

## Abstract

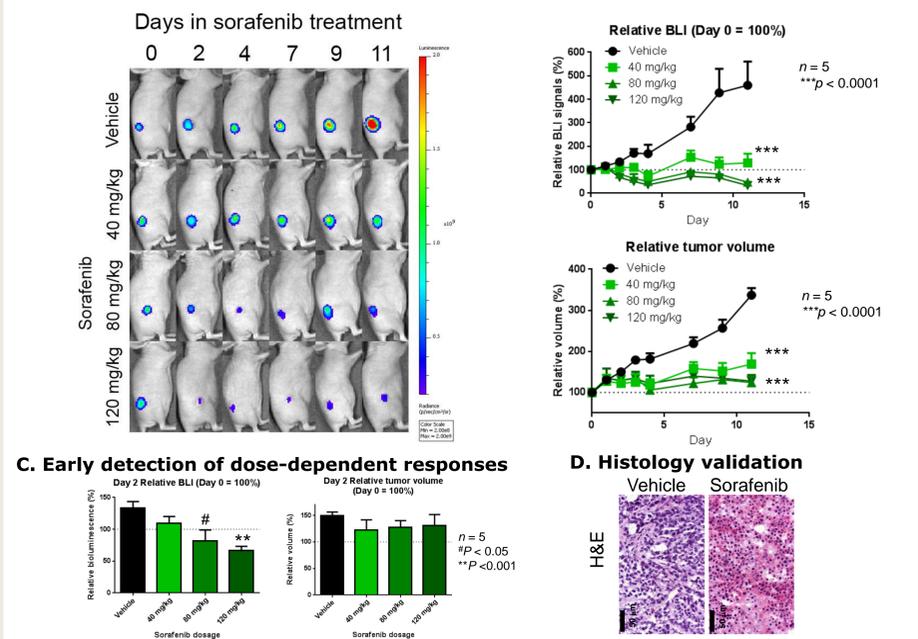
Targeted cancer therapy aims to block key signaling pathways that are critical for tumor cell growth and survival. The blockage eventually results in cell death via apoptosis and tumor growth suppression. This strategy has proven to be safe and efficacious, and the FDA has approved several targeted therapeutics in the past decade. Encouraged by the success in clinical development, many academic and pharmaceutical researcher are in active pursuit of the improvement of next generation targeted anti-cancer drugs. As a result, many new chemical and biological entities are emerging from initial screening of in vitro, in vitro and/or in silico selection processes. From the perspective of drug development, it poses a great challenge on the next stage of in vivo validation and demands a robust, accurate, and efficient method for assessment of these candidates in living animal models.

Cancer cells are known to have abnormally increased cellular metabolism, and in the early stages of effective drug treatment, cancer cells show decreases in metabolism and proliferation. These events occur prior to overt signs of cell destruction and bioluminescence imaging technology can be used to detect early changes in tumor viability in response to targeted therapy when using luciferase-expressing tumor cell lines. However, with many therapeutic treatments, particularly high-dose treatments, these early changes in tumor viability cannot be seen by bioluminescence imaging. We applied non-invasive near infrared (NIR) fluorescence imaging to address this challenge, using PerkinElmer's imaging agents; highly metabolic cancer cells have accompanying elevations in receptors for bombesin and transferrin on their surface that can be readily imaged using our fluorescent agents, BombesinRSense™ 680 [BR680] and Transferrin-Vivo™ 750 [TV750], respectively. In this report, we demonstrate the synergistic use of fluorescence (FLI) and bioluminescence imaging (BLI) to profile tumor metabolism and vitality, respectively, in response to a targeted anti-cancer drug, sorafenib. Sorafenib is a clinically approved tyrosine kinase inhibitor effectively blocking VEGFR, PDGFR and Raf signaling in cancer. At a higher daily dose of 120 mg/kg, the drug effectively decreases viability of HCT116-luc human colon xenograft tumors within 48 hours, coinciding with the loss of bioluminescence as well as BR680 and TV750 fluorescent signals in tumors. Interestingly, at a lower dose of 40 mg/kg, BR680 signal reduction can be observed early (within 48 hours), but no significant reduction of tumor viability by BLI and tumor volume was observed until a week later. These results suggest the potential use of metabolic fluorescent imaging agents as robust, efficient and early biomarkers for pre-clinical development of targeted cancer drugs.

## 1 HCT116/luc tumor is responsive to sorafenib treatment by BLI and tumor volume assessment

### A. Bioluminescence imaging of tumor viability

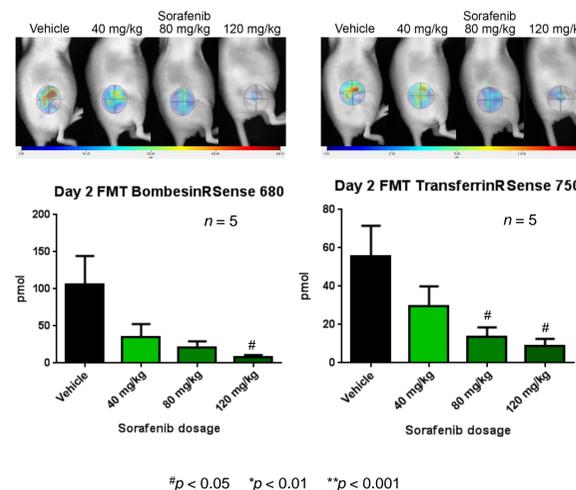
### B. Sorafenib suppresses tumor growth



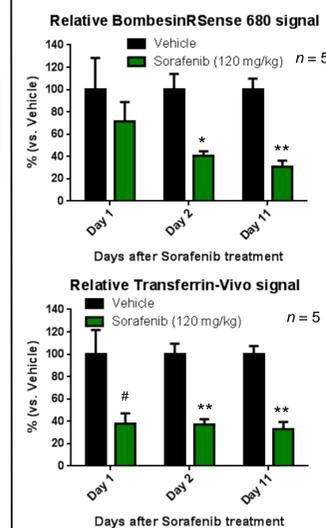
**A.** Longitudinal bioluminescence imaging (BLI) of HCT116-luc tumors in response to daily treatments of 120, 80 or 40 mg/kg sorafenib. Oral gavage treatments were given daily on day 0-4 and day 7-11. **B.** Relative tumor BLI signals and tumor volumes of each treatment group were calculated using the day 0 signals as 100% ( $p$ -value calculated by two-way ANOVA, bar: s.e.m.). **C.** Relative BLI signals of each dose group on day 2 ( $t$ -test, bar: s.e.m.); relative tumor volumes on day 2 (one-way ANOVA, bar: s.e.m.). **D.** H&E staining of tumor tissue sections harvested from the vehicle and sorafenib-treated (120 mg/kg) tumors on day 10. The drug effectively induced necrosis and regression in tumors. Bar: 50  $\mu$ m.

## 2 Metabolic tumor imaging of the sorafenib dose response using fluorescent imaging agents

### A. Fluorescence molecular tomographic (FMT) imaging of tumor metabolic changes in response to sorafenib treatment



### B. Longitudinal imaging



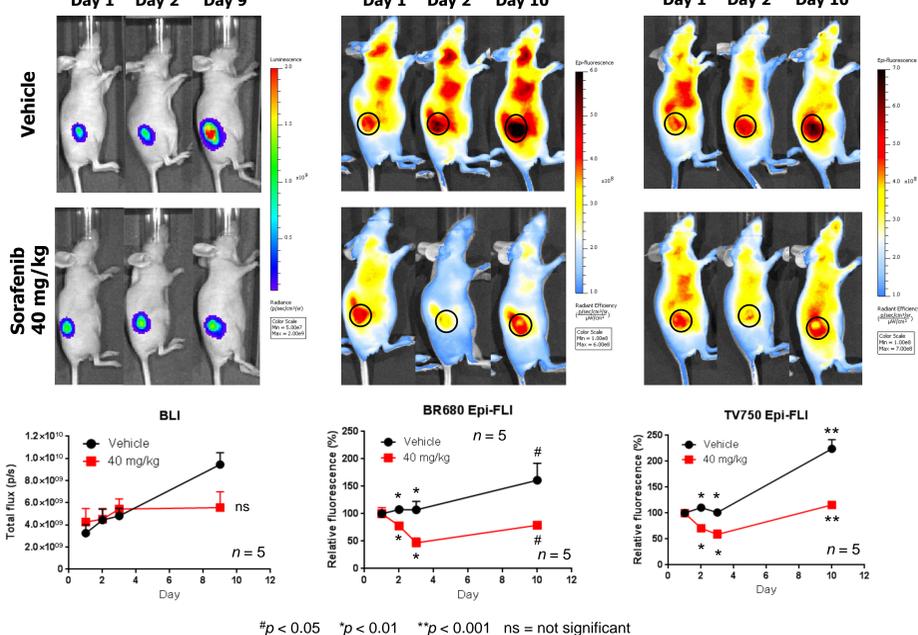
BombesinRSense 680 and Transferrin-Vivo 750 detect early tumor metabolic changes in response to sorafenib treatment. **A.** PerkinElmer's FMT 4000 fluorescence molecular tomography imaging system was used to detect BombesinRSense 680 (BR680) or Transferrin-Vivo 750 (TV750) uptake in the HCT116-luc tumors treated with different doses of sorafenib ( $t$ -test, bar: s.e.m.). **B.** FMT imaging of BR680 and TV750 tumor signals in subcutaneous HCT116-luc tumors treated with vehicle or 120 mg/kg sorafenib daily ( $t$ -test, bar: s.e.m.). Graphs represent longitudinal metabolic changes in HCT116-luc tumors in response to the treatment.

## 3 FLI, but not BLI, can detect early tumor metabolic changes following moderate dose sorafenib treatment

### A. Luciferase BLI imaging

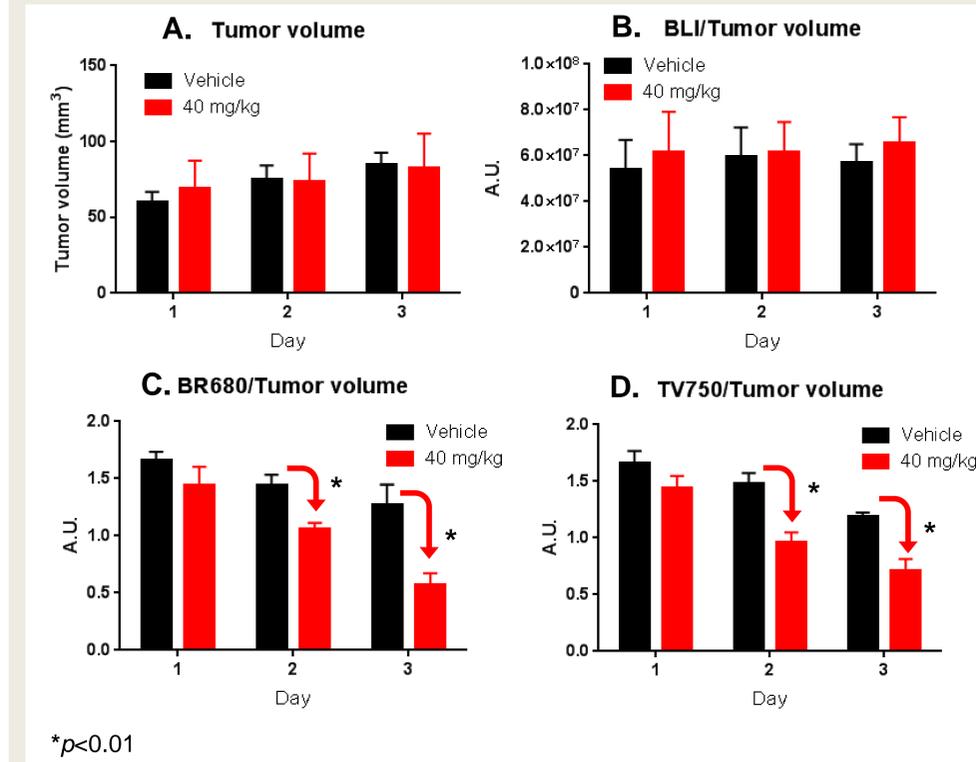
### B. BombesinRSense 680

### C. Transferrin-Vivo 750



BLI and FLI monitoring of tumor changes in response to a moderate dose of sorafenib. **A.** BLI imaging of tumors in animals treated with vehicle or 40 mg/kg sorafenib (bar: s.e.m.) using PerkinElmer's IVIS Spectrum CT imaging system. **B.** Epi-fluorescence imaging (epi-FLI) of BR680 uptake in tumors showing images and quantification ( $t$ -test, bar: s.e.m.). **C.** Epi-FLI of TV750 uptake in tumors showing images and quantification ( $t$ -test, bar: s.e.m.).

## 4 Quantification of the early therapeutic response to a moderate dose of sorafenib with tumor size normalization



To more accurately assess the impact of treatment between day 1 and day 3, taking into account modest increases in tumor size, the quantitative BLI and FLI results were normalized to tumor volume. **A.** Tumor volume during the first three days of treatments. **B.** BLI signals normalized against tumor volume data. **C.** BR680 epi-FLI signals normalized against tumor volume data ( $t$ -test,  $n = 5$ , bar: s.e.m.). **D.** TV750 epi-FLI signal normalized against tumor volume ( $t$ -test,  $n = 5$ , bar: s.e.m.).

## Summary

In these studies, we demonstrated that bioluminescence and fluorescence imaging can work synergistically in order to obtain a comprehensive picture of tumor viability and metabolic status. PerkinElmer's IVIS imaging systems have been widely adopted for pre-clinical imaging of bioluminescent tumor xenografts that stably express firefly luciferase. Since firefly luciferase is only expressed in tumor cells but not present in any normal tissues, bioluminescence imaging (BLI) shows high sensitivity, broad dynamic range and excellent specificity. More importantly, the need of ATP for luciferase-mediated light production can make BLI a useful indicator for assessing tumor viability in response to different anti-cancer treatments, especially when conventional tumor volume measurement fails to indicate any difference. In this report, we used BLI imaging to perform a dose-titration study and identified the minimal effective dosage of sorafenib that suppresses but does not reduce tumor burden or viability (assessed by BLI). Statistically significant changes were seen on day 2 post-treatment with 80-120 mg/kg sorafenib treatment, with 40 mg/kg showing little or no effect on tumor burden at this time point.

For visualizing early metabolic changes in tumors, we used non-invasive near infrared (NIR) fluorescence imaging agents to monitor tumor's metabolic responses to a targeted therapeutics. Due to the spectral wavelength in the NIR, and the accompanying low tissue absorbance, NIR fluorescent imaging generally shows enhanced deep tissue penetration. Bombesin- and transferrin-targeted (BRS680 and TV750, respectively) imaging agents, showed the ability to detect day 2 sorafenib efficacy at a dose of 40 mg/kg, lower than needed for BLI detection. BR680 signal reduction can still be observed early within 48 hours, but no significant reduction of tumor size or BLI signal was observed until a week later. In addition to metabolic imaging, PerkinElmer provides a variety of NIR fluorescent imaging agents for multiplex imaging of biophysiological changes in tumor. We believe the collaborative use of both imaging methods is of significance for pre-clinical anti-cancer drug development.