Introduction

The study aims to establish an orthotopic liver cancer model in nude mice and evaluate the anti-tumor efficacy of Sorafenib. Liver cancer is a leading cause of cancer deaths worldwide, accounting for approximately 830,000 deaths each year. Hepatocellular carcinoma (HCC) is the most prevalent malignant cancer of the liver (80%) and its incidence and mortality have been increasing. Advanced HCC has no effective treatment or cure, despite the discovery of promising novel therapeutic drugs. Sorafenib, a multi-tyrosine kinase inhibitor is widely used as a first-line treatment in HCC patients. However, several clinical studies showed that a considerable percentage of patients with HCC are insensitive to sorafenib. Therefore, there is an ever-increasing need for better treatment options. Animal models have become increasingly important in the study of HCC, as they serve as a critical bridge between laboratory-based discoveries and human clinical trials. Orthotopic implantation of HCC cells into the liver is critical to providing relevant micro environment for development of tumors and proper evaluation of potential therapeutics. This orthotopic model will provide a reproducible model for testing anti cancer activity of therapeutics in a relevant HCC model.

Experimental Procedures

A total of twelve (12) athymic female nude mice (9-10 weeks old) were used for the study. Following the anesthesia (isofluorane), a parallel incision (1 cm) to the linea alba was made in the abdominal wall of the mouse to expose the liver. Each mouse was injected orthotopically into the liver with $1x10^6$ HepG2-Luc cells (JCRB cell Bank) suspended in 1:1 Matrigel/PBS (20 µL). The injection was performed very slowly, and the needle was kept for at least 30 seconds. After injection, the abdominal wall and skin incision were closed by suture using 3-0 surgical sutures and 9 mm autoclips, respectively. Tumor growth in the liver was monitored using IVIS Lumina III (PerkinElmer) in vivo imaging system. Mice were injected with D-luciferin (XenoLight, PerkinElmer; 150 mg/kg body weight, i.p). Mice were imaged bi-weekly for 10 minutes after the D-luciferin injection. On day 7, mice were randomly allocated into two groups (N=6/Group). Mice in Group 1 received Vehicle (0.1 ml 1:1:8 DMSO/Cremphor EL/PBS). Mice in Group 2 were administered with Sorafenib 62 mg/Kg. Vehicle and Sorafenib were administered via oral gavage once every two days for a total of six times (Table 1). Mice were euthanized on day 25. Upon autopsy, mice were screened visually

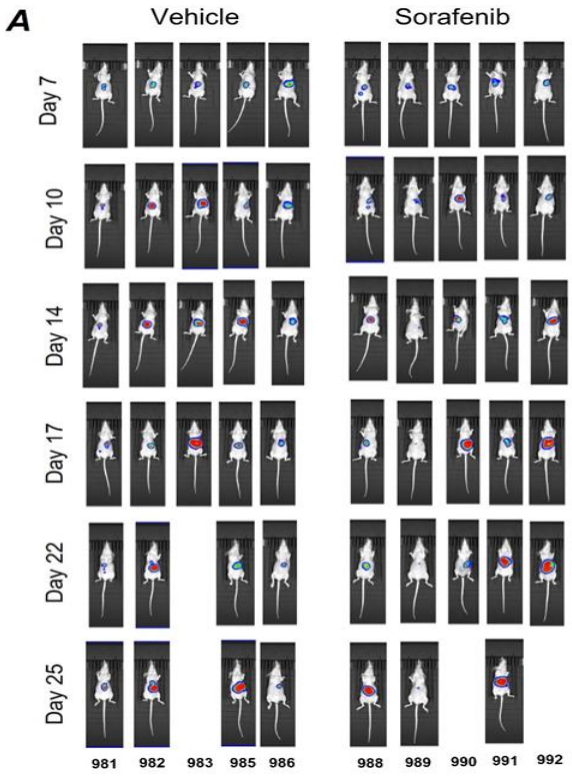
for metastatic lesions in the spleen, lungs, kidneys, lymph nodes, and peritoneum. The liver was excised, formalin-fixed, and sectioned for H&E staining.

Results & Conclusion

Orthotopic hepatocellular carcinoma (HCC) establishment success rate was 75% (Figure 1). Hematoxylin and Eosin (H&E) staining verified the growth of tumors in mouse liver (Figure 2). 2 out of 12 mice died due to surgical complications and 1 mouse developed tumors outside the liver (on the outside muscle of the upper abdominal wall). Only 1 out of 5 established tumors regressed in the Sorafenib treatment group. No metastatic lesions were noted in organs.

Group	N	Mouse strain	Day of tumor cell inoculation	Tumor cell line/cell number/route/ volume	Treatment	Treatment route/volume	Readout
1	6	Athymic Nude	Day 0	HepG2-Luc, 1x 10^6 cells/0.02 mL PBS + 50 % matrigel, intrahepatic	Vehicle (1:1:8 DMSO/Cremphor EL/PBS)	p.o/5 mL/kg	Body weights (Twice a week). IVIS imaging (once a week after tumor cell implantation, followed by twice a week for the duration of the study)
2	6	Athymic Nude			Sorafenib	p.o/5 mL/kg	

Table 1. Study design. Upon tumor establishment, mice were randomly allocated into two groups. Mice in Group 1 were administered with Vehicle (0.1 ml 1:1:8 DMSO/Cremphor EL/PBS). Mice in Group 2 mice were administered with Sorafenib 62 mg/Kg. Vehicle and Sorafenib were administered via oral gavage once every two days for a total of six times.



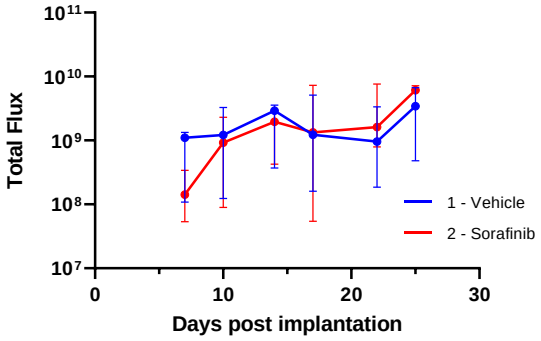


Figure 1. Assessment of liver tumor establishment and growth by in vivo bioluminescence imaging. Imaging was performed using IVIS Lumina III imaging system (PerkinElmer). Photographs of mice at various time points show tumor establishment and tumor growth in mouse liver (A). Time-course F-Luc bioluminescence quantification of mice treated with Vehicle or Sorafenib (B). One mouse in the vehicle treatment group and two mice in the Sorafenib treatment group were found dead on days 20 and 23, respectively.

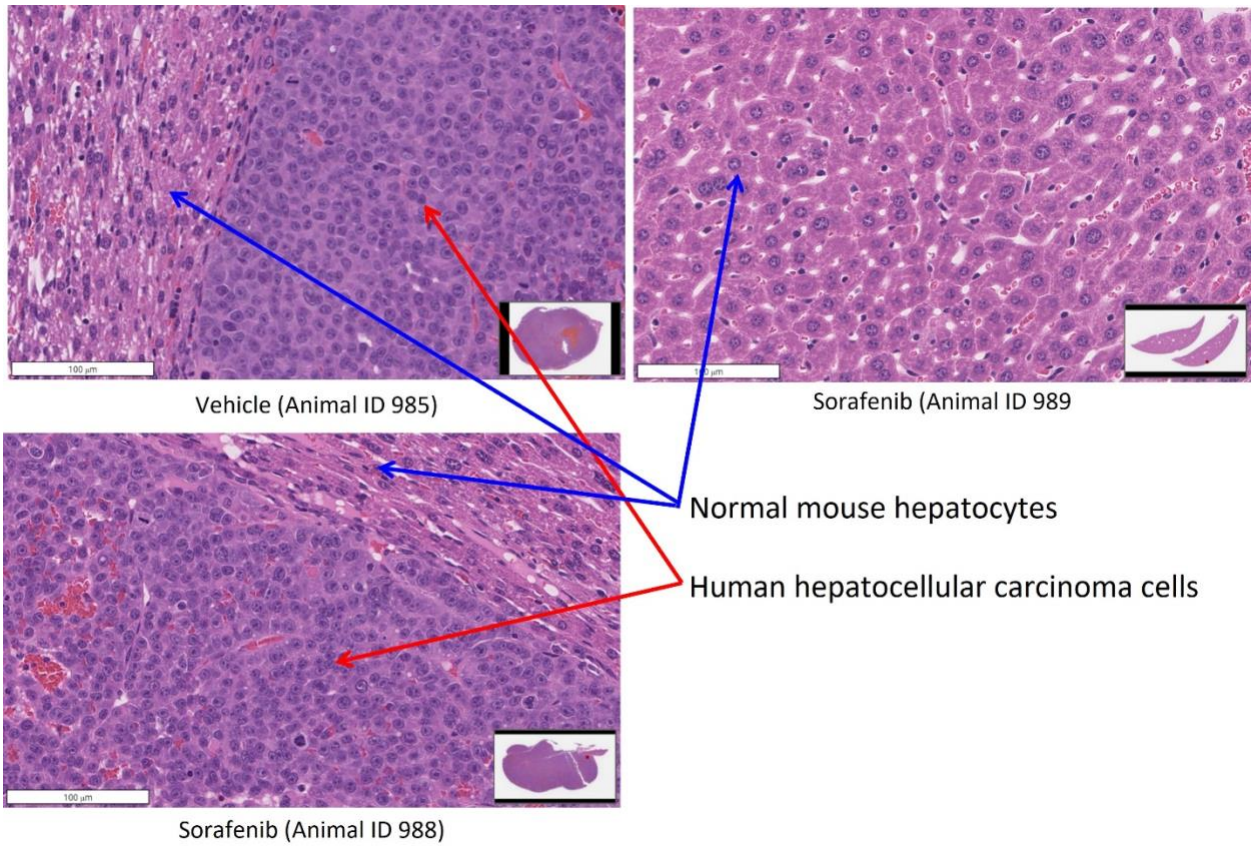


Figure 2. Hematoxylin and Eosin (H&E) sections of the liver show human HCC growth in mouse liver. Blue arrowheads point to normal mouse hepatocytes and red arrowheads point to human hepatocellular carcinoma cells in the mouse liver. Insets show the images of the whole section.