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