(CD133+) (CECp) and activation markers (CD106+) are also measured. For CECs, 50 ul of blood is stained with the indicated MAbs; after RBC lysis, flow cytometry (FC) is performed for total CEC and CECp. For CTCs, 20 ml of blood is subjected to immunomagnetic capture using anti-EpCAM ferrofluid, followed by FC for EpCAM, CD45, and nucleic acid content. A Cox proportional hazards model was used to determine whether numbers of or changes in CEC and CTC at weeks 0, 3 and 12 could predict outcome. Markers of the ER, HER2 and VEGF pathways will be evaluated in archival tumor tissue, and plasma VEGF and bFGF will be measured.

Results: Twenty-eight pts have enrolled. Data is available on 24 pts: median age 49 yrs (32–77), median ECOG PS 0 (0–1). Preliminary toxicity and response data has been reported (Traina, BCRT 2005). The combination was generally well tolerated; one patient withdrew due to Gr 3 headaches. There were no other Gr 3–5 toxicities. 24 pts are evaluable for response: 1 pt had a partial remission, 12 pts have stable disease (SD) >6 mo; 4 pts have SD; 7 pts had progression. 22 patients were followed for up to 52 weeks (median 25 wks, range 5–52). Changes in CEC at 3 and 12 weeks were the best predictors of response (HR = 1.12 per unit change, p = 0.083, for 3-week; HR = 1.16 per unit change, p = 0.068, for 12-week change from baseline). When both 0–3 week and 3–12 week change were used as predictors in a model, hazard ratios were 1.20 (p = 0.040) and 1.13 (p = 0.184) respectively. If patients are divided into two equal sized groups based on combined hazard score, 6 of 7 progressions occurred in the high risk group (p = 0.088, log-rank test).

Conclusions: Combination L and B appears well tolerated. Additional safety and efficacy data is anticipated. This promising preliminary data indicate that CEC may serve as surrogate markers of response and progression. Updated data including CTC will be presented.

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Occult brain metastases in her-2-positive breast cancer patients

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Aim: The aim of the prospective study was to evaluate the frequency of occult brain metastases in breast cancer pts with HER-2 receptor overexpression, and to analyze overall survival and the cause of death of patients (pts) after whole brain radiotherapy (WBRT).

Material and Methods: MRI screening examination of the brain was performed in 60 HER-2 positive breast cancer pts currently treated in Breast Cancer Clinic with trastuzumab and chemotherapy because of visceral metastases and/or locoregional failure. In case of pts with occult brain metastases detected, the irradiation to the brain 30 Gy in 10 fraction was undertaken. Then, control MRI was planned to be performed 3, 6, 9, 12 months after radiotherapy in order to assess the extent of regression of metastases.

Results: In 20 (33%) pts occult brain metastases were detected: in 7 – solitary, in 13 – numerous. Pts with brain metastases were younger than those without them (median age at primary breast cancer 48 years vs 52 years), and more often with distant metastases to lungs and/or liver (18/20 pts). Median time from recurrence of the disease (visceral metastases/locoregional failure) to brain metastases was 9 months, mean 11 months. From among 10 patients with time of observation of at least 1 year after WBRT, 5 pts are still alive without symptoms of brain metastases, 5 patients died: 4- due to progression in viscera and only 1- because of progression in brain.

Conclusion: Our prospective study confirms high percentage of occult brain metastases in HER-2 positive breast cancer pts. Mean time of detection of occult brain metastases does not exceed 1 year from recurrence of the disease. Brain metastases after WBRT undertaken during asymptomatic period are not the main cause of death of breast cancer patients. In most cases those patients die of visceral metastases. It seems that it is reasonable to introduce MRI screening of the brain in HER-2 positive breast cancer pts with disseminated disease for early detection and irradiation of brain metastases before neurological symptoms appear. Longer follow-up period is necessary in order to assess the cause of death of pts.

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Breast cancer with HER2/neu over-expression – are we dealing with a heterogeneous disease?

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Background: HER2/neu over-expression (HER2+) occurs in approximately 25% of breast cancers and is often associated with a more aggressive disease. In this study we examined the effects of hormone receptor (HR) expression on clinical characteristics and natural history in HER2+ breast cancer.

Methods: A retrospective review of 137 patients (pts) with HER2+, metastatic, breast cancer was performed. HER2+ was defined as positive if immunohistochemical staining was +3 or if FISH was positive. HR was defined as positive if either estrogen or progesterone receptors staining were positive, regardless of intensity.

Results: Median age was 48 (range 24–80). At the time of diagnosis 60% were 50 years old or younger. The vast majority (92%) had invasive ductal carcinoma and 70% had grade III disease. Positive HR staining was found in 55 (40%) of the pts and negative HR staining in 82 (60%). No significant differences between the study groups were found in age, tumor histology, grade and number of involved metastatic sites at time of first recurrence.

At first recurrence, HR positive (HR+) pts had significantly lower rates of liver or brain metastasis compared to HR negative (HR-) pts (23% vs. 48%, respectively, p = 0.004) but significantly higher rates of soft tissue, bone or lung metastasis (93% vs. 74%, respectively, p = 0.006). Overall thru-ought the course of the disease, 96% of the HR+ pts developed soft tissue, bone or lung metastasis, compared with 85% in the HR- group (p = 0.043), and 50% developed liver or brain metastasis compared with 72% in the HR- group (p = 0.012).The mean disease free interval in the HR+ group was 24.5 months, compared to 15.4 months in the HR- group (p = 0.023). The mean overall survival was 101.4 months in the HR+ group, compared to 63.8 months in the HR- group (p = 0.015).

Conclusions: Compared to HER2+ HR+ breast cancer patients, patients with HER2+ HR- disease have a greater propensity for liver and CNS involvement and a markedly shorter disease free interval and reduced overall survival. These results suggest that metastatic breast cancer with HER2/neu over-expression is a heterogeneous disease, with HER2+, HR positive tumors, having a distinct and favorable biological nature compared with HER2+, HR negative tumors.

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Phase III trial of oral ibandronate and intravenous zoledronic acid in breast cancer patients with bone metastases: comparative bone turnover marker and safety data

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Bisphosphonates are a standard treatment for metastatic bone disease. Ibandronate is a single-nitrogen, non-cyclic bisphosphonate available in intravenous and oral formulations. In phase III trials, both formulations showed similar efficacies for reducing skeletal events, and tolerability profiles were comparable to placebo. Bone turnover markers are prognostic indicators of skeletal events. In this study, oral ibandronate and intravenous zoledronic acid were compared regarding their effects on bone turnover markers and safety profiles. The study was a 12-week, head-to-head, randomized, open-label phase III trial. Breast cancer patients with advanced disease and confirmed bone lesions received either oral ibandronate 50 mg daily (n = 128) or intravenous zoledronic acid 4 mg (n = 126) every 4 weeks. The primary endpoint was change in serum cross-linked C-terminal telopeptide of type I collagen (S-CTX) concentration at study end. Other assessments included levels of urinary CTX, and serum levels of bone specific alkaline phosphatase, amino-terminal procollagen propeptides of type I collagen, and osteocalcin. For the safety analysis, all AEs were recorded. Treatment with oral ibandronate was associated with comparable and statistically non-inferior reductions in bone marker levels to intravenous zoledronic acid. In addition, the number of patients with high S-CTX levels at baseline that were reduced to normal or low levels after treatment were 26/26 (100%) for ibandronate compared with 19/22 (86.4%) for zoledronic acid. The proportion of patients who experienced AEs was lower in the ibandronate group than the zoledronic acid group (65% vs 76%). In particular, there was a markedly lower incidence of AEs during the first 3 days of the study (8% vs 47%) explained by a lower incidence of acute-phase response AEs (e.g. pyrexia or flu-like illness). Overall, the

data suggest that a convenient oral ibandronate dose of 50 mg/day has a similar efficacy to intravenous zoledronic acid for suppressing tumor-induced bone resorption, but it is associated with a lower incidence of AEs following treatment. Effects on bone markers may indicate the comparable efficacy of the two bisphosphonates for the prevention of skeletal-related events. Thus, oral ibandronate may provide similar benefits to intravenous zoledronic acid for metastatic breast cancer patients, but with a superior tolerability profile.

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Biological functions of brain metastasis from breast cancer

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Secondary to the increased survival of breast cancer patients following chemotherapy, cerebral metastases have recently become a significant clinical problem, with an incidence of 30–40%.

The aim of this study was to characterize functional phenotypes that might enhance brain metastasis in human breast cancers. We used a computer program (PIANA) to build a protein interaction network for a collection of 19 proteins identified by MALDI-TOF. We were able to associate this network of proteins into 8 functional groups.

The METABRE gene analyses of 4 brain metastases made with U133plus2 Affymetrix chips has been used to assess differentially expressed genes in brain metastases compared with a pool of breast tumors, after normalization using the RMA (Robust Multichip Averaging) algorithm. From this analysis we obtained 5.235 candidate genes, 2.467 overexpressed (> 2 fold) and 2.768 underexpressed (< 2 fold). We matched these genes with the PIANA brain network.

As a result we found 179 proteins, 122 overexpressed and 57 underexpressed, in the brain metastasis network belonging to the following functions: 23 protein folding and chaperones; 9 ubiquitination; 36 signal transduction and receptors; 13 kinases; 4 immunological; 5 protein transport; 4 peptidases; 12 structural; 9 cell adhesion; 22 DNA binding, repair and transcription; 8 REDOX; 4 carbohydrate and 7 lipid metabolism. Ten of these proteins belong to the METABRE specific brain metastasis signature: ARFGAP, RNF25, EHMT2, TOP1, RNPC2, eIF-3, MCM4, GRP 94, FN14, and INHA. Some of these are being validated in tissue samples with specific antibodies.

These results provide evidence that the characteristic phenotype of brain metastasis includes specific cell-cell and cell-matrix adhesion, a cohort of stress-inducible proteins, REDOX and detoxification pathways, and lipid and glucose metabolism.

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400 Poster

A multicenter phase II study of epirubicin with low-dose trastuzumab as a first line treatment in Her-2 overexpressing metastatic breast cancer: preliminary results

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Aims: To evaluate the activity and cardiac safety of the combination of epirubicin (E) with low-dose trastuzumab (LD-H) in patients with HER-2 overexpressing metatatic breast cancer.

Patients and Methods: This was a two step study: In the first step, H was given at a loading dose of 2 mg/kg on day 1, followed by 1 mg/kg weekly; in the second step (⩾12 objective responses/21 patients), the dose of H was mainatined to 1 mg/kg weekly. E was administered at 90 mg/m^2 on day 1 every 3 weeks. After 6–8 courses of this combination, H was administered as a single agent for a maximum of 52 weeks. To assess cardiotoxicity, pts were evaluated for the Left Ventricular Ejection Fraction (LVEF) at baseline, every two cycles during E and LD-H, and every three months during LD-H alone. Either ultrasonography or angioscintigraphy were used. Cardiotoxicity was defined as the appearance of signs or symptoms of congestive heart failure in ⩽10% of patients at an E dose of 720 mg/m^2 or in ⩽20% of patients at an E dose > 720 < 1000 mg/m^2.

Results: Twenty-one pts entered the first step: median age was 55 years (41–70 years), hormonal status was positive in 9 pts and negative in 10. Eight pts had received prior adjuvant anthracyclines, and 8 pts prior endocrine therapy. The majority of pts had > 2 organ sites of involvement with visceral lung metastases predominating. A median of 6 cycles (range 1–18) was administered with 134 cycles evaluable for toxicity. The regimen was well tolerated, with grade 3/4 neutropenia, alopecia, and thrombocytopenia occurring in 55%, 25% and 10% of the pts, respectively. Six episodes of cardiotoxicity were observed (an asymptomatic decrease in LVEF ⩾15% in 4 pts and an asymptomatic decline of LVEF at ⩽ 50% in 2 pts). At the time of analysis, 12 (57%) pts achieved a partial response, 6 (%) had stable disease, and 3 (%) had progressive disease. The median time to progression was 9.8 months (C.I.95%: 5. 5–14.1) and the median overall survival was not reached.

Conclusions: These preliminary results show that the combination of E plus LD-H possesses good antitumor activity, with limited cardiotoxicity. The Protocol Committee recommended to enter the second step of the study, maintaining the dose of H at 1 mg/kg weekly. Accrual is continuing; an update will be presented at the meeting.

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Subjective assessment of breast cancer related symptoms, activity levels and quality of life of patients with metastatic breast cancer under treatment with Anastrozole

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Introduction: Third generation aromatase inhibitors have earned their place in first-line therapy for advanced breast cancer with proven superiority over tamoxifen. Particularly relevant in this setting are quality of life and activity levels of the patients from a patient perspective, based on objective response-parameters.

Material and Methods: Over a period of 12 months, a total of 466 patients with metastatic breast cancer either to one (n = 272) or multiple sites (n = 167) were questioned in 3 monthly intervals, and the responses were analysed, for the following:

1. Subjective breast cancer related symptoms based on a score from 1 (no symptoms) to 4 (severe symptoms).
2. Personal activity levels based on a score from 1 (full activity, no symptoms) to 5 (bedridden, unable to provide for oneself).
3. Quality of life based on a score of 1 (excellent) to 7 (very poor).

Results: The median age was 61.7 years (35–94). At the start of the 12-month period 31% of the patients was asymptomatic. After 12 months this percentage had increased to 41% and the degree of reported moderate to severe symptoms reduced in this timeframe from 41% to 28% of syptomatic patients with an average score reduction from 2.8 to 2.2. A worsening of symptoms during the therapy was seen in 151/466 (32.4%) of patients. In 73/466 (15.7%) this reduction was first reported after 3 months of therapy had been completed. The percentage of patients with restricted activity was lower after 12 months 46.2% compared to 57.2% at the start of therapy. The group who classified their quality of life as excellent increased over this time-period from 3.6% to 12.8%, reflected in an increase in average score from 3.0 to 3.7.

Conclusion: The aromatase inhibitor anastrozole is a highly effective palliative treatment for metastatic breast cancer demonstrated by a reduction in symptomatic disease and a corresponding increase in activity levels and quality of life in the majority of patients over a period of 12 months.

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Brain metastases in HER-2 positive metastatic breast cancer (MBC) patients

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Background: Several recent reports suggested relatively high risk of brain relapse in HER2-positive breast cancer patients. This phenomenon has been attributed to either an aggressive behavior of this tumor type and/or an increased survival following trastuzumab therapy without brain protection owing to insufficient penetration of this drug to CNS. In this study we