

**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-1 $\beta$ , 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.

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POSTER

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

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**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.

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POSTER

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

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**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<sup>TM</sup> 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, cytostatic, 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 progressed 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 malignancies in the routine setting. Contrasting its high specificity 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.

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POSTER

#### Fully Automated Molecular Diagnostic System for Personalized Therapy on Colorectal Cancer

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**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<sup>®</sup> 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