has not been established yet. The aim of this study was to determine that pyrosequencing (PSQ) might be used to achieve MGMT promoter methylation using one to thirteen year-old archival tissue samples as a clinical biomarker in routine practice.
**Material and Methods:** The study set included 141 formalin-fixed paraffin-embedded (FFPE) glial tumours from the archives of the pathology department from 1997 to 2010. Quantitative measurement of MGMT gene promoter DNA methylation employed PSQ of PCR products amplified from bisulfite converted DNA.
**Results:** PSQ data were obtained from all 141 samples. The mean of all cases was 14.0±16.8%, and methylated cases were 32.3±14.9%. A value of percentage of methylation (PM) of each year was not significantly different ($p = 0.771$) and didn't show any linear increasing or decreasing pattern according to the age of the FFPE block. Thirty one (41.3%) out of 75 GBM were methylated. Average PM of methylated and unmethylated cases were 35.8±14.7% and 3.2±1.8% respectively ($p < 0.001$). Eight (36.4%) out of 22 anaplastic astrocytoma were methylated,with 31.8±15.5% average PM. Eight (42.1%) out of 19 astrocytoma were methylated with 22.4±15.1% average PM. A tendency was observed toward an increasing pattern of average PM with WHO grade ($p = 0.063$) in astrocytic tumours. Anaplastic oligodendroglioma showed that 4 out of 7 cases (57.4%) were methylated and average PMs were 30.0±8.5% and 4.7±1.1% in methylated and unmethylated cases, respectively. A total of five out of eight cases (62.5%) of oligodendroglial tumour were methylated, and 28.0±8.1% and 4.7±1.2% were the respective average PM of methylated and unmethylated oligodendroglial tumours ($p = 0.024$). A correlation was observed between average PM and WHO grade ($p = 0.038$) and bimodal distribution between methylated and unmethylated cases, using 9% cut-off value ($p < 0.001$).
**Conclusions:** The study showed that a quantitative approach for MGMT promoter methylation gave better results than the classical gel-based methylation specific polymerase chain reaction reported by various researchers on FFPE tissue samples from old archives. The PSQ method can be used for a large scale retrospective trial, but cut-off value and calculation method of the PM should be validated.

Table 1. Methylation status of glial tumours

| | Methylated | | Unmethylated | | Total(%) | p value |
|---|---|---|---|---|---|---|
| | Cases, n (%) | Mean±SD | Cases, n (%) | Mean±SD | | |
| Glioblastoma | 31(41.3) | 35.8±14.7 | 44(58.7) | 3.2±1.8 | 75(100) | <0.001 |
| Anaplastic astrocytoma | 8(36.4) | 31.8±15.5 | 14(63.6) | 3.3±2.2 | 22(100) | <0.001 |
| Astrocytoma | 8(42.1) | 22.4±15.1 | 11(57.9) | 3.4±2.6 | 19(100) | 0.001 |
| Anaplastic oligodendroglioma | 4(57.1) | 30.0±8.5 | 3(42.9) | 4.7±1.1 | 7(100) | 0.005 |
| Oligodendroglioma | 1(100) | 20.0±0.0 | 0 | 0 | 1(100) | |
| Pilocytic astrocytoma | 0 | 0 | 9(100) | 3.0±1.3 | 9(100) | |
| ETC | 0 | 0 | 7(100) | 2.7±1.1 | 7(100) | |
| Total | 52 | 32.3±14.9 | 89 | 3.3±1.9 | | |

**1422** POSTER

**Observational Cohort Study of Plasma Levels of Biomarkers in Patients With Non-small Cell Lung Cancer Treated With Bevacizumab**

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**Background:** Antiangiogenic drugs have shown increased response rate and survival in NSCLC. No biomarkers have been described predictors of response in these patients (Pt). The aim of this study is to investigate the usefulness of the quantification of soluble VEGF, bFGF, E-selectin, and ICAM1 as biomarkers in advanced NSCLC Pt treated with Bevacizumab (BV).
**Material and Method:** This is an observational cohort study, concurrently, consecutively selected Pt. We included NSCLC Pt (squamous excluded), stageIV, treated with BV plus chemotherapy and without any previous antiangiogenic therapy. Plasma samples were collected before cycle 1 and after completion of cycle 2 (week 7). Levels of selected biomarkers were analyzed using commercially available ELISA kits (R&D Systems and Biolegend). We collected clinical and radiological data at the beginning, after the second cycle and at the end of treatment.
**Results:** We present preliminary results of 15Pt: media age was 56.9 (range 45−75; 8 male, 7 female). Basal ECOG: 0 = 9Pt; 1 = 5Pt; 2 = 1Pt. Histological subtypes were 7 adenocarcinomas, 5 large cell carcinomas and 3 not otherwise specified. Chemotherapy regimens used with BV were platinum-based doublet or monotherapy (Pemetrexed, Docetaxel) or Erlotinib. Only 4Pt presented mild adverse effects (2 hypertension; 1 bleeding; 1 thrombosis). Causes of end of BV were progression of disease (11Pt), adverse effect (2Pt), minor surgery (1Pt) and transfer to another hospital (1Pt). Measures of VEGF in the week 7 samples show consistently higher levels than baseline (207.2±52.5 vs 74.2±86.5 pg/mL). ICAM1 values also were slightly higher in the second sample (230.9±81.6 vs 209.8±63.4 ng/mL). However bFGF levels were lower in the second samples (32.3±16.5 vs 78.0±23.8 pg/mL). VEGF levels were different between Pt with clinical stability and clinical weakness (220.9±49.7 vs 152.2±7.1 pg/mL). These differences were also found in the radiological evaluation (214.5±53.1, stable vs 192.5±53.9 pg/mL, clinical weakness). At the end of the treatment, we also found differences in sICAM1 levels in both the clinical response (263.1±58.1, stable vs 142.6±75.2 ng/mL, weakness), and in the radiological evaluation (238.2±80.2, disease control vs 227.3±86.4 ng/mL, progression).
**Conclusions:** Changes in both VEGF and ICAM-1 levels could be used to monitor response to antiangiogenic treatment in NSCLC patients; in addition to standard clinical and radiological evaluations.

**1423** POSTER

**Polymorphism Analysis in the AVADO Randomised Phase III Trial of First-line Bevacizumab (BEV) Combined With Docetaxel in HER2-negative Metastatic Breast Cancer (mBC)**

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**Background:** In the E2100 trial, single nucleotide polymorphisms (SNPs) in the promoter region of VEGF (VEGF −2578, VEGF −1154) were reported to correlate with overall survival (OS) in BEV-treated patients (pts) with mBC [Schneider, JCO 2008]. In a retrospective analysis of the AViTA trial of BEV in pancreatic cancer, SNPs in the VEGFR-1 gene appeared to correlate with efficacy [Lambrechts, ESMO 2009]. We retrospectively analysed data from the BEV–docetaxel mBC AVADO trial [Miles, JCO 2010] to explore potential relationships between genetic variability in the VEGF signalling pathway and efficacy.
**Methods:** In AVADO, 736 pts with HER2-negative mBC were randomised to BEV 7.5 mg/kg, BEV 15 mg/kg or placebo (PLA), each combined with docetaxel 100 mg/m$^2$. The primary endpoint was progression-free survival (PFS). A panel of 26 candidate SNPs in genes involved in angiogenesis and tumourigenesis was evaluated in germ line DNA using kinetic PCR. Simple Cox regression analysis (no adjustment for multiple testing) was performed to correlate genotypes with PFS and OS.
**Results:** Demographics and efficacy in the genetics population (n = 336) were consistent with the overall study population. In the PLA group, the VEGF −2578 C/A polymorphism correlated with PFS: each additional C allele was associated with a 23% decrease in risk of progression or death (HR = 0.727, $p = 0.043$). With BEV 7.5 mg/kg, there was an indication of potential treatment by genotype interaction ($p = 0.02$). VEGF −1154 A/G also correlated with PFS (HR = 1.4, $p = 0.015$) but only in the BEV 7.5 mg/kg arm, with a non-significant treatment interaction. No other correlations were seen between efficacy and VEGF −2578, VEGF −1154, VEGFR-1 SNP rs9582036 or other SNPs.
**Discussion:** Although correlations between several SNPs and efficacy have been proposed in previous studies of BEV, we observed only a weak correlation between the VEGF −2578 SNP and PFS, driven by an effect in the PLA arm. Thus, our analysis of germ line DNA samples did not confirm findings from E2100. Likewise, our data do not confirm previous findings for VEGF and VEGFR-1 SNPs. Further biomarker research to identify pts most likely to benefit from BEV continues. AVADO (NCT00333775, sponsored by Roche) has completed accrual.

**1424** POSTER

**Methods of Identification and Diagnosis of Lung Cancer Using Classification Systems**

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**Background:** The goal of the study was to develop a blood test to detect non-small cell lung cancer (NSCLC) along with a robust statistical method for multimarker data mining.

**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**

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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.

**1426** 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™ 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**

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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® 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