analysed using MSD® technology. In addition, drug effects and targets within the tissue were evaluated using immunohistochemical staining against key phosphoproteins (e.g. pAkt, pMAPK, pmTOR) and numerous other cancer relevant molecules (e.g. p53 and Ki67).
Results: The model was validated using 28 colon, 32 NSCLC and 12 breast cancer specimens. The slices remained viable for at least 4 days and showed a good response to cytotoxic treatment in ATP and caspase 3/7 assay. Analysis of signalling pathways revealed individual differences in drug response, which was supported by IHC staining.
Conclusions: Deciding which drug should enter the clinical trial phase is one of the most critical and expensive parts in drug development. Thus, new models are urgently needed to improve the success rate of drugs in clinical trials. In this study we established a standardised protocol for the preparation of viable tumour tissue slices which allows the testing of anti-cancer agents in a preclinical model. The model was established for colon, NSCLC and breast cancer and tested with various anti-cancer agents used in standard therapy. The model both allowed evaluating target expression, functional drug-effects and inhibition/activation of specific pathways in parallel as well as determination of heterogeneity among patients. The use of such organoid cultures bears great potential for studying tumour responses to anticancer drugs because the complex environment of the primary cancer tissue is being maintained.

1014 POSTER DISCUSSION

Genomics-based selection and characterization of pre-clinical oncology testing models and prediction of patient response in clinical trials

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The current paradigm in cancer drug discovery is to target molecular lesions rather that histopathological cancer types. We used patterns of copy number abnormalities (CNAs) to identify genomic subgroups of several cancer types. This information was used to rationally select panels of pre-clinical testing models. We used high-resolution comparative genomic hybridization (CGH) to create a database of genomic profiles of tumors that includes hundreds of tumors and cells lines. A computational algorithm was developed to process gene copy number data to identify distinct groups within a cancer type and assign cell lines and xenografts to appropriate groups. The analysis was performed for NSCLC, SCLC, colorectal cancer, and melanoma, resulting in creation of panels of characterized pre-clinical models.
A proactive approach to genomic biomarker discovery involves copy number analysis of model systems with known sensitivity to drug candidates. Once a CNA is found that correlates with the sensitivity of the model system to the candidate compound, it can be tested as a predictor of drug response in clinical trials by using FISH. We have implemented this early biomarker discovery strategy for a number of our oncology drug candidates. For example, we applied integrative genomics to identify predictors of sensitivity of SCLC to Bcl-2 family inhibitors. Our CGH screen of SCLC cell lines followed by genome-wide analysis of aberrations has identified a novel gain on 18q21−23 that is associated with sensitivity to ABT-263, a first-in-class Bcl-2 family inhibitor. Our data suggest that the 18q21−23 copy number may be a clinically relevant predictor for sensitivity of SCLC to Bcl-2 antagonists. As the finding is being validated in clinical trials, we use circulating tumour cells (CTCs) from SCLC patients, as biopsies are rarely available. Preliminary results show increased Bcl-2 copy number in some patients.
In summary, our use of high-resolution copy number profiling in cancer has resulted in rational genomics-based selection of preclinical testing models and identification of a stratification marker for therapy with Bcl-2 inhibitors.

1015 POSTER DISCUSSION

Activity of the MAPK signalling cascade correlates to gemcitabine sensitivity in pancreatic cancer cells

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Introduction: Pancreatic cancer is a leading cause of cancer-related death in the world. Gemcitabine remains to be the gold standard of therapy. However, the effectiveness of this treatment has been hindered by increasing numbers of patients exhibiting resistance. Research has hinted at a relationship between the Ras/Raf/Mek/Erk pathway and chemoresistance, as this pathway induces a strong survival signal in cancer cells. The purpose of this study was to establish a molecular background of pancreatic cancer cell lines and to identify the molecular signature of gemcitabine.
Methods: PANC-1, MIA-PaCa-2 and BxPC-3 pancreatic cancer cells were seeded into 96 well plates at 5×10^3 cells/well and treated with gemcitabine (1 uM to 10 mM) for 48 h. IC50 of gemcitabine for each cell line was then determined using MTT or Alamar Blue assays. MAPK specific protein arrays from R&D Systems were used to screen cells pre- and post-gemcitabine treatment (10 uM, 48 h) to create a list of protein affected by gemcitabine treatment. In addition, Western Blot analysis was used to confirm the results of the protein arrays.
Results: MTT and Alamar Blue assays showed in order of gemcitabine sensitivity, PANC-1>BxPC-3>MIA-PaCa-2 (see table 1). Screening with protein arrays demonstrated that basal levels of MAPK related proteins differ between cell lines and this relates to gemcitabine sensitivity. Comparison of untreated cells and gemcitabine treated cells created a shortlist of proteins with greater than 20% change in expression for each cell line. Differences in expression levels in proteins from this shortlist were then quantified using Western Blot analysis. Results showed that gemcitabine resistant PANC-1 cells exhibit higher basal Erk activity than sensitive MIA-PaCa-2 cells. Currently work is underway using the MEK inhibitor U0126 to knock out Erk signalling. Preliminary results suggest a relationship between Erk activity and gemcitabine sensitivity.

	PANC-1	MIA-PaCa-2	BxPC-3
Kras	Mutant	Mutant	Wild type
IC50 (uM)	300	60	120
Untreated (%GAPDH)			
tErk	78.5±17.6	60.8±9.8	98.5±13.4
pErk	27.1±6.9	21.3±3.4	9.2±4.1
Gemcitabine (10 uM)			
tErk	83.4±9.1	60.7±17.8	53.2±11.0
pErk	45.6±17.4	36.0±5.6	12.9±1.8

Conclusions: Results show that Erk activity may be important in cellular resistance to gemcitabine and sensitivity may be increased through its inhibition. This suggests that combining gemcitabine with an inhibitor of Erk may enhance gemcitabine efficacy.

1016 POSTER DISCUSSION

Prognostic and predictive significance of BRAF mutation in patients with metastatic colorectal cancer treated with 5-fluorouracil-based 1st line chemotherapy

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Background: The genetic events underlying colorectal cancer (CRC) are well characterized and include common somatic mutations in the *APC*, *TP53* and *KRAS* genes, followed in frequency by *PIK3CA* and *BRAF* mutations. Activating mutations in the *BRAF* oncogene deregulate growth factor pathways, stimulate cell proliferation, and promote metastasis. As previously reported by our group, patients with metastatic CRC primary tumors harbor *BRAF* mutations have an increased risk of disease progression and death. In the present study the significance of *BRAF* mutations was evaluated in an independent group of patients with metastatic CRC.
Material and Methods: The *BRAF* (V600E) mutation was determined by allelic discrimination, using Real-Time PCR, in 150 primary tumors from patients treated for metastatic CRC at a single institution. The underlying *BRAF* mutation was correlated with time to tumor progression (TTP) and overall survival (OS).
Results: The median age of this patients' population was 64.5 years (range 23−81) and 63% were male. All patients received 5-FU-based 1st line chemotherapy, 33% in combination with oxaliplatin, 18% with irinotecan and 49% with both drugs; bevacizumab or cetuximab was added to chemotherapy in 22% and 7% of patients, respectively. *BRAF* mutations were present in 12 (8%) cases. Patients with *BRAF* mutated primary tumors had a median OS of 14 months compared to patients with wild-type primary tumors whose median OS was 30 months ($p < 0.0001$). In addition, TTP was 2.6 months for patients with *BRAF* mutated primary tumors whereas it was 10.3 months for patients with *BRAF* wild-type primary tumors ($p < 0.0001$). Cox regression analysis uncovered *BRAF* mutations as prognostic factor for decreased OS (Hazard Ratio [HR] 4.4, 95% CI 2.3−8.2). Patients with *BRAF*-mutant tumors had significantly lower TTP (HR 2.98, 95% CI 1.6−5.4) than those whose primary tumors carried only wild-type *BRAF*.