for bladder cancer available in clinics. UCA1 (urothelial cancer associated 1 gene) is a non-protein-coding RNA reported as up-regulated in bladder carcinoma, influencing cell growth and promoting invasion. It is a very sensitive and specific unique marker for bladder cancer. A growing body of data suggests the outstanding clinical utility of early non-invasive molecular diagnostics. In this research, we report the setting up and validation of UCA1 identification in urine samples, along with its successful use in clinical diagnosis.
Materials and Methods: We used a standard RT-PCR analysis with specifically designed primers for UCA1 identification in routinely obtained urinary samples. Validation assays were based on the assessment of TBP (TATA Box Binding Protein) expression. Primer and test specificity were demonstrated by sequencing of amplified UCA1 and TBP fragments.
Results: Test specificity was assessed on 20 negative samples (with no tumor cells detected by standard cytology evaluation) and perfect matching (100% of correlation) was obtained (i.e. no UCA1 expression in urinary samples devoid of tumor cells). Test repeatability and reproducibility were demonstrated on matching independent triplicates from samples with both high and low UCA1 expression. The assay sensibility was demonstrated by correlation of the results of UCA1 expression (samples with high and low UCA1 expression) with the results obtained by standard microscopy diagnosis. Finally, 30 samples were compared by the standard cytology approach with the results obtained with the RT-PCR-based method. Four parameters were assessed for concordance: sensitivity (concordance between two tests: 100%), specificity (67%), positive predictive value (75%) and negative predictive value (100%).
Conclusion: Our results demonstrated very good correlation of this non-invasive assay with the widely used invasive cytology analysis evidencing thus the reliability and interest of use of UCA1 testing for urothelial cancer diagnosis. Our ongoing large-scale study will (i) help in better understanding the clinical signification of low UCA1 expressing samples, (ii) enable validation of potential use of this assay for predicting of bladder cancer recurrence and (iii) support to further acknowledge the standardization of this diagnostic approach.

PP24

CXCR4: a predictive marker of bone metastases in breast cancer patients

E. Sacanna, T. Ibrahim, M. Gaudio, L. Mercatali, R. Ricci, E. Scarpi, M. Ricci, P. Serra, D. Amadori. *Osteo-Oncology Center, I.R.S.T., Italy*

Background: CXCR4, a chemokine cell receptor, is expressed by several tumor histotypes and, together with its ligand CXCL12, is involved in tumor growth, angiogenesis, and homing of cancer cells to distant sites. Our objective was to investigate the possible predictive role of this biological marker in bone metastatization in breast cancer patients.
Materials and Methods: CXCR4 expression was evaluated by immunohistochemical staining in paraffin-embedded tissue sections of primary breast cancers from 40 individuals: 11 disease-free (DS) at 110 months (83–138), median age 61 years (range 48−78) and 29 with relapsed disease, median age 67 years (range 42−87). In the latter group, 10 had visceral metastases (VM), median age 68 years (range 52−86) and 19 had bone metastases (BM), median age 66 years (range 42−87).
Results: CXCR4 was detected in the cytoplasm and/or nucleus of tumor cells. 13% of all samples showed strong nuclear staining and 25% strong cytoplasmic staining. In particular, cytoplasmic expression was observed in 9% of samples from DF patients, in none of the samples from those with VM ($p = 0.048$), and in 47% of sections from BM patients ($p = 0.011$). Considering either nuclear or cytoplasmic CXCR4 expression, sensitivity was observed in 18% of DF patients, 10% of VM patients (n.s.) and 53% of BM patients ($p = 0.044$). However, no relation was observed between CXCR4 expression and disease-free or overall survival in the last subgroup.
Conclusion: Our preliminary results suggest that cytoplasmic CXCR4 expression in the primary tumor could be a predictive marker of bone metastases in breast cancer patients. A larger study is ongoing to confirm these results.

PP33

Functional characterization of CYP2C8 promoter polymorphisms

V. Selvarajan. *National Cancer Centre, Singapore*

Background: The objective of the present study was (1) to determine polymorphic variations in the CYP2C8 gene in three distinct healthy Asian subjects (Chinese: N = 101, Malay: N = 91 and Indian: N = 90, populations), and (2) to functionally characterize high frequency CYP2C8 promoter polymorphisms.
Materials and Methods: Screening for genetic variations in the promoter, exons and exon-intron junctions of CYP2C8 gene was performed by PCR followed by direct DNA sequencing. Functional characterization of promoter polymorphisms were studied by using various combinations of plasmid constructs containing the identified promoter polymorphic variants. The different constructs were cloned in pGL3 expression vector and investigated for their activity in driving reporter gene expression in transfected HepG2 cells under optimized conditions.
Results: Seven polymorphisms were identified and their allelic frequencies were as follows: 5′-UTR: g.−411C>T (C:0.33;T:0.67), g.−370T>G (T:0.72;G:0.28) and g.−271C>A (C:0.88;A:0.12); intron 2: I.−64A>G (A:0.50;G:0.50), −13insT (Wt:0.87;insT:0.13); intron 7: +49T>A (T:0.47;A:0.53) and 3′-UTR: 24C>T (C:0.62;T:0.38). Haplotype analysis revealed fourteen different haplotypes in Chinese, eighteen in Malays, and twenty one in the Indian population. Two haplotype blocks were inferred in each ethnic group based on the solid-spline algorithm. The promoter construct harboring the single g.−411C>T variant showed approximately 2-fold higher luciferase activity compared with the reference construct ($P = 0.002$). The construct harboring the combined g.−411C>T and g.−271C>A polymorphic variants showed a severe reduction (44-fold) in luciferase activity compared with the construct containing the reference sequence ($P = 0.006$).
Conclusion: Future studies should be done to investigate the influence of CYP2C8 g.−411C>T and g.−271C>A polymorphisms on the disposition CYP2C8 drug substrates.

PP89

Upregulated p38 MAPK signaling in circulating pancreatic cancer cells

G. Sergeant[1], R. van Eijsden[2], J. Allemeersch[2], V. Van Duppen[3], R. Aers[1], T. Roskams[4], N. Ectors[4], P. Van Hummelen[2], J. Van Pelt[5], B. Topal[1]. *[1]Department of Abdominal Surgery, University Hospital Leuven, Belgium; [2]MicroArray Facility, VIB, Leuven, Belgium; [3]Department of Experimental Hematology, University Hospital Leuven, Belgium; [4]Department of Pathology, University Hospital Leuven, Belgium; [5]Department of Hepatology, University Hospital Leuven, Belgium*

Background: Hematogenous cancer cell dissemination is the most important route of metastasis in pancreatic ductal adenocarcinoma. Our aim was to identify gene expression profiles of circulating tumor cells (CTC) immediately after resection of the primary tumor.
Materials and Methods: CTC were isolated from whole blood by density centrifugation (Oncoquick®) followed by negative selection fluorescence activated cell sorting combining a dump channel, anti-CD45, anti-CD34 and 7-AAD viability staining to exclude all hematologic (G) and non-viable cells. Four subgroups were obtained for each patient: two sorted fractions (CTC and G), the original tumor (T) and non-tumor pancreatic control tissue (P). RNA was isolated from all samples. After double linear amplification of RNA, microarrays (whole genome affymetrix genechip HG-U133_Plus_2) were run. The robust multi-array (RMA) analysis was run on the probes that had at least 4 out of 6 detection calls. On this list of probe sets, a filter was applied selecting genes a 2-fold up- or down-regulation in the comparisons of CTC versus T, AND in CTC versus P, AND CTC versus G, using uncorrected p-values ($p < 0.001$). Resulting data were analyzed with 'Ingenuity Pathways Analysis' software.
Results: In 6/10 patients the samples from all four subgroups reached the RNA quality standards set for microarray analysis. From 46,467 probes a set of 8,152 probe sets were retained. After application of the filter, 1,059 probe sets were retained, of which 572 were eligible for function and pathway analysis. Most molecules were involved in genetic diseases, inflammatory response, cancer, cell-to-cell signaling and cellular movement. The pathway with the highest ratio of molecules that met cut-off criteria was p38 MAPK signaling. In this pathway transforming growth factor beta 1 (TGFβ1) and MYC associated factor X (MAX) were significantly upregulated in the CTC fraction compared to the T, P and G groups. S100A8 was found to be strongly upregulated in CTC.
Conclusion: Gene expression profiles can be obtained from circulating tumor cells without a priori selection markers. S100A8, TGFβ1 and MAX are upregulated in CTC of patients with PDAC. These genes are involved in p38 MAPK signaling which is responsible for increased CTC motility.

PP67

Early alpha-fetoprotein response predicts treatment efficacy of anti-angiogenic therapy in combination with metronomic chemotherapy for advanced hepatocellular carcinoma

Y.-Y. Shao[1], Z.-Z. Lin[2], C. Hsu[2], Y.-C. Shen[2], C.-H. Hsu[2], A.-L. Cheng[2]. *[1]National Taiwan University Hospital, Yunlin Branch, Taiwan; [2]National Taiwan University Hospital, Taiwan*

Background: Sorafenib and other molecular-targeted agents with anti-angiogenic activity have shown moderate clinical benefit in patients with advanced hepatocellular carcinoma (HCC). However, the biomarkers

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.
The study was supported by the grant of NSC 98-3112-B-002-038.

PP35

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

S. Siddiqui[1], Y.-H. Hsiao[2], W. Wang[3], Y.-g. Man[4]. *[1]Pathology Graduate, Howard University Hospital, USA; [2] 2Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; [3]China-Japan Union Hospital, Jilin University, China; [4]Armed Forces Institute of Pathology and American Registry of Pathology, USA*

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.
Supported in part by grant 2006CB910505 from the Ministry of Chinese Science and Technology Department, grants DAMD17-01-1-0129, DAMD17-01-1-0130, PC051308 from Congressionally Directed Medical Research Programs, grant BCTR0706983 from The Susan G. Komen Breast Cancer Foundation, and grant 2008-02 from US Military Cancer Institute and Henry M. Jackson Foundation.

PP95

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

M. Sirisawad[1], L. Tee[2], H. Mamuszka[2], J.J. Buggy[1]. *[1]Pharmacyclics, USA; [2]Exiqon, USA*

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

M. Spahn[1], S. Kneitz[2], C.-J. Scholz[2], N. Stenger[3], T. Rüdiger[4], P. Ströbel[5], H. Riedmiller[6], B. Kneitz[6]. *[1]Department of Urology and Pediatric Urology, University Hospital Würzburg, Germany; [2]Microarray Core Unit, IZKF (Interdisciplinary Center for Clinical Research), University of Würzburg, Germany; [3]Department of Urology and Paediatric Urology, University Hospital Würzburg, Germany; [4]Institute of Pathology, Community Hospital Karlsruhe, Germany; [5]Institute of Pathology, University Medical Center Mannheim, University of Heidelberg, Germany; [6]1. Department of Urology and Paediatric Urology, University Hospital Würzburg, Germany*

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