266 POSTER

Monitoring of pancreatic ductal adenocarcinoma in mouse models via multiparametric magnetic resonance imaging

I. Heid[1], M. Trajkovic-Arsic[1], M. Gretzinger[2], Y. Kosanke[2], A. Steingoetter[3], E.J. Rummeny[2], R.M. Schmid[1], J.T. Siveke[1], R. Braren[2]. *[1]Klinikum Rechts der Isar Technical University of Munich, Second Department of Internal Medicine, Munich, Germany; [2]Klinikum Rechts der Isar Technical University of Munich, Institute of Radiology, Munich, Germany; [3]University and ETH Zurich, Institute for Biomedical Engineering, Zurich, Switzerland*

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease due to the late diagnosis and the lack of effective chemo/radiotherapy. The development of strategies for early tumor detection and evaluation of new treatments in models with high predictive value is of outmost importance. To characterize the spontaneous course of tumor progression, we monitored tumor growth kinetics and physiology in genetically engineered mouse models (GEMMs) of PDAC by multiparametric magnetic resonance imaging (MRI).

For generation of conditional endogenous PDAC models, *Ptf1a*$^{+/Cre}$ mice were crossed to *Kras*$^{+/LSL-G12D}$*(K)Tgfa(T), K;p53(P)*$^{+/fl}$, *K;P*$^{fl/fl}$, *K;P*$^{+/R172H}$*;T* and *K;P*$^{+/fl}$*;T* GEMMs and subjected to serial T_2-weighted (T_2w) MRI from 4−6 weeks of age onwards. Upon detection of solid tumor, diffusion-weighted imaging and dynamic contrast enhanced-MRI protocols were implemented. Survival analysis and histopathological characterization of PDAC were performed and correlated with MRI data.

Tumor growth kinetics as determined by semi-automated segmentation of T_2w data showed good correlation with the animal genotype. In *K;P*$^{+/fl}$ animals, tumors were first detected at 12−15 weeks of age while significant tumor burden was observed around week 20. Tumor appearance was highly heterogeneous, with cystic and solid tumor parts clearly distinguishable. Additional TGFa overexpression led to earlier onset, faster tumor growth and significantly shorter lifespan. Large premalignant IPMN lesions, seen in TGFa expressing models, appeared hypointense compared to PDAC. Deletion of both p53 alleles led to development of PDAC already at 4 weeks of age. These tumors appeared more homogeneous and were histologically confirmed as highly fibrotic well differentiated PDAC. ADC values correlated well with the histological grading of tumor. Tumor perfusion revealed differences among the tumors and allowed identification of spontaneous necrosis.

Conclusions: Using non-invasive multiparametric MRI we characterized PDAC development and progression in mouse models. This information is valuable for correct interpretation of spontaneous and drug-induced changes in tumor physiology when conducting preclinical therapy studies. Strong heterogeneity in tumor morphology emphasizes the necessity for an individualized tumor assessment and response monitoring.

267 POSTER

Early detection of pro-apoptotic effects of chemotherapy using a caspase-3 fluorescent probe in human breast tumorgraft models

M.E. Legrier[1], H. Ligeret[2], C. Renauleaud[1], M. Lassalle[1], O. Déas[1], A. Beurdeley[1], S. Cairo[1], M. Massonneau[2], J.G. Judde[1]. *[1]XenTech, Research, Evry, France; [2]QUIDD, Research, Saint Etienne du Rouvray, France*

Background: Apoptosis is a programmed cell-death process believed to play an important role in tumor response to chemotherapy. As an early effector in the apoptotic cascade, caspase-3 (CASP3) activation is an attractive target for *in vivo* molecular imaging. We used a noninvasive molecular imaging technology to study early detection of CASP3 activity *in vivo,* as a potential marker of therapeutic efficacy in a panel of human breast tumorgraft models.

Methods: Human breast tumor xenografts were established directly onto *Nude* mice from patient tumor material (Marangoni et al. Clin Cancer Res 2007; 13: 3989–3998). Ten breast tumor models were characterized for their response to Adriamycin/Cyclophosphamide (A/C) treatment, defining two groups of 5 responders and 5 non-responder tumors. *In vivo* measurement of tumor CASP3 proteolytic activity was carried out 6−72 h after A/C treatment in groups of three mice bearing subcutaneous tumors. QCASP3.2 probe, a FRET probe emitting in the near-infrared wavelength (QUIDD, France) was injected intravenously (7 nmol) and fluorescence imaging was performed 6−24 h later with an optical imaging system (IVIS-Lumina II, Caliper, CA). For *in vitro* assays, tumor protein extracts were prepared in lysis buffer containing protease inhibitor cocktail, and incubated during 1 h at 4ºC. Total proteins were quantified by the microBCA protein assay kit (Pierce, Rockford, IL). Caspase activity was measured on synthetic substrate Ac-DEVD-AFC for caspases 3/7 (AnaSpec, San Jose, CA). Enzymatic reactions were allowed to proceed after 30 mn at room temperature. Fluorescence intensity was measured at different time points on a PerkinElmer LS 50B spectrofluorometer (PerkinElmer Life Sciences, Norwalk, CT). Specificity of the fluorometric signal was confirmed by adding specific caspase inhibitor to the reaction mixture (casp3 inhibitor, z-DEVD-fmk). Levels of cleaved CASP3 and PARP were assessed in parallel by western blotting.

Results: Basal levels of CASP3 activity were variable between tumors. Increased level of CASP3 activity was detected *in vivo* in tumors of mice treated with chemotherapy 48h post-treatment. This increase was confirmed *in vitro* by enzymatic and western blotting analyses. Increased CASP3 activity in tumors was correlated with their sensitivity to A/C treatment as assessed by the extent of tumor growth inhibition 10−20 days later.

Conclusion: The QCASP3.2 probe is efficient in early detection of tumor apoptosis *in vivo.* The data confirm that drug-induced tumor apoptosis can be an early marker of antitumor efficacy. This approach will allow molecular imaging assessment of tumor apoptotic response to therapy in preclinical models. Quantitative imaging of apoptosis *in vivo* with the QCASP3.2 probe will facilitate dynamic screening of compound's activity and the assessment of tumor CASP3 activity as an early predictor of therapeutic efficacy in various tumor models.

268 POSTER

An in vivo-like tumor stem cell-related glioblastoma in vitro model for drug discovery

S.S. Jensen[1], C. Aaberg-Jessen[1], A. Nørregaard[1], B.W. Kristensen[1]. *[1]Institute of Clinical Research, Dept. of Pathology, Odense, Denmark*

The discovery of tumor stem cells being highly resistant against therapy makes new demands to model systems suitable for evaluation of the effects of new drugs on tumor stem cells. The aim of the present study was therefore to develop an in vivo-like in vitro glioblastoma model for testing the effects of new drugs on tumor cells including tumor stem cells. Implantation of glioblastoma cells into organotypic brain slice cultures has previously been published as a model system, but not using a stem cell favourable environment.

Organotypic corticostriatal rat brain slice cultures were prepared and cultured in a serum containing medium replaced after three days with a serum-free stem cell medium. Thereafter fluorescent DiI labelled glioblastoma spheroids from the cell line U87 and the tumor stem cell line SJ-1 established in our laboratory were implanted into the brain slices between cortex and striatum. The growth and invasion of the tumor cells were monitored for six days by confocal microscopy followed by processing for histology and immunohistochemical staining. In addition, in vivo xenografts were performed in BALB/c nude mice with U87 cells and SJ-1 cells.

The results showed an expansive growth of the U87 implants, but no invasion of cells into the brain tissue, neither in vitro nor in vivo. In contrast, SJ-1 was clearly invasive both in vitro and in vivo, but not very expansive. The co-cultures and brains with xenografts were immunohistochemically stained with anti-human vimentin confirming the results obtained with hematoxylin-eosin staining and confocal microscopy. Both in vitro and in vivo, U87 implants had a very high proliferation index, whereas the invasive phenotype of SJ-1 only had a low index as shown by Ki-67 immunohistochemistry. Immunohistochemistry for the stem cell marker CD133 revealed only a few positive cells in contrast to staining for the proposed tumor stem cell marker podoplanin showing a more pronounced staining.

In conclusion, we have established an in vivo-like in vitro co-culture model system that enables us to monitor tumor growth and invasion using a stem cell favourable environment. We propose that this model system could play a role in testing of new drugs targeting tumor stem cells.

269 POSTER

Monitoring radiation therapy in orthotopic mouse models by bioluminescence imaging

M. Baugher[1], M. Ziemke[2], E. Trachet[1], P. McConville[2]. *[1]MIR Preclinical Services (Charles River Laboratories), Oncology Pharmacology Services, Ann Arbor MI, USA; [2]MIR Preclinical Services (Charles River Laboratories), Imaging Services, Ann Arbor MI, USA*

Introduction: Radiation therapy is a standard of care for many cancer indications including brain and prostate. Preclinical methods for using localized radiation (+/−chemotherapy) against tumors in deep tissues may increase clinical relevance and predictive power of models. The ability to non-invasively detect tumor burden and radiation response in deep tissue tumors would be an additional advantage. We describe application of localized radiation therapy in two orthotopic tumor models with parallel luciferase driven imaging of tumor burden and response.

Methods: Luciferase-expressing human prostate and brain tumor lines were used in orthotopics [prostate: PC-3M-luc-C6 prostate carcinoma cells (5×10^5 cells in 20 μl) injected into the dorsal prostatic lobe of male nu/nu mice; brain: U87MG-luc glioma cells (10^6 cells in 10 μl) injected by stereotax 2 mm right lateral and 1 mm anterior to the coronal suture at a depth of 2 mm]. At staging (prostate: Day 7; brain: Day 27) animals were imaged using bioluminescence. The tumor total light output (photons/sec) was used to select mice to go into untreated and fractionated radiation treated [2.5 Gy (QD × 5;2off) × 2 wk] groups (prostate: n = 10; brain: n = 8) matched for mean tumor burden. A lead shielded holder localized the dose to the prostate or brain regions. Bioluminescence imaging was used to assess tumor burden and treatment response time course in each model. **Results and Discussion**: Radiation therapy at 2.5 Gy on a (QDx5;2off) × 2 wk schedule was tolerated, producing 3% and 13% mean body weight loss in the prostate and brain tumored mice respectively. Bioluminescence imaging enabled a highly sensitive and quantitative end point for tumor burden in both cases, and confirmed localization of the tumor growth to the tissues in question. Radiation resulted in approximate tumor stasis in both cases, with T/C values of 6% (4 weeks post-Rx) and 43% (1 week post-Rx) in the prostate and brain tumor models, respectively. **Conclusion:** Bioluminescence imaging coupled with localized radiation treatment enables a quantitative model for deep tissue tumor targeted combination chemotherapy/radiation treatment strategies in preclinical models. This approach may facilitate development of new molecules for use with radiation, including optimization of schedule and timing.

270 POSTER

Human primary colon tumorgraft models possess similar clinical response characteristics

E.M. Bruckheimer[1], S.J. Strawn[1], M.J. Wick[2], F. Nieves[2], M. Hidalgo[1], D. Sidransky[3], E.K. Rowinsky[1]. *[1]Champions Biotechnology, Preclinical Development, Baltimore, USA; [2]START, Preclinical Development, San Antonio, USA; [3]Johns Hopkins University, Dept. of Otolaryngology, Baltimore, USA*

One of the key challenges facing oncology drug development is the high attrition rates of compounds that enter the drug development pipeline, where very few achieve successful approval and marketing, often due to the inability of preclinical xenograft studies to predict clinical trial results. Champions Biotechnology, in an effort to enhance the value of preclinical compounds and accelerate oncology drug development, has developed a novel preclinical platform derived from Biomerk Tumorgraft™ models; an innovative approach that utilizes the implantation of primary human tumors in immune-deficient mice in a manner that preserves the biological properties of the original human tumor. Biomerk Tumorgrafts™ differ from traditional xenograft models in that they are not maintained in tissue culture, and are instead exclusively passaged *in vivo*. Additionally, the Biomerk Tumorgraft™ models: (a) maintain the fundamental genotypic features of the original cancer, (b) represent the genetic heterogeneity of the cancer, (c) do not change over several passages, (d) and retain cancer stem cells and stromal components. In the current study Champions evaluated the response of colon tumorgraft models to EGFR inhibitors and assessed the superiority of these models over traditional xenograft models, based on historical data, in predicting clinical outcomes. In brief, K-ras status was indicative of resistance/sensitivity to the EGFR inhibitor Cetuximab. For mutant K-ras models 7/8 models showed intermediate/strong resistance where as 1/1 K-ras WT modes showed sensitivity. There was one K-ras mutant model which did not respond as predicted and it was determined that it possessed a PI3K mutation, which may rendered it sensitive to Cetuximab. Together, these results demonstrate that Champions Biomerk Tumorgraft™ models represent a novel preclinical *in vivo* platform capable of predicting the clinical effectiveness of preclinical drug candidates which have the potential to accelerate and enhance oncology drug development.

271 POSTER

Antitumor effect of zalypsis (PM00104) in a human pancreatic adenocarcinoma orthotopic model

P. Aviles[1], M.V. Cespedes[2], M.L. Soto-Montenegro[3], M.J. Guillén[1], J.J. Vaquero[3], P. Alamo[2], C. Cabrera[2], R. Mangues[2], M. Desco[3], C. Cuevas[1]. *[1]PharmaMar S.A., Preclinical, Colmenar Viejo (Madrid), Spain; [2]Institut de Recerca del Hospital de San Pau, Oncogenesis and Antitumor Drug Group, Barcelona, Spain; [3]Hospital General Universitario Gregorio Marañón, Unidad de Medicina y Cirugía Experimental, Madrid, Spain*

Background: Pancreatic ductal adenocarcinoma is the fourth cause of death in the Western world and, despite the advances in cancer therapies, limited therapeutic benefit (palliating symptoms, better quality of life) may be offered to patients: the overall 5-year survival rate remains <5%. PM00104 (a Jorumycin and Renieramycins-related new synthetic alkaloid) is a strong transcriptional inhibitor that shows in vivo antitumor activity against a broad panel of human-derived tumors such as bladder, hepatocellular carcinoma, multiple myeloma or neuroblastoma. Currently, PM00104 clinical development includes phase II single agent trials (cervix/endometrium, and multiple myeloma) as well as phase I combination trials (with carboplatin).
The in vivo antitumor activity of PM00104 has been investigated in a human pancreatic orthotopic model (NP9).
Material and Methods: Athymic nude female mice were orthotopically implanted with NP9 tumors. Fifteen days after implantation, tumor bearing mice were randomly allocated ($N = 10$/group) into either treatment (PM00104 at 1 mg/kg) or control (placebo) groups. Treatments were initiated (Day 0) and administered for 3 consecutive weeks (q7dx3; days 0, 7, 14). On days 0 and 20, animals were subjected to [^{18}F]FDG (2-deoxy-2-[^{18}F]fluoro-D-glucose)-positron emission tomography (PET)/computed tomography (CT) imaging; [^{18}F]FDG-PET images and CT scans were analyzed quantitatively. On Day 21, animals were sacrificed, tumors dissected free, weighed, and frozen or fixed and paraffin embedded and sectioned. Sections were then processed for H&E staining (morphology/necrosis/mitotic bodies) or western blotting (caspase 8; caspase 9; PARP) and quantitatively analyzed. Statistical differences between groups were determined using two-tailed Mann–Whitney *U* test.
Results: Results showed that PM00104 (q7d × 3 at 1 mg/kg) induced statistically significant tumor reduction compared to placebo-treated animals by CT analysis ($P = 0.034$; on Day 20). Reductions in tumor size were associated to changes in metabolic rates: [^{18}F]FDG uptake (Day 20 *vs.* Day 0) was statistically reduced ($P = 0.019$) in PM00104-treated animals. Also, PM00104 treatment significantly increased the % necrosis ($P = 0.025$) and the number of mitotic bodies ($P = 0.020$) compared to placebo-treated animals. Tumor cell death was caspase-independent.
Conclusion: PM00104 has demonstrated in vivo antitumor activity in a human pancreatic orthotopic model (NP9), as reflected by metabolic, size-related and increased necrosis.

272 POSTER

Humanisation of xenograft models to optimally assess the c-Met: HGF paracrine axis

R. Kumari[1], R. Argent[1], D. Onion[1], P. Clarke[1], S. Watson[1], P. King[2], M. Page[2]. *[1]University of Nottingham, Division of Pre-Clinical Oncology & PRECOS, Nottingham, United Kingdom; [2]OrthoBiotech, Oncology Research & Development, Beerse, Belgium*

Introduction: Aberrant c-Met activation is implicated in the development of many cancers, and is therefore an attractive therapeutic target. Hepatocyte growth factor (HGF) signalling to c-Met generally occurs in a paracrine manner, with HGF being secreted by the stroma, and c-Met being expressed by epithelial cells, demonstrating synergy between different cell types within the tumour microenvironment. The aim here was to investigate optimal paracrine signalling developed by humanisation of xenograft models using orthotopic models with human stroma admixed with tumour epithelial cells.
Methods: Human T24 bladder cells and MGLVA-1 gastric carcinoma cells were either admixed with MRC5s/tumour conditioned mesenchymal stem cells (tc-MSCs) (3:1 ratio) or used alone for in vivo administration. T24 cells were injected into the bladder wall and MGLVA-1 cells were injected intrasplenically. Tumours collected at termination were formalin-fixed and preserved in RNA*later.* Gene expression levels of c-Met and HGF were assessed by quantitative PCR, and c-Met, phospho c-Met and Vimentin protein levels were examined histologically.
Results: Increased tumour take-rate was observed in the liver following intrasplenic injection of MGLVA-1 cells and the bladder with T24 cells in the presence of human stroma. In addition, vimentin staining confirmed that human stroma was maintained for the duration of the tumour growth. With human stroma present HGF expression was observed consistent with phospho c-met expression and a more invasive phenotype.
Conclusion: Humanisation of xenograft models with human stroma such as MRC5 or tc-MSCs results in optimal HGF:c-Met paracrine signalling.