PP 23

The role of vascular endothelial growth factor (VEGF) and VEGF-receptors genotyping in guiding the metastatic process in radically resected gastric cancer patients

M. Scartozzi, C. Loretelli, A. Mandolesi, E. Galizia, A. Bittoni, L. Faloppi, R. Giampieri, M. Pistelli, I. Bearzi, S. Cascinu. *Oncologia Clinica, AO Ospedali Riuniti Ancona, ANCONA, Italy*

Background: In radically resected gastric cancer the possibility to predict the site of relapse could be clinically relevant for the selection of post-surgical management. We previously demonstrated that tumour integrins genotyping is involved in determining the metastatic sites. Preclinical studies suggested that tumour angiogenesis may also be crucial for the metastatic process of gastric cancer cells. We then investigated the role of VEGFs and VEGF receptors genotyping in determining either peritoneal carcinosis or hematogenous metastases in radically resected gastric cancer patients.
Materials and Methods: Genotyping for VEGF-A, VEGF-C and VEGFR-1,2,3 was carried out on pT4a radically resected gastric tumours recurring with either peritoneal-only carcinosis or hematogenous metastases. Tumour genotyping for integrins was also performed according to our previous findings.
Results: 101 patients fulfilled the inclusion criteria: 57 with peritoneal carcinomatosis only and 44 with hematogenous spread only. At multivariate analysis, intestinal histology and the AC genotype of rs699947 (VEGFA) showed to independently correlate with hematogenous metastases, whereas diffuse histology and the AA genotype of rs2269772 (ITGA) independently correlated with peritoneal-only diffusion ($p = 0.001$).
Conclusion: Our results seem to indicate that combining information from genotyping of rs699947 (VEGFA, AC), rs2269772 (ITGA, AA) and tumour histology could allow clinicians to individuate gastric cancer at high risk for recurrence either with peritoneal or hematogenous metastases. The selection tool deriving from this analysis may allow an optimal use of the available treatment strategies in these patients.

PP 51

Register trial of sorafenib (S) for patients (pts) with metastatic uveal melanoma (metUvMel)

M.E. Scheulen, B. Nokay, H. Richly, A-C. Hoffmann, J. Kalkmann, J. Stattaus, N. Bornfeld, M.H. Schuler, J. Hense. *Innere Klinik (Tumorforschung), West German Cancer Center, University Hospital Essen, Essen, Germany*

Background: There is no effective systemic therapy for patients (pts) with metastatic uveal melanoma (metUvMel). Due to the paucity of clinical trials pts are frequently offered individual treatment options. We have analyzed the outcome of pts treated for metUvMel with the oral multikinase inhibitor sorafenib (S) in an IEC-approved register trial of the West German Cancer Center, a national reference center for uveal melanomas.
Materials and Methods: Pts with metUvMel were treated with S [at a dose of 400 mg bid in the first group (G1) and of 200 mg bid in the second group (G2)]. Overall survival (OS) and time to symptomatic progression (TTPsymp) were studied as primary outcome parameters. Secondary outcomes included time to radiologic progression (TTPrad) according to CT and safety. In addition, digital contrast-enhanced NMR (DCE-NMR) and contrast-enhanced ultrasound (CEUS) of liver metastases were performed in selected pts.
Results: A total of 62 pts (median age 63 yrs, range 35−83 yrs; 31 female [50%], 31 male [50%], ECOG 0 44 pts [71%], ECOG 1 15 pts [26%], ECOG 2 2 pts [3%]) were included in the analysis on an intent to treat basis (ITT). Fortysix pts had no prior systemic treatment (74%). Metastatic sites included liver in 60 pts (97%) in addition to other organs in 20 pts (32%). Only two pts (3%) had exclusively extrahepatic metastases. Following 4 CTC-Grade 4 toxicities [hand-foot-syndrome (HFS) and/or diarrhea] in the first 31 pts (G1) (13%), the starting dose of S was reduced to 200 mg twice daily in the subsequent 31 pts (G2) effectively preventing further severe side effects. Median OS was 10.8 mths (CI 6.1–15.1 mths) in G1 and 14.0 mths (CI 6.4-nd mths) in G2, median TTPrad was 5.0 mths (CI 3.1−8.9 mths) in G1 and 4,5 mths (CI 1.5−6.0 mths) in G2 and TTPsymp was 4.1 mths (CI 3.1−5.2 mths) in G1 and 7.1 mths (CI 3.2–10.6 mths) in G2 (Wilcoxon 0.043), respectively.
Conclusion: S at a dose of 200 mg bid is safe in pts with metUvMel predominantly metastatic to the liver and seems to be effective with a median OS of more than 10 mths. Treatment results are encouraging in an orphan malignant disease without established palliative systemic treatment options. A multicenter randomized discontinuation trial with S in pts with metUvMel will be performed.

PP 72

Angiogenesis-related cytokines as potential predictive biomarkers in a phase II trial evaluating everolimus efficacy in locally advanced or metastatic Transitional Carcinoma Cell

E. Seront, S. Rottey, B. Sautois, L. D'Hondt, J-L. Canon, J-M. Vandenbulcke, N. Whenham, J-C. Goeminne, O. Feron, J-P. Machiels. *Cliniques Universitaires Saint Luc − Institute of Experimental and Clinical Research (IREC) − UCL, Zaventem, Belgium*

Background: Dysregulation of mammalian Target of Rapamycin (mTOR) pathway and subsequent angiogenesis activation play a role in Transitional Carcinoma Cell (TCC). Despite growing interest for mTOR inhibitors in cancer, there is up to now no potential biomarker able to predict disease control. Here, we evaluated in a phase II trial the efficacy of the mTOR inhibitor everolimus in patients with palliative TCC after failure of platinum-based therapy and we explored potential angiogenesis-related plasma proteins as predictive biomarkers.
Materials and Methods: Patients with locally advanced or metastatic TCC received everolimus 10 mg/day continuously until progressive disease (PD) or unacceptable toxicity. Primary endpoints were control disease rate, including complete response (CR), partial response (PR) or stable disease (SD) at 8 weeks. Plasma samples were collected on day 1 (baseline before treatment), day 28 (during treatment), and at PD. A screening of 55 angiogenesis-related proteins was performed in plasma by cytokine arrays and most significant results were confirmed with dedicated ELISA.
Results: 37 patients (pts) were included. Confirmed PR was observed in 2 pts and SD in 8 pts, resulting in a disease control rate of 27% at 8 wks. Analyzing changes in plasma cytokine concentrations between baseline and day 28, we found that everolimus induces globally an increase in the angiogenesis inhibitor angiostatin (+ 114%; $p < 0.0001$). In pts with controlled disease, we observed an early decrease in two markers of tumor vessel maturation, namely angiopoietin-1 (−84%; $p = 0.01$) and PDGF-AB (−64%; $p = 0.0036$) compared to pts with non-controlled disease. The tumor endothelial cell marker endoglin showed an increase in pts with non-controlled disease (+19%; $p = 0.06$) compared to pts with controlled disease (−17%; $p = 0.27$). Moreover, baseline levels of angiopoietin-1 were much higher in pts with controlled disease than in pts with non-controlled disease (+267%; $p = 0.011$). Interestingly, in the 2 pts with PR, an increase in angiopoietin-1 (+800%), PDGF-AA (+300%) and PDGF-AB (+400%) levels were observed at the time of PD (vs measurements at day 28).
Conclusion: Everolimus exerts antitumor activity in advanced TCC, through a likely anti-angiogenic activity. Angiopoietin-1 appears as a potential biomarker to predict and track such response and should be considered in further clinical trials.

PP 60

Targeting hyaluronan in tumor stroma. Interim translational and biomarker evaluations of pegylated hyaluronidase (PEGPH20) in animal models and patients with advanced solid tumors

S. Dychter, R. Ramanathan, J. Infante, L. Rosen, D. Von Hoff, M. Shepard, P. Jiang, D. Maneval, P. Selvam, G. Frost. *Halozyme Therapeutics, Inc., San Diego, United States*

Background: Hyaluronan (HA), a component of the tumor matrix, is present in many solid tumors (T). T's characterized by the accumulation of HA have high interstitial fluid pressure (IFP) that inhibits penetration of chemotherapeutics. PEGPH20 (P) has been shown to remove HA-rich T extracellular matrices in vitro and in vivo xenograft T models. P-mediated depletion of HA in HA+ xenografts reduced T IFP/water content, inhibited T growth, and increased efficacy of chemotherapy. Phase 1 (Ph1) trials currently utilize biomarkers to evaluate P in vivo activity.
Materials and Methods: Two Ph1 studies in patients (PTS) with advanced solid T's refractory to prior therapy assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of single-agent IV P. Dosing in Study 1 was $1\times$/21 d and in Study 2 is $2\times$/wk $\times$ 4 wks and $1\times$/wk thereafter in combination with dexamethasone. Biomarkers evaluated include histochemistry to identify HA status of T's, Apparent Diffusion Coefficient (ADC)/Dynamic Contrast Enhancement (DCE)-MRI of T/normal tissue to assess impact on T water diffusion, and serum HA to demonstrate enzymatic activity of P.
Results: Activity of P was reflected by detection of HA catabolites in plasma. Systemic exposure of HA catabolites increased after single treatment and was dependent on the dose of P. HA concentrations were elevated 100-fold in patients treated with 50 μg/kg P. Repeat dosing with P resulted in sustained plasma concentrations of HA catabolites. ADC-MRI results suggested increased diffusion within T, and DCE-MRI (Ktrans) analysis suggested elevated T perfusion within 24 hours of P dosing. Histochemistry with a HA binding protein demonstrated the ability of P to reduce pericellular and stromal HA after 4 wks of treatment. Plasma and T HA results were consistent between murine xenograft models and humans.

Conclusion: P targets T matrix, resulting in T-associated HA reduction. Ph1 studies have shown in vivo activity of P as reflected by elevated plasma HA catabolites and increased T water diffusion and T perfusion by ADC/DCE-MRI. The use of histochemistry as a predictor of P efficacy as well as HA plasma catabolites, histochemistry and ADC/DCE-MRI to monitor response to P will be investigated Phase 2 trials.

PP 19

Highly sensitive detection of microRNA and mRNA from FFPE tissue and blood samples by expression microarray

H. Sudo, T. Satoko, U. Yoji, K. Toshihiko, I. Makiko, N. Osamu, A. Hideo, N. Hitoshi. *Toray Industries, Inc., Kanagawa, Japan*

Background: Gene expression profiling of readily available clinical samples, such as blood or FFPE tissue, is a promising method to discover novel diagnostic markers. As RNA is subjected to degradation even in properly-collected tissue samples, it is more difficult to obtain intact RNA from FFPE or body fluid samples for diagnostic analysis. 3D-Gene™ is highly sensitive gene expression microarray, featuring the unique micro-columnar structure on the platform substrate and the beads agitation system during the hybridization reaction. Using 3D-Gene™, we achieved highly sensitive and reproducible detection of mRNA or miRNA from FFPE tissue samples.
Materials and Methods: Total RNA was extracted from human serum, plasma and frozen or FFPE tissue samples, with the recommended protocol for each sample. For mRNA detection, total RNA was reverse-transcribed to cDNA and labeled with fluorescent dye directly or after the amplification. For miRNA detection, total RNA was labeled with fluorescent dye directly. These pretreated target nucleotides were hybridized to 3D-Gene™ while the hybridized buffer containing target nucleotides was agitated by beads during hybridization. The hybridized microarrays were washed and scanned for image acquisition.
Results: The result was highly correlated with the expression profiles from frozen tissue samples. Furthermore, exosomal miRNA from serum or plasma was also detected with high sensitivity and reproducibility. From these analyses of FFPE tissue or blood samples, we found potential miRNA biomarkers for various cancers. (i) Using 3D-Gene™, we detected mRNA expression profile from FFPE samples with high reproducibility. We also showed high correlation of the expression profiles between FFPE and frozen tissue samples. Furthermore, microRNA obtained from frozen as well as FFPE tissue samples was reproducibly detected at atto-molar level. Some miRNA biomarkers for various cancers were found from FFPE samples. (ii) Serum and plasma are suggested to contain microsomes in which miRNA is enclosed. miRNA from serum and plasma samples were detected with high sensitivity and reproducibility with 3D-Gene™. Some miRNA biomarkers for various cancers were found from patients' sera.
Conclusion: The Application of our 3D-Gene™ for the gene expression analysis of clinical samples could bring a formally unexplored venue in the biomarker discovery and diagnostic field.

PP 105

Multiple gene signatures: some putative answers on the why and the how

W. Talloen, L. Bijnens. *Janssen Pharmaceuticals group, Beerse, Belgium*

Background: With the completion of the sequencing of the human genome and with the emergence of biotechnologies such as microarrays, we have entered the post-genomic era with much hope to harvest some of the fruits hidden in the genomic text. At the same time, the current difficulties faced by pharma research to discover generally applicable block-buster drugs have lead to think in terms of personalized medicine. Consequently, high hopes are on clinical opportunities for gene-based prediction of illness or drug response using post-genomic tools. The -omics revolution was also warmly welcomed by statisticians as its data properties imposed new and interesting statistical challenges. For example, the quest for biomarkers in the context of personalized medicine has made many statisticians think about classification models that are robust against overfitting for generation of molecular signatures.
Materials and Methods: Here we propose three biological scenarios where multiple gene signatures may outperform single gene markers; (1) inhibition or catalyzation, (2) downstream effects and (3) upstream effects. A simulation study is set up to mimic three different biological scenarios, and each of the three datasets is analyzed using various algorithms including PAM, Random Forest, Support Vector Machines, CART, etc.
Results: The algorithms under study perform clearly differently between the three scenarios.
Conclusion: This presentation discusses how genes can be aggregated into one composite index (i.e., the marker) so as to reflect the underlying biology, as categorized using the three previously proposed biological scenarios.

PP 58

Plasma microRNAs in breast cancer detection

C. Thompson, H. Heneghan, L. Li, R. Leidner. *Case Western Reserve University, Cleveland, OH, USA*

Background: Circulating microRNA (miRNA) expression levels have been proposed as a potential biomarker for cancer detection due to their key role in cancer and stability in the circulation. In fact, a circulating miRNA-based test for colorectal cancer detection is already in clinical trials. However, the field is much less advanced in breast cancer.
Materials and Methods: Using the Illumina human miRNA microarray, we interrogated the expression level of 1145 miRNAs in the plasma of 18 breast cancer patients prior to tumor resection, 17 patients after tumor resection and 20 mammography-screened controls. Controls were matched to pre-resection cases on age and race. We excluded 245 miRNAs due to low expression across all samples. Differences in expression levels between pre-resection cases and controls were assessed via a pooled t-test.
Results: Thirty six of the remaining miRNAs were differentially expressed between pre-resection breast cancer cases and controls ($p < 0.01$). Using a single ratio-normalized miRNA level, with 100% specificity we were able to correctly identify 13% of the cases. Increasing the signature to 2 miRNAs ratios allowed us to correctly classify 50% of cases. Further signature modeling using 6 ratios yielded a test with 89% sensitivity at 100% specificity. This signature held up to random permutation testing ($p < 0.01$). We noted that this signature was better at detecting ER+ breast cancers, where it correctly identified 100% of the ER+ cases. Furthermore, the expression levels were highly correlated with stage (lowest in in-situ cases and highest in stages 3 and 4), and returned to baseline levels in post-resection samples.
Conclusion: Overall, our data provides compelling evidence of the potential of miRNAs to be used as a minimally invasive screening test for breast cancer, conceivably as an adjunct to improve mammographic accuracy. Confirmation of preliminary results in a larger sample size is underway.

PP 76

Biomarker discovery using multiplexed in solution proximity extension assays: a case–control study for early detection of colorectal cancer

S.B. Thorsen, M. Lundberg, E. Assarsson, N. Gee, B.S. Nielsen, I.J. Christensen, H.-J. Nielsen, N. Brünner, J. Stenvang, S. Fredriksson. *University of Copenhagen, IVS, Patobiology, Frederiksberg, Denmark*

Background: One of the challenges in early detection of colorectal cancer (CRC) is the limited success with screening. We here report a study, focusing on discovery of biomarkers for early detection of CRC, using EDTA plasma samples from a case–control group collected from a larger endoscopy study. We demonstrate that the combination of biomarker discovery and molecular technique development is one lead to discover new diagnostic biomarkers for CRC.
Materials and Methods: We have established and validated a high throughput multiplex in solution proximity extension assay (PEA) platform and demonstrate simultaneous quantification of 96 different proteins in 1 μL sample. The PEA employs two primary antibodies, linked to two different DNA strands; upon simultaneous and proximal binding to a target protein the two strands can be connected. The DNA strands now form a PCR amplicon detectable by real-time qPCR. The amplification ability of the DNA strands drive the sensitivity and lowers sample consumption, while supporting multiplexing capabilities based on the oligonucleotide design. The PEA technology possesses all the required qualities for a biomarker discovery tool. From a literature study investigating interesting molecular pathways relevant for CRC, we designed four biomarker panels. In total, we measure 150 different protein markers, of which many have never been reported in human plasma. Using this multiplex PEA discovery tool, we test the biomarker potential for each protein. Our case–control study consists of four groups: 74 stage I-IV CRC patients, 74 adenoma patients, 74 patients with other diseases, and 74 healthy individuals. All patients and individuals have been age and gender matched.
Results: We have previously applied an earlier version of the assay (PLA) and successfully demonstrated detection and quantification of 74 different protein markers in CRC and matched healthy individuals. This demonstrated the feasibility and potential for the assay and identified putative biomarkers [1]. We will present assay validation results and biomarker potential for all panels included in the new PEA high-throughput assays.