consent statement is reviewed by a properly constituted Ethics Committee (EC) / Institutional Review Board (IRB). The EC/IRB decision concerning the conduct of the study is made in writing to the investigator.

The investigator agrees to make required progress reports to the EC/IRB, as well as report any serious adverse reaction (SAR) and suspected unexpected serious adverse reaction (SUSAR). The investigator also informs the EC/IRB of reports of serious adverse reactions (provided to him/her by the GBG Forschungs GmbH) in other clinical studies conducted with the study drug if deemed necessary by the GBG Forschungs GmbH as the sponsor. The EC/IRB must be informed by the sponsor of the termination of the study.

The sponsor is responsible for all communications with and seeking necessary approvals from the competent regulatory authorities of the study.

15.7 Declaration of Helsinki

This study is to be performed in accordance with the Declaration of Helsinki (Sommerset West, 2008), as described in Appendix 1.

15.8 Modification of the Protocol

Any modifications to the protocol which may impact on the conduct of the study, on the potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects, will require a formal amendment to the protocol. Such an amendment will be agreed upon by the Protocol Board, and approved by the EC/IRB prior to implementation, and reported to the health authorities in accordance with local regulations.

Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by the Protocol Boardand will be documented in a memorandum. The EC/IRB may be notified of administrative changes at the discretion of the investigator.

15.9 Study Documents

All information concerning the study drug and trial conduction, such as scientific data and material not previously published are considered confidential and shall remain the sole property of GBG Forschungs GmbH.

The investigator agrees to use the information provided for the conduct of this study only and to use it for no other purposes unless she/he obtains the written consent of the GBG Forschungs GmbH as the sponsor.

15.10 Case Report Forms

All key study information must be recorded in the patient's hospital notes. Study procedures will be fully online documented on the electronic CRFs provided through the GBG own EDC-System (electronic data capture) MedCODES. Before submission of the CRF data, the investigator will be prompted with an interface to enter his/her login data to sign the document electronically according to the FDA regulatory known as "21 CRF Part 11". The investigator will add the study number, patient number and medical interpretation of results to the relevant laboratory reports, and will sign and date such reports.

The CRFs, as well as the protocol, are confidential. The CRFs remain the property of the GBG Forschungs GmbH at all times. On the CRFs, patients should be identified by their patient number.

Patients' data entries may only be made by the persons registered on the form "Delegation of responsibilities and signature list of investigators and medical staff".

For details concerning the CRF submission process, please refer to the application manual and electronic training material.

15.11 GCP Documents

The following documents are collected from the investigator's site:

- Signed Investigator's Agreement,
- Curricula vitae of all investigators and medical staff,
- Name and address of the laboratories,
- List of laboratory reference ranges and a quality certificate,
- Form "Delegation of responsibilities and signature list of investigators and medical staff",

- Any other relevant GCP documents.

15.12 Archiving

After completing the study, the GBG Forschungs GmbH will retain all study documents for at least ten years after the completion of the study.

The investigator shall arrange for the retention of the patient identification codes, patient files and other source data until at least ten years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least two years have elapsed since the formal discontinuation of the clinical development of the product. These documents need to be retained for a longer period of time if required by the competent authorities or by agreement with the GBG Forschungs GmbH as the sponsor.

Patient files and other source data shall be kept for the maximum period of time permitted by the hospital, institution or private practice.

After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the sponsor or their representatives. Essential documents must be retained for ten years after completion of the study. The investigator will appoint individuals responsible for the storage of essential documents and access to the documents will be restricted to those people. Any alterations to essential documents must be traceable. The investigator must contact the sponsor before destroying any study-related documentation.

15.13 Use of Information and Publication

To allow for the use of the information derived from this clinical study and to ensure compliance to current regulations, the investigator is obliged to provide the sponsor with complete test results and all data obtained in this study. This information is only made available to physicians and to the competent authorities, unless the sponsor is under legal obligation to pass it on a third party. The final statistical trial report will be prepared by the responsible biostatistician and the final medical report by the coordinating investigator and the sponsor.

The final study report will be a publication in a peer-reviewed journal under the responsibility of the Protocol Board.

No publication of the study will be released without approval of the Protocol Board. The Protocol Board will review the manuscript to prevent forfeiture of patent rights to data not in the public domain. The authorship list will be agreed upon by the investigators prior to publication. The names on the author list will be mentioned according to the participation in the design of the protocol as well as according to the input of the number of eligible and

evaluable patients accrued by the investigators at each site. Interim abstracts will be presented according to the statistical plan and in agreement with the Protocol Board.

The data are owned by GBG. However, GBG are only allowed to use these for any purposes after approval by the principal investigator.

15.14 Finance and Insurance

Details on finance and insurance will be outlined in a separate agreement between the investigator and the sponsor.

16. SUBSTUDIES

16.1 Pharmacogenetic Substudy

TITLE OF STUDY	Genetic Markers from peripheral blood to predict tumor biology, treatment response and prognosis
CHAIRS	Peter A. Fasching, Lothar Häberle
SUMMARY	Over the decade "pharmacogenetics", the study of the role of inheritance in drug response phenotypes, has evolved into "pharmacogenomics" – with a steady migration from a focus on single genes, often genes that encode proteins involved in pharmacokinetics – to the group of genes encoding all of the proteins in "pathways" that include both pharmacokinetic (PK) and pharmacodynamic (PD) variation, to the recent incorporation of genome-wide techniques such as genome-wide association (GWA) studies (Weinshilboum, R. M. and L. Wang Annu Rev Genomics Hum Genet 2006). In this context pharmacogenetics was not only able to provide with genetic markers, that could predict the prognosis of breast cancer patients (Azzato EM et al, JNCI 2010, Fagerholm R et al, Nat. Genetics 2008, Schroth W et al. JAMA 2009), but also the toxicity of treatments such as aromatase inhibitors (Ingle JN, J Clin Oncol 2010) Furthermore there is evidence, that given a specific germline genetic pattern within an individual, that the tumor, which will develop in this organism has distinct molecular patterns (Garcia-Closas M. et al. PLoS Genet 2008, Reeves GK etal, JAMA 2010). Both, the direct involvement of genetic markers in the metabolism and the pharmacokinetic of a drug, and the influence of the inherited genetic trait on the molecular profile of the tumor could have an influence on an individual's prognosis. Aim of this study is therefore to perform genetic association for pharmacogenetic studies • associate the germline genotype of the patient with the treatment response in both randomization arms • associate the germline genotype of the patient with the long term prognosis of the patients in both randomization arms • associate the germline genotype of the patient with the

	molecular profile of the tumors • associate the germline genotype of the patient with breast cancer risk, in an exploratory setting according to molecular profile of the tumor
STUDY TYPE	Prospective, multicentre, observational substudy
OBJECTIVE	Primary objective: • To associate the germline genotype of the patient with the treatment response in both randomization arms. Secondary endpoints: • To associate the germline genotype of the patient with the long term prognosis of the patients in both randomization arms. • To associate the germline genotype of the patient with the molecular profile of the tumors.
INCLUSION CRITERIA	All patients eligible for the GeparX study having collected a whole blood sample (8-10 ml EDTA or CPDA).
ETHICAL CONSIDERATIONS	All patients will have given informed consent to provide a prespecified amount of extra blood before entering the GeparX study with the informed consent form. Participation on the clinical trial is still possible if a patient does not agree to provide extra blood samples. Results on pharmacogenomics tests will only linked to clinical data after irreversible anonymisation of the clinical data. Patients will not be informed about the laboratory results due to their experimental character.
STATISTICAL CONSIDERATIONS (Dr. Lothar Häberle, Statistician)	Power calculations are based on the study population of 950 with an expected drop-out rate of 15%, resulting in a total of 807 patients. These patients are assumed to have a pCR rate in about 55% of the cases. Power calculations are based on assumed 1,000,000 analyzed genotypes and are based on various scenarios with several minor allele frequencies (MAF) and relative risks (RR) per allele for patients with pCR compared to patients without pCR. Power analyses are performed for the Cochran-Armitage test for trend comparing patients with and without pCR for every SNP (ordinal; 0,1,2 minor alleles). Further assumptions concerning the variability don't have to be made. In order to reach a genome-wide significance level of α=0.05, a p-value lower than 5*10-08, is required (Bonferroni adjustment).

<table>
<tr><td></td><td>Power calculation is performed according to published algorithms Slager and Schaid (2001): Slager, S.L. and Schaid DJ., Case-control studies of genetic markers: power and sample size approximations for Armitage's test for trend, Huma Hered 2001; 52: 149 - 153].
The following table shows the power for several MAF and RR
<table>
<tr><td></td><td>RR</td><td>1.3</td><td>1.4</td><td>1.5</td><td>1.6</td><td>1.7</td></tr>
<tr><td>MAF</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0.10</td><td></td><td>0.01</td><td>0.09</td><td>0.36</td><td>0.72</td><td>0.94</td></tr>
<tr><td>0.15</td><td></td><td>0.04</td><td>0.27</td><td>0.69</td><td>0.94</td><td>1.00</td></tr>
<tr><td>0.20</td><td></td><td>0.09</td><td>0.45</td><td>0.86</td><td>0.99</td><td>1.00</td></tr>
<tr><td>0.25</td><td></td><td>0.14</td><td>0.59</td><td>0.93</td><td>1.00</td><td>1.00</td></tr>
<tr><td>0.30</td><td></td><td>0.18</td><td>0.68</td><td>0.96</td><td>1.00</td><td>1.00</td></tr>
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</td></tr>
<tr><td>TRIAL DURATION</td><td>similar to the GeparX Study.</td></tr>
<tr><td>PARTICIPATING CENTERS</td><td>All sites participating in the GeparX study</td></tr>
</table>

16.2 DTC Substudy

Title of study	Influence of denosumab on disseminated tumor cells (DTC) in the bone marrow of breast cancer patients with neoadjuvant treatment
Chairs	Wimberger P, Kuhlmann JD: Department of Gynecology and Obstetrics, Technical University of Dresden, Germany
Background	Recent advances have shown that hematogenic dissemination of tumor cells is an early event in breast cancer patients. Already at primary diagnosis, up to 30% of the patients are positive for the presence of disseminated tumor cells (DTC) in the bone marrow (BM) in the adjuvant setting (Braun, Vogl et al. 2005, Domschke, Diel et al. 2013). It has been shown that the presence of DTC significantly associates with reduced disease free survival (DFS) and overall survival (OS) and, particularly, the persistence of DTC after adjuvant chemotherapy has also been ascertained as an independent predictor for a poor DFS, for cancer-specific survival and OS (Braun, Vogl et al. 2005, Janni, Vogl et al. 2011).

	Moreover, it has been demonstrated that bisphosphonates critically interfere with the bone metabolism by inhibiting osteoclast mediated bone-resorption (Fleisch 1989, Hortobagyi, Theriault et al. 1996, Rodan and Fleisch 1996). In this context, a clinical study has shown that an oral bisphosphonate therapy (clodronic acid, clodronate) for two years, reduces the incidence and number of new bone and visceral metastases in DTC bearing breast cancer patients and improves metastasis-free survival and OS (Diel, Solomayer et al. 1998). These findings were recently validated in an unpublished study of Kasimir-Bauer et al. at the Department of Gynecology and Obstetrics, University Hospital of Essen, Germany. In a cohort of 398 breast cancer patients, prognosis for DTC-positive patients, treated with clodronate, was comparable to the prognosis of DTC-negative patients. Moreover, in apparently disease-free breast cancer patients with persisting DTC, detected 2-10 years after primary diagnosis, oral ibandronate treatment for six months resulted in no further detection of DTC in most of the patients (82%) (Hoffmann, Aktas et al. 2011). However, in patients with remaining DTC-positivity, a treatment with ibandronate for further six months, effectuated complete DTC-negativity in this cohort (Hoffmann, Aktas et al. 2011). RANK-ligands (RANKL) are proteins belonging to the tumor necrosis factor family and are essential for the differentiation of osteoclasts. Similarly to the functional mechanism of bisphosphonates, denosumab, a monoclonal IgG2-anti-RANKL-antibody, likewise interferes with osteoclastic function in the bone. Denosumab inhibits osteoclastic differentiation by binding to RANKL, thereby preventing the interaction between RANKL and its corresponding RANK-receptor (Casas, Llombart et al. 2013). In a currently ongoing clinical trial (D-CARE, NCT01077154), studying patients with high risk early breast cancer, the effect of denosumab treatment for one year on the patient's bone metastasis-free survival, DFS and OS is going to be investigated. However, the effect of denosumab on DTC-positivity in the BM is not going to be addressed in this clinical trial. Therefore, objective of this GeparX linked translational substudy is, whether the application of denosumab, in terms of an add-on neoadjuvant treatment, influences the DTC-status of breast cancer patients in a short term interval of 24 weeks.

Study type	Prospective, multi-center, translational substudy
Objectives	*Primary endpoint of this translational substudy:* Does the application of denosumab in terms of an add-on neoadjuvant treatment eradicate DTCs in the BM of breast cancer patients? *Secondary endpoint of this translational substudy:* Does a potential eradication of DTC by add-on neoadjuvant denosumab treatment influence the rate of pCR?
Inclusion criteria	All patients eligible for the GeparX study having collected a bone marrow aspirate after randomization and before neoadjuvant treatment.
Ethical Considerations	All patients will have given informed consent for bilateral BM aspiration and DTC-testing.
Interventions	BM samples will be collected at baseline (before the beginning of neoadjuvant chemotherapy). Subsequently, patients in both arms with confirmed DTC-positivity at baseline will be subjected to a single follow-up bone marrow aspiration within surgery. For DTC-analysis, bone marrow aspirates, bilaterally aspirated from the iliac crests, with approx. 10 ml per site will be collected. For each site, please draw up 6-7 ml aspirate in a syringe with 1 ml sodium heparine. Then draw up exactly 2 cm air and close the syringe. Invert the several times and ship the samples directly to the laboratory at the Department of Gynecology and Obstetrics, Technical University of Dresden. For DTC analysis the samples will be subjected to density gradient centrifugation (Ficoll, density 1.077 g/ml). Subsequently, after cell-separation, the mononuclear cell (MNC) fraction will be isolated from the centrifuged sample and will be spun onto a total of six glass slides with 1.5×10^6 MNC per slide. Subsequently, the MNC-fraction will be analyzed by immunocytochemistry using the pan-cytokeratin (CK) antibody A45-B/B3. Evaluation of DTC will be performed with the ARIOL-system (Applied Imaging) according to the ISHAGE evaluation criteria. The samples will be stored at the laboratory at the Department of Gynecology and Obstetrics, Technical University of Dresden. A patient will be considered DTC-positive, if at least one CK-positive cell is detectable in one of the two BM aspirates analyzed. In parallel, serum RANKL level will be analyzed in all patients at baseline and at the time point of follow-up BM aspiration by the RANKL soluble

	ELISA kit (Enzo Life Science, Inc)
Sample size	All study patients are supposed to be subjected to DTC-analysis. Given the expected frequency of DTC-positivity (roughly 40%), we expect approx. 310 patients eligible for additional follow-up aspiration, resulting in approx. 600 DTC analyses in total.
Trial duration	Similar to the GeparX study.
Participating centers	All planned centers are planned to be involved into this translational substudy.

16.3 Substudy on urinary miRNA sampling (UMS)

Title of study	**Urinary miRNA sampling (UMS) substudy**
Chairs	Stickeler E: Department of Gynecology and Obstetrics, University Hospital RWTH Aachen, Germany
Summary	MicroRNAs (miRNAs) are important regulators of gene expression. Aberrant expression profiles of miRNAs with subsequent functional consequences on target gene regulation could already be associated with breast cancer. MicroRNAs represent interesting biomarker candidates since they are robust and easy accessible biomolecules. They are can be easily detected in body fluids, e.g. blood derivates and urine. The implementation of distinct miRNA pattern in urine as a liquid biomarker detection represents a novel approach in clinical breast cancer management with implications for early cancer detection, intrinsic subtype characterization, prognostication, treatment response prediction as well as treatment monitoring.
Background	Over the past decade, the pivotal regulatory impact of microRNAs (miRNAs) on gene expression became recognized and is currently explored in a wide number of translational studies. The number of identified miRNA is growing and currently approximately 2000 diverse miRNA are known. Aberrant expression profiles of miRNAs with subsequent functional consequences on target gene regulation in physiological and pathological pathways could already be set in clear association with breast cancer. There is also growing evidence for a highly auspicious potential of specific miRNAs pattern to serve as biomarkers in this disease. MicroRNAs represent interesting biomarker candidates since they are robust and easy accessible biomolecules, which can be isolated from

	various organic matrices. The application of microRNAs profiling as a novel potential biomarker-based tool to complete and improve classical detection procedures offers a range of advantages. In detail, microRNAs as small and stable biomolecules can be easily detected in body fluids, e.g. blood derivates and even urine. Furthermore, they are characterized by highly specific and sensitive expression alterations that account for different pathological states, even at very early stages of cancer progression. The implementation of distinct miRNA pattern in urine as a liquid biomarker detection represents a novel approach in clinical breast cancer management with implications for early cancer detection, intrinsic subtype characterization, prognostication, treatment response prediction as well as treatment monitoring.
Study type	Prospective, non-randomized, open, diagnostic substudy
Objectives	Primary objective: • To evaluate specific microRNA signatures in urine specimen as an innovative tool for subtype-specific diagnosis of breast cancer (Her2 pos. vs. TNBC). Secondary objectives: • To evaluate specific urinary miRNA pattern alterations as a tool for the prediction of pCR in the neoadjuvant subtype specific treatment of primary BC. • To evaluate specific urinary miRNA pattern as a tool for the prognostication for clinical outcome (DFS, OS).
Inclusion criteria	All patients eligible for the GeparX study having collected urine samples.
Ethical Considerations	All patients will have given informed consent for urine sampling.
Interventions	Urine samples will be collected at baseline (before the beginning of neoadjuvant chemotherapy), after 6 weeks of chemotherapy and after 12 weeks of chemotherapy. Please transfer 10 ml of the midstream urine sample into the Urine-Monovette (Sarstedt). The urine samples should be frozen at -20°C to -80° C. The frozen samples will be stored until picked up by a courier service.
Sample size	All study patients are supposed to be subjected to UMS substudy.
Trial duration	Similar to the GeparX study.
Participating centers	All planned centers are planned to be involved into this translational

	substudy.

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16.4 Investigating ABP 980 in combination with Pertuzumab as part of the neoadjuvant therapy in HER2+ primary breast cancer – a Substudy of the GeparX trial

Sub Study Title	**Investigating ABP 980 in combination with Pertuzumab as part of the neoadjuvant therapy in HER2+ primary breast cancer – a Substudy of the GeparX trial**
Study Code	GBG 88
EudraCT Number	2015-001755-72
Sponsor	GBG Forschungs GmbH, Neu-Isenburg
Development Phase	Non-randomized cohort study within the randomized phase IIB GeparX study
Rationale	Monoclonal Antibodies are complex proteins with high molecular weight (MW). Biosimilars have the potential to significantly improve access to expensive agents. Biosimilarity is defined as follows: The biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components. There are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency. ABP 980, a candidate as a biosimilar to trastuzumab, has been shown to be as effective as the reference product Herceptin®, in terms of pCR achievement in early breast cancer (Lilac, NCT01901146). This was the first time this was demonstrated for a biosimilar for trastuzumab in the neoadjuvant setting. ABP 980 has similar binding to $Fc_{\gamma}RIIIa$ as Herceptin®. In vivo and in vitro data confirmed similar function. In a neoadjuvant study randomizing 725 patients, ABP 980 was compared to Herceptin as part of a standard neoadjuvant EC-Paclitaxel regimen and showed comparable pCR rates. Patients receiving Herceptin were randomized to continue after surgery with Herceptin or transitioned to ABP 980. All other short and long term parameters assessed were also not significantly different. Currently the dual blockade of trastuzumab and pertuzumab in combination with chemotherapy is indicated as neoadjuvant therapy in HER2+ primary breast cancer. This substudy will evaluate the safety and efficacy of ABP 980 in combination with pertuzumab as neoadjuvant therapy in the

	treatment of HER2+ primary breast cancer. All patients with HER2+ disease will receive Pertuzumab in addition to ABP 980 throughout the trial.
Primary Objectives of the substudy (Exploratory to Main study)	• To assess the pathological complete response (pCR= ypT0 ypN0) rate of neoadjuvant treatment with ABP 980 and pertuzumab in the overall HER2+ cohort and compare with the results obtained in GeparSepto study. • To compare the pathological complete response (pCR= ypT0 ypN0) rate of nPac 125mg/m² weekly→EC or nPac 125mg/m² day 1,8 q22 →EC plus anti-HER2 treatment (i.e. ABP 980/ pertuzumab in case of positive HER2-status) in patients with early breast cancer.
Secondary Objectives of the substudy (exploratory to main study)	• To assess the pCR rates in HER2+ patients treated with ABP 980 in subgroups according to HR status. • To assess the pCR rate in subgroups by denosumab. • To determine the pCR rates in the overall HER2+ cohort of ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0 for both randomizations. • To determine the response rates on the HER2+ cohort of the breast tumor and axillary nodes based on physical examination and imaging tests (sonography, mammography, or MRI) after treatment in both arms for each randomization. • To determine the breast conservation rate in the HER2+ cohort. • To assess the toxicity and compliance for the HER2+ cohort treated with ABP 980 and by systemic therapy (nabPaclitaxel 125mg/m² continuously vs. 2/3; EC, Denosumab yes vs. no). • To specifically address the incidence of diarrhea and cardiovascular events. • To assess the toxicity with EC and ABP 980/pertuzumab. • To determine loco-regional invasive recurrence free survival (LRRFS), distant-disease-free survival (DDFS), invasive disease-free survival (IDFS), EFS (event free survival) and overall survival (OS) for all HER2+ patient treated with ABP 980/pertuzumab.
Study Design and Treatment	The substudy is a cohort study investigating open label non randomized use of ABP 980 in combination with pertuzumab for subjects that are HER2 positive. In all study arms, treatment will be given until surgery, disease

	progression, unacceptable toxicity, withdrawal of consent of the patient, or termination by the Sponsor.
Eligibility Criteria	Patients will be eligible for the substudy if they comply with the criteria for the main study participation and have a centrally confirmed HER2+ tumor.
Investigational product and formulation	ABP 980 Loading dose: 8mg/kg, thereafter 6 mg/kg, every 3 weeks simultaneously to all chemotherapy cycles. After surgery all patients will change to either the reference product Herceptin or to another approved biosimilar trastuzumab per investigator`s decision/local standard.
Primary endpoint of substudy	**Primary efficacy endpoint:** **Pathological complete response of breast and lymph nodes (ypT0 ypN0; primary endpoint), as defined in the main protocol:** No microscopic evidence of residual invasive or non-invasive viable tumor cells in all resected specimens of the breast and axilla. Pathological response will be assessed considering all removed breast and lymphatic tissues from all surgeries. Patients with negative sentinel node biopsy prior to treatment start and no axilla surgery after neoadjuvant chemotherapy will be counted as pCR if no invasive and non-invasive residual tumor is detected in the removed breast tissue. Patients with histologically/cytologically positive nodes prior to treatment start and no axilla surgery after chemotherapy will be counted as no pCR (preferably axillary dissection instead of sentinel node biopsy is strongly recommended in this situation). Patients with positive sentinel node biopsy prior to treatment start and no invasive and non-invasive residual tumor detected in the removed breast tissue and lymph nodes after chemotherapy will be counted as pCR.
Secondary endpoints of substudy	**Secondary short-time efficacy endpoints, as defined in the main protocol** ypT0/Tis ypN0 is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast and axilla; in case of sentinel node biopsy prior to treatment start, the axillary lymph nodes will be evaluated as described for the primary endpoint. ypT0 ypN0/+ is defined as no microscopic evidence of residual

	invasive or non-invasieve viable tumor cells in all resected specimens of the breast; ypT0/Tis ypN0/+ is defined as no microscopic evidence of residual invasive viable tumor cells in all resected specimens of the breast; patients with a sentinel node biopsy prior to treatment start will be evaluated for $ypT_{(any)}$ ypN0 similarly to the description given for the primary endpoint. Clinical (c) and imaging (i) response will be assessed every 2nd cycle and before surgery by physical examination and imaging tests. Sonography is the preferred examination, however, if sonography appears not to provide valid results or is not performed, MRI or mammography will be considered with decreasing priority. The same imaging method should be considered for the measurement before, during and after treatment. For defined categories of efficacy (complete, partial, stable, or progression), the proportion of patients with success will be determined and appropriate confidence intervals will be calculated. The response categories of the breast are: • **Complete response (CR):** complete disappearance of all tumor signs in the breast as assessed by all available imaging test and palpation. The response of the axillary nodes is not to be considered. • **Partial response (PR):** reduction in the product of the two largest perpendicular diameters of the primary tumor size by 50% or more assessed by imaging test or palpation. In patients with multifocal or multicentric disease, the lesion with the largest diameters should be chosen for follow-up. The response of the axillary nodes is not to be considered. • **Stable disease (NC):** no significant change in tumor size during treatment which means an estimated reduction of the tumor area by less than 50%, or an estimated increase in the size of the tumor area lesions of less than 25%. • **Progressive disease (PD):** development of new, previously undetected lesions, or an estimated increase in the size of pre-existing lesions by 25% or more after at least two cycles of therapy. Breast conservation is defined as tumorectomy, segmentectomy or quadrantectomy as a most radical surgery. Patients in whom success cannot be determined (e.g. patients in whom histology is not evaluable) will be included in the denominator,

	i.e. these patients will affect the success rate in the same way as treatment failures. **LRRFS, DDFS, IDFS, EFS and OS** are defined as the time period between randomisation and first event and will be analyzed after the end of the study by referring to data from GBG patient's registry. Progressions during neoadjuvant treatment are not considered as events unless the patient is not amenable for surgery. **Tolerability and Safety:** Descriptive statistics for the 4 treatments (+anti-HER2-treatment) will be given on the number of patients whose treatment had to be reduced, delayed or permanently stopped. The reason for termination includes aspects of efficacy (e.g. termination due to tumor progression), safety (e.g. termination due to adverse events) and compliance (e.g. termination due to patient's withdrawal of consent). Reasons for premature termination will be categorized according to the main reason and will be presented in frequency tables. Safety by toxicity grades are defined by the NCI-CTCAE version 4.0. AEs of special interest are: Cardiac failure, infusion reactions, pulmonary toxicity, hypersensitivity, infections and infestations.
Statistical Methods **Primary endpoint**	An 'intent-to-treat' (ITT) analysis will be conducted for all patients randomized in the study. In addition, a 'per-protocol' analysis will be conducted; the detailed definition of the per-protocol analysis set will be given in the statistical analysis plan. All HER2+ positive patients will be analysed irrespective of the anti-HER2 treatment according to the general ITT principles. Primary and secondary objectives for this substudy will be assessed in all patients who have received at least one dose of ABP 980. The pCR rates with a 95% CI will be reported and compared between chemotherapy treatment arms using the continuity corrected χ^2-test.
Statistical Methods Sample size	The sample size calculation is based on the primary endpoint of the main study: All patients with HER2+ disease enrolled into the study will receive ABP 980 in addition to pertuzumab and backbone chemotherapy. It is planned to recruit approximately 150 subjects into this substudy.
Statistical Methods Secondary Endpoints	**Secondary short-time efficacy endpoints** (ypT0/Tis ypN0; ypT0 ypN0/+; ypT0/Tis ypN0/+; $ypT_{(any)}$ ypN0, response by physical examination, imaging response, breast conservation) will also be summarized as rates in each treatment group, two-sided 95%

	confidence intervals will be calculated according to Pearson and Clopper, and the continuity corrected χ^2-test will be used to compare between chemotherapy treatment arms. Logistic regressions will be used to adjust odds ratios for minimization factors for primary endpoint and for ypT0/is ypN0. **For LRRFS, DDFS, IDFS, EFS and OS** curves will be estimated using the Kaplan-Meier method, based on the ITT population. 3 year and 5 year survival (and 95%CIs) will be estimated. Univariate and multivariate Cox-proportional hazards model will be used to adjust hazard ratios for minimization factors. Safety and compliance for HER2+ substudy will be reported descriptively in treatment arms. More details will be in the SAP and follow the general safety assessment of the main study.
Study duration	Similar to the main study (until Q IV 2018).
Follow-up Period	As no study specific treatment or investigation is planned after 90 days after surgery, follow up is not part of this study. However, information on subsequent cancer specific treatments and the health status of the patients is collected either based on yearly chart reviews at the sites or based on information deriving from the GBG registry of previous study participants. Information on date and site of recurrences, date and cause of deaths as well as secondary malignancies and long-term side effects will be collected.

17. INVESTIGATOR'S AGREEMENT

Ich habe den folgenden Prüfplan gelesen:

Investigating Denosumab as an add-on to neoadjuvant chemotherapy in RANK/L-positive or RANK/L-negative primary breast cancer and two different nab-Paclitaxel schedules in a 2x2 factorial design (GeparX)

und versichere, dass er alle notwendigen Angaben zur Durchführung der Studie enthält. Ich werde die Studie wie hierin vorgesehen durchführen. Ich werde allen an der Durchführung der Studie beteiligten Ärzten Kopien des Prüfplans und der Arzneimittel-informationen zur Verfügung stellen. Ich versichere, dass eine ordnungsgemäße Dokumentation aller mit der Studie in Zusammenhang stehenden Daten erfolgt.

Sponsor

Datum: 11.04.2019 Unterschrift: _____

Prof. Dr. Sibylle Loibl

GBG Forschungs GmbH

Prüfärztin / Prüfarzt

Datum: _ _ . _ _ . 201 _ Unterschrift: _____

Stempel oder Name in Blockschrift:

18. APPENDICES

18.1 Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53th WMA General Assembly, Washington 2002

55th WMA General Assembly, Tokyo 2004

59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best currentinterventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be

independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of

interest, institutional affiliations of the researcher, the anticipated benefits and potentialrisks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient’s decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

18.2 NCI Common Terminology Criteria

Please use the pdf from the following website:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

18.3 BREAST SURGERY GUIDELINE

Prof. Dr. Thorsten Kühn, Klinikum Esslingen

In a neoadjuvant trial all patients should be treated surgically after completion of chemotherapy, because otherwise neither the primary objective (pCR) nor the secondary objective (rate of breast conservations) can be evaluated properly.

This surgical protocol is in line with the recommendations for standardized pathological characterization of residual disease for neoadjuvant clinical trials of breast cancer by the BIG-NABCG collaboration.[86] Bossuyt V et al. Ann Oncol 2015

1. Timing of surgery

Definitive surgery should be performed 1-14 days after completion (after day 21) of the last chemotherapy cycle. In any case full hematologic recovery to normal limit is recommended. This is the case after having completed all (8 or 12) planned chemotherapy cycles or if no further chemotherapy can be given for reasons related to tumor progression and / or intolerable toxicity according to the protocol. Please make sure that at least 28 days have passed between the last infusion of bevacizumab and the surgery to avoid increased bleeding risk and / or wound-healing complications.

In some trials the primary endpoint might be assessed after one part of the sequential therapy. Another algorithm might be followed then as describe in the respective protocol.

2. Localizing tumor in the breast

Prior to initiating chemotherapy, the breast surgeon should localize the breast tumor exactly. Clip placement into the tumor bed is strongly recommended as a routine procedure in most patients especially in women with a good response to chemotherapy after the first few cycles. The clip can be applied either at the time of diagnosis or within the first cycles of chemotherapy.

Markings on the skin can be performed in addition. With the patient standing or lying down and stretching the arm in 90° to the side, this can be done by measuring the distance from the jugulum to the nipple and taking a rectangle to determine the distance from the jugulum-nipple line to the middle of the palpable mass (see figure 4)[87]

After performing the markings, photographs of both breasts should be taken. The patient should be seen by the breast surgeon at least after every two chemotherapy cycles. Thus the excisional area can be best identified, even if the tumor has become non-palpable.

Prior to surgery stereotactic wire localization of the clip should be performed in cases of a clinically complete response without a palpable lesion.

If a clinically node-positive lymph node is histologically confirmed to be positive by FNA or core needle biopsy, a clip should be placed into the involved lymph node.

3. Extent of the excision, margins

Surgical resection volume is based on preoperative imaging (after completion of chemotherapy). All detectable residual disease should be removed with clear margins. In case of complete radiologic response, the center of the tumor bed should be removed, including any radiologic clips. Specimen radiography is mandatory in patients with non palpable lesions and a clip placement. Orientation of the specimen by the surgeon is imperative. Marking of the excisional cavity to allow targeted boost irradiation is recommended.

4. Breast conservation

Breast surgery after primary chemotherapy should be performed according to the guidelines for breast surgery without prior systemic therapy. Breast conservation techniques include lumpectomy, wide excision (segmental resection) and quadrantectomy.[88] For medium and enlarged breasts dermoglandular flaps can be used to fill the excision site.

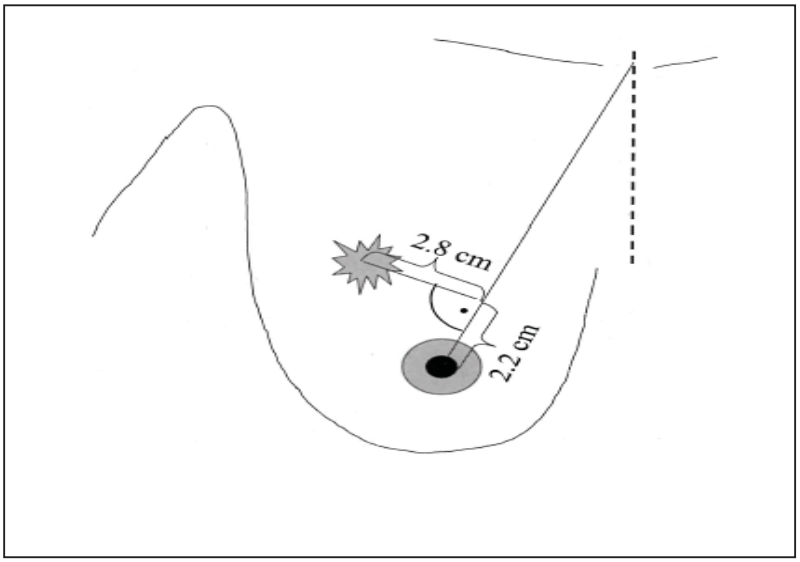
5. Mastectomy and breast reconstruction

If mastectomy is indicated primary or secondary reconstruction can be performed according to current guidelines (hhtp://www.ago-online.de). Due to the majority of these women being high risk patients, and therefore radiotherapy might be indicated, this needs to be discussed in the multidisciplinary team.

6. Surgery of the axilla

It is not recommended to perform SLNB prior to chemotherapy in clinically node negative patients, since assessment of nodal response in the axilla is important. The clinical endpoint of the study cannot be assessed, when a SLNB has been performed before chemotherapy and yielded a positive result. Therefore postchemotherapy SLNB is recommended for patients with initially unsuspicious nodes. In case a SNB has still been done and reveals an involved lymph node prior to surgery the patient has to be treated with axillary dissection at the time of definite surgery. Patients who present with suspicious nodes prior to chemotherapy (palpation and/or ultrasound) should undergo core needle biopsy of the node. If the result is positive the patient should undergo axillary dissection after chemotherapy.

Figure 6: Proposal for identifying a palpable mass after complete clinical response. However clip insertion is recommended in addition.



18.4 Histopathologic examination of the removed tissue from breast and axilla

Prof. Dr. C. Denkert, Institut für Pathologie, Charité, Berlin, carsten.denkert@charite.de

Preamble

The core needle biopsies and resected breast tissues are evaluated histopathologically in strict accordance with the current diagnostic standards for breast pathology, for example as summarized in the National S3 Diagnostic Guideline, Therapy and Aftercare for Breast Cancer of the German Cancer Association, the AGO Organkommission Guideline 'Mamma' or in the relevant international guidelines. [89, 90, 91]

For an update on guidelines for evaluation of specimen after neoadjuvant chemotherapy, we recommend the international recommendations published in 2015:

- Provenzano E, Bossuyt V, Viale G, Cameron D, Badve S, Denkert C, MacGrogan G, Penault-Llorca F, Boughey J, Curigliano G, Dixon JM, Esserman L, Fastner G, Kuehn T, Peintinger F, von Minckwitz G, White J, Yang W, Symmans WF. Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group. Mod Pathol. 2015 Sep;28(9):1185-201. doi: 10.1038/modpathol.2015.74. Epub 2015 Jul 24. PubMed PMID: 26205180.
- Bossuyt V, Provenzano E, Symmans WF, Boughey JC, Coles C, Curigliano G, Dixon JM, Esserman LJ, Fastner G, Kuehn T, Peintinger F, von Minckwitz G, White J, Yang W, Badve S, Denkert C, MacGrogan G, Penault-Llorca F, Viale G, Cameron D; Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration. Recommendations for standardized pathological characterization of residual disease for neoadjuvant clinical trials of breast cancer by the BIG-NABCG collaboration. Ann Oncol. 2015 Jul;26(7):1280-91. doi: 10.1093/annonc/mdv161. Epub 2015 May 27. Review. PubMed PMID: 26019189.

It is the task of pathohistological diagnostics to determine the extent and regression grade of the tumor after chemotherapy. Pathomorphologically determined complete remission (pCR) is one of the most important prognosis factors for relapse-free survival after neoadjuvant chemotherapy. Therefore, the pathological results should be collected in a standardized way.

Special features present in the main where there is no or only very little tumor tissue in evidence after therapy. The following section serves as an only very brief presentation of the generally known diagnostic standards. This is followed by a more detailed review of the special features of histological diagnostics in neoadjuvant chemotherapy.

1. Standardized diagnostics

1.1. Core needle biopsy prior to treatment

Core needle biopsies fixed in buffered formalin and then evaluated histopathologically after paraffin embedding. A frozen section should not be used in this diagnostic procedure in order to avoid methodological problems in grading and in immunohistological testing.

The results produced by the core needle biopsy should include:

a histopathological tumor classification according to the WHO (2012).[92]

the Elston-Ellis histological grading system [93]; the mitosis rate should be determined based on the individual field view size.

hormone receptor expression (given as %), HER-2 status (HER-2 score by means of standardized immunohistochemistry and/or in-situ hybridization).

The following material should be sent to the central pathology laboratory of GBG:

- the paraffin block of the diagnostic core needle biopsy prior to treatment
- a copy of the histology report containing the ER, PR and HER2 status (pseudonymized, with study name, patient number and site number)

1.2 Resected tumor tissue

After completion of neoadjuvant chemotherapy, the tumor is resected, with either breast conservation or mastectomy according to the clinical assessment. The following clinical information is necessary for histological diagnostics:

- confirmation that neoadjuvant chemotherapy has been carried out;
- tumor localization within the specimen;
- tumor size as determined clinically prior to treatment.
- Clinical response
- Information about a possible pretherapeutic sentinel node procedure

The current diagnostic standards should be applied to the histopathological preparation. Special features are necessary when determining the extent of the existing residual tumor and tumor regression.

We strongly recommend to include the Residual Cancer Burden (RCB) in each pathology report for improvement of international standardization. This requires simply to add the tumor cellularity, the size of the tumor bed as well as the size of the largest lymph node metastasis to the histopathology report. From these parameters the RCB can be calculated at any time.

The RCB is further explained on these websites:

http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

http://www.pathology.at/pathologinnen/fachlicheinfo/233-rcb-score

The standardized results pertaining to the resected tumor tissue after completion of neoadjuvant chemotherapy should include:

- a histopathological tumor classification according to the WHO (2012).[92]
- Elston-Ellis[93] histological grading marked 'Condition after chemotherapy'. The mitosis rate should be determined taking due account of the individual field view size.
- Tumor size in two dimensions, where applicable details of multifocality or multicentricity with the size of the residual tumor foci.
- Tumor cellularity, using the standardized evaluation schema developed at the MD Anderson Cancer Center, which is available here: http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3
- Intraductal tumor components: Amount, extent and grading pursuant to WHO.[92]
- R-status with distance from the resection margins in mm, where applicable, data to be given separately for the invasive and intraductal tumor components.
- Hormone receptor status (given as %).
- HER-2 status (HER-2 score).
- Regression grade (see below).[94]
- Lymph vessel invasion, vein invasion.
- Lymph node status, including the size of the largest lymph node metastasis

- yTNM classification.[95]

After conclusion of histological diagnostics, the following material should be sent to the central tumor bank:

- a representative paraffin block of the tumor (If histological examination shows no evidence of residual tumor, a representative block from the former tumor bed with DCIS or inflammatory changes should be sent.)
- a representative paraffin block of one lymph node (if possible with tumor infiltrate, but a negative node will be fine also, if the tumor is nodal negative)
- a copy of the histological report of tumor resection as well as lymph nodes which contains the macroscopic assessement as well as the pathological response (patient name should be crossed out)

Two paraffin blocks will be stored in the central tumor bank of the GBG for translational research project (based on the informed consent of the patient).

2. Special features of the histopathological examination

2.1 Special features applying to section and the macroscopic assessment

Delimitable tumor focus

If a delimitable tumor focus is found, section generally poses no difficulties. At least three tumor blocks are embedded – with larger tumors at least one tumor cross section at the point of maximum diameter. Additionally, tissue from all resection margins and, where applicable, also fibrosed areas adjacent to the tumor are examined.

Weakly delimitated tumor bed

If macroscopically the tumor focus cannot be sharply delimitated, the former tumor bed is examined in order to document the response to preoperative chemotherapy. In addition, the clinical partners should indicate or mark the extent and site / quadrant of the tumor. With this information, the tumor bed is then generally detectable as an irregular fibrosed area. the number of tissue blocks should be based on the pretherapeutic tumor size. Here, too, examination is based on the maximum tumor diameter (as a guide, at least one block per cm of tumor – pre-treatment size) and the distance to all resection margins is documented. Additionally, fibrosed areas adjacent to the tumor bed and in the region of the resection margins suspected of being tumorous should be examined.

No clearly detectable tumor bed

If both macroscopically and on the basis of the clinical information, no obvious tumor bed is detectable, smaller diagnostic excisions should be embedded completely at the discretion of the pathologist.

Where larger amounts of tissue are resected, the first step is exploratory evlauation in the fibrosed areas. Here, too, the extent of the section should be governed by the size of the tumor prior to treatment. As a guide, roughly one block per cm tumor diameter should be. If there is macroscopic evidence of tumor infiltrates, DCIS or inflammatory / regressive changes, a follow up examination should then be made specifically of tissue from this area by extending the section. The histological results should show the total number of blocks examined.

2.2 Regression grade

An overview of typical histological and cytological changes after chemotherapy can be found, for example, in Länger et al.[96] The regression grade is modified using Sinn's method.[94]

- Regression grade 0: no effect
- Regression grade 1: tumor sclerosis with resorptive inflammation and/or cytopathic effects.
- Regression grade 2: extensive tumor sclerosis with only focal / multifocal minimally invasive residual tumor (≤ 0.5 cm).
- Regression grade 3 +: only non-invasive residual tumor in the breast, lymph nodes unaffected
- Regression grade 3 -: only non-invasive residual tumor in the breast, although lymph nodes are affected.
- Regression grade 4: no residual (non-invasive or invasive) tumor in the breast, but affected nodes
- Regression grade 5: no residual (non-invasive or invasive) tumor in the breast and lymph nodes.

In addition we recommend to include the Residual Cancer Burden (RCB), as explained above.

2.3 Special features of regression grades 3 und 4

In this case, histological examination reveals no residual presence of the carcinoma or only evidence of DCIS. If regression grades 3 or 4 are suspected, further tissue should be examined where applicable depending on the macroscopic results so that an adequate histological assessment of the whole former tumor bed can be made. This is because in some cases only very small multifocal residual infiltrates measuring a few millimeters are found after therapy. Also, if the infiltrate is severely inflamed, it can be difficult to distinguish between regressively changed tumor cells and histiocytes. Here, immunohistological examinations should be used to detect or rule out regressively changed residual tumor cells in the region of the sclerotic areas that have changed because of inflammation.

2.4 Assessment of tumor extent / Pseudo-multifocality

Determination of tumor size takes microscopic and macroscopic findings into account. If histological examination reveals several tumor cell islands interspersed with fibrotic zones in a macroscopically clearly identifiable tumor bed, the total macroscopic extent of the tumor and not the largest individual focus should be used for tumor size determination (therapy-induced pseudo-multifocality).[96] Where applicable, the attempt can be made to find immunohistological evidence of individual degeneratively changed tumor cells in the fibrotic areas.

A multifocal tumor should only be diagnosed if there are different tumor foci which, taking due account of the macroscopic findings as well, are not located in a common fibrosed area corresponding to the tumor bed prior to treatment.

2.5 Assessment of the resection margin

If the tumor is only weakly delimitated or discontinuous after treatment, particular caution should be exercised when assessing the resection margins. Where applicable here, discontinuous fibrotic foci should be examined with reference to the resection margin.

2.6 Lymph node examination

When examining lymph nodes, it may be necessary to grade regression separately. Here, too, immunohistological examinations should be carried out, where applicable, to find evidence of small regressively changed portions of residual tumor.

18.5 RADIOTHERAPY AFTER NEOADJUVANT SYSTEMIC TREATMENT

Dr. David Krug, Heidelberg

Introduction

There is Level I evidence supporting the use of neoadjuvant chemotherapy for breast cancer.[97] [98] Although there is no survival benefit related to the preoperative administration of chemotherapy, there are several advantages of this treatment paradigm such as the possibility to monitor treatment response in vivo, the rapid resting of novel drugs or clinical trial designs and higher rates of breast-conserving surgery. Response to neoadjuvant treatment, especially pathologic complete response (pCR) is a very important prognostic factor although its impact differs according to the molecular subtype.[99]

Although the evidence supporting neoadjuvant treatment in breast cancer is compelling, there are no results available from prospective randomized controlled trials addressing the role of radiotherapy in patients who have been treated with neoadjuvant chemotherapy and surgery. Practically all studies on this subject are retrospective, single-institutional reports. Due to this lack of evidence, most professional societies recommend to base the indication for radiotherapy either on the pretherapeutic staging[100] [101] [102] or the worst staging irrespective of its timepoint.[103]

It is very important to keep in mind that patients receiving neoadjuvant treatment are typically a negative selection at high risk of recurrence when compared to the general breast cancer patient population. Historically, neoadjuvant chemotherapy was almost exclusivelyused in those patients with advanced tumor and/or nodal stage, however there is an increasing use not only among those patients[104], but also among early stage-breast cancer patients with high risk-features such as triple negative- or HER2-positive-tumors[105].

This chapter will focus on the treatment of patients with intermediate- and high-risk features. Topics such as partial breast irradiation or omission of adjuvant radiotherapy in patients with low risk-disease will not be addressed.

The reader is kindly referred to national and international radiotherapy guidelines for recommendations regarding treatment planning and target volume definition.[100, 101, 106 107 108]

Breast-conserving surgery

Adjuvant radiotherapy to the operated breast is considered standard after breast-conserving surgery. The EBCTCG-meta-analysis published in 2011 showed a 15% reduction in the recurrence risk and a 4% improvement in breast cancer-mortality by the addition of whole-breast irradiation to breast-conserving surgery.[109] An additional boost dose to the tumor bed further reduces the relative risk of local recurrence by approximately 35% but does not

improve survival[110]. The benefit of the tumor bed boost was highest in patients younger than 50 years with an absolute benefit of 12% compared to only 3% for women older than 60 years. However, most of the included patients in the EORTC-trial had pT1pN0-disease without high risk-features.[110]

Since the advent of these trials, locoregional recurrence rates have drastically declined due to advances in imaging, surgery, systemic therapy and radiotherapy.[111]

Neoadjuvant treatment increases the likelihood of breast-conserving surgery by approximately 30%.[98] When excluding studies which used definitive radiotherapy without surgery, no increased risk of local recurrence was found in a meta-analysis (HR 1.13, p = 0.46). Similarly, there was no significantly increased risk of locoregional recurrence for initial mastectomy candidates who became eligible for breast-conserving surgery after neoadjuvant treatment (HR 1.34, p = 0.21), although patient numbers were small.

Several retrospective reports have analyzed the risk of local recurrence after neoadjuvant chemotherapy and breast-conserving surgery. Chen et al. published their experience on 340 patients treated between 1987 and 2000 at MDACC, mostly with anthracycline-based regimens[112]. Adjuvant radiotherapy was given to the whole breast at a dose of 50 Gy in 25 fractions followed by a boost to the tumor bed of 10 Gy in 5 fractions. The 5-year incidence of local and locoregional recurrence was 5% and 9%, respectively. Both the clinical staging before treatment and the pathological staging after treatment did significantly influence the risk of locoregional recurrence. The same group of researchers developed a prognostic index based on the clinical nodal stage, pathologic tumor size after treatment, tumor regression pattern and lymphovascular invasion. While patients with a score of 0 had 5-year locoregional recurrence-free rates of 97%, patients with a score of 3 had a rate of 58%.[113]

As shown in a retrospective analysis of the neoadjuvant treatment arms of NSABP18/B27[114], pathologic nodal involvement after chemotherapy is propably the most important predictor of locoregional recurrence. While locoregional recurrence rates after a median follow up of 12 years were generally below 10% in patients with breast-conserving surgery and adjuvant radiotherapy to the whole breast, patients with pathologically involved nodes had recurrence rates up to 22%. However, many publications including the NSABP-analysis showed that the risk of locoregional recurrence is also determined by the clinical stage at first diagnosis. [112] [114] [115] [116]

<u>Recommendations:</u>

Whole-breast irradiation is the standard treatment after breast-conserving surgery regardless of the timing of systemic therapy. The typical dose and fractionation would be 50-50.4 Gy in 25-28 sessions. While hypofractionation is a very good option for patients with early stage- and low risk-breast cancer, there is limited evidence to support its use in patients who have

had chemotherapy, let alone in patients with neoadjuvant chemotherapy. Thus, hypofractionated radiotherapy after neoadjuvant chemotherapy should not be performed outside of clinical trials such as the german ARO HYPOSIB-trial (NCT02474641). Since most patients who receive neoadjuvant chemotherapy possess high risk-features either in terms of tumor size or molecular features, the use of a tumor bed boost is usually indicated. In most cases, it is delivered as an additional percutaneous irradiation of 10-16 Gy in 5-8 sessions with photons and/or electrons. The ongoing german randomized controlled trial ARO HYPOSIB-trial compares hypofractionated whole breast-radiation with a simultaneous integrated boost to either normofractionated or hypofractionated radiotherapy of the breast with a sequential boost. Furthermore, there is the possibility to apply the dose as multi-catheter interstitial brachytherapy. Initial reports suggest that intraoperative radiotherapy as a boost is safe and yields high rates of local control. There are no data available on the role of boost irradiation in patients with a pCR of the primary breast tumor.

Post-mastectomy radiotherapy (PMRT)

The EBCTCG meta-analysis on PMRT published in 2014 showed an absolute benefit of about 8% on breast cancer mortality regardless of the number of involved lymph nodes (1-3 vs. 4 or more).[117] However, the trials included in this publication used outdated systemic therapy and radiotherapy techniques. There is an ongoing debate on the role of PMRT in the context of contemporary systemic therapy since several reports have shown low recurrence rates for subgroups such as patients with pT1/2pN1 or pT3pN0-disease.[118] [119]

The most comprehensive prospective analysis on the risk of locoregional recurrence after neoadjuvant chemotherapy and mastectomy is a publication on the neoadjuvant treatment arms of NSABP B18/B27 by Mamounas et al.[114] As defined in the study protocol, the use of PMRT was not allowed. The 10 year-risk of locoregional recurrence was 12.6%. On multivariate analysis, clinical tumor size > 5 cm, clinical lymph node involvement at first diagnosis and pathologically positive lymph nodes after neoadjuvant treatment (when compared to patients with a pCR) were significant predictors of a locoregional recurrence. Patients with pathologically positive lymph nodes had the highest overall risk of recurrence. There were no recurrences for patients with clinically involved lymph nodes who developed a pCR, however this subgroup was very small (n = 32) and paradoxically, the recurrence rate was higher for patients who were clinically node negative and had a pCR.

A meta-analysis published in 2012 summarized the available literature on PMRT.[120] Besides NSABP B18/B27 (no patient received PMRT in these studies), 23 single-institutional (most of them from MDACC), retrospective studies were included in this report. Most of these studies showed that there is a benefit of PMRT, especially for those patients with advanced disease at presentation and/or residual disease after chemotherapy. However, several publications

showed that PMRT improves locoregional control and survival even in subsets of patients with pCR.[116] [121] Other subgroups that have been shown to benefit from PMRT are young patients < 35 years and patients with cT3N0-tumors.[122] [123]

Other publications have suggested that PMRT might not be beneficial in patients with ypN0-status, however those studies included a considerable amount of patients with cN0-staging.[124] [125]

The NSABP B-51/RTOG 1304-trial (NCT01872975) will address the question whether treatment response can be used to guide radiotherapy treatment decisions based on the therapeutic response in patients with clinically positive lymph nodes (cN+, fine needle aspiration or core needle biopsy) who have pathologically negative lymph nodes (ypN0) after standard neoadjuvant systemic treatment. Patients with mastectomy will be randomized to radiotherapy to the chest wall and regional lymph nodes or to no radiotherapy at all.

Recommendations

In the absence of data from prospective randomized controlled trials, it is advised to base the indication for radiotherapy on the worst staging either before or after neoadjuvant chemotherapy.[103] PMRT is strongly recommended in the presence of pathologically involved lymph nodes after chemotherapy. Due to the high prevalence of adverse factors among patients with an indication for neoadjuvant chemotherapy, PMRT is also advised in patients with clinically involved lymph nodes at first diagnosis and those with cT3/4-tumors. PMRT should be performed in patients with positive resection margins if re-resection is not possible. In the case of R1- or R2-resection, an additional boost dose of 10 (-20) Gy should be applied. At the moment, there is insufficient evidence to recommend de-escalation of locoregional radiotherapy based on a favorable response to neoadjuvant chemotherapy.

Regional nodal irradiation (RNI)

In most prospective trials of PMRT, radiotherapy portals did not only include the chest wall, but also the regional lymph nodes in the supra-/infraclavicular area and those along the internal mammary artery (IMN). In the absence of prospective data, regional nodal irradiation was also recommended in patients with 4 or more involved lymph nodes after breast-conserving surgery.

Recently, results from two large prospective randomized phase III-trials addressing regional nodal irradiation after upfront surgery have been published in the New England Journal of Medicine.

The MA.20 trial[126] randomized about 1800 patients after breast-conserving surgery to whole-breast irradiation (WBI) alone or WBI plus regional nodal irradiation (supra-/infraclavicular

area and IMN). Patients either had lymph node involvement (90%, most of those with 1-3 positive nodes), T3-disease or T2-disease with inadequate lymphadenectomy along with other risk factors. All patients received systemic treatment, 90% had chemotherapy. After a median follow up pf 10 years, RNI improved locoregional control, distant control and disease-free survival, but had no impact on overall survival.

The EORTC 22922-10925-trial[127] enrolled over 4000 patients and randomized them to WBI/chest wall irradiation alone or combined with RNI (supra-/infraclavicular area and IMN). Patients were eligible if they were either lymph node positive (mostly pN1) or if they were lymph node negative and had a medial or central tumor location (45% of patients). Most patients had breast-conserving surgery and adjuvant systemic treatment (55% received chemotherapy). Again, RNI improved locoregional control, distant control and disease-free survival. There was a strong trend towards improvement of overall survival (p = 0.06).

In both trials, toxicity was mildly elevated in the RNI groups, mostly in terms of acute and chronic skin fibrosis, lymphedema and pneumonitis. There was no increase in cardiac mortality or secondary malignancies. Since both trials used comprehensive nodal irradiation including IMN, there is no possibility to isolate the impact of irradiation of any of the separate lymph node areas. The only prospective randomized trial that has studied the role of IMN-irradiation after PMRT separately from the supra-/infraclavicular region showed no benefit in terms of survival, but was likely underpowered.[128]

Evidence for RNI in the context of neoadjuvant chemotherapy is very limited. A publication on PMRT +/- RNI including 464 patients showed inferior locoregional control when RNI was omitted.[129] A recent publication from Korea found a significant benefit of radiotherapy to the IMN after neoadjuvant chemotherapy for disease-free survival and locoregional control[130]. However, two studies showed no effect of RNI on locoregional control or survival in patients with ypN0[131 132]. As stated above for PMRT, these two studies included a large amount of cN0-patients.

The NSABP B-51/RTOG 1304-trial (NCT01872975) will randomize patients with clinically positive lymph nodes who convert to ypN0 after breast-conserving surgery to WBI alone or WBI + RNI.

The Alliance A11202-trial (NCT01901094) will enroll patients with clinically positive lymph nodes (cN+) and a positive sentinel lymph node-biopsy after chemotherapy (ypN+) and randomize them to axillary dissection followed by PMRT/WBI + RNI or to PMRT/WBI + RNI with inclusion of the full axilla (level I-III).

Recommendations

As for post-mastectomy radiotherapy, the indication for RNI should be evaluated on the basis of the worst available staging either before or after neoadjuvant chemotherapy. All patients

with pathologically involved lymph nodes (ypN+) or 4 or more involved lymph nodes at diagnosis should receive RNI. RNI should be strongly considered in patients in patients with 1-3 involved axillary lymph nodes at first diagnosis (pN1) or those with clinically involved lymph nodes with an unknown number of involved nodes (cN+), especially in the case of advanced lymph node involvement. There is insufficient evidence to generally support the irradiation of the internal mammary nodes, this should be discussed on a case-by-case basis. Axillary lymph node levels I and II should only be included into the clinical target volume in the case of positive axillary resections margins or macroscopic residual tumor without axillary dissection. Omission of RNI in node-positive patients with ypN0 or pCR outside of clinical trials is discouraged. The standard dose and fractionation for RNI would be 50-50.4 Gy in 25-28 sessions.

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