predictive of the efficacy of these agents remain elusive. Previously, serial alpha-fetoprotein (AFP) measurement has been found to be useful in prognostication and monitoring treatment response in HCC patients undergoing systemic chemotherapy. Whether AFP changes during therapy are able to predict treatment efficacy of anti-angiogenic therapy in advanced HCC patients is still unknown.
Materials and Methods: Advanced HCC patients who had been enrolled in three prospective phase II clinical trials evaluating a combination of anti-angiogenic therapy (sorafenib, bevacizumab, or thalidomide) and metronomic oral 5-fluorouracil preparations (tegafur/uracil or capecitabine) as the first-line systemic therapy for advanced diseases were included. Early AFP response was defined as a decline in level of more than 20% from baseline after 2 to 3 weeks of treatment. Baseline AFP level and AFP response were analyzed for their associations with treatment efficacy and survival outcome.
Results: A total of 107 patients were enrolled. Baseline AFP level was elevated in 85 (79%) patients. Patients with normal baseline AFP levels, compared to those with elevated levels, had a better disease control rate (77% vs. 39%, $p = 0.001$), median progression-free survival (PFS, 4.0 vs. 2.0 months, $p = 0.024$) and overall survival (OS, 10.7 vs. 4.2 months, $p = 0.013$). Seventy-two patients were evaluable for early AFP response, and 12 (17%) of them were classified as early AFP responders. Early AFP responders, compared to non-responders, had a better overall response rate (33% vs. 8%, $p = 0.037$) and disease control rate (83% vs. 35%, $p = 0.002$). Median PFS (AFP responders vs. non-responders, 7.5 vs. 1.9 months, $p = 0.001$) and OS (15.3 vs. 4.1 months, $p = 0.019$) were also longer in AFP responders. By multivariate analysis, AFP response remained a significant independent predictor for better PFS and OS.
Conclusion: Early AFP response can predict treatment efficacy and survival of advanced HCC treated with anti-angiogenic targeted therapy and metronomic chemotherapy.
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PP35

Dual use of single WT-1 immunohistochemistry in evaluation of ovarian tumors: a preliminary study of 20 cases

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Background: Our previous studies revealed that a single Wilms' tumor 1 (WT-1) immunohistochemistry could be used to evaluate both the myoepithelai cells and blood vessels of human breast tumors. As the human ovarian tissue is rich in blood vessels and WT-1 has been suggested to be a biomarker for ovarian tumors, our current study intended to assess whether a single WT-1 immunohistochemistry may have dual use in evaluation of the epithelial cells and microvascular density of ovarian tumors.
Materials and Methods: Consecutive sections were prepared from 20-ovarian tumors with co-existing normal and neoplastic components. Consecutive sections were subjected to immunohistochemistry with a mouse monoclonal antibody against human WT-1 protein. To confirm the specificity and sensitivity of WT-1 immunostaining, two adjacent sections from each case were subjected to immunohistochemistry for a well defined ovarian tumor marker, CA125, and a blood vessel specific marker, CD34. From each case, 4−5 randomly selected areas were photographed, and the percentages of positive cells for these molecules were compared.
Results: Distinct immunoreactivities to WT-1 were co-localized with CA 125 in a vast majority of the ovarian tumor foci. Distinct WT-1 expression was also seen in a vast majority of morphologically distinct endothelial cells that were strongly positive for blood vessel marker CD34. WT-1 immunoreactivities appeared to be substantially higher in small vessels near invasive than in normal or pre-invasive lesions, suggesting that WT-1 expression may correlate with tumor progression or invasion.
Conclusion: Our findings suggest that a single WT-1 immunohistochemistry may be used to assess both the tumor cells and micro-vascular density in ovarian tumors. More importantly, the development of agents to target WT-1 expression in vascular structures may have significant therapeutic value.
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PP95

Predictive mRNA and microRNA markers of response to the HDAC inhibitor PCI-24781 in colorectal tumors

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Background: PCI-24781 is an oral HDAC inhibitor currently in clinical trials for treatment of solid and hematological malignancies. It has demonstrated very good activity as a single agent in lymphoma with a good safety profile. In solid tumors, there have been several documented stable diseases (SD) but no PRs or CRs to date, as has been noted for other HDAC inhibitors previously. As with EGFR inhibitors, it is possible that clinical success in solid tumors depends upon the selection of the most sensitive tumor type and the most likely responder population within that indication. Interestingly, however, many of the SDs have been durable, with the longest duration of SD (8 months) being observed in a rectal cancer patient. This correlates well with preclinical data showing very good activity of PCI-24781 in colorectal cancer (CRC) cell lines & xenograft models. We therefore examined the activity of PCI-24781 in primary CRC tumors to identify predictive markers of efficacy.
Materials and Methods: Primary CRC samples were obtained from patient biopsies, plated in soft agar and treated with PCI-24781 and the percentage of cell growth inhibition (%GI) was calculated. RNA from these tumors were profiled on whole genome human microarrays, as well as on microarrays containing all known human microRNAs. Validation of mRNA and microRNA hits was performed by RT-PCR. siRNA was used to knock down these mRNA and miRNAs and changes in sensitivity to PCI-24781 as well as in the gene expression profiles were analyzed.
Results: In metastatic primary tumors from heavily pretreated patients, about 38% of the tumors could be classified as resistant to PCI-24781. From the mRNA profiles in the primary tumors resistance markers was identified and validated in a second independent set of primary tumors by RT-PCR. siRNA knockdown of resistant markers sensitized the cells to PCI-24781. From the miRNA profiles, a predictive signature consisting of 6 miRNAs was obtained, two of were also found to be differentially expressed in a separate analysis of colorectal tumor lines. siRNA knockdown of these miRNAs influenced the mRNA expression profile and sensitivity to PCI-24781.
Conclusion: Predicitive mRNA and miRNA markers of resistance to the HDAC inhibitor PCI-24781 in primary human CRC tumors have been developed. Some of these mRNA and miRNAs were shown to be functionally important in the mechanism of action of PCI-24781 and may be useful as predictive markers for patient stratification in clinical trials.

PP27

Expression of microRNA-221 is progressively reduced in aggressive prostate cancer and metastasis and predicts clinical recurrence

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Background: Emerging evidence shows that microRNAs (miR) are involved in the pathogenesis of a variety of cancers, including prostate carcinoma. Little information is available regarding miR expression levels in lymph node metastasis of prostate cancer or the potential of miRs as prognostic markers in this disease. Therefore, we analyzed miR signatures in prostate carcinoma metastasis and studied the role of miR-221 as a novel prognostic marker in prostate cancer.
Materials and Methods: We analysed the global expression of miRs in benign and hyperplastic prostate tissue (BPH), primary prostate carcinoma (PCA), and corresponding metastatic tissues by micro-array analysis. Ninety two samples of radical prostatectomies were subsequently investigated by qRT-PCR to validate the associations between the expression of miR-221, various clinicopathologic factors, and patient survival.
Results: Consistent with the proposal that some microRNAs are oncomirs, we found aberrant expression of several miRs, including the down-regulation of miR-221, in prostate carcinoma metastasis. In a large study cohort, the miR-221 oncomir was progressively down-regulated in aggressive forms of prostate carcinoma. Down-regulation of miR-221 was associated with clinicopathological parameters, including the Gleason score and the clinical recurrence during follow up. Kaplan Meier estimates

and Cox proportional hazard models showed that miR-221 down-regulation was linked to tumor progression and recurrence.
Conclusion: Our results suggest that progressive miR1-221 down-regulation is a hallmark of metastasis and a novel prognostic marker in prostate carcinoma. This suggests that miR-221 has potential as a diagnostic marker and therapeutic target.

PP59

Prognostic significance of αB-crystallin, vimentin and HSP 27 association in primary breast cancer

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Background: αB-crystallin is a heat shock protein, which function as stress-induced molecular chaperones to inhibit the aggregation of denatured proteins. Previous studies have identified αB-crystallin as a marker of poor prognosis for breast cancer and have suggested that it is an excellent marker for tumours of basal origin. We have considered that αB-crystallin binding proteins, vimentin and HSP27 also show a similar association with poor prognosis.
Materials and Methods: Tissue Micro Arrays of 0.6 mm cores of 246 breast cancers were stained with antibodies to αB-crystallin, vimentin, HSP27 (antibody ERD5) and HSP27 82P and scored using the Quick Score Method. The results stored with the Aperio Pathology Database were then subsequently compared with clinical and pathological parameters.
Results: Expression of αB-crystallin was associated with vimentin [$P < 0.001$ Fishers exact test (FET)]. αB-crystallin expression was linked to a low expression of the estrogen receptor and reduced survival ($P < 0.001$ (FET), $P = 0.002$ Kaplan Meier Log Rank (KM) respectively). Vimentin expression was associated with estrogen receptor (ER) negative cancers and poor survival ($P < 0.001$ (FET), $P = 0.002$ (KM Log Rank) respectively). In contrast to αB-crystallin, low expression of HSP27 was associated with low ER and progesterone receptor (PGR).
Conclusion: Increased expression of the protein chaperon, αB-crystallin and its binding partner, vimentin were linked to reduced survival. A similar association was not found for HSP27 expression. The potential functional significance of this interaction for metastasis will be discussed in the context of other predictive markers for breast cancer.

PP118

Detection and quantification of EGF receptor phosphorylation in formalin-fixed tumor sections by selected/multiple reaction monitoring mass spectrometry

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Background: The epidermal growth factor receptor (EGFR) is a drug target in several cancers, but suffers from a lack of molecular biomarkers to facilitate the selection, monitoring and dosing of patients. Mass spectrometry (MS) has emerged as a sensitive method to track not only the EGFR, but to monitor specifically sites of phosphotyrosine (pY) on the EGFR and the protein components of its signaling network that may serve as biomarkers of EGFR expression and activity. Major challenges in the development and application of MS as a means to discover and assay biomarkers, and in particular phosphorylation-type protein features related to drug target modulation, include (i) the preservation of protein-phosphorylation in patient samples, and (ii) the detection and quantification of such features in minute, heterogeneous patient samples. To address these challenges we have combined Liquid Tissue technology, which enables solubilization of protein from cells obtained by laser microdissection of formalin fixed patient samples, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation.
Materials and Methods: Liquid Tissue technology was used to solubilize protein from formalin fixed tissue samples. Solubilized, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation. This approach was applied to measure features of the EGFR network in formalin fixed tissue culture cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples.
Results: EGFR peptides were measured by direct SRM/MRM analysis of trypsin-digested, liquefied samples from formalin fixed cultured cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples. Enrichment of phosphorylated peptides by using titanium dioxide resins enabled the measurement of EGFR phosphorylation sites reflecting activated EGFR.
Conclusion: These results provide proof of concept for a robust approach to monitor in tumors the EGFR and other phosphorylation-associated drug targets and biomarkers, and which may offer superior dynamic range and quantification over traditional immunohistochemistry-based methods.

PP94

The Chernobyl Tissue Bank − a model for integrating "omics" research on single blocks of tissue

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Background: The Chernobyl Tissue Bank (CTB) was established in 1998 to collect, store and distribute biological samples from patients resident in the regions of Ukraine and Russia contaminated by fallout from the Chernobyl accident and who developed thyroid cancer. Patients give generic (broad) informed consent for thyroid cancer research; access to biomaterials is approved by an external review panel. A sample of blood for extraction of DNA, serum and samples of both frozen and formalin fixed paraffin embedded (FFPE) tumour and normal thyroid tissue are provided from each patient.
Materials and Methods: The current collection includes 2493 cases of thyroid cancer and adenoma. RNA and DNA are extracted from the same frozen tissue block and are distributed to researchers in aliquots of 5 μg (RNA) and 3 μg (DNA), permitting multiple projects to have access to material from the same block of tissue. A frozen section is taken from each block prior to extraction and the relative proportions of epithelial, stromal, lymphoid cells are assessed. Quality assurance (QA) is carried out by Aglient Bioanlyser (RNA–RIN) and 10 kb gel (DNA), enabling samples only of the highest quality to be provided to projects that require this e.g. Affymetrix 3′ array.
Results: A recent QA audit showed that the average RIN was 8.5 (range 6.4−9.4). There was no significant degradation over a 10 year period of storage as a frozen block prior to extraction. 1631 aliquots of RNA and 703 of DNA from tissue, 136 aliquots of DNA from blood and 5921 sections from FFPE blocks have been issued to researchers worldwide. The research projects supported by the CTB range from single gene investigations to complex projects using a variety of array based platforms. One example is Genrisk-T, an EC funded project is currently combining mRNA array, bac array (on RNA and DNA extracted from a single frozen tissue block) and germline SNP data with miRNA (from FFPE material of the same case) and clinicopathological data on an age-matched series of 50 patients who were exposed to radiation and 50 who were born after 1/1/87 and have developed spontaneous thyroid cancer at a young age. The aim of the study is to identify radiation related changes and novel genes in thyroid cancer.
Conclusion: The CTB is being used by others (e.g. the Wales Cancer Bank) and by clinical trial groups as a paradigm for a tissue bank to support integrated "omics" research on other tumour types.

PP85

Genotyping of microsatellite alterations and EGFR somatic mutations in exhaled breath condensate of NSCLC patients

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Background: A common goal in treatment of NSCLC patients is to individualize genetic and epigenetic events which can be used as early diagnostic marker and which could be easily and time-saving investigated. We recently demonstrated the possibility of studying microsatellite alterations (MAs) in the DNA of exhaled breath condensate (EBC). The aim of the present study was to verify whether MA analyzed in DNA from EBC can be used to detect tumor susceptibility in high risk subjects studying microsatellite alterations and whether it can be useful to detect EGFR more common mutations in lung cancer.
Materials and Methods: 59 subjects entered the study: 41 with NSCLC and 18 with non-neoplastic diseases. All subjects underwent allelotyping on DNA from whole blood, EBC, and lung tissue removed for histologic diagnosis by analyzing a panel of five microsatellites (D3S2338, D3S1266, D3S1300, D3S1304, D3S1289) located in chromosomal region 3p. Among the overall series, 23 patients were also investigated for EGFR mutations in exons 18−21 on DNA from EBC and paraffin embedded tumor tissues.
Results: MAs in DNA from tumor tissues and EBC of each patient with cancer presented an overlapping profile of loss of heterozygosity (26%)