Materials and Methods: DNA was extracted from 5 μM paraffin-embedded sections containing at least 50% of tumoral cells, derived from primary or metastatic lesions. Exon 2 of KRAS and exon 15 of BRAF were amplified by PCR and analysed by direct sequencing. PIK3CA status was analysed by Pyrosequencing. PTEN espression was analyzed by immunohistochemistry using a Dako monoclonal antibody diluted 1:100 (cut-off 5%). PFS was estimated by Kaplan–Meier method and curves were compared by log-rank test. Hazard Ratios (HR) were estimated according to Cox multiple regression model.
Results: Patient characteristics were as follows: Male/Female = 35/29; primary site: rectum/colon = 13/51. KRAS, BRAF and PI3K mutations were present in 28.1%, 21.3% and 15.1% of CRC lesions, respectively, while PTEN positivity in 34.7%. KRAS WT was associated with median trend towards a higher PFS: 5.1 (95% CI 3.3−6.2) vs 3.1 months (95% CI 2.3−5.5), $p = 0.85$. BRAF WT was associated with higher PFS: 5.1 (95% CI 2.9−6.5) vs 3.4 months (95% CI 1.4−5.1), $p < 0.01$. PI3K WT was associated with higher PFS 5.3(95% CI 3.6−6.5) vs 2.2 months (95% CI 1.1−3.3), $p < 0.01$. High PTEN expression was associated with PFS 6.7 (2.8−8.7)/3.4 (2.6−5.3) months, $p = 0.031$. Moreover, the multivariate analysis of PFS indicated BRAF and PI3K as potential independent predictors of clinical benefit: HR (95% CI) 3.14 (1.38–7.16), $p = 0.006$ and 6.68 (2.17–20.52), $p = 0.0009$, respectively.
Conclusion: Our results seem to confirm that comprehensive molecular dissection of the EGFR signaling pathways should be considered to better select mCRC patients for CTX based therapies.

PP 40

Tumor microenvironment and prognosis in breast carcinoma

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Background: Multiple lines of evidence indicate that tumor microenvironment (TME) influences the breast cancer growth and metastasis and therefore could be promising prognostic parameter. The aim of the present study was to determine the prognostic significance of immune response, by quantifying mediators and immune cells, and (lymph)angiogenesis, by quantifying (lymph)angiogenic mediators and vessels, in the primary breast tumors.
Materials and Methods: The study included 135 pN0M0 breast cancer patients, who received no adjuvant therapy. Median follow-up of the natural disease course was 107 months. Indirect quantification of TME components was done by measuring expression of 19 (lymph)angiogenic factors and cytokines on protein level, in cytosolic fraction of tumors using classical biochemical method for ER and PR, and ELISA for others, and on mRNA level, by applying RT-qPCR in RNA fraction of the tissue samples. Direct quantification of TME parameters was done on cellular level, by performing IHC staining of FFPE tissue slides, using antibodies against CD3, CD8, CD45RO, CD68, CD105 and podoplanin. IHC positively stained cells were quantified as number of cells per tissue surface, in the hotspot regions of center of tumor (CT) and invasive margin (IM), by HISTOLAB software analysis of scanned tissue slides.
Results: DFI analysis on protein level showed that high ER (>60 fmol/mg), IL8 (>120.3 pg/mg) and PAI (>4.97 pg/mg) levels in tumors were associated with unfavorable prognosis ($p = 0.003$, $p > 0.001$, $p = 0.05$), contrary to high bFGF (>64.6 pg/mg) values in tumors ($p = 0.007$). On mRNA level high IL1β (dCt < 20.3) and IL8 (dCt < 21.9) values in tumors were linked with unfavorable prognosis ($p = 0.02$, $p = 0.02$), contrary to high VEGF-A values (dCt < 12.1, $p = 0.05$). Finally, on cellular level high density of blood (but not lymphatic) vessels at IM and high density of CD8+ infiltrates both in CT and IM, indicate a good prognostic groups. Combined status of classical prognostic parameters and analyzed biomarkers improved their individual prognostic impact and provided better separation of patients into prognostic groups.
Conclusion: This study suggests that accessing ER, IL8, PAI, bFGF, IL1β, VEGF-A levels, as wells as estimating degree of vascularisation and lymphocyte infiltrates, in the primary tumors, may be important for classifying patients with pN0M0 breast carcinoma into different prognostic groups and may help the individualization of the breast cancer targeted therapy.

PP 48

TRIB3: a prognostic factor and involved in hypoxia sensitivity in breast cancer patients

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Background: Tribbles homolog 3 (TRIB3) is a hypoxia induced pseudokinase involved in the regulation of several signaling pathways involved in cell survival and/or cell stress. Here, we determined the correlation between breast cancer prognosis and TRIB3 protein levels and established the role of TRIB3 in cell survival after hypoxia and/or radiotherapy.
Materials and Methods: TRIB3 mRNA and protein were quantified in breast cancer cell lines and in a breast cancer patient cohort ($n \geqslant 95$) using qPCR and a new specific avian antibody against TRIB3. Correlation between TRIB3 mRNA and protein were investigated and in the patient cohort prognostic and predictive value of both measurements were determined. In addition, we used siRNA-mediated knockdown of TRIB3 in a colony-forming assay after hypoxia and radiotherapy.
Results: We found that TRIB3 mRNA levels did not correlate with protein levels in breast cancer cell lines neither in the human breast cancer material. We validated our earlier finding that high TRIB3 mRNA denotes a poor prognosis, but found that high TRIB3 protein levels were associated with a good prognosis in breast cancer patients. We also show that knockdown of TRIB3 resulted in an increased survival under hypoxic conditions. Furthermore, we have indications that TRIB3 is relevant for hypoxia induced cell death in cells with AKT knockdown.
Conclusion: Our results presented here indicate that these data on mRNA levels do not necessarily translate to protein, nor to function, of all genes. Whereas mRNA levels of TRIB3 are related with a poor prognosis, TRIB3 protein is associated with a good prognosis in human breast cancer patients, possibly due to the fact that TRIB3 is involved in hypoxia tolerance.

PP 47

Prediction of non-responders to chemoradiation in HPV-positive head and neck cancers by gene expression profiling

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Background: Human papillomavirus (HPV) has been shown to have a causal role in the development of Head and Neck Squamous Cell Carcinoma (HNSCC). In one study, 22% of tumors were HPV+, with 87% being of the HPV16 subtype. HPV-related HNSCC represents a distinct and well-defined pathology that is associated with a better response to radiation and more favorable prognosis. However, approximately 15% of the patients do not respond positively to radiation or chemoradiation, thus suffering unnecessary morbidity and delay to receive effective therapy.
Materials and Methods: 11 patients with laryngeal and oropharyngeal HPV+ HNSCC were included in the study, 5 of which had a complete response to treatment and 6 who were non-responders. RNA was extracted from prospectively collected, pre-treatment tumor specimens and subjected to gene expression analysis using Affymetrix Human Exon 1.0ST arrays. HPV-status was confirmed by detection of HPV16 E7 with RT-PCR.
Results: ANOVA ($p > 0.05$) and a 2-fold cutoff were used to identify 118 altered genes, including 112 genes over-expressed in the complete responders compared to the non-responders. Interestingly, in the complete responders, over-expressed were associated with T cell proliferation (PTPRC, ITGAL, IL6ST, CD3E, CORO1A) and antigen processing/presentation (HLA-F, HLA-DRA, HLA-DQA2, PSMB9, ERAP1, CD74). Utilizing Ariadne Pathway Studio to characterize the data, changes in gene expression were enriched in genes encoding proteins involved in regulating cell processes such as presentation of endogenous peptide antigen, lymphocyte adhesion, and T-cell related processes. Further, genes associated with the Gene Ontology group “response to virus” were up-regulated in complete responders.
Conclusion: The data obtained in this study suggested that differences in response to chemoradiation therapy were related to immune response prior to treatment. These data can potentially lead to an assay that can be used clinically to predict HPV+ HNSCC patients that will not benefit from chemoradiation and who may benefit from earlier surgical intervention.

PP 30

Prospective comparison of Recurrence Score, uPA/PAI-1, central grade and molecular subtyping in early breast cancer: first results from the WSG-Plan B trial (interim analysis)

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Background: For decision support regarding adjuvant chemotherapy in early breast cancer,the recurrence score (RS) multi-gene assay and invasion factors uPA/PAI-1 are included in national and international guidelines. We present first correlation analysis of RS, uPA/PAI-1 and molecular subtypes by protein expression of the first pre-planned trial, WSG Plan-B.
Materials and Methods: The Plan-B trial ($n = 2.448$) is evaluating anthracycline-free adjuvant chemotherapy ($6 \times$ TC) versus $4 \times$ EC − $4 \times$ Doc in Her 2 neg. breat cancer. RS is used as selection criterion

for chemotherapy or hormonal treatment alone; uPA/PAI-1 is obtained as an optional risk factor. Central grading, Ki-67 and luminal subtype are performed by an independant pathologist.

Results: The trial was performed from april 2009 to july 2011. We present data for RS, central grade, Ki-67 and uPA/PAI-1. In preliminary data RS was weakly positively correlated with PAI-1, Ki-67 and central grade. There was only a weak concordance between RS and uPA/PAI-1, using either standard RS (<18; >30) or the cut-offs within the Plan-B trial (<11; >25). While RS high risk was predictive of high risk by uPA/PAI-1, grade and luminal B subtype, the converse was not found.

Conclusion: This first results comnpare prospectively risk groups according to RS, Ki-67 and uPA/PAI-1 and molecular subtypes. Further evaluation within the Plan-B trial will help to clarify the clinical significance and meaning as prognostic and predictive factors of these findings.