KRAS, mutations. The frequency of EML4-ALK fusion transcripts is around 5% or less in Caucasians patients. Crizotinib (PF-02341066) is an ALK and MET inhibitor that has demonstrated outstanding activity in monotherapy in patients with EML4-ALK translocations: 57% objective response in 82 patients with a median duration of treatment of 5,7 months and a predicted 67% PFS rate at 6 months.
Many other abnormalities linked to tyrosine kinase receptors could be used for selection of specific therapy such as amplification or activation mutation of HER2, HER3, HER4, FGFR1, FGFR2, KDR .... However correlation of presence of such abnormalities and clinical response are still not firmly documented, although some interesting case reports have been documented. Table 1 provides a summary of specific alterations and their potential corresponding drug. DNA repair markers are also potential predictors of chemotherapy bases therapies (ERCC1, MSH2, BRCA1, PARP).

Table 1

Molecular alteration	Potential Drugs
EGFR mutation	Erlotinib, gefitinib New pan-HER inhibitors
EML4-ALK translocation	Crizotinib
HER2 mutation or amplification	Trastuzumab Lapatinib
PI3K mutation or amplification	GDC-0941 XL-147 XL-765 PX-866 BEZ-235 BKM120
MET amplification	XL184 ARQ917
RAS and RAF mutations	Sorafenib AZD6244; GSK1120212; AS703026

Thursday, 18 November 2010

Poster Sessions

Animal models

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Multi-modality in vivo imaging of bone metabolism and tumor growth in a mouse model of bone metastasis

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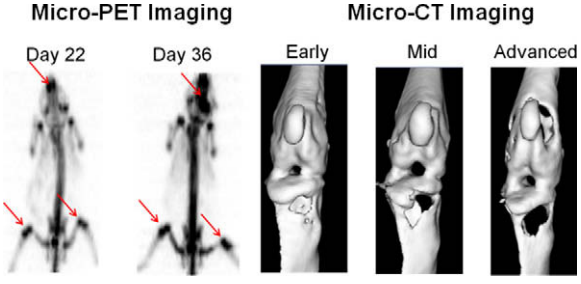
Background: The ability to visualize and quantify early stages of bone involvement in mouse models of bone metastasis would provide a platform for development of new agents targeted at inhibition or treatment of bone metastases. While micro-CT imaging provides a qualitative assessment of bone erosion and bioluminescence imaging using luciferase expressing tumor lines enables monitoring of tumor burden, there is a need to track bone lesion progression as well.
Materials and Methods: Optical imaging using biphosphonate fluorescent probes and 18F-NaF PET imaging both provide readouts for hydroxyapatite (HA) activity, a key feature of bone metastasis. Female nu/nu mice underwent intracardiac inoculation with MDA-MB-231-luc-D3H2LN human mammary adenocarcinoma cells (10^5 cells in 100 µl). On Day 14, all mice were imaged using bioluminescence and enrolled on study based on incidence of luciferase signals at bone sites. Subsequent bioluminescence imaging was used to monitor growth of bone metastases. Micro-CT (see figure: right panel) was used to assess the extent of corresponding bone lesions. 18F-NaF PET imaging (45 min uptake) (see figure: left panel), and fluorescence imaging (24 h after Osteosense 750 administration) were used to characterize HA activity.
Results: Both 18F-NaF PET imaging and fluorescent imaging using Osteosense highlighted localized bone signals that were associated with bioluminescent tumor signals and micro-CT visualized bone lesions from approximately Day 17. Bioluminescence imaging showed the greatest sensitivity to disease progression in both the mandible and hindlimb bones. The PET and fluorescence imaging approaches showed bone involvement (presumably through HA), indicating osteoblastic activity.
Conclusion: The combination of PET, fluorescent imaging of bone remodeling mechanism coupled with bioluminescent imaging of tumor growth and microCT imaging of bone anatomy, enables quantitative, non-invasive means for characterizing bone metastasis. Importantly, the use of complimentary imaging methods can provide assessment of novel therapeutics against both metastatic tumor growth and bone lesion progression.



261 POSTER

Effects of isoflurane anesthesia on bioluminescence measurements: impact on pharmacological assessment of anti-tumor activity of chemical entities

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Bioluminescence imaging (BLI) has been used for several years in oncology research to quantify tumor growth. In order to allow immobilization during data acquisition, animals are usually anesthetized by isoflurane gas inhalation. However, anesthesia leads to modifications of many physiological parameters, and particularly decreases in heart rate, blood pressure and blood flow, leading to decrease of substrate availability. Thus, it can be postulated that anesthesia will impair bioluminescence read outs. This may in turn profoundly affect interpretation of compound activity based on BLI.
We have investigated the difference in BLI measurements performed in conscious and anesthetized animals using subcutaneous xenografts (HCT116 colorectal and U87MG glioblastoma) and orthotopic (U87MG cells injected directly in the brain) models. Moreover, functional tumor vessel mapping and hypoxia stage were obtained using casting technology and HypoxyProbe™, respectively. Anti-tumor activity of 5-fluorouracil and temozolomide were assessed in the HCT116 xenograft and U87MG orthotopic models, respectively.
Our results have clearly demonstrated that in all tested tumor models, tumor growth and efficacy of different compounds were not affected by the regular anesthesia procedure used for bi-weekly BLI assessments. We found a good correlation between caliper and BLI over a wide range of tumor size in conscious mice. When BLI was measured in anesthetized mice, signal intensity dropped significantly up to 70%, impairing the assessment of the antitumor efficacy of 5-FU. A clear correlation to functional tumor vessel density was evidenced.
In the U87MG tumors cells implanted intracranialy, we could clearly follow tumor growth over time using BLI readouts. Isoflurane did not impair BLI measurements or the efficacy profile of temozolomide.
In conclusion, we can say that BLI measurements in conscious tumor bearing mice offers a better alternative for resolving pharmacologic queries without the confounding effects of anesthesia. Obviously, more extended studies over a wide range of tumor types will be needed to reinforce these conclusions. Moreover, a more quantitative approach to assess vessel density and distribution in the casts using micro-CT technologies would be pivotal.

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Establishment of patient-tumor derived xenograft models for testing anticancer agents

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Human xenograft tumor models established by transplantation of human tumor cell lines into immunodifficient mice have been routinely used for preclinical test of anticancer agents. But such tumor models have a relatively low transplantability, limited number of cell lines available for certain tumor types, and limited correlation with clinical findings. Recently, we have developed patient-tumor derived xenograft tumor models by transplanting human fresh tumor tissues into nude mice, and which have been used for test of clinically used anticancer drugs as positive

controls. A total of 215 tumor samples excised from patients have been transplanted for patient-tumor derived model development; and a total of 78 xenograft tumor models have been established with a tumor taking rate of 36%. The tumor taking rates of different tumor types were non-small cell lung cancer (40%), small cell lung (50), colorectal (46%), gastric (37%), ovarian (40%), renal cell carcinoma (15%), and acute lymphocytic leukemia (20%). The tumor taking rates were higher in the later passages than earlier passages for the various tumor types, ranged from approximately 50–100%. The positive control drugs tested against the patient-tumor derived models included paclitaxel, docetaxel, irinotecan, doxorubicin, 5-FU, gemcitabine, and tarceva; they produced tumor inhibition rates ranged from 30−70%, which were consistent with their clinical findings. The patient-tumor xenograft tissues from all 5 generations presented a similar histopathological morphology and genomic profile to human primary tumors. These results suggest that patient-tumor derived xenografts provide a unique renewable source of tumor material for test of novel anticancer agents and may predict more relevant clinical response rate and higher correlation with clinical findings than use of xenograft models established from long-term cultured cancer cell lines. Especially, they have advantages for test of target-oriented therapeutics in new drugs development programs.

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A non-invasive bioluminescent imaging technique for monitoring shutdown of the tumour vasculature

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In the preclinical development of vascular disrupting agents (VDA) a key pharmacodynamic endpoint is demonstration of shutdown of the functional tumour vasculature. This is usually done by injecting a dye which labels the functioning blood vessels, e.g. Hoechst 33342, immediately prior to sacrifice and evaluating vascular profile density in tumour cryosections. This is not ideal as it only gives indication of shutdown at a fixed time-point, and shutdown is not assessed over time in the same animal. With non-invasive bioluminescent imaging (BLI) techniques detection of tumour deposits is reliant upon the conversion of an injected substrate, luciferin by luciferase present in the tumour cells resulting in light emission. We hypothesise that by closing down the tumour vasculature with a VDA the amount of luciferin that can reach the tumour cells will be restricted, and thus reduce the emitted light.

In this study we used non-invasive BLI to assess the effect of a novel colchicine-derivative VDA, ICT-2552 on a subcutaneously implanted DLD-1 human colorectal adenocarcinoma cell line engineered to express luciferase.

Optimal shutdown time was initially determined using BLI and compared to the Hoechst 33342 method at 1, 4 and 24 hours post-treatment, using different mice for each time point. Both methods indicated vascular shutdown at 1 and 4 hours, with re-establishment by 24 hours. In a further experiment, Cisplatin which should not affect the vasculature was included as a control agent, with evaluation at 1 and 24 hours post-treatment. As seen previously, both methods indicated reductions in tumour vasculature in the ICT-2552 group at 1 hour ($p < 0.05$), with recovery by 24 hours. No significant change was seen for Cisplatin.

Having demonstrated that BLI can be reliably used to measure vascular shutdown, a further BLI study was carried out monitoring shutdown in the same animal over time at 1, 6 and 24 hours following treatment with either ICT-2552 or 2 more standard agents which should not affect the tumour vasculature, 5-Fluorouracil and Doxorubicin. Vascular shutdown was seen at 1 and 6 hours in those mice treated with ICT-2552 ($p < 0.05$) whilst signals remained stable or increased in those mice treated with the control drugs.

In conclusion, we have demonstrated that BLI is a realistic alternative to invasive methods in evaluating vascular shutdown, with the advantage of being able to follow the same animal throughout a study thus reducing animal use.

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Establishment of a targeted fluorescence guided colonoscopy for a xenograph orthotopic colorectal cancer model

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Introduction: Many GI tract tumors are derived from early metaplastic and dysplastic changes of the mucosa that so far are missed by conventional endoscopic imaging approaches in 25−30% of patients. Potential applications for near-infrared contrast agents are detection of gastrointestinal tumors by fluorescence-guided endoscopy. Despite many published orthotopic tumor models of CRC none of them is appropriate for monitoring xenograph tumor growth by an fluorescent-guided endoscopy.

Material and Methods: Our xenograph orthotopic tumor model is implanted in the Colon descendens of nude mice. Tumor growth and spread out of metastasis were monitored by Bioluminescence Imaging. Endoscopy started on day 1 after surgery and was repeated once a week. For monitoring size and grading of the tumors in the lumen a score sheet was prepared and validated. In addition fluorescence-guided endoscopy was established by combining the rigid endoscope (STORZ) with a fiber probe (Manu Kea). For the fluorescence endoscopy the animals received a targeted fluorescene imaging agent (IntegrisSense 680, VISEN) 24 hours before imaging. This potent, selective non peptide molecule is an $\alpha_v\beta_3$ antagonist and a Near- Infrared- fluorochrome. With this technique we are able to visualize and to quantify the integrin $\alpha_v\beta_3$ expression in colorectal tumors.

Representative mice were also imaged in a μCT. At the end of each study the colons including the tumors were taken out for further immunohistochemical analysis including HE, Cytokeratin, CD31 and Ki67 staining.

Results: Tumor growth of different cell lines could be monitored by endoscopy and bioluminescence imaging. We could combine the fiber endoscope with the rigid endoscope and we could clearly distinguish healthy mucosa from early stages of tumor tissue. The small tumors could also be detected by negative contrast in the μCT post rectal injection of BaSO4 (positive contrast agent). Accordingly, immunohistochemical analysis of the tissues revealed human cells growing in the colon, which were positive for PanCytokeratin, CD31 and Ki67.

Conclusion: Tumor growth of different human colorectal cell lines could be monitored by non invasive Bioluminescence imaging as well as endoscopy. Our new established orthotopic CRC tumor model in the colon descendence now provides the basis for further pre-clinical studies to validate new contrast agents and new therapy targets to obtain clinically relevant data.

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Rac1 is a therapeutic target for pancreatic cancer prevention and treatment

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Pancreatic cancer reveals very high mortality rates due to the late diagnosis, the early metastatic spread and the lack of efficient therapies. Thus, elucidation of pancreatic carcinogenesis as well as development of new treatment strategies are urgent goals. We established several conditional mouse model of Pancreatic Ductal Adenocarcimoma (PDAC), using frequent genetic alterations found in human PDAC: activation of oncogenic $Kras^{G12D}$ and EGFR signaling together with inactivation of tumor suppressor p53. These mice show complete spectrum of clinically relevant preneoplastic lesions: PanIN and IPMN, which progress to invasive and metastatic PDAC. RAS-related C3 botulinum substrate 1 (Rac1) is a Ras- and EGFR-effector molecule. Rac1 is up regulated in several human cancers including PDAC and is known to control cellular motility, proliferation and survival. The aim of this study was to investigate the role of Rac1 in the development of PDAC and its possible application as a therapeutic target.

$Kras^{G12D/+}$(K), Ela-Tgfa (T), and $p53^{R172/+}$ (P) mouse models were crossed with $Ptf1a^{+/Cre}$ and $Rac1^{fl/fl}$ (R) mice. The tumor onset and progression in the resulting pairs of mouse models (wt-R; K-KR; KT-KTR; KP-KPR) were histologically characterized as well as with ex vivo acinar transplants, Western blot, expression microarrays, RT-PCR and survival analysis.

Rac1 m-RNA and protein levels were up regulated in all analyzed mouse models (K, KT) of PDAC. Deletion of Rac1 in these models led to a strong impairment of PanIN and IPMN (KTR) development as well as a reduction of inflammatory infiltrates, desmoplastic stroma formation and cytokine secretion in KR and KPR models. In addition, acinar epithelial explants lacking Rac1 were unable to undergo Acinar to Ductal Metaplasia (ADM) in 3D cultures after TGFa stimulation. ADM of wild-type acini was similarly impaired after treatment with inhibitors of actin polymerization, supporting a potential role of Rac1 in actin-dependent plasticity during ADM. Furthermore, treatment of primary mouse tumor cell lines with the Rac1-specific inhibitor NSC23766 in vitro resulted in a strong negative effect on adherent and anchorage-independent cell growth.

In conclusion, we show that Rac1 is essential for ADM and development of preneoplastic lesions in several mouse models of PDAC and propose Rac1 as a new target for prevention and treatment of PDAC.