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.