Results: A total 43 patients were enrolled (isolated mediastinal LN:13 patients, isolated pulmonary nodule: 30 patients). Eighteen patients (42%) were confirmed to have benign lesion (group A) and 25 patients (58%) confirmed to have metastasis (group B). The disease free interval (median duration from initial operation of breast cancer to the detection of mediastinal LN or lung nodules) was similar between two groups (7.8 months (A) vs 9.5 months (B), p = 0.386). Between two groups, initial T stage (p = 0.145) and N stage (p = 0.749) was not different. Hormone receptor positivity was more prevalent in group A (72.2% vs 40.9%, p = 0.048) and triple-negativity was more prevalent in group B (16.7% vs 40%, p = 0.113). The mean size of the largest lesion was bigger in group B than in group A (20.8 mm vs 14.4 mm, p = 0.024). There was no difference in the number of lesions between two groups (2.17 (A) vs 2.76 (B), p = 0.361). PET was performed in 29 patients (67%). Metastatic lesions had significantly higher maximal SUV than that of benign lesions (6.42 vs 3.41, p = 0.021). mSUV more than 6.0 could define the lesion to be metastasis with the sensitivity of 50% and the specificity of 92% by ROC curve.
Conclusions: The biologic subtype, size of lesion and maximal SUV on PET could help physician to differentiate metastasis from benign lesion in breast cancer patients who present new isolated mediastinal LN or pulmonary nodule during surveillance.

5088 POSTER

Analysis of factors predicting response to second-line trastuzumab-based therapy in patients (pts) with Her2-positive advanced breast cancer (ABC)

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Background: Upfront use of trastuzumab (T), either alone or in combination with chemotherapy or aromatase inhibitors, is well established in Her2-positive ABC. Upon disease progression, pts may be switched to lapatinib plus capecitabine. Others however may be candidates for continued antibody therapy. Finding the optimal treatment approach therefore is pertinent. We tried to identify factors predicting efficacy of second-line T-based therapy.
Methods: 97 pts treated with >1 line of T-based therapy were identified from a breast cancer database. Her2-status was determined by immunohistochemistry and re-analyzed by FISH if a score of 2+ was gained. Time to progression (TTP) on second-line therapy was defined as primary study endpoint. Secondary endpoints consisted of response rate (RR), clinical benefit rate (CBR; CR+PR+SD >6 months), overall survival (OS), development of brain metastases, and cardiac toxicity.
Response was evaluated every three months (m) using UICC criteria. TTP and OS were estimated using the Kaplan-Meier product limit method. Multivariate analyses (Cox proportional hazards model, multinomial logistic regression) were applied in order to identify factors associated with TTP and RR.
The following variables were included: age, initial tumor stage, grading, endocrine receptor status, prior non T-containing palliative chemotherapy, metastatic sites, and clinical benefit from T-based first-line therapy. *p* values <0.05 were considered to indicate statistical significance.
Results: Second-line TTP was median 7 m (95% CI 5.74−8.26) and first-line 8 m (95% CI 6.25−9.74) (n.s.). RR on second-line was 30.9% (44.3% first-line). In the multivariate models, none of the factors included could independently predict for activity of second-line treatment. OS was 43 months (95% CI 37.92−48.09).
A significant deterioration of cardiac function was observed in three patients; 40.2% developed brain metastases on second-line T or during follow-up after a median 21 m (95% CI 13.86−28.14).
Conclusions: Trastuzumab in multiple lines showed considerable activity. None of the variables investigated correlated with activity of second-line therapy. In order to predict for activity of second-line T, evaluation of other factors known to confer trastuzumab-resistance (p95Her2, PTEN-loss) appears necessary.

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Shorter Overall Survival (OS) in HER2-positive (HER2+) metastatic breast cancer (MBC) patients (pts) treated with trastuzumab (T) ± chemotherapy (CT) and overexpressing HER3 by immunohistochemistry (IHC)

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Background: Mechanisms of resistance to T in HER2+ MBC pts are still poorly understood. Both EGFR and HER3 form heterodimers with HER2. We investigated the role of HER3 protein expression by IHC in HER2+ MBC pts treated with T ± CT.
Material and Methods: From 04/99 and 03/06, 76 consecutive HER2+ MBC pts were treated at our institution but tumor tissue was available, for this analysis, from 42 pts. HER2 was evaluated by IHC (MoAb CB11) and scored according to DAKO Herceptest. HER3 status was evaluated by IHC on 3.5 μm section of formalin-fixed, paraffin embedded tissue using a mouse MoAb (clone RTJ1; Novocastra United Kingdom; used at 1:20). The immunostaining reactivity was citoplasmatic. Tumors were considered HER3+ if >50% of tumor cells were positive.
Results: At median follow up of 26.5 months (3.7−99.6) from the start of T, 42 pts were evaluable for OS and incidence of CNS metastases and 40 for response to T and TTP. Median age of pts was 53 years (23−77). We observed 25/40 responses (CR+PR) to T ± CT (62.5%) and CNS metastases in 20/42 pts (47.6%); median TTP from the start of T was 9.6 months (1.3−80+). Median OS from the start of T was 29.6 months (3.7−99.6+). Twenty-one pts were HER3+ with a cut-off of 50%. HER3 overexpression was not significantly correlated with response to T, TTP, incidence of CNS metastases; OS from start of T was shorter in HER3+ tumors compared to HER3 tumors (28.2 vs 42.7 months; p = 0.152). These data confirm the notion that HER2/HER3 heterodimer is the major oncogenic unit in HER2+ MBC.
Conclusions: In this limited series of pts, a worse OS was observed in HER2+ MBC with HER3+ status by IHC. Approaches to target HER2/HER3 signaling may be warranted.

5090 POSTER

Micrometastatic tumor cells in blood and bone marrow of patients with primary breast cancer: extended surrogate marker-panel for multi-gene expression analysis

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Background: We have validated a new preanalytical enrichment and molecular detection method using embedded tumor cell calibrators (ETC) for quantitative gene expression analysis of circulating tumor cells (CTC) and bone marrow tumor cells (DTC). Here we present results of an extended surrogate marker panel.
Methods: Samples from patients were divided in native probes and matched calibrator probes containing either 2 or 10 breast carcinoma tumor cells (ETC). The high affinity antibodies BM7 (MUC-1) and VU1D9 (EpCAM) coupled to 4 μm dynabeads (Invitrogen®) were used for immunomagnetic tumor cell enrichment from $1x10^7$ bone marrow (BM) cells and 10 ml peripheral EDTA-blood of patients with primary breast cancer and metastatic disease. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis. We used end point RT-PCR (Adnagen®) and real-time quantitative RT-PCR approaches with the epithelial markers cytokeratin19 (CK19), mammaglobin1 (MG1), MUC1 and EpCAM for tumor cell identification. The phenotype of micrometastatic cells was determined by expression analysis of the markers ALDH1, TKTL1, Survivin, CXCR4, HIF-1, HER2 and CD276 (B7-H3).
Results: Positivity rate of ETC controlled RT-PCR on the basis of CK19, MG1, and EpCAM was 8.6% in 16/187 patients with primary breast cancer, 24% for DTC analysis in BM and 61.1% in patients with metastatic disease. During a 12 to 24 months follow-up of 114 patients of the primary breast cancer group CTC positivity was determined in 9.6% of the patients, however only one of these had been positive before operation. In three patients with multimarker expression early metastasis was clinically confirmed later. Progressive disease in metastatic breast cancer patients was characterized by elevated marker levels of CXCR4 (86%), survivin (58%) and CD276 (50%).