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Advancing bioluminescence imaging technology for evaluating anticancer agents in MDA-MB-435 subcutaneous, mammary orthotopic and subrenal capsule tumor models

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In vivo bioluminescence imaging (BLI) has emerged as a sensitive and quantitative imaging modality gaining popularity due to the capacity to conduct non-invasive longitudinal studies monitoring tumorgenesis, metastasis, regression and response to anticancer agents. Diligent optimization of imaging parameters applied to relevant disease models is critical to realize the potential of BLI as a tool to reliably evaluate anticancer agents. Luciferase (Luc), a widely used optical reporter gene, stably integrated into tumor cells is replicated with every cell division enabling spatiotemporal analysis of tumor growth via BLI. Luc produces light in the presence of the substrate luciferin, oxygen and ATP; light photons are emitted and can be externally detected and quantified using sensitive light imaging systems. We describe the importance of luciferin kinetics as it relates to detection of peak bioluminescence signals and quantification of primary tumor burden and metastatic lesions arising from tumors of the same cellular origin but implanted in distinct locations in the mouse. MDA-MB-435-Luc mammary carcinoma cells were implanted subcutaneously (sc) in the flank, orthotopically in the mammary fat-pad or surgically in the subrenal capsules of mice. All models displayed high take rate (>90%) while the subrenal grafts showed a higher occurrence of lung metastasis (85%) compared to mammary fat-pad (20%) or sc (<5%). Using an IVIS 100 (Xenogen®), we collected multiple images between 5–30 minute intervals for an exposure time of 5–30 seconds to determine peak bioluminescent signals for each model. Results indicate the post-luciferin time interval to reach peak bioluminescence was variable depending on the model and tumor size. In the sc flank and mammary model the peak bioluminescence signal occurred within a narrow time interval and a strong correlation was demonstrated between the tumor mass and peak bioluminescence intensity. In contrast to the sc flank and mammary models, the time interval to reach peak bioluminescence intensity in the primary tumor and lung metastasis of the subrenal capsule model was highly variable spanning 5–30 minutes depending on the mouse. Capture of the peak bioluminescence intensity was essential to optimize signal to noise and accurately quantitate tumor burden. Failure to detect the peak bioluminescence signal may underestimate tumor burden and lead to incorrect evaluation of therapeutic response. Upon identifying the optimal imaging parameters for various MDA-MB-435-Luc models we evaluated the antitumor activity of Taxol® and AG-024322 (CDKi) and demonstrated the utility of BLI as a tool to reliably evaluate anticancer agents. Advancing BLI technology in relevant disease models creates an opportunity to improve preclinical evaluations of anticancer agents.

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Animal study management software: novel technology to optimize cancer research in vivo

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Efficacy and preliminary safety tests performed in animals for promising drug candidates represent a critical bottleneck in cancer research and form the basis for the pivotal decision to pursue full-scale preclinical development. Remarkably, most animal studies are still performed like they were 20 years ago, using a combination of paper scientific notebooks and electronic spreadsheets. These manual methods are highly inefficient, are prone to problems with data integrity, accuracy and accessibility. The inefficiencies intrinsic to a single screening study are multiplied with each additional study conducted and constitute a serious but avoidable problem for cancer research organizations; that of prolonged discovery time for new drugs. Data collection for animal studies commonly entails technicians measuring and recording results on paper, entering the data into spreadsheets, and later transferring the data into statistical software for further analysis. Spreadsheets do manage measurement data, but do not manage detailed information on study design, conditions, animal models or projects. A lack of consistent data formatting convention within spreadsheets makes the analysis and inter-study comparison quite challenging and time consuming. Results from completed studies are pasted into the research notebook, which is later microfiched and archived out of reach. The manual recording of data into notebooks and the transcription to spreadsheets, statistical programs and databases is prone to errors. Problems with data integrity and security due to accidental destruction, notebook and datasheet loss, file spreadsheet deletion or change, theft and intentional falsification have also been identified as serious concerns. Commercially available systems, such as Studylog Systems' Study Director®, IPA's Labcat and Tumor Tracker automate study processes such as study design, data collection, task management, data analysis, graphing, report generation, and enable enterprise-wide access to study information for current and archived studies. Animal study management software improves process efficiency, data integrity and security, and data accessibility. This software also increases the transparency of study data and processes, facilitating personnel oversight, IACUC compliance, and inter-study comparability. The advent of study automation technology provides the research enterprise with valuable new means to significantly decrease the time to discovery of novel therapies.

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Antitumour activity of new compounds from N-sulfonyluracil and benzothiazole group with hyperthermia on the growth of mouse tumours

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Aim: The purpose of this study is to investigate antitumour activity of newly synthesized compounds from N-sulfonyluracil and benzothiazole group. These types of compounds showed potent inhibitory activity on the growth of human tumour cell lines *in vitro*. In this study we have investigated antitumour activity of 5-bromo-1-(metanesulfonyl)uracil on the growth of transplantable mouse mammary carcinoma (TMMCa) *in vivo* and antitumour effect of newly synthesized benzothiazoles on the growth of mouse fibrosarcoma (FsaR), melanoma (B16-F10), and squamous cell carcinoma (SCCVII). Also some of these new agents have been applied with local hyperthermia (LTH).
Material and Methods: In this study we used a mouse mammary carcinoma and fibrosarcoma and melanoma. Tumour cells were injected into the mouse footpad of the right hind leg. Tumour bearing mice have been treated with new compounds as a single agent or in combination with hyperthermia (43.0ºC/60 min). The end point was tumour growth time (TGT). TGT is the time needed for tumour volume to grow five times over the treated volume measured by calliper and calculated by the formula $A \times B \times C \times \pi/6$. 5-bromo-1-(metanesulfonyl)uracil has been synthesized at Rudjer Boskovic Institute and benzothiazole have been synthesized at Faculty of Chemical Engineering and Technology, University of Zagreb.
Results: The obtained data show that examined 5-brom-1-(metansulfonyl)-uracil has suppressed tumour growth of TMMCa in comparison to control group. When 5-brom-1-(metanesulfonyl)uracil has been combined with local hyperthermia, antitumour activity of this derivative was enhanced. Benzothiazole compounds have shown good antitumour activity against melanoma B16 and fibrosarcoma as well as against SCCVII carcinoma.
Conclusion: The obtained data show that new antitumour compounds (*N*-sulfonyluracil and benzothiazole derivatives) can reduce tumour growth time in mice.

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Cancer tissue model to study anti cancer drug effects and to identify predictive markers

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Background: The current model systems to study anticancer drug sensitivity such as cell lines, isolated tumour cells and animal models only poorly predict clinical response. One reason is that the drug response of tumour cells is greatly influenced by their cellular and non-cellular environment. Therefore, model systems which allow studying drug effects on primary tumour cells within different compartments of an individual tumour *ex vivo* provide a great advance for research on response prediction or the investigation of efficacy of novel treatment strategies.
Material and Methods: We prepared 200 μm thick tissue slices from freshly excised tumour samples from primary breast cancer and lung metastases and cultivated them in the presence or absence of anticancer drugs for 4 days. To visualize viability, cell death, and expression of surface molecules in different compartments of non-fixed primary breast cancer tissues we established a method based on confocal laser scan microscopy using mitochondria- and DNA-selective dyes and fluorescent-conjugated antibodies. Proliferation and apoptosis was assessed by