Using non-invasive fluorescence and ultrasound imaging to assess the biological and functional cardiotoxic effects of 5-fluorouracil in mice

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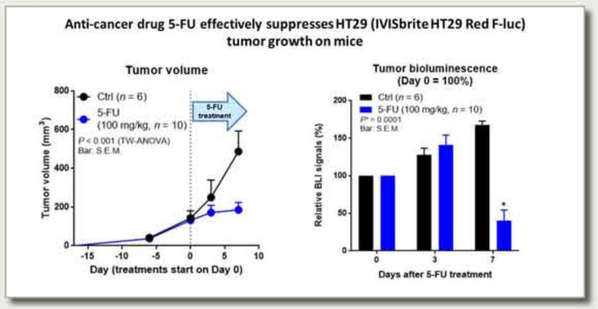
Abstract

Objective: Fluorouracil (5-FU) is a commonly used chemotherapeutic for treatment of solid tumors. However, the life-saving drug is known to be cardiotoxic, though its underlying mechanisms are still elusive. One of the challenges for assessing this type of toxicity is to non-invasively measure biomarkers associated with heart toxicity while still allowing determination of drug effects on heart physiology. In this study we explored a novel non-invasive multi-modal approach combining fluorescence imaging ("FLI"; FMT®-4000, PerkinElmer) and robotic ultrasound ("US"; Vega, SonoVol) to assess drug-induced biological and functional cardiac changes.

Methods: Female nu/nu mice bearing ~450 mm³ HT29 (IVISbrite™ HT29 Red F-luc) subcutaneously implanted tumors were split into 3 groups treated daily with saline or 5-FU (50 or 100 mg/kg). These HT29 tumor xenografts showed ~70% regression by volume within a week of daily 100 mg/kg 5-FU treatments for this study. To assess heart toxicity, animals were i.v. injected with FLI probe cocktail AMT750 which simultaneously detects biomarkers of cell viability, matrix metalloproteinase (MMP) secretion, and iron metabolism. To capture the drug's immediate cardiotoxic effects, AMT750 was delivered 2 hours following first 5-FU treatment, and the next day mice were imaged by FMT. Of note, the FMT imaging involves a systemic deep tissue approach that is not limited to heart imaging, rather, the imaging can be effectively extended to visualize drug-induced injury in other organs, or tissues such as tumors. To measure long-term physiologic changes in cardiac function, we performed M-mode US imaging at various timepoints after the drug treatment began. M-mode US imaging was used to assess cardiac function, and we compared controls to mice treated with 50 or 100 mg/kg of 5-FU at 2, 4, or 7 days. For consistency, US imaging was performed after 2-3 minutes of 2% isoflurane exposure. M-mode datasets were then segmented using the onboard AI software to obtain several physiological metrics of heart function.

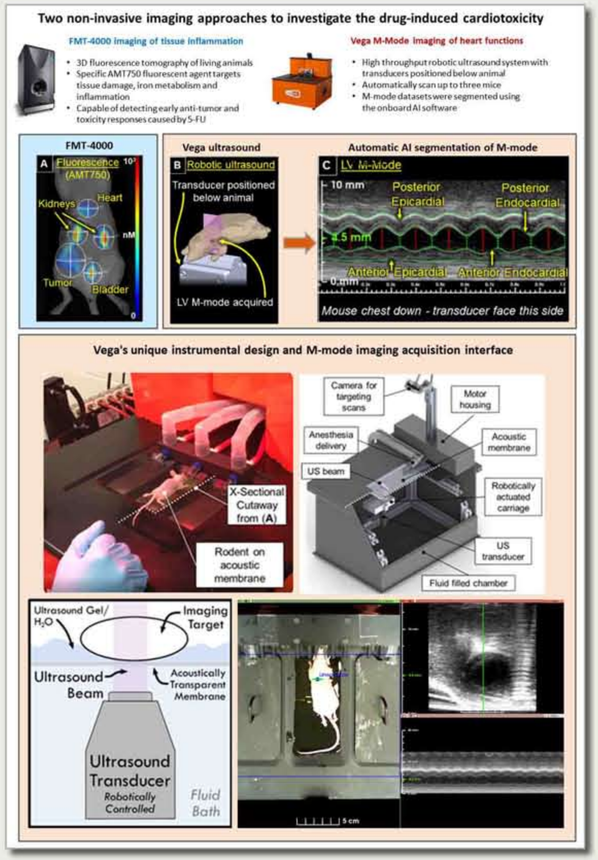
Results: Within one or two days after the first 50 mg/kg and 100 mg/kg 5-FU treatments, we observed abnormal AMT750 fluorescence increases in the tumors, stomach, and intestines, as well as dramatic 30 and 70 pmol increases in the heart, as compared to controls. These results suggest the drug induces significant early biological changes associated with its ultimate cardiotoxicity. Interestingly, after a week of treatment there were significant, overt physiological effects on cardiac function; mice treated with 50 mg/kg 5-FU showed elevated heart rates and increase in cardiac output after 4 days, and 100 mg/kg 5-FU caused more significant decreases in stroke volume (~35%) and cardiac output (~40%) compared with the control group.

1 HT29 Red F-luc tumor model



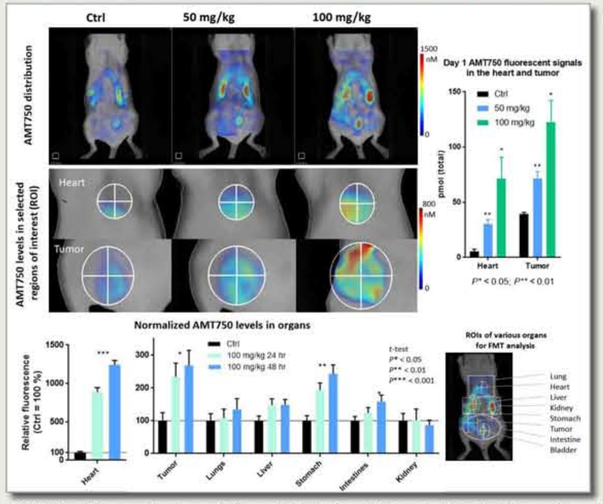
Tumor model: IVISbrite™ HT29 Red F-luc subcutaneous tumor on nu/nu mice. The dashed line indicates the beginning of 5-FU treatment. The tumor cells carry a red-shift luciferase gene and tumor viability can be measured by bioluminescence signals.

2 Dual-modality imaging overview



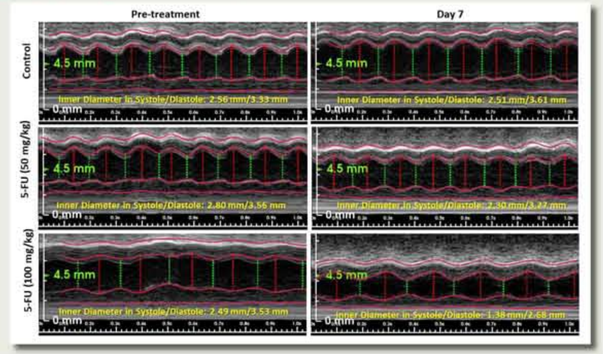
We use two **non-invasive** imaging approaches to investigate the cardiotoxicity induced by fluorouracil (5-FU), a commonly used chemotherapeutic agent, in a mouse tumor model. **Fluorescence molecular tomography (FMT® 4000, PerkinElmer)** enables early detection of cytotoxic and tissue inflammatory responses in the heart using the specific AMT750 fluorescent agent cocktail [IVISense™ Annexin-V 750, IVISense MMP750 FAST and IVISense Transferrin Receptor 750]. **Robotic ultrasound (Vega, SonoVol)** was used to measure drug-induced biological and functional cardiac changes after repeated 5-FU treatments.

3 FMT imaging of AMT750 reveals dose-dependent anti-tumor efficacy and organ toxicity within one or two days of 5-FU



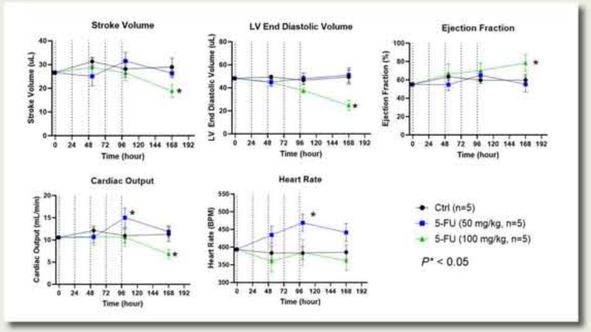
AMT750 is a fluorescent probe cocktail specifically designed to target cell death, inflammation and metabolic changes. AMT750 was injected 2 hours after 5-FU dose to capture early biological changes that can be imaged at 24 hours (day 1). Within one day after treatments, higher AMT750 signals were observed in the tumors and hearts in a dose-dependent fashion.
In a separate study, elevated AMT750 signals were observed during the first two days of 5-FU treatments in various organs.

4 M-mode imaging of heart functions reveals functional changes after repeated 5-FU treatments



Nu/nu mice were repeatedly treated with 5-FU, and the effects of the treatment were noticeable in the 100 mg/kg group on day 7 with the decreased internal diameter in both systole and diastole.

5 Quantitative analysis of heart function metrics

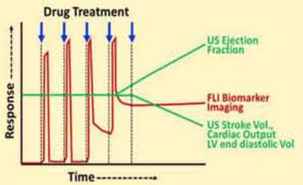


Nu/nu mice were repeatedly treated with 5-FU, as indicated by the dashed lines. The M-mode tracked changes in the function of the heart (*P<0.05 vs. Ctrl, two-way ANOVA).

Summary

Paradigm for combined FL/US imaging to evaluate an anti-tumor drug's toxicity and efficacy

Non-invasive preclinical imaging offers opportunities to quickly and non-invasively characterize biological and physiological changes in animals undergoing therapeutic treatment. The present studies provide evidence for the utility of pairing fluorescence tomography (FMT4000, PerkinElmer Inc) and ultrasound imaging (Vega, SonoVol) approaches to characterize both anti-tumor efficacy and off-target toxicity, including cardiotoxicity. A fluorescent imaging agent cocktail, AMT750, was used to explore biomarker changes in 5-FU treated tumor-bearing mice, and a unique high through-put ultrasound imaging system further characterized observed cardiac effects through M-mode imaging and automated analysis.



It is remarkable that in these studies as little as a single administration of 5-FU induced detectable biomarker changes in stomach and intestines, previously characterized to show injury following much longer 5-FU dosing regimens. Importantly, poorly understood clinical cardiac toxicity was also detected consistently in all mice. Ultrasound imaging further determined that recurrent injections of high-dose 5-FU (100 mg/kg) eventually yielded statistically significant decreases in functional cardiac measurements such as stroke volume (SV) and cardiac output (CO). In these mice, ejection fraction (EF) appeared to increase, however this was likely driven by the substantial reduction in end diastolic volume (EDV) in this cohort.