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.

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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.

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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.

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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.