that low expression of Microtubule-associated protein tau (MAPT) is a predictive marker for response to anthracycline based chemotherapy with or without taxane. In contrast, it has recently been shown, that high expression of MAPT may indicate response to endocrine therapies. Here, we have analyzed the RNA expression of MAPT in formalin fixed paraffin embedded breast cancer tumour tissues of patients treated within two neoadjuvant chemotherapy trials (TECHNO/PREPARE trial) to evaluate the prognostic value in this setting.

Patients and Methods: Breast cancer patients (?cT2, N0-N3, M0) received neoadjuvant chemotherapy of 4 cycles of epirubicin and cyclophosphamide followed by 4 cycles paclitaxel (PREPARE trial). In Her-2/neu positive breast cancer patients trastuzumab was additionally administered (TECHNO trial). Samples of an initial group of 57 patients (34 TECHNO/23 PREPARE) were prepared for first analysis. RNA was successfully isolated from the tissue samples by an automated system based on magnetic beads (Siemens Medical Solutions Diagnostics GmbH). RNA expression of ESR1, Her-2/neu and MAPT expression was determined by quantitative RT-PCR. MAPT expression was correlated to histopathological findings.

Results: RNA expression of MAPT significantly correlated with ESR1 expression (Spearman $r = 0.677$; $p < 0.0001$), but not with Her-2/neu expression. Median expression of MAPT was 4 fold higher ESR1 positive tumors. Of the 57 patients included in the first analysis, 10 patients showed a pathological complete response (pCR), 4 in the PREPARE group and 6 in the Her-2/neu positive TECHNO group. There was a significant difference of MAPT-RNA expression between those patients with histopathologically complete response after neoadjuvant chemotherapy and those with no or partial response in the first cohort (Mann-Whitney-U, $p = 0.019$). Patients with a complete response showed significantly lower levels of MAPT-RNA. Interestingly, this difference was prominent in the group of Her-2/neu positive tumors (TECHNO trial) treated in addition with trastuzumab with all responding tumors exhibiting a 4 fold lower median MAPT expression (Mann-Whitney-U, $p = 0.009$). In contrast, no statistically difference was seen in the PREPARE group (Mann-Whitney-U, $p = 0.59$) with only half of the tumors being below the median MAPT expression.

Conclusion: These results validated the initial hypothesis that low expression of MAPT indicates sensitivity to chemotherapy on RNA level in clinical routine paraffin tissue. Moreover, low MAPT-expression was particularly informative in Her-2/neu positive breast cancer patients treated with anthracyclin-based chemotherapy containing paclitaxel and trastuzumab.

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External and internal assurance of the determination of the prognostic factors uPA and PAI-1 for the ongoing NNBC 3-Europe trial

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Background: The biomarkers urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 (plasminogen activator inhibitor-1) are suitable tools to predict which patient with node-negative breast cancer would benefit from chemotherapy and which not. Based on this finding, a still ongoing multi-center trial, the NNBC 3-Europe trial, is conducted, which is supported by the German AGO, the German Breast Group and the European EORTC PathoBiology Group. Recruitment centers are allowed to select risk assessment criteria either by using established clinical and histomorphological criteria or by using uPA/PAI-1 measurements in primary tumor tissue extracts.

External Quality Assessment (EQA) of total protein and the uPA and PAI-1 determination in the cytosol of the primary tumors are performed by the Department of Chemical Endocrinology of the University Hospital Nijmegen, The Netherlands.

Material and Methods: Quality assessment samples are being prepared centrally as a lyophilised cytosol. In the nine participating laboratories the vial contents are reconstituted as instructed and assayed as part of the next routine batch. After an initial pilot phase the qualtity control included the monitoring of the between- and the within-laboratory variations.

In order to get some more information on the homogeneity of the uPA/PAI-1 expression within the tumor tissue, 30 different tumor samples were cut up to nine pieces and each piece analyzed individual for the level of these biomarkers.

Results: The median values of the coefficient of variation (CV) between labs are for uPA and PAI-1 15%. The median of the within-lab between-run CVs are for uPA 10%, for PAI-1 12% ranging from 0.0 and 18%. For quality assessment of total protein measurement, all CVs values are acceptable.The analyses of different pieces of the same tumor revealed homogenous and heterogenous distribution of uPA and PAI-1 levels within the tumor.

Conclusions: In order to get a representative value of the invasion factors, a minimum of 100 mg tumor weight preferably from different areas of the tumor periphery are recommended. The NNBC 3-Europe trial shows that inclusion of patients based on biological testing of fresh frozen tumor material is feasible. The quality assurance showed acceptable performance.

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Increasing Her2/Centromere17(CEP17) ratio predicts for greater sensitivity to trastuzumab based therapy in metastatic breast cancer (MBC)

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Background: Recent preliminary results suggest that a higher Her2/CEP17 ratio (Her2/CEP17R) predicts for improved response to trastuzumab (T) and that some H/17R non-amplified cancers with polysomy CEP17 may respond to T. We sought to examine the relationships between primary tumour Her2 gene copy number (Her2GCN), Her2/CEP17R and polysomy CEP17 and time to progression (TTP) with T based therapy in MBC.

Methods: Cases of Her2 positive MBC treated with T were identified from our databases. Data were extracted from case notes on previous treatments for MBC, TTP on T and primary tumour immunohistochemistry for ER, PgR, Ki67, EGFR and Her2 expression. FISH analysis was performed on freshly cut sections using Her2/CEP17 probe mix (Abbott-Vysis PathVysion®). Statistical analysis of TTP by categorical variables was by Kaplan–Meier log rank test. Cox regression with continuous covariates log(Her2GCN) and log(CEP17) was used to explore their combined prognostic content.

Results: Data were available on 169 patients with MBC treated with T between 1999 and 2007. 61/169 (36%) had T monotherapy (Tm) and 108 (64%) combination T+chemotherapy (Tc). Prior chemotherapy regimens for MBC: 0 = 117/169 (69%), 1 = 41 (24%) and >1 = 11 (7%). No differences were seen for TTP between Tm vs Tc ($p = 0.78$) or by line of treatment ($p = 0.88$; logrank). Her2/CEP17R and to a lesser degree Her2GCN had positive relationship with TTP when categorised as borderline/low ($\leqslant 2.2$ and $\leqslant 6$), moderate ($2.2–\leqslant 7$ and $6–\leqslant 20$) and high amplification (>7 and >20; $p = 0.004$ and $p = 0.14$ respectively by logrank). Polysomy 17 (CEP $\geqslant 3$; 64/169 (38%)) predicted for shorter TTP versus tumours with CEP17 < 3 ($p = 0.056$; logrank). Polysomy 17 (CEP $\geqslant 3$) did not influence TTP in cancers with Her2/CEP17R $\leqslant 2.2$, but numbers were small. Cox regression showed no evidence of an interaction between log(Her2GCN) and log(CEP17) ($p = 0.60$). Their combined prognostic information was best carried in a single variable: log(Her2/CEP17R). Fitting this single variable yielded a hazard ratio for TTP of 0.68 for each unit increase in log(Her2/CEP17) ($p = 0.001$).

Conclusion: Her2/CEP17R is positively associated with the duration of benefit from T containing regimens for MBC. We found no convincing evidence for benefit from T in patients with Her2 non amplified tumours and polysomy CEP17. These data on the relative benefits of T based therapy may help to guide patient information, treatment choices and follow up strategies in patients with Her2+ MBC.