Materials and Methods: We quantified levels of 59 plasma analytes using multiplexed immunoassays in patients with NSCLC (225 men, 114 women), asthma (AST; 67 men, 112 women) and normal controls (NOR; 122 men, 165 women) and used a support vector machine (SVM) to analyze the complete data (N = 791) after a random partition into training (N = 402) and test (N = 389) data sets.
Results: We developed seven SVM models that classified subjects to NSCLC, AST or NOR: 1) 59 biomarkers, both genders for NSCLC, AST and NOR, 2) 59 biomarkers, both genders for NSCLC and NOR, 3) best subset of 4 variables for NSCLC and NOR, 4) 59 biomarkers for males; examined for NSCLC and NOR in both genders or males only, 5) Best subset of 5 variables selected from 59 biomarkers, males only; examined in NSCLC and NOR, males and females or males only, 6) 59 biomarkers, females only; examined in NSCLC and NOR, males and females or females only, and 7) best subset of 3 variables selected from 59 biomarkers, females only; examined in NSCLC and NOR males and females or females only. When SVM classified subjects to NSCLC, AST or NOR, 7 biomarkers in the best reduced model (I-TAC, MMP-7, HGF, MMP-8, IL-2, MIP-1β, IL-4) had an accuracy of 0.9 (SE: 0.015). Restricting to NSCLC versus NOR produced 4 markers [EGF, sCD40 ligand, IL-8 and MMP-8; sensitivity 0.93 (0.014), specificity 0.87 (0.02)]. Stratifying on genders [males: EGF, IL-8, sFAS, MMP-9 and PAI-1, females: EGF, sCD40 ligand], yielded the sensitivity and specificity of 1 (0).
Conclusions: The study identified biomarkers and combinations thereof useful in diagnosing lung diseases such as NSCLC. We developed a method for mining test data that comprise a plurality of biomarker measures for the subset of biomarkers in a human test subject and evaluating the test data using the electronic representation of the trained SVM and outputting a classification of the human test subject based on the evaluating step. The method is widely applicable to development of test kits comprising agents for detecting biomarkers and combination of biomarkers.

1425 POSTER

Effect of Preoperative Neutrophil-lymphocyte Ratio on the Surgical Outcomes of Middle and Lower Bile Duct Carcinoma

Y. Iso[1], M. Ishizuka[1], T. Sawada[1], M. Kato[1], J. Kita[1], M. Shimoda[1], K. Kubota[1]. *[1]Dokkyo Medical University, Gastroenterological Surgery, Tochigi, Japan*

Background: The current standard of treatment for the middle and lower bile duct carcinoma (MLBDC) remains surgical resection as no alternative effective treatment exists. But if resected, the long-term prognosis is poor. The simple biomarkers to predict response or toxicity have not been identified, which are applicable to all community oncology settings worldwide. The use of inflammatory markers based on differential white-cell counts, such as the neutrophil/lymphocyte ratio (NLR), may be simple and readily available biomarkers. This study aimed to determine whether the NLR is a predictor of surgical outcomes in patients with MLBDC.
Materials and Methods: We enrolled 70 MLBDC patients who had undergone pancreatoduodenectomy (PD) at a single institution between April 2000 and March 2011. In 10 patients, PD with extended hepatectomy was performed because carcinoma invaded hepatic hilus. And 5 patients underwent PD with portal vein resection due to portal invasion.
Results: Of these 70 patients, 45 (64.3%) patients had a normal NLR and 25 (35.7%) had an elevated NLR (NLR > 5). Patients with an elevated NLR had a significantly worse overall survival (OS) than did patients with a normal NLR. Cox regression analysis revealed that elevated NLR was an independent predictor of OS (P = 0.01).
Conclusions: An elevated NLR is an independent predictor of OS in patients with MLBDC. Preoperative NLR measurement in MLBDC patients may be a simple method for identifying patients with a poor prognosis who can be enrolled in further trials of surgical resection.

1426 POSTER

Circulating Tumour Cells: a Valuable New Tool to Monitor the Clinical Course of Patients With Epithelial Neoplasms in the Routine Setting

C.M. Kurbacher[1], M.E. Schmidt[2], P.N. Arenz[3], W.J. Nagel[4], R. Stark[4], H. Hanitzsch[4], J.A. Kurbacher[5]. *[1]Medical Centre Bonn-Friedensplatz, Gynecologic Oncology, Bonn, [2]Medical Centre Bonn-Friedensplatz, Urologic Oncology, Bonn, [3]Medical Centre Bonn-Friedensplatz, Medical Controlling, Bonn, [4]Medical Centre Bonn-Friedensplatz, General Urology, Bonn, [5]Medical Centre Bonn-Friedensplatz, Gynecology and Obstetrics, Bonn, Germany*

Background: Circulating tumour cells (CTC) in the peripheral blood of cancer patients (pts) are an indicator of a poor prognosis and have also been successfully used to monitor therapy (Tx). Currently, the CellSearch™ system (CS; Veridex, Raritan, NJ, USA) is the only FDA-approved technique for CTC detection. Despite its prognostic and predictive merits gained in numerous trials, there are only few data existing elucidating the value of CS in the routine setting. We thus report on our single-institution experiences in the clinical use of CS in pts with various epithelial tumours.
Methods: A total of 394 samples have been analyzed (breast cancer, BC: 266; prostate cancer, PC: 70; colorectal cancer, CRC: 10; ovarian cancer, OC: 25; others: 23). CTC-negativity and -positivity were distinguished using a threshold of >3 (CRC) or >5 CTCs (all others) in 7.5 mL venous blood. 35 endocrine, cyctostatic, immunologic, or combined Tx in BC (n = 23), OC (n = 7), and PC (n = 3) were monitored by CS performed prior to and 6−8 weeks after Tx initiation. The first radiologic re-evaluation was performed 12 weeks after start of Tx and repeated every 3 months, if indicated. The response status was scored according to RECIST.
Results: In all but 4 cases (all BC), CS was considered as evaluable, resulting in an assay success rate of 99%. 19 BCs (7.1%) had a 1−5 CTCs, and 21 (7.9%) had >5 CTCs. The corresponding results were 4 (5.7%) and 10 (14.3%) for PC and 4 (16%) and 0 (0%) for OC. In 35 pts monitored by CS, 19 progessed while 16 did not progress on Tx. All progression-free pts showed constantly normal or declining CTC values. In only one pt, the CTC count did not drop into the normal range. In contrast, 13 of 19 pts showing disease progression had increasing CTC counts. Moreover 3 pts with a pathological CS did not normalize while being on Tx. Notably a CTC within the normal range indicated progression in 2 cases whereas a decrease within the normal range was associated with response to Tx in 4 pts.
Conclusions: CS is a valuable and robust tool to determine CTCs in the peripheral blood of pts with various epithelial malinancies in the routine setting. Contrasting its high specifity compared to other methods the sensitivity of CS is relatively low which may result in a considerable number of false-negative measures. When regarding our own experiences, we thus conclude that the occurrence of any CTC detected by CS must be taken seriously.

1427 POSTER

Fully Automated Molecular Diagnostic System for Personalized Therapy on Colorectal Cancer

S. Kitano[1], J. Myers[2], M. Ratain[2], W. Liu[2], M. Amano[1]. *[1]Toppan Printing Co. Ltd., Life Science Research Laboratory, Chiba, Japan; [2]University of Chicago, Department of Medicine, Chicago, USA*

Background: *KRAS*, *BRAF,* and *PIK3CA* mutations are strong molecular predictors for efficacy of cetuximab and panitumumab in colorectal cancer (CRC). We have developed a novel, simple, sensitive and fully automated DNA mutation detection system (Toppan Genotyping Analyzer, TGA) based on Invader Plus® technology. This system includes the DNA extraction process from blood and frozen tissue. Here we report the feasibility study of our system, comparing it to direct sequencing (DS) in the detection of *KRAS*, *BRAF* and *PIK3CA* mutations.
Material and Methods: Assays were set up using plasmids containing major *KRAS* (G12A, G12C, G12D, G12R, G12S, G12V and G13D), *BRAF* (V600E) and *PIK3CA* (E542K, E545K, E545G, H1047L and H1047R) mutations. Sensitivity and accuracy of the detection method were evaluated with plasmids and cancer cell lines with *KRAS* or *BRAF* mutations. DNA samples were extracted from frozen (n = 70) and formalin fixed, paraffin embedded (FFPE) samples (n = 70). Also, the frozen tissue samples were tested for fully automated extraction-detection procedure.
Results: Sensitivity and accuracy assays showed that the TGA system can detect mutations at a 5% level. The chip-based assay system allows for simultaneous analysis of 23 mutations in one hour (including the PCR process). The comparison results between TGA and DS are shown in Table. All *KRAS*, *BRAF* and *PIK3CA* mutations detected by DS in both frozen (total number of mutation, n = 33) and FFPE (n = 27) samples were also successfully (100%) detected by the TGA. In the samples shown to be wild-types by DS, however, the TGA was able to detect additional mutants in the frozen (n = 7) and FFPE (n = 10) samples. In addition, TGA was able to detect *KRAS* mutations directly from crashing rice-grain sized CRC frozen tissue.

Table: Frequency of *KRAS*, *BRAF* and *PI3K* mutations.

	Frozen		FFPE	
	DS	TGA	DS	TGA
KRAS	27/70 (38.6%)	26/70 (37.1%)	28/70 (40.0%)	21/70 (30.0%)
BRAF	3/70 (4.3%)	2/70 (2.9%)	1/70 (1.4%)	1/70 (1.4%)
PIK3CA	10/70 (14.3%)	5/70 (7.1%)	8/70 (11.4%)	5/70 (7.1%)

Conclusions: In terms of detection of *KRAS*, *BRAF* and *PIK3CA* mutations, TGA is a highly sensitive and accurate system compared to DS. It also possesses several other advantages including its all-in-one chip reaction, simple procedure and excellent reproducibility. The versatility in detecting mutations in DNA samples with different fixative forms as well as

frozen tissue samples makes the TGA a useful system that can be widely applied in both diagnosis and research areas. We hypothesize that TGA is superior to DS for predicting efficacy of molecularly targeted agents, since it has greater sensitivity for detection of mutations associated with drug resistance.

1428 POSTER

Evaluation of EpCAM Protein Expression in Human Cancers as Therapeutic Target for Catumaxomab Treatment

G. Spizzo[1], D. Fong[1], M. Wurm[2], C. Ensinger[3], P. Obrist[4], C. Hofer[3], G. Mazzoleni[5], G. Gastl[6], P. Went[7]. *[1]Ospedale Franz Tappeiner, Onco-Haematologic Day Hospital, Merano, Italy; [2]Oncotyrol GmbH, Division of Haematology and Oncology, Innsbruck, Austria; [3]Pathology, Department of Pathology, Innsbruck, [4]Pathology Zams, General Hospital Zams, Zams, Austria; [5]Pathology, General Hospital Bolzano, Bolzano, Italy; [6]Haematology and Oncology, Department for Internal Medicine, Innsbruck, Austria; [7]Pathology, Department of Pathology Zürich, Zürich, Switzerland*

Introduction: The Epithelial Cell Adhesion Molecule (EpCAM) is a cell surface protein with oncogenic features expressed on healthy human epithelia and corresponding malignant tumours. Our work group recently explored molecular changes following EpCAM overexpression in commercially available cancer cell lines. For the first time we could show that EpCAM overexpression was associated with the downregulation of the Wnt signaling inhibitors. Moreover, an increase of proliferation and chemosensitivity to Docetaxel was associated with EpCAM overexpression. Similarly, in the clinical setting we observed that EpCAM overexpression detected by immunhistochemistry was associated with a poor prognosis in breast cancer patients and other tumour entities. These observations have promoted EpCAM to a 'druggable' target. As such, Catumaxomab was approved by the European Union for intraperitoneal use in patients with EpCAM-expressing cancer. However, no consensus exists on how and when to evaluate EpCAM expression in these cancer patients.
Material and Methods: EpCAM expression was assessed by a well established immunohistochemical staining protocol in 2291 primary tumour tissues and in 108 metastases using the EpCAM-specific antibody clone VU1D9. A total immunostaining score (TIS) was calculated as the product of a proportion score and an intensity score. Four expression subgroups (no, weak, moderate and intense) were defined. As described previously, the term 'EpCAM overexpression' was reserved for tissues showing a TIS-value >4.
Results: EpCAM was highly expressed in most tumours of gastrointestinal origin and in some carcinomas of the genito-urinary tract. However, hepatocellular carcinomas, clear cell renal cell cancer, urothelial cancer and squamous cell cancers are frequently EpCAM negative. EpCAM expression in breast cancer depends on the histological subtype, as lobular histology shows usually no or weak expression. Most metastases and particularly peritoneal lesions are EpCAM positive and they frequently reflect the expression phenotype of the primary tumour.
Conclusion: EpCAM expression is detected on adenocarcinomas of various primary sites. If EpCAM-specific antibodies (such as Catumaxomab) are intended to be used in cancer patients, we recommend prior immunohistochemical evaluation of EpCAM expression particularly in patients with renal cell cancer, hepatocellular carcinoma, urothelial carcinoma, breast cancer and squamous cell carcinomas.

1429 POSTER

FCGRIIa-131 and FCGRIIIa-158 Polymorphisms − Distribution and Clinical Outcomes of Cetuximab-based Chemotherapy in Japanese Patients With Metastatic Colorectal Cancer (mCRC)

K. Yamazaki[1], H. Fukushima[2], T. Yoshino[3], T. Nishina[4], S. Yuki[2], S. Kadowaki[5], E. Shinozaki[6], T. Yokota[7], S. Kajiura[8], T. Yamanaka[9]. *[1]Shizuoka Cancer Center, Division of Gastrointestinal Oncology and Endoscopy, Shizuoka, [2]Hokkaido University Graduate School of Medicine, Department of Gastroenterology, Sapporo, [3]National Cancer Center Hospital East, Department of Gastroenterology and GI Oncology, Chiba, [4]National Hospital Organization Shikoku Cancer Center, Department of Gastroenterology, Matsuyama, [5]Saitama Cancer Center, Division of Gastroenterology, Saitama, [6]Cancer Institute Hospital of Japanese Foundation for Cancer Research, Department of Medical Oncology, Tokyo, [7]Aichi Cancer Center Hospital, Department of Clinical Oncology, Aichi, [8]Graduate School of Medicine and Pharmaceutical Science University of Toyama, Department of Gastroenterology and Hematology, Toyama, [9]National Kyushu Cancer Center, Institute for Clinical Research, Fukuoka, Japan*

Background: Polymorphisms in fragment C receptor (FCGR) are expected as a predictive biomarker of cetuximab (Cmab). Previous studies have convincingly confirmed the distributions (dists) of FCGR polymorphisms in Western population and shown the existence of linkage disequilibrium (LD) between FCGRIIa and FCGRIIIa polymorphisms. Meanwhile, the dists in Asian population have been unknown but a few studies for non-cancer patients have suggested the difference in dists between Asian and Western populations. We investigated the dists of FCGR polymorphisms and their association with clinical outcomes of Cmab based chemotherapy in Japanese mCRC patients.
Materials and Methods: Ninety-three patients with irinotecan/oxaliplatin/5-FU-refractory mCRC and treated by Cmab plus irinotecan or Cmab monotherapy were retrospectively registered from 8 centers in Japan. FCGR polymorphisms were determined from genomic DNA extracted from peripheral blood samples based on the Multiplex allele-specific PCR method. Comparisons according to FCGR polymorphisms were evaluated using Fisher's exact test for response rate (RR) and log-rank test for progression-free survival (PFS) and overall survival (OS) curves.
Results: The dists of FCGRIIa HH/HR/RR and FCGRIIIa VV/VF/FF were 68/30/2% and 4/40/56%, respectively (Table). The absence of LD between FCGRIIa and FCGRIIIa polymorphisms was confirmed (GENEPOP, $p = 0.526$; Linkdis, $p = 0.146$). Of 74 patients with KRAS wild-type and treated by Cmab plus irinotecan, no difference according to FCGR polymorphisms was observed in either RR (IIa: HH 37% vs. HR/RR 36%, p=1.00; IIIa: VV/VF 39% vs. FF 35%, $p = 0.81$) or PFS curves (IIa: HH vs. HR/RR, $p = 0.60$; IIIa: VV/VF vs. FF, $p = 0.06$) or OS curves (IIa: HH vs. HR/RR, $p = 0.65$; IIIa: VV/VF vs. FF, $p = 0.30$).
Conclusions: This study clarified an ethnic difference in the frequencies of FCGR polymorphisms. The polymorphisms did not influence the clinical outcomes of Cmab based chemotherapy in Japanese patients with mCRC.

FCGRIIa-131	FCGRIIIa-158			
	VV	VF	FF	Total
HH	3	28	32	63 (68%)
HR	1	9	18	28 (30%)
RR	0	0	2	2 (2%)
Total	4 (4%)	37 (40%)	52 (56%)	93

1430 POSTER

Quantitative Analysis of PTEN-dependent Glycoprotein Patterns Reveals Predictive Biomarker Signature for Response of Human Patients to Docetaxel Therapy in Metastatic Castration Resistant Prostate Cancer (mCRPC)

M. Kälin[1], I.L. Cima[2], R. Schiess[3], P.J. Wild[4], N. Fankhauser[2], A. Templeton[1], R. Aebersold[3], W. Krek[2], T. Cerny[1], S. Gillessen[1]. *[1]Kantonsspital St. Gallen, Medical Oncology, St. Gallen, [2]ETH Zürich, Institute of Cell Biology, Zürich, [3]ETH Zürich, Institute of Molecular Systems Biology, Zürich, [4]University Hospital Zürich, Department of Surgical Pathology, Zürich, Switzerland*

Background: Since 2004 chemotherapy with docetaxel has been the standard therapy in progressive mCRPC. Unfortunately only a subgroup of patients responds to this treatment. As rebiopsy is rarely done in mCRPC, predictive serum biomarkers for therapy response would be of great value. We recently presented a novel platform for human biomarker discovery and validation based on a large-scale quantitative analysis of *N*-linked glycoproteins of the Phosphatase and Tensin homolog (Pten) conditional knockout mouse model for prostate cancer progression. This work delivered biomarker signatures for PTEN-status, Gleason sum and diagnosis in localized prostate cancer (Cima et al., PNAS 2011). This model has also revealed a prognostic biomarker signature in patients with mCRPC (manuscript submitted). To screen our biomarker set for factors for response to treatment with docetaxel in mCRPC patients seemed a reasonable step towards the vision of a personalized cancer medicine.
Methods: In serum samples from 40 patients with mCRPC who underwent chemotherapy with docetaxel we measured 13 proteins with ELISA and 66 different proteins by selected reaction monitoring (SRM) mass spectrometry. Random forest algorithm was applied to establish a multifactor signature predictive for response. Therapy response was defined as at least stable disease biochemically (PSA increase <25% over baseline) and by imaging after three cycles of therapy with docetaxel.
Results: Serum samples of 40 patients with mCRCP under chemotherapy with docetaxel were retrospectively analyzed. We identified four factors correlating significantly ($p < 0.05$) with therapy response in a univariate analysis. Additionally we performed a random forest analysis identifying combined predictive biomarker signatures. Intriguingly the serum concentration of two identified factors in combination significantly predicted whether patients with mCRPC responded to taxane therapy or not with an accuracy of 85% in a confusion matrix.