Conclusions: The survival of patients with advanced RCC can be predicted by evaluating their SUVmax using ^{18}F-FDG-PET/CT. ^{18}F-FDG-PET/CT has potency as an "imaging biomarker" to provide helpful information for the clinical decision-making.

624 POSTER

Establishment of a large panel of "early" colon carcinoma xenografts as a preclinical tool for identification of predictive biomarkers

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Cancer is the result of an accumulation of several genetic and environmental factors promoting tumour growth. Therefore, only a quite small fraction of the patients derive benefit from novel targeted drugs. This is, to a large extent, due to the molecular variability among the tumours of the same classification. Each tumour is individual, and every patient can, at least to some extent, react differently to a particular treatment.
Human tumour xenografts directly derived from patient cancer specimen can provide a preclinical research alternative considering both heterogeneity and individuality of malignomas. Xenografts allow to test novel antitumour agents in a fast and standardised manner and provide sufficient tumour tissue, even post treatment, for the search of corresponding predictive biomarkers.
It was the aim of our project to establish a relevant number of human colon carcinoma xenograft models to perform a preclinical biomarker study. 240 primary colon carcinoma tissue samples were collected during two years by a network of four clinics using a standardised procedure. Tumour pieces were transplanted onto immunodeficient mice immediately after surgery. A panel of 148 stably passagable colon cancer xenografts could be established as permanent tumour models. These patient-derived colon cancer models feature a high coincidence with the original tumour regarding histology and genome-wide gene expression profiling.
In ongoing experiments these models are subjected to an extensive characterization, including gene expression analysis, sequencing for mutations, and determination of response to classical as well as novel targeted compounds. Interim analysis of available results determined the following response rates: Oxaliplatin 7%, Cetuximab 25%, and Bevacizumab 3%. An integrated data analysis will be performed and should lead to the identification of candidate markers of response or resistance for final characterization and validation in clinical studies.

625 POSTER

Optimization of microRNAs detection in urine samples of patients with bladder and prostate cancers

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Background: Detection of cancer in its early stages is a key factor in improving patients' survival rate and lowering the cost of treatments. Unfortunately, the current methods for bladder/prostate cancer diagnosis are suffering from low sensitivity and specificity. Therefore, the discovery of novel tumor markers with high specificity and sensitivity is of great interest in cancer research.
MicroRNAs (miRNA, mir) are small endogenously-produced, non-coding RNAs with an important role in regulating gene expression. Recent studies show that miRNAs expression profiles represent significant tumor-specific changes that are unique for most cancers. The latest achievement in detecting miRNAs in peripheral blood was an important step to utilize miRNAs as a novel class of tumor markers. Detection of cancer specific miRNAs in urine would be another step to achieve. The latter is especially important for early detection and screening of the patients with bladder and prostate cancers.
Purpose: Detection and optimization of mir-21, mir-141, mir-127 and mir-205 oncogenic miRNAs in urine of patients with bladder and prostate cancers.
Methods: 4 ml of urine samples from patients as well as age-matched bladdere/prostate cancer free volunteers (control group) were aliquoted in eppendorf tubes and stored in −80ºC. RNA extraction was carried out using 2 distinct methods; Trizol and RNX solution. RNA concentration and optical absorption in 260 and 280 nanometer were measured by Nanodrop instrument. Presence of mir-21, mir-141 and mir-205 were quantified in fresh and frozen samples by real-time RT-PCR.
Results: miRNA extraction from different samples by Trizol and RNX were compared and optimized. After doing some modifications in extraction method and adding a protease K treatment step, extracted RNAs were used in real time RT-PCR. Presence of mir-21, mir-141 and mir-205 was detected in the urine of control and patient groups. The level of mir-21 in extracted RNAs using a modified Trizol method was significantly higher than RNX method. Interestingly, the levels of mirRNAs expression were much higher in the frozen urines compared to the fresh ones. Mir-21, mir-141 and mir-205 showed a differential pattern of expression in normal persons compared to the cancer patients.
Conclusions: We have succeeded to set-up a protocol to easily detect and quantify miRNAs in urine samples. Based on our preliminary data, microRNAs seem to be good biomarkers for early detection of cancers.

626 POSTER

Molecular characterization of circulating tumor cells using a highly sensitive method of enrichment based on the CellSearch CTC profile kit

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Background: The concept of "liquid biopsy" refers to analysis of rare cells in the blood such as Circulating Tumor Cells (CTC) and represents a powerful tool for molecular characterization of tumors for which biopsies are not available. An urgent need exists for improved technologies for isolation and molecular characterization of CTCs for low EpCAM-expressing cancers. Herein, we demonstrate a new approach for isolation and characterization of CTCs.
Materials and Methods: We developed a highly sensitive method for CTC characterization based on integration of the following platforms: CellSearch CTC Profile Kit for CTC isolation, immunofluorescent analysis using LSC for CTC detection and enumeration, and an automated platform for FISH analysis. The efficiency of CTC isolation by CellSearch Profile Kit was compared to that of FDA-cleared CellSearch CTC enumeration kit from Veridex, LLC. CTCs isolated from the blood of patients with non-small cell lung cancer (NSCLC), breast and prostate cancers were subjected to FISH analysis for detection of c-Met and IGF1R amplification, PTEN deletion and TMPRSS2-ERG fusion.
Results: In a side-by side comparison using blood from prostate cancer patients (EpCAM-positive), Profile Kit/LCS method recovered up to 470% more CTCs compared to standard CellSearch CTC enumeration kit. Zero CTCs were recovered by standard CTC Kit, whereas $\geqslant$39 CTCs were recovered by the Profile Kit in 4 prostate cancer cases, and $\geqslant$29 CTCs were recovered in 3 hepatocellular carcinoma (EpCAM-low). CTC detection in patients with head and neck, renal cell, basal cell, prostate, NSCLC, sebaceous gland and ovarian cancers demonstrated that the frequency of successful CTC detection was consistently higher with Profile Kit/LSC method: 79 of 90 (88%) were CTC-negative using standard CTC kit, while only 37% of patients were CTC-negative by Profile Kit/LSC method. We further validated an integrated method of CTC analysis by FISH. Using new method, NSCLC, prostate and breast cancer CTCs were interrogated by FISH and found to carry genetic abnormalities in c-Met, IGF1R, PTEN and TMPRSS2-ERG.
Conclusion: We developed a new method that offers higher CTC recovery and provides a broader capability for downstream molecular characterization of cancers. We report for the first time on the ability to conduct FISH characterization of NSCLC and breast cancer CTCs for abnormalities in c-Met and IGF1R at the single cell level.

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PI3K- and ERK-pathway biomarker comparison by IHC, IF/AQUA™ and RPPA upon AKT inhibition

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Background: Identification of robust mechanism-based biomarkers is increasingly important in preclinical and clinical studies. Classical immunohistochemistry (IHC) is an operator-biased technique that does not provide precise protein quantification. In the current study we have evaluated the reliability of immunofluorescence (IF)/AQUA™ and reverse phase protein arrays (RPPA) methodologies in measuring markers of PI3K pathway inhibition. Our ultimate goal is to incorporate these biomarkers in the phase I study with GDC-0068.
Methods: We have used xenografts from trastuzumab resistant breast BT474-Tr and PC3 prostate cells. Ten PI3K- and ERK-pathway biomarkers were analyzed upon treatment with a pan-AKT inhibitor, GDC-0068.

We have assessed the phosphorylation status of AKT-473, MAPK and S6–240/4 at serial time points and compound dosages in both xenografts and mouse skin (surrogate tissue). Other relevant biomarkers like phosphorylation of PRAS40, nuclear translocation of FoxO3a or phosphorylation of the eiF4E/4G complex, together with the proliferation related Ki67 and Cyclin D1, were investigated. IHC/H-Score, IF/AQUA™ and RPPA/densitometry techniques were used in order to quantify these protein levels.
Results and Conclusions: GDC-0068 induced profound biomarker change along the PI3K pathway. Across the three platforms, the data significantly correlated at time points/doses for which GDC-0068 was active. As observed for other AKT catalytic inhibitors, AKT-473 was consistently hyperphosphorylated in a dose response manner and therefore useful for assessing the compound presence in the tumor. FoxO3a nuclear shift was persistent throughout the study, indicating constant AKT inhibition. Other biomarkers such as P-S6–240/4 and Ki67 were reduced upon acute AKT inhibition but their response was not maintained after long-term GDC-0068 administration. AQUA™ and RPPA are quantitative techniques that provide unbiased and reproducible biomarker evaluation.

628 POSTER

Stem cell marker expression profile in colon cancer xenografts and their corresponding cell lines

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The isolation and characterization of tumorigenic colon cancer stem cells may help to devise novel diagnostic and therapeutic procedures. In the present study we characterized a panel of 15 human colon carcinoma cell lines and their corresponding xenografts for their expression of 5 different potential stem cell markers: CD133, CDCP1, CXCR4, CD24 and CD44. Detection of the different surface markers was done by flow-cytometry (FACS). In 8 out of 15 models mRNA expression of the investigated markers was determined using a gene expression array (HU133 plus 2.0). Tumor growth behavior in correlation to CD133 expression was determined in SW620, when CD133+, CD133− and unsorted cells were injected subcutaneously (sc) into NOD/SCID mice. All five determined so called stem cell markers showed different but distinct expression patterns in the examined tumors. CD133 was highly expressed (>85% of positive cells) on 3 out of 5 patient-derived cell lines whereas in long-term culture based models CD133 expression ranged from 0−<20%. In 12/15 cell lines more then 80% of the cells were positive for CD24 and 13/15 were positive for CD44 to an extent of 70% and more. 11/15 cell lines expressed CDCP1 on $\geqslant$ 83% of their cells. CXCR4 was expressed exclusively on 3 cell lines (94L, SW480 and A293). Analyses of the corresponding xenografts revealed a significant reduction of cell numbers expressing the investigated surface markers. Gene expression analyses disclosed a strong correlation between CD133 and CD44 (0.952; p-value <0.005) and CD24 (0.81; p-value <0.005). Within the investigated xenografts small subpopulation of double (CD133/CD44 or CD133/CD24) and triple (CD133/CD24/CD44) positive cells could be described. In vivo growth behavior studies of SW620 revealed a CD133 dependent tumor growth in vivo, as CD133 positive subclones of SW620 showed significantly higher take rates and doubling times then the unsorted cell line. In contrast CD133 negative subclones induced significantly lower take rates and doubling times in comparison to the unsorted cell line. Tumors of all three cell types were analyzed as described above and showed the same expression pattern of the five investigated surface markers. Our data strongly recommend CD133 as a potential stem cell marker within the investigated colon carcinoma panel. Further studies will elucidate its role as a potential therapeutic target.

629 POSTER

Genetic polymorphisms of transforming growth factor-beta1 and estrogen metabolizing enzyme in estrogen receptor-positive and -negative infiltrating ductal breast carcinoma

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Background: Interactions between the transforming growth factor and estrogen metabolizing enzymes play important role in maintaining reproductive homeostasis. The aim of this study was to examine the effect of single nucleotide polymorphisms (SNPs) in the CYP1A1–6235T/C (rs4646903), SULT1A1–638G/A (rs9282861), TGFB1–509C>T (rs1800469) and TGFB1−29T>C (rs1982073) gene on the risk, progression and response to neoadjuvant chemotherapy in a cohort of estrogen receptor (ER)-positive and -negative infiltrating ductal breast carcinoma patients.
Material and Methods: This study included 178 women with ER-positive and negative histologically confirmed infiltrating ductal breast carcinoma, who received two-four cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute. The control group consisted of 290 unrelated women with benign breast pathologies. Genotyping was performed on genomic DNA using polymerase chain reaction and restriction fragment length polymorphism.
Results: Both ER-positive and -negative patients groups carrying SULT1A1(A/A) genotype were found to be significantly associated with increased risk of ductal breast carcinoma (OR = 2.02; p = 0.002 and OR = 1.88; p = 0.03, respectively). We showed the protective effect concerning this disease for the CYP1A1(T/T) and SULT1A1(G/G) genotypes independent of patients estrogen receptor status. In addition the ER-positive women with the TGFB1–509(T/T) genotype had a significantly lower risk of developing ductal breast carcinoma (p = 0.01) while a trend to the same association was observed among the ER-positive TGFB1−29(T/T) carriers (p = 0.051). There was also trend for association between the ER-positive TGFB1–509(C/C) genotype and large tumor size (p = 0.057). We found that the TGFB1–509(C/C) and SULT1A1(A/A) genotypes were non-statistically significant related with a poorer response to chemotherapy in ER-positive women (p = 0.09 and p = 0.06, respectively). Both ER-positive and negative the carriers of the CYP1A1(T/T) genotype showed an association with better response to neoadjuvant chemotherapy compared to the carriers of the other genotypes although the differences did not reach statistical significance (p = 0.09 and p = 0.06, respectively).
Conclusions: These findings suggest that genetic polymorphisms in TGFB1–509C>T and TGFB1−29T>C may modify individual susceptibility to ER-positive infiltrating ductal breast carcinoma. Further studies are needed to clarify the effect of SNPs analyzed in this work on prognosis and the efficacy to neoadjuvant chemotherapy in ER-positive and negative patients with infiltrating ductal breast carcinoma.

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Microarray based expression profiling of BRCA1 mutated breast tumours using a breast cancer specific array to identify a profile of BRCA1-deficiency

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Background: The BRCA1 tumour suppressor gene is mutated in a significant proportion of hereditary breast cancer cases. In addition, downregulation of BRCA1 mRNA and protein expression is reported in approximately one third of sporadic breast cancers. BRCA1 is strongly implicated in the maintenance of genomic stability by its involvement in multiple cellular pathways including DNA damage signalling, DNA repair, cell cycle regulation, protein ubiquitination, chromatin remodelling, transcriptional regulation and apoptosis. To date, gene expression profiling has identified: (1) at least five breast cancer subtypes and that (2) BRCA1 mutant tumours segregate with basal-like breast cancers. These studies also provide evidence that breast cancers with germline mutations in BRCA1 are different from non BRCA1-related tumours. The main aim of this study is to investigate the underlying biology of BRCA1-mutated breast cancer.
Methods: Extensive gene expression profiling and data analysis were performed on a cohort of 70 FFPE (Formalin Fixed Paraffin Embedded) derived BRCA1 mutated breast tumours and matched sporadic controls using the Almac Breast Cancer DSA™ research tool. Functional analysis was performed by DAVID and METACORE. Validation of gene targets was performed by both qRT-PCR and Western blotting.
Results: A list of differentially expressed transcripts has been derived from the comparison of these BRCA1 mutant breast tumours and matched sporadic controls. Functional analysis of this gene list has identified the key genes and molecular pathways that are deregulated in these tumours. BRCA1 deficiency was associated with deregulation of pathways involved in: (1) immune response, (2) metastasis and invasion, (3) cytoskeletal remodelling, (4) spindle assembly and chromosome separation, (5) apoptosis and survival. Validation of the key genes underlying this BRCA1-deficient breast cancer profile has been performed.
Conclusions: This approach has revealed a set of transcripts that could potentially be used to identify both hereditary and sporadic breast cancer