frozen tissue samples makes the TGA a useful system that can be widely applied in both diagnosis and research areas. We hypothesize that TGA is superior to DS for predicting efficacy of molecularly targeted agents, since it has greater sensitivity for detection of mutations associated with drug resistance.

1428 POSTER

Evaluation of EpCAM Protein Expression in Human Cancers as Therapeutic Target for Catumaxomab Treatment

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Introduction: The Epithelial Cell Adhesion Molecule (EpCAM) is a cell surface protein with oncogenic features expressed on healthy human epithelia and corresponding malignant tumours. Our work group recently explored molecular changes following EpCAM overexpression in commercially available cancer cell lines. For the first time we could show that EpCAM overexpression was associated with the downregulation of the Wnt signaling inhibitors. Moreover, an increase of proliferation and chemosensitivity to Docetaxel was associated with EpCAM overexpression. Similarly, in the clinical setting we observed that EpCAM overexpression detected by immunhistochemistry was associated with a poor prognosis in breast cancer patients and other tumour entities. These observations have promoted EpCAM to a 'druggable' target. As such, Catumaxomab was approved by the European Union for intraperitoneal use in patients with EpCAM-expressing cancer. However, no consensus exists on how and when to evaluate EpCAM expression in these cancer patients.
Material and Methods: EpCAM expression was assessed by a well established immunohistochemical staining protocol in 2291 primary tumour tissues and in 108 metastases using the EpCAM-specific antibody clone VU1D9. A total immunostaining score (TIS) was calculated as the product of a proportion score and an intensity score. Four expression subgroups (no, weak, moderate and intense) were defined. As described previously, the term 'EpCAM overexpression' was reserved for tissues showing a TIS-value >4.
Results: EpCAM was highly expressed in most tumours of gastrointestinal origin and in some carcinomas of the genito-urinary tract. However, hepatocellular carcinomas, clear cell renal cell cancer, urothelial cancer and squamous cell cancers are frequently EpCAM negative. EpCAM expression in breast cancer depends on the histological subtype, as lobular histology shows usually no or weak expression. Most metastases and particularly peritoneal lesions are EpCAM positive and they frequently reflect the expression phenotype of the primary tumour.
Conclusion: EpCAM expression is detected on adenocarcinomas of various primary sites. If EpCAM-specific antibodies (such as Catumaxomab) are intended to be used in cancer patients, we recommend prior immunohistochemical evaluation of EpCAM expression particularly in patients with renal cell cancer, hepatocellular carcinoma, urothelial carcinoma, breast cancer and squamous cell carcinomas.

1429 POSTER

FCGRIIa-131 and FCGRIIIa-158 Polymorphisms − Distribution and Clinical Outcomes of Cetuximab-based Chemotherapy in Japanese Patients With Metastatic Colorectal Cancer (mCRC)

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Background: Polymorphisms in fragment C receptor (FCGR) are expected as a predictive biomarker of cetuximab (Cmab). Previous studies have convincingly confirmed the distributions (dists) of FCGR polymorphisms in Western population and shown the existence of linkage disequilibrium (LD) between FCGRIIa and FCGRIIIa polymorphisms. Meanwhile, the dists in Asian population have been unknown but a few studies for non-cancer patients have suggested the difference in dists between Asian and Western populations. We investigated the dists of FCGR polymorphisms and their association with clinical outcomes of Cmab based chemotherapy in Japanese mCRC patients.
Materials and Methods: Ninety-three patients with irinotecan/oxaliplatin/5-FU-refractory mCRC and treated by Cmab plus irinotecan or Cmab monotherapy were retrospectively registered from 8 centers in Japan. FCGR polymorphisms were determined from genomic DNA extracted from peripheral blood samples based on the Multiplex allele-specific PCR method. Comparisons according to FCGR polymorphisms were evaluated using Fisher's exact test for response rate (RR) and log-rank test for progression-free survival (PFS) and overall survival (OS) curves.
Results: The dists of FCGRIIa HH/HR/RR and FCGRIIIa VV/VF/FF were 68/30/2% and 4/40/56%, respectively (Table). The absence of LD between FCGRIIa and FCGRIIIa polymorphisms was confirmed (GENEPOP, $p = 0.526$; Linkdis, $p = 0.146$). Of 74 patients with KRAS wild-type and treated by Cmab plus irinotecan, no difference according to FCGR polymorphisms was observed in either RR (IIa: HH 37% vs. HR/RR 36%, p=1.00; IIIa: VV/VF 39% vs. FF 35%, $p = 0.81$) or PFS curves (IIa: HH vs. HR/RR, $p = 0.60$; IIIa: VV/VF vs. FF, $p = 0.06$) or OS curves (IIa: HH vs. HR/RR, $p = 0.65$; IIIa: VV/VF vs. FF, $p = 0.30$).
Conclusions: This study clarified an ethnic difference in the frequencies of FCGR polymorphisms. The polymorphisms did not influence the clinical outcomes of Cmab based chemotherapy in Japanese patients with mCRC.

FCGRIIa-131	FCGRIIIa-158			
	VV	VF	FF	Total
HH	3	28	32	63 (68%)
HR	1	9	18	28 (30%)
RR	0	0	2	2 (2%)
Total	4 (4%)	37 (40%)	52 (56%)	93

1430 POSTER

Quantitative Analysis of PTEN-dependent Glycoprotein Patterns Reveals Predictive Biomarker Signature for Response of Human Patients to Docetaxel Therapy in Metastatic Castration Resistant Prostate Cancer (mCRPC)

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Background: Since 2004 chemotherapy with docetaxel has been the standard therapy in progressive mCRPC. Unfortunately only a subgroup of patients responds to this treatment. As rebiopsy is rarely done in mCRPC, predictive serum biomarkers for therapy response would be of great value. We recently presented a novel platform for human biomarker discovery and validation based on a large-scale quantitative analysis of *N*-linked glycoproteins of the Phosphatase and Tensin homolog (Pten) conditional knockout mouse model for prostate cancer progression. This work delivered biomarker signatures for PTEN-status, Gleason sum and diagnosis in localized prostate cancer (Cima et al., PNAS 2011). This model has also revealed a prognostic biomarker signature in patients with mCRPC (manuscript submitted). To screen our biomarker set for factors for response to treatment with docetaxel in mCRPC patients seemed a reasonable step towards the vision of a personalized cancer medicine.
Methods: In serum samples from 40 patients with mCRPC who underwent chemotherapy with docetaxel we measured 13 proteins with ELISA and 66 different proteins by selected reaction monitoring (SRM) mass spectrometry. Random forest algorithm was applied to establish a multifactor signature predictive for response. Therapy response was defined as at least stable disease biochemically (PSA increase <25% over baseline) and by imaging after three cycles of therapy with docetaxel.
Results: Serum samples of 40 patients with mCRCP under chemotherapy with docetaxel were retrospectively analyzed. We identified four factors correlating significantly ($p < 0.05$) with therapy response in a univariate analysis. Additionally we performed a random forest analysis identifying combined predictive biomarker signatures. Intriguingly the serum concentration of two identified factors in combination significantly predicted whether patients with mCRPC responded to taxane therapy or not with an accuracy of 85% in a confusion matrix.

Conclusions: Our recently presented biomarker-platform derived from a Pten conditional knockout mouse model showed high feasiblity for the identification of predictive markers for therapy response to docetaxel chemotherpay in human patients with mCRPC. The analysis of the biomarker signature combining two of these candidate biomarkers therefore warrants further investigation in a bigger collective of patients.

1431 POSTER

Application of Native Fluorescence of Blood Plasma in Colorectal Cancer Detection: Results of a Prospective Study

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Background: Fluorescence spectroscopy of biomolecules is considered a promising method to *in vivo* discriminate normal tissue from malignant tissue in various sites, including breast, cervix, lung, and colon. In the present work we investigated the possible role of the native fluorescence of blood plasma in discriminating patients with colorectal cancer from subjects of a control population. Approval for this research was obtained from the Ethics Committee of our Institute; the study was registered in ClinicalTrials.gov with the code NCT01286064.
Methods: In this preliminary phase, the study involved 100 subjects: 50 healthy subjects with negative result from colonoscopy (40% male and 60% female; mean age 58.0) and 50 patients bearing colorectal adenocarcinoma (44% male and 56% female; mean age 60.2). All participants gave written informed consent and completed questionnaires on their diet, lifestyle and medical history. Blood samples were collected from all the subjects and plasma fluorescence spectrum was analyzed using a conventional spectrofluorimeter.
Results: The intensity of the fluorescence emission peak around 615–635 nm of the collected blood samples was significantly different between patients bearing colorectal cancer (median value 14.94 a.u., mean 16.01±4.87 a.u.) and healthy subjects (median value 13.35 a.u., mean 14.06±3.79a.u.), with the minimum p level at 623 nm ($p < 0.0001$). Data on height and weight, alcohol use, red meat and vegetables intake, smoking status, concomitant illness and familial tumour history were used with the fluorescence intensity at 623 nm for setting up a neural network classifier designed to perform automated diagnosis. Not all the variables were included in the network input, because some of them did not add any significant improvement to the discrimination. Variables retained as input data over intensity of fluorescence were body mass index, sex and familial tumour history. The neural network capability in discriminate healthy subjects from patients bearing colorectal cancer was tested by ROC analysis, which resulted in an AUC of 0.81.
Conclusion: According to our results, a possible application of the fluorescence measurements of blood plasma in colorectal cancer detection would seem justified. Work is in progress to asses the true clinical value of the test on a larger number of subjects.

1432 POSTER

Pharmacogenetic Assessment of Toxicity After Docetaxel Chemotherapy in Breast Cancer

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Background: Taxanes are the most active agents in the treatment of breast cancer. However, the utility of taxane-based therapy is limited principally by gastrointestinal and hematological toxicity, hypersensitivity and cumulative neurotoxicity. To understand why only some patients experience severe adverse effects the metabolic pathways of this drug have to be unraveled in detail. Docetaxel is metabolized by CYP3A4 and CYP3A5 and is a substrate for the ATP binding cassette multidrug transporters ABCB1. The aim of our study was to evaluate the association between docetaxel-toxicity and genetic polymorphisms related to its metabolism through peripheral venous blood sampling in patients with breast cancer undergoing chemotherapy.
Materials and Methods: We studied 100 patients (age 53.3±8.5DS) affected by breast cancer under treatment with docetaxel as adjuvant or metastatic therapy; we genotyped them for selected polymorphisms and ABC-transporters that may influence cellular sensitivity to taxanes: CYP3A4* 1B (A > G), CYP3A5* 3 (G > A) and ABCB1 (1236 C > T; 3435 C > T). SNPs (single nucleotide polymorphisms) were carachterized by pyrosequencing. The statistical survey was conducted by SPSS 14.2 software.
Results: We observed a significant association between patients homozygous for ABCB1 polymorphisms and a lower toxicity after therapy with docetaxel. For CYP3A4* 1B and CYP3A5* 3, although without statistical significance ($p > 0.005$) we can dimostrate a greatest exposure to the toxicity of docetaxel, presumably due to increased production of reactive metabolites.
Conclusions: We suggest that CYP3A4, CYP3A5 and ABCB1 might affect taxane toxicity therefore representing, if confirmed in a larger cohort of patients, a toxicity predictive biomarker. In the future, studies with SNP chips and other studies on the transcriptome, proteome and metabolome level should be performed in order to identify signatures differentiating between patients with high or lower toxicity linked to docetaxel chemotherapy.

1433 POSTER

Diagnostic Ability of TPSa and CPSa in a Patient Cohort Referred to a Danish Urological Department

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Introduction: Both total-PSA (tPSA) and complexed-PSA (cPSA) have been advocated for diagnosis of prostate cancer (PCA). However it remains unclear which of these two PSA forms has the best diagnostic efficiency.
Materials and Methods: 1423 consecutive patients referred to the Department of Urology from general practitioners during June 2005 to August 2006 were included in the study. 161 patients with previously known Prostate Cancer (PCA) were excluded, leaving 1262 patients for diagnostic procedures. Of these, 299 patients were diagnosed with PCA and 963 patients were found without PCA at the time of inclusion. Blood samples were collected in tubes with gel separation, centrifugated and the serum frozen within 1 hour for later analysis tPSA and cPSA were measured by the Bayer/Siemens chemiluminescent assays on an ADVIA Centaur automated analyzer.
Results: tPSA and cPSA levels among the 299 PCA patients ranged from 0.06–5920.50 μg/l and 0.06–4908.70 μg/l, respectively with medians of 13.39 μg/l and 10.86 μg/l. tPSA and cPSA levels in 963 patients without PCA at the time of investigation ranged from 0.06–233.49 μg/l and 0.06–83.82 μg/l, respectively with medians of 2.81 μg/l and 2.10 μg/l. The sensitivity of tPSA and cPSA were 97.7% and 97.3%, respectively ($p > 0.05$). The specificity of tPSA and cPSA were 60.4% and 65.1%, respectively ($p > 0.05$). PVpos of tPSA and cPSA were 39.3% and 42.2%, respectively ($p > 0.05$). PVneg of tPSA and cPSA were 99.0% and 98.9% respectively ($p > 0.05$). Efficiency of tPSA and cPSA were 68.1% and 71.8%, respectively ($p > 0.05$).
Conclusion: The diagnostic ability of tPSA and cPSA is similar ($p > 0.05$). The tPSA and cPSA concentrations among patients referred to the Department of Urology from general practice were surprisingly high indicating late referral.

1434 POSTER

Impact of KRAS Mutations (Krasmut) on Clinical Outcome in Stage IV Non-small Cell Lung Cancer (NSCLC) Patients (pts) and Their Relationship With Other Biomarkers

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Background: Kras accounts for 90% of RAS mutations in lung adenocarcinoma and approximately 97% of Krasmut in NSCLC involve codons 12 or 13. Kras tumour status cannot be easily predicted on the basis of smoking history alone. Krasmut status might help in the prediction of clinical outcome for pts receiving different treatments. The role of Krasmut as a predictor of response for pts with stage IV NSCLC treated with chemotherapy alone is poorly understood. Emerging data suggest that Krasmut are negative predictors of benefit from both adjuvant chemotherapy and anti-EGFR-directed therapies.
Material and Methods: From August 2009 to January 2011 we analyzed Krasmut in samples from 114 stage IV NSCLC pts. We analyzed different types of Kras point mutations in codons 12 and 13 by direct DNA sequencing from paraffin-embedded tumour tissue (PETT). We also used DNA sequencing from PETT to analyze other mutations (EGFR) and mRNA gene expression to evaluate BRCA1and RAP80 levels. We evaluated the presence of Krasmut according to histological subtype.
Results: Krasmut were found in 21.9% (25 /114). Out of pts harboring Krasmut the median age was 59 y, 64% were male. According to smoking status 8% were never smokers, 32% former smokers and 60% current smokers. According to histology 72% were adenocarcinoma, 12% squamous cell carcinoma and 8% bronchioloalveolar carcinoma. According to PS ECOG 44% were PS0, 32% PS1 and 24% PS2. The distribution of