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