recently discovered class of genes transcribing small non-coding RNAs, namely microRNAs, was found to play important regulatory roles in normal development and physiology in plants and animals. Mature microRNAs are 20−22 nucleotides molecules that can regulate gene expression through RNA interference effecter complex (RISC) mediated mRNA degradation and translational suppression via complimentary pairing to predominantly 3′-untranslated region (3′-UTR) of their targeted messenger RNAs. Increasing number of studies has demonstrated a perturbation of the normal expression patterns of microRNAs in many human cancers.

Main Message: In order to investigate the microRNAs expression signatures in pediatric malignancies, we profiled a panel of pediatric tumor xenografts and cell lines belonging to 6 diagnostic categories using an in-house developed microRNA microarray. This panel included rhabdomyosarcoma (RMS), neuroblastoma (NB), Ewing's sarcoma, osteosarcoma, Wilm's and brain tumors. Each of our microRNA microarrays contains 336 human microRNAs probes based on Sanger Center's microRNA registry release 7. By unsupervised hierarchical clustering using all high quality data the cancers were primarily grouped according to their diagnosis. Using a parametric statistical analysis we identified 18 miRNAs that were differentially expressed between RMS and NB several of which were confirmed by real-time RT-PCR methods. We subsequently confirmed the expression of several cancer-specific microRNAs in the corresponding clinical tumor samples indicating their potential use for the diagnosis of these cancers.

In order to investigate the potential targets and functional roles of these microRNAs, we performed parallel gene expression profiling study on the same set of xenograft samples using Affymetrix U133 Plus 2 chips, and calculated the correlations between the expression of microRNAs and messenger RNAs. This resulting data matrix will identify potential targets of miRNA which will require experimental validation. Furthermore, we performed knock down and over expression studies with several NB and RMS specific miRNAs. We found that the over expression of mir-133a caused a profound growth suppression and induction of apoptosis in a RMS cell line.

Conclusions: Our findings showed the potential use of microRNAs for the diagnosis of pediatric cancers. Furthermore our results will reveal new insights into the function, and identify potential therapeutic uses of these small RNAs in these pediatric cancers.

S20

Novel biomarkers for prognosis and therapy response in ovarian cancer

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Introduction: New biomarkers for diagnosis, prognosis, monitoring and prediction of therapeutic response in ovarian cancer are needed.

Main Message: Recently, we have delineated the complete organization of the human kallikrein locus, which comprises 15 genes with significant homology. We have produced recombinant proteins and developed ELISA methodologies for quantitative assessment of all kallikrein proteins in serum and tissue extracts. We found that some tissue kallikreins, including KLK5, KLK6, KLK8, KLK10, KLK11 and KLK13 are over-expressed in ovarian cancer tissue, in comparison to normal ovarian tissue. We found that these KLKs have prognostic value in ovarian cancer. More recently, we have analyzed panels of kallikreins, as well as other biomarkers, in our efforts to develop a multiparametric prognostic and predictive model for this disease. We developed a combined marker which provided an area under the curve (AUC) of 0.97 for discriminating normal from cancer groups. Univariately, KLK5 and KLK6 were positively associated with progression. Increasing levels of KLK13 were associated with chemotherapy response with an odds ratio of 2.32. The predictive power of KLK13 to chemotherapy response was improved with a panel of 5 biomarkers with AUC of 0.75. After adding other clinical parameters, the AUC increased to 0.91. This data suggests that a group of kallikreins and multiparametric combinations with other biomarkers and clinical parameters can significantly assist with ovarian cancer classification, prognosis and response to chemotherapy.

Conclusions: In conclusion, human tissue kallikreins represent novel biomarkers for ovarian cancer diagnosis, prognosis and prediction of response to chemotherapy. These biomarkers, assessed individually and/or in combination with other biomarkers, may assist with delivery of individualized treatments in ovarian cancer.

S21

Biomarkers of brain tumors to temozolamide treatment

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Introduction: Glioblastomas are the most malignant brain tumors with a median survival of less than 15 months despite modern therapies. The accumulation of diverse aberrations in regulatory processes enables tumor cells to bypass the effects of most classical therapies available. Molecular alterations underlying such mechanisms comprise aberrations on the genetic level, such as point mutations of distinct genes, or amplifications and deletions, while others result from epigenetic modifications such as aberrant methylation of CpG islands in the regulatory sequence of genes.

Main Message: In a candidate gene approach the epigenetic inactivation of the MGMT gene was evaluated as a predictive factor for benefit from the alkylating agent temozolomide that was added concomitant and adjuvant to radiotherapy. MGMT is a repair enzyme known to rapidly revert the highly toxic O6-methylguanine to its native state, guanine, in a suicide reaction – hence, blunting most of the treatment effect of the alkylating drug. The translational

research effort accompanying the randomized clinical trial EORTC26981/NCIC CE.3 indeed showed that the benefit from TMZ was mainly confined to patients whose tumors carried an epigenetically inactivated MGMT gene. At 2 years, 46% of the patients treated with TMZ/radiotherapy and whose tumors had a methylated MGMT promoter survived, compared with only 14% for the patients with an unmethylated MGMT status (overall log-rank P = 0.0001). Prospective validation of this factor for prediction of benefit from TMZ is ongoing.

However, even in the cohort of patients with a methylated MGMT overall survival remains unsatisfactory and extremely variable, indicating additional mechanisms of treatment resistance. A first step is the identification of relevant molecular mechanisms driving the aggressive biological behavior of glioblastoma. We investigated glioblastoma gene expression profiles and identified new independent mechanisms of resistance to this treatment that may be targeted as part of an improved trial design.

Conclusions: To date, the test for the MGMT-methylation status is the only tool available that may direct the choice for alkylating agents in glioblastoma patients, but many others may hopefully become part of an arsenal to stratify patients to respective targeted therapies within the next years.

S22

Biomarkers of brain tumors to EGFR-TKI

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Glioblastoma is one of the most aggressive human cancers with a median survival of about 15 months despite optimal therapy with surgery, radiation, and chemotherapy. Reasons for this dismal track record of current therapeutics are unknown and are likely to include disease-specific genetic abnormalities, limited penetration of therapeutics across the blood-brain barrier, and the difficulty to obtain serial tissue samples for the monitoring of tumor cell response to therapy. Since about 40% of primary glioblastomas harbor amplification of the EGFR gene locus and often express a mutant EGF receptor with constitutively activity due to an in-frame truncation within the ligand-binding domain (EGFRvIII), inhibition of EGFR signaling presents a molecularly compelling strategy for the treatment of glioblastoma. We and others have observed clear antitumor activity of small molecule EGFR tyrosine kinase inhibitors (EGFR TKIs) in 10−20% of glioblastoma patients. Clinical response was highly correlated with coexpression in tumor cells of the tumor suppressor PTEN and EGFRvIII. We have also identified missense mutations in the extracellular domain of EGFR as novel mechanisms for oncogenic EGFR conversion in glioblastoma. These observations raise a number of questions for the clinical evaluation and optimal deployment of EGFR kinase inhibitors in glioblastoma which will be discussed in this presentation.

S23

Biomarkers of lung cancer response to EGFR-TKI

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Introduction: More than three years have passed since mutations of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) were discovered in patients with lung cancer who had dramatic clinical responses to treatment with gefitinib. The other common genomic changes that arise in lung cancer that have an impact on EGFR-TKI sensitivity include KRAS mutations, secondary T790M mutations in EGFR, and MET amplification. The retrospective studies have shown that EGFR mutations are closely associated with response while EGFR copy number and EGFR detection by immunohistochemistry are most closely associated with a prolongation in time to progression and survival in randomized studies of EGFR-TKI versus placebo. These retrospective studies have now led to prospective studies incorporating these different biomarkers of response and outcome into trials using EGFR-TKIs as therapeutic agents.

Main Message: Prospective studies of lung cancer patients with EGFR mutations treated with gefitinib and erlotinib have reported a response rate of approximately 80%, a median time to progression in excess of approximately one year, and a median survival in excess of two years. This has led to ongoing trials in Japan comparing patients with EGFR mutations being treated with either chemotherapy or gefitinib and the development of commercial tests to determine if the DNA from tumors retrieved from patients with adenocarcinoma have a mutation of the EGFR. The EGFR copy number assessments by FISH have been prospectively incorporated into trials that have been recently reported. One trial called INVITE compared 196 patients older than 70 years of age with non-small cell lung cancer who were randomly assigned to either treatment with gefitinib or vinorelbine. A lung cancer specimen was required for entry onto the study to determine biomarkers and EGFR copy number could be assessed in 158 of the 196 patients' tumors. Increased EGFR copy number was not associated with increased response or survival benefit in patients given gefitinib compared to those given vinorelbine. A second trial called INTEREST studied patients with non-small cell lung cancer previously treated with one or two chemotherapy regimens. 1466 patients were randomized to treatment with either gefitinib or docetaxel given every 3 weeks. Lung cancer specimens were not required for entry onto the study to determine biomarker status. Once again, there was no difference in outcome between the two arms and no relationship between EGFR copy number determined by FISH and response rate, time to progression, or survival on either arm.

The genomic change associated with resistance to treatment with gefinib and erlotinib is a DNA mutation which changes the threonine to methionine at the 790th amino acid of EGFR known as the (T790M) mutation as well as amplification of the MET oncogene. The T790M mutation in EGFR is responsible for approximately half