EVALUATION OF 5-FLUOROURACIL EFFECTS ON TUMOR GROWTH
IN A HCT116-LUC2 ORTHOTOPIC COLORECTAL CANCER MODEL IN MICE.

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Introduction
In testing novel anticancer therapies, models that most faithfully reflect the human disease must be used as much where possible. In this regard, experimental orthotopic models are developed because the in vivo microenvironment surrounding cancer cells exerts a significant influence on the tumor’s growth and metastasis as well as its response to treatment. The purpose of this study was to validate an orthotopic model of colorectal cancer in nude mice by characterizing the behavior of a human colon cancer cell line, HCT116-luc2 and evaluating effects of a standard chemotherapeutic, 5-fluorouracil (5-FU), using in vivo bioluminescence imaging.

Material and Methods
Animals: male Swiss nude mice (7 weeks old) were housed in a controlled environment with temperature (20-24°C) and 12 hours light /dark cycle (light off at 7:30 pm).

Tumor induction: HCT116-luc2 cells were first injected subcutaneously into the flank region of Swiss nude mice to obtain a subcutaneous tumor. This tumor was excised in small fragments, implanted onto the caecum of the host mouse. After 10 days, an orthotopic human colorectal carcinoma model was obtained. Since luciferase protein is expressed in HCT116-luc2 cells, this model allows in vivo tumor visualization by bioluminescence imaging.

Bioluminescence measurements: Mice were injected intraperitoneally with 100 mg/kg luciferin. Five minutes after luciferin injection, mice were anaesthetized with isoflurane placed under the CCD camera and kept under isoflurane anesthesia (1.5%-2.0%) during 1 to 5 minutes for imaging. The total photon flux emitted from specific regions (HCT116-luc2 tumor cells) was computed by integrating the photon flux over the entire emission area as described by drawing a circle on the image and using LivingImage software (Figure 1: Xenogen). In vivo luciferase activity is presented in photons per second. Bioluminescence values include both metastasis and primary tumor signal.

Study design: The study involved 3 groups of 7 animals each, control group (Water for Injection) and two 5-fluorouracil treated groups, 20 mg/kg and 60 mg/kg. 5-fluorouracil or its vehicle were administered three times a week for 5 weeks. The tumor growth was assessed by bioluminescence before the start of treatment and once a week all over the 5-week treatment period.

Results: Effects of 5-FU on tumor growth and metastatic proliferation over 5 weeks

• Control group: a marked tumor growth occurred. Liver, kidney, spleen, stomach and lung metastasis were identified as distant colony formation (Figure 2).
• Treated groups: reduction of the tumor growth and distant metastasis inhibition (Figures 3 and 4).

Conclusion
Under our experimental conditions, tumor growth and dissemination achieved after implantation of HCT116-luc2 cells and the reduction in tumor size after treatment with a reference compound, 5-FU, support the relevance of this model of HCT116-luc2 orthotopic colorectal cancer in mice for assessment of antitumor effects of test compounds.