receptor (ER) and hormone resistance biology. Because of a very low tumor take in immunodeficient mice, most *in vivo* models of estrogen-dependent human breast tumors are derived from human cancer cell lines. We report here the establishment and the characterization of new primary human luminal breast cancer xenografts directly obtained from fresh human tumor samples.

Methods: As of December 2009, 453 fresh human BC samples have been engrafted in the interscapular fatpad of *nude* mice, of which 405 were retained for further studies (32 were non infiltrating or non-breast carcinoma, and 16 were axillary metastatic lymph node from a simultaneously engrafted primay tumor). ER was expressed in 313 tumors (77.3%), progesterone receptor in 175/291 informative tumors (60.1%), Her2 in 39/315 tumors (12.4%), and overall 60 tumors were triple negative. Validation of the xenografts was obtained by a large phenotypic and genotypic profiling including: pathological and immunohistochemical (IHC) examination, dedicated gene expression (RT-qPCR), genomic (BAC CGH arrays) and transcriptomic (Affymetrix u133 RNA chips) analyses, and therapeutic assessment (estrogen deprivation, ovariectomy, LHRH agonists, letrozole, tamoxifen, fulvestrant).

Results: Among the 405 human xenografted tumors, 8 luminal models have been established (2%), 7 from ER+/PR+ tumors and 1 from an axillary relapse of an ER−/HER2+ tumor. In all tumor/xenograft pairs, histopathological analyses showed an impressive morphological concordance. One had a strong mucinous component, and all of them were grade II/III tumors. Out of the 7 ER+/PR+ models, 3 were also HER2 positive. RNA expression by RT-qPCR confirmed ER, PR and HER2 status for the 7 ER+/ER+ tumors, and confirmed the ER+ status of the ER-/HER2+ derived tumorgraft. CGH arrays analyses demonstrated striking similarities of the genomic profile between the original tumors and their corresponding xenografts. Array CGH analyses were also performed at several passages, showing stable profile of the tumors during sequential *in vivo* passages. Transcriptomic profiling is ongoing. Therapeutic characterization of the xenografts showed that tamoxifen had a delayed but significant anti-tumor effect, whereas fulvestrant was the most efficient hormone therapy with durable complete responses observed in 3/3 evaluable models. Updated and extended results will be presented during the meeting.

Conclusions: We have durably established and characterized 8 primary human luminal BC xenografts. In order to identify new therapeutic approaches of hormone resistant BC, we have now planed to decipher in these well-defined preclinical models the molecular variations associated with emergence of resistance to hormone therapies.

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Preclinical antitumor assessment of bendamustine in human primary uveal melanoma xenografts

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Background: Uveal melanoma (UM) is the most common primary cancer of the eye, with a pejorative outcome due to metastatic death in up to half of the patients. Apart complete resection of metastasis, few alkylating agents such as temozolomide and fotemustine were used in metastatic UM patients with a slight efficacy. Bendamustine hydrochloride, which is both an alkylating and an anti-metabolite cytotoxic drug, has been shown to possess clinical activity in cancer patients refractory to alkylating-based chemotherapy. The purpose of this study was therefore to determine the efficacy of bendamustine in primary human UM xenografts.

Materials and methods: Four well characterized models of human UM, obtained from patients after enucleation (primary tumors)(MP41, MP46, and MP80) or liver surgery (metastatic tumors)(MM26), were used for the *in vivo* experiments (Némati et al, CCR 2010). Bendamustine was administered intraperitoneally (IP) at a dosage of 11 mg/kg day 1 to 5 every 28 days; temozolomide was administered IP at a dose of 40 mg/kg day 1 to 5 every 28 days and fotemustine was administered IP at a dosage of 30 mg/kg every three weeks. Tumor growth inhibition (TGI) was calculated to measure the efficacy of various tested compounds.

Results: Bendamustine induced a TGI between 44% and 49% in the four human UM xenografts, as shown in the Table 1. Moreover, when bendamustine was compared to temozolomide and fotemustine, it appears less efficient than fotemustine in all tested tumors and more efficient than temozolomide in 2/4 xenografts.

Conclusions: Using 4 human UM models, bendamustine was less efficient than standard chemotherapies administered in metastatic patients. These data are correlated to the results of the only one clinical study evaluating bendamustine efficacy in relapsed or refractory metastatic UM patients and showing 11/11 progressive diseases (Schmidt-Hieber et al, Melanoma Res 2004). These data also suggest that human primary UM xenografts constitute relevant preclinical models for pharmacological assessment of new therapeutic compounds and new combination of treatments.

UM models	Bendamustine	Temozolomide	Fotemustine
MP41	49	38	64
MP46	46	93	94
MP80	49	14	75
MM26	48	95	96

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Myxoid liposarcoma tumors with different chimera subtypes xenografted in nude mice are characterized by different response to trabectedin and gene expression profile

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Background: Trabectedin is a marine alkaloid isolated from Ecteinascidia turbinata, that is approved in Europe for the 2nd line of therapy in soft tissue sarcomas (STS). Among different STS, myxoid liposarcomas (MLS) are particularly sensitive to trabectedin, a clinical finding possibly related to the drug ability to block the trans-activating activity of the FUS-CHOP chimera gene, that represents the MLS pathogenic lesion. Different chimera subtypes seem to share different response to trabectedin in clinical setting. To define if this can be related to a different pattern of sensitivity to trabectedin tumor myxoid liposarcomas type II and type III were xenografted in nude mice, treated with trabectedin and analyzed for their gene expression profile.

Material and Methods: Fragments of type II and type III MLS were transplanted s.c. in female athymic NCr-nu/nu mice. Xenografts were established and characterized by morphology and molecular biology. Trabectedin 0.15 mg/kg was injected i.v. weekly for three times. The growing tumor masses were measured with a Vernier caliper. Drug efficacy was calculated as T/C %, where T and C are the mean tumor weights of treated and control groups, respectively. Whole gene expression experiments were performed with dual color labeling protocols and hybridized onto 44K oligos-array platforms commercially available. Analysis was performed with "R" package software. Pathway analysis was performed using Metacore software. qRT-PCR was used for data validation. Statistical analysis was performed using *Graphpad software.*

Results: The responsiveness to trabectedin in type II MLS xenografts was very high (T/C = 8%) whereas type III MLS xenografts appeared much less sensitive (T/C = 42%) to trabectedin. Gene expression analysis of both type II and type III subtypes identified a large subset of genes which expression is modulated by trabectedin in a dose dependent manner. Pathway analysis revealed that trabectedin treatment modulated different molecular pathways in the two FUS-CHOP subtypes models.

Conclusions: The overall data suggest that nude mice xenografted with different FUS-CHOP subtypes are associated with different sensitivity to trabectedin, mirroring clinical evidence. The differences appear to be related to a different modulation of gene expression by trabectedin.

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NC-001 induces tumor growth discontinuation and necrosis in a xenograft renal cancer rat model

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Of all cancer malignancies in the world, renal cancer constitutes about 3%, which corresponds to 100,000 cases annually worldwide. The majority of renal cancer (75%) starts in the proximal tubular epithelial cells in the kidney, and is referred to as clear cell renal cell carcinoma (CCRCC). At diagnosis, about one third of the patients are presented with metastases. Subsequently, half of the patients who seemed to have a localized disease initially, will develop metastases even if the original tumor is successfully removed. Several new therapies are emerging in clinical trials, with mainly anti-angiogenic properties. These are combined and compared with conventional therapies, explicitly interferon-alfa and interleukin-2. However, none of these show true curative potential, although significant retardation of the disease have been reported for specific patient categories.

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.

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

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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

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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

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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