**Conclusion:** We showed that MD is an essential procedure for *KRAS* testing by DS when samples show tumour areas less than 50 or 70%; in contrast, MD may not be necessary for the Luminex method.

**1143** POSTER

**KRAS Mutational Status Strongly Impact Progression Free Survival of Patients Treated With Platinum Based Chemotherapy in NSCLC − Final Results of a Multicenter Prospective Study**

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**Background:** KRAS mutations in NSCLC are supposed to indicate a poor prognosis and poor response to anticancer treatment. However, such evidence is only drawn from retrospective series giving controversial results. Moreover, it is possible that the various KRAS mutations differently affects prognosis, carcinogenesis and drug response as demonstrated in preclinical setting.
Aim of this study is to prospectively assess the prognostic value of KRAS mutations in NSCLC patients treated with a first line platinum containing regimen. This is a properly planned ancillary study within the TAILOR trial (NCT00637910) which is mainly focused on the second line.
**Methods:** Tissue and blood samples were collected at diagnosis in the whole cohort of registered patients. KRAS status was centrally determined with standard direct sequencing and KRAS genotype was assessed by real time PCR. The primary hypothesis is a difference in PFS according to KRAS mutational status; the impact of the three more frequent KRAS substitutions (G12C, G12V, and G12D) was also explored. The analysis was planned at occurrence of 200 events (HR $\geqslant$ 1.49, power 80%, 2-tailed alpha 10%),in a Cox model adjusting for Performance Status and radical surgery.
**Results:** Out of 565 patients registered, 341 (60.5%) were evaluable for KRAS and 85(25%) were mutated.
At a median follow-up of 17 months KRAS mutated patients showed a statistically significant worse PFS (HR 1.42 95% CI 1.06–1.94; p = 0.02). No differences among doublets were observed in KRAS mutated patients. The most frequent KRAS mutations were: G12C (36.4%), G12V (21.1%), G12D (16.4%), others (25.9%). Prognostic differences among variants are observed. Final genotype analyses are ongoing.
**Conclusions:** This is the first prospective, pre-planned and adequately sized evaluation of KRAS in NSCLC. Patients mutated for KRAS seem to have a higher risk of progressing. These results suggest that KRAS mutation epidemiology in this setting highly differs from that of colon cancer. Clinical data suggest that tailored strategies for these patients are warranted and our preclinical studies will help in clarifying the molecular mechanisms.

**1144** POSTER

**Using Single-cell Network Profiling (SCNP) Signatures to Predict Response to Induction Therapy and Relapse Risk in Pediatric Patients With Acute Myeloid Leukemia (AML)**

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**Background:** In pediatric AML, cytarabine-based combination regimens result in 80−90% complete remission (CR) rate but ultimately only half of the patients achieve long term remissions. The need for accurate prediction of two separate outcomes is of interest: 1. response to induction therapy, which offers guidance on patient specific induction therapy and 2: early relapse (CR-Rel), which allows for consolidation therapy decisions. Current prognostic factors (e.g., cytogenetics, FLT3 ITD) are not completely predictive of response or outcome for individual patients. SCNP is a functional evaluation measuring the effects of multiple modulators (including drugs) on signaling pathways at the single-cell level.
**Methods:** SCNP assays were analyzed for 67 BM samples from pediatric AML patients enrolled in POG (now COG) trial 9421 (46 CR and 21 NR). 80 signaling nodes (i.e., the combinations of modulators and intracellular activated proteins) were investigated including the PI3K, JAK/STAT, DNA damage response and apoptosis pathways. Basal and modulated protein levels in leukemic blasts were measured, and nodes were examined by univariate and multivariate analyses.
**Results:** DNA Damage and Apoptosis nodes (e.g., Etoposide or AraC+Daunorubicin $\rightarrow$ c-PARP and p-Chk2, p = 0.001) and induced phosphorylation (p-) levels of PI3K/MAPK pathway members S6 and ERK (Flt3 $\rightarrow$ p-S6, p = 0.04) showed higher levels in CR samples. Induced apoptosis was also associated with risk of relapse. Thapsigargin, a calcium modulator, induced higher levels of p-Erk, p-CREB and p-S6 in patients with CCR as compared to CR-Rel samples (p = 0.02). More importantly, in multivariate analysis, combination of 2−5 nodes (representing apoptosis, Jak/Stat and PI3K pathways) resulted in classifiers with good performance characteristics (bootstrap adjusted AUC 0.80–0.86) in predicting response to induction therapy and risk of relapse. The model predictions remain significant (p < 0.04 for both models) after adjusting for any one of the clinical covariates e.g., cytogenetics, FLT3-ITD, WBC, cytogenetics and age.
**Conclusion:** This study showed that performing quantitative SCNP under modulated conditions could serve as the basis for developing improved predictive tests for response to induction chemotherapy in pediatric patients with newly diagnosed AML. Additionally, the biology revealed could prove useful in determining alternative therapeutic strategies. Independent validation is ongoing.

**1145** POSTER

**Drug Resistance Induced by Plasmatic Concentrations of Paclitaxel and Carboplatin in Cancer Cell Lines**

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**Background:** Several proteins, as PgP and MRP family, are involved in the resistance to chemotherapy of the tumour cells. PgP (mdr1) and MRP family are members of the ATP-binding-cassette (ABC) transporters family. ABC transporters are a protein family able to transport a wide variety of substrates such as lipids, bile salts, toxins, and antigen-presenting peptides. This transport process is carried out across the membrane against a concentration gradient and gained from ATP hydrolysis. Antineoplastic drugs from natural sources such as taxanes, vinca-alkaloids, antracyclines, and epipodophyllotoxins are some of the ABC transporters substrates.
**Material and Methods:** We have studied the MDR1 and MRP3 expression in 6 cell lines, 3 of non small cell lung cancer (NSCLC), 1 of breast cancer, 1 of gastric cancer and other one of seminoma, after being exposed to plasma concentrations of Paclitaxel, Carboplatin, and the combination of both.
**Results:** After chemotherapy, we observed that paclitaxel induced MDR1 and carboplatin induced MRP3 in NSCLC cell lines. The association of both drugs increased significantly the expression of MDR1, and very few the expression of MRP3. Paclitaxel induced MDR1 in all cell lines derived from other tumours. Carboplatin did not induce MDR1 as previously, nor MRP3 in gastric cancer cell line.
**Conclusions:** Plasma concentrations of paclitaxel induced MDR1 expression but not MRP3 in NSCLC and other tumours derived cell lines. However, carboplatin produced overexpression of MRP3 but not MDR1 in the same cell lines.
The combination of both drugs was not able to activate a new resistance mechanism in the studied cell lines, but it was able to improve the resistance mechanism induced by each one of the drugs individually. This fact resulted in an increase of MDR1 expression with paclitaxel and MRP3 expression with carboplatin.

**1146** POSTER

**Pro-angiogenic Factor Cyr61 is Linked to Colorectal Cancer Development and Prognosis**

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**Background:** Angiogenic factor Cysteine-rich 61 (Cyr61) is a member of the CCN protein family that has been implicated in diverse biological processes such as cell adhesion, proliferation, angiogenesis, and tumorigenesis. An altered expression of Cyr61 is found to be associated with several human cancers. However, the correlation of expression of Cyr61 protein and clinical features of colorectal cancer remains unknown.
**Material and Methods:** Cyr61 expression in colorectal cancer and normal tissues was evaluated by Western blot analysis. Immunohistochemical staining was carried out using Tissue Microassay(TMA) to examine the

expression status of Cyr61. Correlations of Cyr61 over-expression with various clinicopathologic factors were also determined. Statistical analysis was performed to explore the links between expression of the Cyr61 and clinicopathological parameters.
**Results:** On Western blot analysis Cyr61 up-regulation was observed in colorectal cancer tissues (17/21,80.9%). In 234 colorectal cancers, tumour tissue microarray revealed significantly up-regulated Cyr61 protein expression in colorectal cancer tissues versus normal tissues adjacent to tumour. Cyr61 expression was high in 136 of 234 cases of colorectal carcinomas (58.1%). Cyr61 over-expression was significantly associated with TNM stage ($P = 0.012$) and regional lymph node involvement ($P = 0.018$). Kaplan–Meier survival analysis showed that over-expression of Cyr61 was related to poor survival of colorectal cancer patients ($P = 0.031$). But significant associations were not found between Cyr61 expression versus tumour grade, age and gender.
**Conclusions:** Our results suggest that Cyr61 is highly expressed in colorectal carcinomas and Cyr61 may play a role in the progression of colorectal cancers. Also, Cyr61 might be a new molecular marker to predict the prognosis and serve as valuable targets for therapeutic intervention of patients with colorectal carcinoma.

**1147** POSTER

**Inter-reader Agreement in Response to Therapy Evaluation of Advanced Lung Cancer: Benefits of a Volume-derived Imaging Biomarker**

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**Background:** Imaging-based endpoints are used to assess cancer response to therapy with lesion size-based criteria such as RECIST. Recent initiatives from interdisciplinary communities investigate other imaging biomarkers such as lesion volume. As discordance in the response evaluation is a critical issue, the expected benefit of a novel biomarker should be an improvement of the inter-reader agreement. The goal of this study is to evaluate the impacts of a volume-based measurement on the inter-reader variability.
**Material and Methods:** A retrospective study was performed on 10 patients having at least one Non-Small Cell Lung Cancer (NSCLC) lesion. These patients were followed over time with an average of 7 Computed Tomography (CT) studies. 3 readers delineated the volume of each lesion at each time point. Volume was automatically computed after a semi-automatic segmentation completed slice-by-slice with help of a manual tool. From the volume delineation, Longest Axial Diameter (LAD) and Spherical Equivalent Diameter (SED) were extracted. For each patient 2 response evaluations were performed according to RECIST thresholds based on LAD and SED. Quantitative inter-reader variability was analyzed relying on non-parametric statistics of Bland-Altman limit of agreement. Inter-reader agreement of the Best Overall Response was analyzed using Kappa coefficient.
**Results:**The variability in the measure was reduced from 26% (LAD) to 21% (SED). This benefit in measurement brought an improvement in the inter-reader agreement from Kappa = 0.15 (LAD) to 0.55 (SED).
**Conclusions:** We measured a reduction of quantitative variability using SED instead of LAD and an improvement of the inter-reader agreement.

**1148** POSTER

**DAB2 Interactive Protein (DAB2IP) Methylation in Serum DNA of Non-Small-Cell Lung Cancer (NSCLC) Patients (p) With Epidermal Growth Factor Receptor (EGFR) Mutations**

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**Background:** DAB2IP loss promotes primary tumour growth by activating Ras and drives metastasis through NFkB, serving as a signaling scaffold to coordinately regulate these pathways. DAB2IP is frequently methylated in lung cancer, and methylation in the m2a region is a key regulatory factor for DAB2IP expression in prostate cancer. We examined DAB2IP methylation in cell lines and in serum from erlotinib-treated NSCLC p with EGFR mutations.
**Material and Methods:** In human lung, breast and colorectal cancer cell lines, we analyzed DAB2IP promoter methylation in regions m2a and m2b by methylation-specific PCR (MSP) and bisulfite genomic sequencing. In circulating serum DNA from 152 erlotinib-treated NSCLC p with EGFR mutations, we analyzed methylation in the m2a and m2b promoter regions of DAB2IP by MSP. Methylation status was correlated with clinical outcome.
**Results:** Methylation was detected in the m2a region of 42 (27.63%) p, and in the m2b region in 51 (33.55%) p. There were no major differences in clinical characteristics (age, gender, smoking history, EGFR mutation type, metastatic sites) between p with methylation in the m2a region and p with methylation in the m2b region. Overall progression-free survival (PFS) was 15 months (m), and median survival (MS) 28 m for all 152 p. For the 41 p with bone metastases (mets), PFS was 14 m for 30 p without methylation in the m2a region vs 8 m for 11 p with methylation in the m2a region (P = 0.01), and MS was 23 m vs 10 m, respectively (P = 0.19). For the 57 p with distant mets but no lung mets, PFS was 18 m for 36 p without methylation in the m2a region vs 10 m for 21 p with methylation in the m2a region (P = 0.01), and MS was 24 m vs 16 m, respectively (P = 0.03). No differences in either PFS or MS were observed according to the methylation status of the m2b region.
**Conclusions:** Methylation in the m2a region of DAB2IP in serum DNA correlates with PFS and MS to erlotinib in NSCLC p with EGFR mutations with non-lung mets. Surveillance of DAB2IP methylation status in circulating DNA could be a useful tool to predict outcome to erlotinib in EGFR-mutated NSCLC p with non-lung mets.

**1149** POSTER

**Patient-derived Tumourgrafts − Models for a Systemic Cancer Biology Research**

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**Background:** Cancer is a complex genetic disease leading to a high variety of phenotypes among the different individuals. Each tumour presents a very specific pattern of molecular changes and responses to drugs. The correct identification of predictive biomarkers selecting the most appropriate therapies and avoiding unnecessary treatments for an individual patient is still a challenge. Patient-derived tumourgrafts allow preclinical investigations in a clinically relevant way. We performed investigations to improve the understanding of cancer complexity and to draw rational conclusions for therapy decisions.
**Methods:** The tumour models were established by direct transplantation of surgical specimens to immunodeficient mice and were maintained in early passages. A high congruence between original patient sample and xenograft could be proven both at gene and protein level. The following tumourgrafts are available: 10 breast, 28 colo-rectal, 25 lung, 6 ovarian, 10 sarcomas, 25 ALL, 5 AML. We will show examples for which purposes the models are appropriate and focus on non-small cell lung (NSCLC) and colon cancer.
**Results:** The xenografts were characterized for response towards clinically used cytotoxic and novel targeted drugs. The analysis for mutations revealed that all NSCLC models were *EGFRwt*, 5/25 were *KRASmut* and 12/25 were *P53mut*. None of the mutations correlated with response to therapy. In the colon cancer xenografts *KRAS, BRAF* and *PIK3CA* mutations predicted resistance to Cetuximab.
Using Affymetrix based gene profiling we identified a potential set of 20 genes which were differentially expressed between Oxaliplatin responder and non-responder.
In a preclinical Phase II study, the response of 22 NSCLC xenografts to a novel Epothilone was evaluated; *P53* mutations and overexpression of a cytochrome P450 enzyme were identified as potential biomarkers for the stratification of patients. The individual comparison of responses of colon cancer patients and their derived xenografts resulted in a congruence in 5 out of 5 patients included.
**Conclusions:** Patient-derived xenografts are a valuable model system to address clinically relevant questions in a standardized and strictly controlled fashion. They show a high concordance with the clinical specimens concerning marker expression and response to therapy.

**1150** POSTER

**Analysis of Biological Markers, Tumoral Predictors and Clinical Features as Prognosis Factors to Cnemotherapy Response in Metastatic Carcinomas of Unknown Primary Site**

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**Background:** The authors investigated prognosis factors to chemotherapy response such as clinicopathological features: age, gender, performance