In addition, the activity of Akt and MAPK pathways was analysed. Akt signaling is important in many breast cancers and 8 out of 12 tumor models depicted activated Akt, some of which also had highly activated p70S6K. The mutational status of PI3Kα and PTEN was assessed with PTEN protein expression also evaluated by Western Blot. Modifications in the PI3Kα activity and its negative effector PTEN modulate Akt kinase activity and downstream signaling.
Finally, the estrogen-dependency of the ER-positive models MAXF 713 and MAXF 1398 and their growth properties in dependence of subcutaneous versus orthotopic implantation were assessed. The xenograft models are routinely implanted subcutaneously, which sustains tumor growth. Nevertheless we found that of the 12 MAXF models as studied, 4 grew faster when implanted orthotopically into the mammary fat pad.
In summary, a unique collection of patient-derived mammary xenograft models has been characterised comprehensively in order to enable a pre-study selection of suitable tumors for in-vivo efficacy testing.

635 POSTER

Novel mechanisms of taxane and platinum resistance in Oesophagogastric Cancer Cells

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Background: Platinum analogues and taxanes are clinically effective in oesophagogastric (OG) cancer treatment. Nevertheless, resistance (primary or acquired) remains a major therapeutic problem. We developed platinum and docetaxel resistant cell line models and used gene expression profiling for hypothesis generation regarding predictive biomarkers for resistance and novel modes of action/resistance to facilitate target discovery and provide insight into mechanisms of cross resistance to inform optimal cytotoxic/targeted therapy strategies.
Material and Methods: Resistant daughter cell lines (n = 14) were generated from 3 parental OG cancer cell lines (OE33, OE21, AGS) by exposure to increasing concentrations of oxaliplatin, cisplatin and docetaxel. Cell viability was measured by the MTT assay. Drug resistance and cross resistance was determined. Gene expression analysis was performed on wild type and resistant daughter cell lines using Exon 1.0 Affymetrix microarrays. Gene expression data were normalized and statistically significant changes identified in GeneSpring GX v10.0.1. Ingenuity Pathway Analysis (IPA) software and GSEA gene sets were utilized to identify key biological pathways altered in resistant cells.
Results: Cisplatin and oxaliplatin cross resistance was observed in 5 out of the 6 platinum resistant cell lines. Docetaxel cross resistance was observed in only 1 out of 6 oxaliplatin and cisplatin resistant cell lines with 2 out of 6 lines showing increased sensitivity to docetaxel. Preliminary analysis demonstrates that the primary difference in gene expression between cell lines is tissue type (gastric vs. oesophageal) and histology (squamous vs. adenocarcinoma). Gene expression analysis of the oxaliplatin resistant lines identified 107 candidate genes involved in oxaliplatin resistance. Biological pathway analysis highlights DNA mismatch repair, down-regulation of cell cycling and anti-apoptotic response as potential mechanisms of oxaliplatin resistance.
Conclusions: Significant differences at the transcriptional level within the panel of OG cell lines are consistent with observed clinical heterogeneity of these tumours. No common mechanisms of resistance to all drugs were identified. We are currently processing further technical replicates in order to perform a comparative study (sensitive versus resistant) within each cell line and for each drug, to characterize gastric vs. oesophageal and adenocarcinoma vs. squamous cell carcinoma- dependent mechanisms of resistance and/or cross resistance.

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CD9 decreases adhesion and improves chemotactic response of B acute lymphoblastic cells: role in pathogenesis of TEL/AML1 leukemia?

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B lineage acute lymphoblastic leukemia (B-ALL) is the most common cancer in childhood and 25% displayed the *TEL/AML1* rearrangement. Previously, we have shown that this subset of B-ALL is characterized by five biological processes and 14 genes. We investigated the role of the *CD9* gene, which is involved in the motility process and is differentially down-regulated in *TEL/AML1* positive B-ALL. Indeed, the expression of CD9 has been correlated with the risk of metastases or a poor clinical outcome in various types of cancer. Because of a good overall prognosis of the *TEL/AML1* positive B-ALL and the special occurrence of very late relapses, we decided to study the impact of the expression of CD9 by motility assays in the context of *TEL/AML1* positive B-ALL.
Leukemic blasts isolated from bone marrow of patients and B-ALL cell lines that expressed or not CD9 (REH CD9+, Raji CD9− and Raji transfected with CD9) were used for the assays. For adhesion assay, 96-well plates were coated with superfibronectin (1 mg/ml). $5 \times 10e5$ cells/well were allowed to attach for 1h30 with or without blocking CD9-antibody (Ab). Adherent cells were quantified using MTS for 2h30 at 37ºC with absorbance measure at 540 nm. For migration assay, cells were seeded on 5mm transwell microporous polycarbonate membranes. $2 \times 10e6$ cells treated with different blocking Abs (CD9-Ab, CXCR4-Ab, IgG control) were loaded in the upper chamber while 100 ng/ml CXCL12 (B-ALL specific chemokine CXCR4 ligand) was added to the medium of the lower chamber. After 5 hours, the migrated cells were recovered from the lower chamber, numbered and analysed by FACS.
We showed that the expression of CD9 decreased the ability of lymphoblasts to attach to an extracellular matrix component both in B-ALL cell lines and in patients cells. This effect was reversed by CD9 blocking Ab. Conversely, migration was increased in CD9 positive cell lines whereas blocking CD9 reduced the chemotactic migration through membranes. A synergic effect with CXCR4 expression was also shown.
This study pointed out the importance of the CD9 expression on the ability of B-ALL cells to attach and migrate. Moreover, CD9 and CXCR4 seemed tightly linked in the chemotactic response to CXCL12. Because CXCL12-CXCR4 axis is known to facilitate metastasis and CD9 confers particular adhesion and migration properties to lymphoblasts, we raised the issue of a role of CD9 in the pathogenesis of the late extramedullary relapses of *TEL/AML1* B-ALL.

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Prediction of the human pharmacokinetics (PK) and pharmacodynamics (PD) of MLN9708, an investigational proteasome inhibitor

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The investigational drug MLN9708 is a modified dipeptidyl boronic acid that is a potent, reversible and specific inhibitor of the 20S proteasome. MLN9708 is currently being evaluated in first-in-human phase I trials of hematologic malignancies and solid tumors. The current work reports on the prediction of human blood MLN9708 PK and PD based on pre-clinical studies. In aqueous solutions MLN9708 is immediately and completely converted to MLN2238. Pre-clinical studies were performed using MLN2238 and human PK results were measured as MLN2238 concentration. The PK of MLN2238 in whole blood and plasma and the PD of proteasome inhibition in whole blood were characterized in immunocompromised mice, Sprague–Dawley rats, Beagle dogs and Cynomolgous monkeys. In all preclinical species tested, MLN2238 displayed a bi-exponential profile in whole blood and plasma following IV dosing, with a rapid initial disposition phase and a slow terminal disposition phase. Projection of human PK profiles was performed according to a modification of the method of Wajima et al. (Wajima T. et al. 2004. J Pharm Sci 93:1890−1900), using the experimental whole blood and plasma PK data from rat, dog and monkey. Projection of human whole blood PD profile (20S specific activity vs. time) was derived using the predicted human whole blood PK profile and in vitro proteasome inhibition parameters determined in monkey.
The human projected PK and PD were compared to the preliminary PK and PD results of the MTD cohort (1.76 mg/m^2) from the first-in-human IV study of MLN9708. A bi-exponential human plasma PK profile was predicted. For a dose of 1.76 mg/m^2, a C_{max} of 122 ng/mL an initial disposition phase $t_{1/2}$ of 0.25 hr, and a terminal disposition phase $t_{1/2}$ of 3.1 days were projected. In the clinical MTD cohort (n = 6), C_{max} was 319 ng/mL the initial disposition phase $t_{1/2}$ was 0.20 hr, and a terminal disposition phase $t_{1/2}$ was 1.8 days. Maximal whole blood proteasome inhibition was predicted to occur immediately following bolus injection of MLN9708, with 70% inhibition anticipated at a dose of 1.76 mg/m^2. At all doses maximum whole blood proteasome inhibition was observed in the first post dose sample (5 min), with 55% inhibition observed in the MTD cohort.
Conclusion: The projected PK and PD profiles of MLN9708 were similar to those observed in the MTD cohort of the FIH study of MLN9708.