We have shown that the toxicity of the fungal toxin NC-001, previously known to be highly and specifically toxic to proximal tubular cells, also extends to cancer cells evolving from these. Presently we are evaluating NC-001's efficacy *in vivo*. Therefore, a subcutaneous renal cancer xenograft model in athymic, radiation-treated rats (RNU, Charles River) was developed utilizing a human metastatic CCRCC cell line (SKRC-17). Also, an automated peritoneal dialysis system was constructed for renal replacement since NC-001 induces total renal failure in the animals through its effect on normal proximal tubular cells. When treating CCRCC-bearing RNU-rats with NC-001, administered i.p. with the dialysis fluid (10 mg NC-001 per L) for the first 48 hours of a 10 day period, tumor growth was completely halted and extensive areas of necrosis developed in treated animals compared to untreated controls. Untreated rats had tumors weighing 4.5 g with 10−20% necrosis (n = 6), while the tumor mass after NC-001 treatment was 1 g with 40−80% necrosis (n = 5). Thus, NC-001 seems to reduce the tumor load with about 90% in 10 days.
In summary, our results indicate that NC-001 has an impact on tumor growth by directly inducing necrosis within the tumor and therefore has potential of a truly curative treatment of metastatic CCRCC. The only known side effect of NC-001 treatment, loss of kidney function, can be well managed by dialysis while waiting for a renal transplantation.

280 POSTER

Bioluminescent imaging in evaluation of therapeutic strategies against cancer: Focus on orthotopic xenograft tumor models with spontaneous metastases

Y. Xu[1], E. Roschmann[2], O. Buiakova[1], D. Grass[1]. [1]*Taconic Cranbury, Scientific Operations, Cranbury, USA;* [2]*TaconicArtemis, Business Development, Cologne, Germany*

Background: More predictive small animal models for compound assessment are needed. We have used *in vivo* and *ex vivo* bioluminescent imaging (BLI) technology to create oncology models to evaluate compound efficacy in mouse models of orthotopic tumor growth and spontaneous metastases. Orthotopic tumor models are more relevant with respect to host-tumor interactions, characteristic disease progression, metastatic potential and response to therapy than the commonly used (subcutaneous) models for preclinical drug selection.
Materials and Methods: Several human tumor cell lines that were genetically modified to express firefly luciferase were inoculated orthotopically. *In vivo* BLI was performed using an *In Vivo* Imaging System (IVIS®) weekly or twice weekly to follow primary and metastatic tumor growth. Metastatic tumor load was also assessed at the end of the study by *ex vivo* tissue BLI. Effects of gemcitabine and docetaxel in the orthotopic models were evaluated.
Results: We have established several orthotopic tumor models progressing to distant spontaneous metastases using human light producing tumor cell lines. In one case, BxPC-3-luc2 human pancreatic adenocarcinoma cells were inoculated orthotopically. Using BLI, we followed the kinetics of tumor growth *in vivo*. At the end of the study, distant metastases were identified and measured by *ex vivo* BLI in the lymph nodes, liver, spleen, lung, femur and diaphragm. Metastases were detected in all of the vehicle treated animals in at least 2 of the evaluated tissues. Interestingly, we did not see a significant effect of gemcitabine (the first line treatment for pancreatic cancer in the human) on the growth of the BxPC-3-luc2 tumors. However, treatment with 20 mg/kg docetaxel effectively inhibited both the primary tumor growth and the development of metastases.
Using a similar approach we have established orthotopic xenograft models with spontaneous metastases for the prostate (PC-3M-luc), breast (MDA-MB-231-luc) and ovarian (SKOV-3) cancers. Importantly, in all of these orthotopic models, the location of the metastases mimics the metastatic sites observed in human patients (diSibio & French, 2008, Arch Pathol Lab Med: v.132, P. 931).
Conclusions: Our platform is highly sensitive and allows for the performance of quantitative and high throughput *in vivo* assessment of potential anti-neoplastic therapies and is especially valuable for evaluating effects of test compounds on spontaneous metastases.

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Investigation of the effect of the sequence-selective DNA cross-linking agent SJG-136 on canine tumour cells in vitro

M. Mellinas-Gomez[1], V.J. Spanswick[2], A. Stell[1], J.A. Hartley[3], D.E. Thurston[3], S. Baines[1]. [1]*The Royal Veterinary College, Veterinary Clinical Sciences, London, United Kingdom;* [2]*University College London, London, United Kingdom;* [3]*Spirogen Ltd, London, United Kingdom*

Introduction: SJG-136 is a novel sequence-selective DNA cross-linking agent that causes minimal distortion of the helical structure such that the cross-links persist. SJG-136 is an effective cytotoxic agent in rodent and human tumour cell lines and is currently in phase II clinical trials in humans. The aim of this study is to evaluate whether SJG-136 is effective in killing canine cancer cells *in vitro*.
Materials and Methods: Canine cell lines representating the main canine cancers, 2 oral melanoma (LmeC and KmeC), 2 skin melanoma (CmeC1 and CmeC2), 2 mast cell tumour, (C2 and ARCE), mammary carcinoma (CFMg), hemangiosarcoma (DEN), osteosarcoma (D17), connective tissue tumour (A72) cell lines were exposed to SJG-136 for 1 hour and 96 hours. Growth inhibition was investigated using SRB and MTT assays to calculate the concentration causing 50% inhibition, IC_{50}. Formation of inter-strand cross-links was measured in 4 melanoma and 2 MCT cell lines using a modified single cell gel electrophoresis (Comet) assay to calculate the concentration causing 50% decrease in Comet tail moment (XL_{50}) after 1 hour of incubation. The effect of SJG-136 on the cell cycle was examined with flow cytometry in 3 melanoma cell lines after 1 hour of exposure and 96 hour post-incubation.
Results: SJG-136 showed potent and selective cytotoxicity with IC_{50} that ranged from <0.03 nM in KmeC and C2 to 17.33±2.33 nM in CmeC1, after 96 hour incubation; and from 4.73±2.22 nM in KmeC to >1000 nM in CmeC1, following 1 hour incubation. DNA cross-links were measured in 6 cell lines, with a linear increase in cross-link formation as the greater drug concentration. The IC_{50} and XL_{50} for 5 cell lines were correlated ($R^2 = 0.9131$); the CmeC1 diverged from this correlation. No significant repair (unhook) of DNA inter-strand cross-links was observed over 48 hour post-incubation. Accumulation of cells in the G2-M phase was observed from 24 hour post-incubation in KmeC and LmeC; in contrast, an accumulation in S phase was observed in CmeC2.
Conclusion: These preliminary data suggest that SJG-136 might be a useful cytotoxic agent for the treatment of canine neoplasias, particularly melanoma and mast cell tumours.

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Set up of a xenogenic, orthotopic and bioluminescent model of localized lung cancer in mice: a stepwise approach

P. Mordant[1], B. Lahon[2], Y. Loriot[3], Y. Castier[4], J.C. Soria[5], E. Deutsch[6]. [1]*UPRES 2710 − Institut Gustave Roussy, Service de Chirurgie Thoracique − Hôpital Bichat, Paris, France;* [2]*UPRES 2710 − Institut Gustave Roussy, Service de Chirurgie Thoracique − Hôpital Bichat, Paris, France;* [3]*UPRES 2710 − Institut Gustave Roussy, Departement de Médecine − Institut Gustave Roussy, Villejuif, France;* [4]*Université Paris 12, Service de chirurgie thoracique − Hôpital Bichat, Paris, France;* [5]*Université Paris 11, Département de Médecine − Institut Gustave Roussy, Villejuif, France;* [6]*UPRES 2710, Département de Radiothérapie − Institut Gustave Roussy, Paris, France*

Background: In the past decades, usual preclinical models of human non-small cell lung cancer (NSCLC) yielded conflicting conclusions. Recently, the disappointing clinical results of otherwise promising targeted therapies underlined the need for more relevant preclinical models. We used a stepwise approach to set up an xenogenic, orthotopic and bioluminescent model of localized intra parenchymatous lung cancer in mice.
Material and Methods: We used athymic *nude* mice and luciferase-positive A549 lung adenocarcinoma cell line (A549-luc). In group 1, animals underwent subcutaneous injection of cells in the right flank (n = 15). In group 2, animals underwent general anesthesia, tracheal intubation, mechanical ventilation, and left thoracotomy to surgically implant a 1 mm^3-piece of luciferase-positive tumor in the parenchyma of the left lung (n = 25). In group 3, animals underwent general anesthesia, chest wall incision, and transpleural injection of cells in the parenchyma of the left lung (n = 25). In group 4, cells were diluted in a solution containing contrast media and mouse sarcoma proteins. Then, animals underwent general anesthesia, radioscopic assessment and percutaneous injection of the solution in the parenchyma of the left lung (n = 30). Bioluminescent in vivo imaging was performed weekly until the end of the experiments, defined as cachexia, dyspnea, or clinical worsening. Xenograft implantation rate was defined as the number of tumour on imaging 2 weeks after implantation or injection. Subsequent locoregional extension, lymphatic and hematogenous metastasis, and 2-month cancer-related mortality were also assessed.
Results: Group 1 was characterized by no perioperative mortality, high implantation rate (100%), neither loco-regional nor metastatic extension, and 2-month mortality of 7%. Group 2 demonstrated high perioperative mortality (60%), low xenograft implantation rate (24%), neither loco-regional nor metastatic extension, and no specific 2-month mortality. Group 3 yielded intermediate results, with perioperative mortality of 36%, implantation rate of 36%, lymphatic extension rate of 19%, and 2-month mortality of 31%. Group 4 was associated with perioperative mortality of 7%, implantation rate of 65%, lymphatic extension rate of 13%, metastasis rate of 36%, and a 2-month mortality of 40%.
Conclusions: Both percutaneous and transpleural orthotopic injection of A549-luc cells in the parenchyma of mouse lung induces localized

tumor, followed by lymphatic extension and specific mortality. Percutaneous injection and adjunction of mouse sarcoma proteins are associated with decreased perioperative mortality and increased hematogenous dissemination. This model will be of interest to study oncogenesis and to assess new treatments.

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New preclinical models of metastatic colon cancer: towards bridging the gap between bench and bedside therapeutic outcomes

C. Hackl[1], G. Francia[1], S. Man[1], P. Xu[1], R.S. Kerbel[1]. [1]*Sunnybrook Health Sciences Centre, Molecular and Cellular Biology, Totonto, Canada*

Background: In 2007, the small molecule receptor tyrosine kinase inhibitor sorafenib was FDA-approved for the treatment of advanced hepatocellular carcinoma (HCC). In contrast, little is known about the efficacy of adjuvant sorafenib for early stage HCC. As HCC is an intrinsically chemotherapy-resistant malignancy and as most patients suffering from HCC have reduced liver function thus not tolerating conventional chemotherapy, the impact of sorafenib-based regimens for this malignancy in earlier stages of disease progression may be important as a means to improve the clinical management of this highly lethal malignancy.
Methods: The human HCC cell line Hep3B was transfected with a hCG.pIRES vector and β-hCG expressing variants were obtained by puromycin selection. Analysis of β-hCG expression enables in vivo monitoring of relative tumor burden. Cells were orthotopically injected into the right lower lobe of the liver in a total of 50 CB17 SCID mice. Control vehicle or Sorafenib (15 or 30 mg/kg) was administered by daily gavage starting either immediately after wound healing (day 7) before circulating β-hCG was detected or after evidence of established tumors as determined by β-hCG analysis (days 14−21). Monitoring was carried out by analysis of β-hCG secretion, survival analysis and endpoint necropsy. Tissue was preserved for immunohistochemistry.
Results: All control animals needed to be sacrificed within 65 days due to primary tumor burden and ascites. No animal of this group showed local or distant metastasis. In contrast, all four dosing regimens of sorafenib significantly inhibited primary tumor growth, inhibited the formation of ascites and prolonged overall survival. However, possibly as a result of the prolonged survival, 56% (19/34) of the animals treated with sorafenib developed local, mesenteric and omental lymph node metastasis and 21% (7/34) developed secondary liver metastases. Metastatic cell lines were re-adapted to cell culture for future analysis.
Conclusions: Sorafenib prolongs survival and successfully controls primary tumor growth in an orthotopic model approximating early-stage HCC. However, it does not inhibit the development of secondary liver metastases or local and distant lymph node metastasis. The nature of these secondary growths will be addressed in follow-up experiments. Future analyses will also include adjuvant therapy of microscopic metastases following resection of the primary. Furthermore, experiments will be repeated using MHCC97-H as a second HCC cell line. Results of this ongoing study will be presented at the conference.

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Establishment and characterization of individualized patient-derived low passage human tumor models: Development, validation and evaluation for clinical correlation analysis

V. Estrada[1], M. Moore[1], F. Nieves[1], K. Papadopoulos[2], A. Tolcher[2], A. Patnaik[2], A.J. White[2], R. Drengler[2], L. Smith[2], M. Wick[1]. [1]*South Texas Accelerated Research Therapeutics (START), Preclinical Research, San Antonio TX, USA;* [2]*South Texas Accelerated Research Therapeutics (START), Clinical Research, San Antonio TX, USA*

The majority of patients with advanced solid tumors die from an absence of effective therapy. This is despite three decades of drug development using the current human tumor model screening platform derived from ex-vivo passaged cancer cells. While cell-derived models are useful for high throughput lead candidate identification and early stage preclinical single agent and combination optimization, these lines represent a cross section of different tumors but physically represent only a fraction of genetic and biological abnormalities which are now known to play a role in the pathogenesis and progression of human cancers. Patient-derived tumor models passaged only a few times in vivo retain physical and molecular characteristics of human cancer and may prove essential in identifying disease biomarkers and drug targets in later stage development.
To address this unmet need, we have implanted tissue from donor patients into immunocompromised mice to develop low passage models more representative of human cancer. To date one hundred seventy-four samples have been implanted over thirteen tumor types with a model development success rate of approximately sixty percent:

Tumor Type	No. of Models	%Total
Brain	8	5%
Breast	16	9%
Gastrointestinal (esophagus, colon)	(3, 18)	12%
Genitourinary (Renal, Bladder)	(5, 1)	4%
Head & Neck	16	9%
Hematopoietic	10	6%
Lung	16	9%
Neuroendocrine	3	2%
Ovary	32	18%
Prostate	2	1%
Pancreas	6	3%
Sarcoma	15	9%
Skin (melanoma, vulva)	(22, 1)	13%

Molecular and clinical outcome data was collected from donor patients and compared with data obtained from model characterization with excellent correlation. Several low passage models including colorectal, lung and ovary cancers and melanoma were screened for sensitivity to relevant standards of care with results correlating to clinical response 70% of the time. Taken together, these results demonstrate the ability to generate improved models of human cancer which retain molecular and clinical characteristics which may be used for patient drug sensitivity screening and improved oncology drug development.

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Constitutive overexpression of Id-1 in mammary glands of transgenic mice results in precocious and increased formation of terminal end buds, enhanced alveologenesis, delayed involution

D.H. Shin[1], S.H. Jang[1], K.S. Jang[1], B.C. Kang[2], H.J. Kim[3], S.H. Oh[4], G. Kong[5]. [1]*College of Medicine, Pathology, Seoul, South Korea;* [2]*College of Medicine, Laboratory Animal Medicine, Seoul, South Korea;* [3]*College of Medicine, Collene of Medicine, Seoul, South Korea;* [4]*National Cancer Center, Division of Basic and Applied Sciences, Gyeonggi-do, South Korea;* [5]*College of Medicine, Department of Pathology, Seoul, South Korea*

Inhibitor of differentiation-1 (Id-1) has been shown to play an essential role in cell proliferation, invasion, migration and anti-apoptosis. However, the effect of Id-1 in mammary gland development in in vivo remains unknown. Here, we analyzed the effect of Id-1 overexpression in mammary gland development of MMTV-Id-1 transgenic mice during virgin, pregnancy and involution. In virgin mice, overexpression of Id-1 led to precocious development and delayed regression of terminal end buds (TEBs) compared with wild type mice. The number of BrdUpositive cells, an indicator of cell proliferation, and the expression of Wnt signaling molecules, β-catenin and cyclin D1, which regulate ductal extension and TEB formation in virgin, were statistically higher in Id-1 transgenic mice than in wild type mice. Id-1 also had an effect on the formation and proliferation of lobuloalveolar structures during early and mid-pregnancy. The Id-1 transgenic mice had more lobulated and prominent alveolar budding than wild type mice and had significantly greater counts of lobuloalveolar structures in early pregnancy. The expression of BrdU, β-catenin and cyclin D1 was also predominantly increased in Id-1 transgenic mice. Moreover, Id-1 transgenic mice showed delayed involution in mammary gland development. Id-1 regulated the expression levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax, and resulted in delay of apoptotic peak during postlactational involution. Taken together, our results suggest that Id-1 plays a pivotal role in mammary gland development through Wnt signaling-mediated acceleration of precocity and alveologenesis and Bcl-2 family members-mediated delay of involution.

Clinical methodology

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Tailored dosing of tasisulam-sodium (LY573636-sodium) to reduce hematologic toxicity and improve therapeutic index

D. de Alwis[1], A. Cleverly[2], K. Chow[2], I. Troconiz[3], R. Ilaria[4]. [1]*Eli Lilly & Co Ltd, Pharmacokinetics, Surrey, United Kingdom;* [2]*Eli Lilly & Co Ltd, Oncology, Surrey, United Kingdom;* [3]*University of Navvara, Oncology, Pamplona, Spain;* [4]*Eli Lilly and Company, Oncology, Indianapolis, USA*

Background: Tasisulam (LY573636) is an acylsulfonamide with novel anti-cancer activity across a broad range of cell lines that induces apoptosis by a mitochondrial-mediated mechanism.
Material and Methods: In a phase I study and four subsequent phase 2 studies, tasisulam was administered by a 2-hour infusion using a lean