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Original Articles

Preclinical comparison of regorafenib and sorafenib efficacy for hepatocellular carcinoma using multimodality molecular imaging

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ABSTRACT

Sorafenib has been used as a clinical targeted therapy for hepatocellular carcinoma (HCC) for more than a decade. In 2017, regorafenib was approved for HCC treatment and has since been reported to prolong the survival of advanced HCC patients after treatment failure with sorafenib. However, there has been no direct systematic comparison of the therapeutic effects of regorafenib and sorafenib against HCC. In this study, we comprehensively compared the therapeutic effects of sorafenib and regorafenib against HCC *in vitro* and *in vivo* using multimodality molecular imaging, which can show molecular and cellular differences at early stages. The side effects of sorafenib and regorafenib were also systematically evaluated. The data showed that compared with sorafenib treatment, regorafenib exerted stronger antitumor and antiangiogenic effects and significantly increased the survival rate of HCC mice. Sorafenib but not regorafenib treatment caused body weight loss and liver and kidney dysfunction, while regorafenib but not sorafenib treatment caused hypertension. Our study may provide an experimental basis for the guidance of clinical HCC targeted treatment with regorafenib and sorafenib.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Its incidence rate has markedly increased in the U.S, and the disease has become the second leading cause of cancer-related death worldwide in the past decades [1,2]. HCC targeted therapy is evolving as an extraordinarily promising approach [3,4], whereby a drug recognizes and specifically binds to the target and causes death of cancer cells without affecting normal liver tissue [5,6]. The mechanisms of HCC targeted therapy involve the inhibition of specific growth factor receptors and their corresponding signaling pathways [7]. In the clinic, there are only two HCC-targeting drugs approved by the Food and Drug Administration (FDA) worldwide, sorafenib and regorafenib [8].

Regorafenib is a novel oral multikinase inhibitor [9], which was developed to target multiform kinases involved in the angiogenic, tumor growth-promoting, and tumor microenvironment signaling pathways, including vascular endothelial growth factor receptors (VEGFR1, 2, and 3), platelet-derived growth factor receptor beta (PDGFR-β), fibroblast growth factor receptor 1 (FGFR1), etc [10,11]. In preclinical and clinical phase I–III trials, regorafenib has demonstrated potent antiangiogenic and antitumor effects [12]. Sorafenib is a small molecule inhibitor that inhibits tumor cell proliferation and

Abbreviations: HCC, Hepatocellular carcinoma; BLI, Bioluminescence imaging; FMI, Fluorescence imaging; CTA, CT angiography

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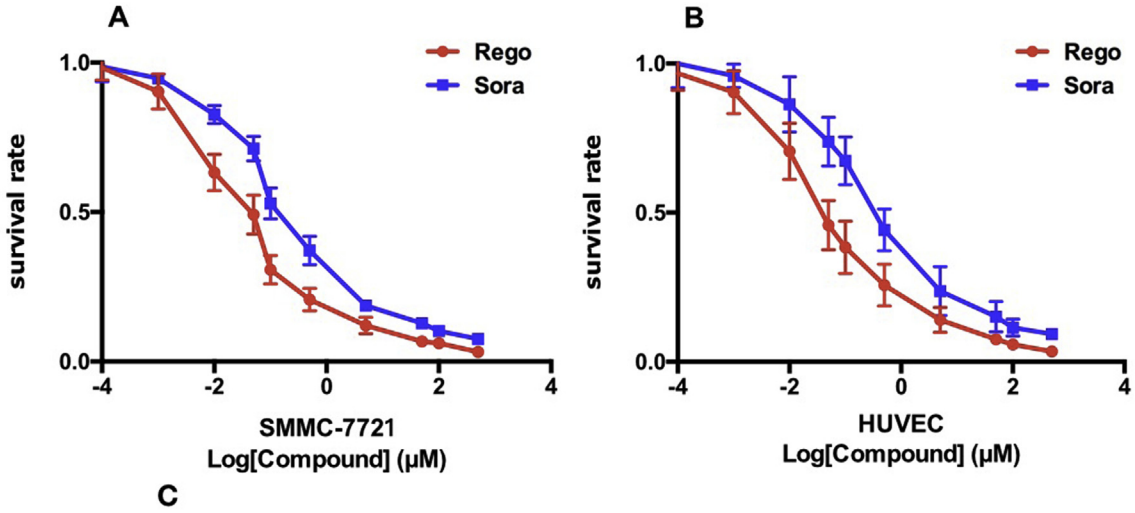

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Item		SMMC-7721	HUVEC
IC50 ± SEM (μM)	Rego	0.0229 ± 0.0117	0.0491 ± 0.0238
	Sora	0.1222 ± 0.0634	0.2871 ± 0.0858
P value (Rego & Sora)		P<0.05 (*)	P<0.01 (**)

Fig. 1. Effects of regorafenib and sorafenib on the viability of SMMC-7721-fLuc cells and HUVECs. The drug inhibition relative survival rate of SMMC-7721 cells (A) and HUVECs (B) after treatment with regorafenib and sorafenib at different concentrations were obtained using the MTT assay. (C) IC_{50} values. (*$P < 0.05$; **$P < 0.01$).

angiogenesis. It can promote the apoptosis of cells in extensive tumor models [13]. In preclinical experiments, sorafenib reduced tumor angiogenesis and proliferation and increased tumor cell apoptosis in a human HCC mouse xenograft model [14,15]. Sorafenib was approved by the FDA in 2007 for the treatment of unresectable HCC [16]. In the following decade, it has become a first-line HCC targeted drug for single and combination therapy [17–19]. A study completed in the early 2017 showed that regorafenib, as a second-line medicament, prolonged survival by 2.8 months in HCC patients who progressed on sorafenib therapy [20]. Prior studies have suggested that some side effects of regorafenib were less severe than those of sorafenib [21]. Therefore, regorafenib was approved by the FDA for the treatment of HCC. Gradually, it has been listed in many countries as a new targeted drug for HCC. It brings hope to patients with HCC, especially to advanced HCC patients who no longer respond to sorafenib [22]. Based on existing reports, there are certain advantages in using sorafenib, which has been approved for a longer time and used widely. Consequently, there are more existing data so that treatment effects can be predicted and side effects and risks may be controlled [23,24]. Regarding regorafenib, it can be effective for subsequent treatment after patients stop responding to sorafenib treatment. However, a higher cost of regorafenib is a critical factor to be considered. Because there have been no direct comparative studies on the anti-HCC effects of regorafenib and sorafenib, it is actually difficult to choose the most suitable clinical drug for patients at the current stage. Therefore, it is necessary to find sensitive methods to evaluate the drug treatment efficacy at an early stage and select the suitable drug according to the patient's illness status, physical condition, endurance capacity for side effects, and economic means [25]. Once the gap is filled, patients and doctors will be more aware of specific choices of right therapeutic drugs.

Molecular imaging can reflect physiological and pathological changes in the organism at the molecular level. Compared with other *in vivo* imaging techniques, optical molecular imaging has many advantages, such as its high sensitivity, good specificity, intuitive results, rapid measurement, low cost, etc. The approach has been widely used in tumor studies and in the development of new drugs [26–28]. To overcome the problem of the high scattering of fluorescence signals in biological tissues, multimodality molecular imaging was developed, based on bioluminescence imaging (BLI) and fluorescence imaging (FMI), combined with computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET)/single photon emission tomography (SPECT), etc [29]. Multimodality molecular imaging combines the advantages of various imaging techniques and provides researchers with more comprehensive and accurate information on the disease progression, tumor monitoring, drug development, and therapeutic evaluation. Multimodality molecular imaging has shown a great potential in preclinical and clinical studies [30].

The objective of the current study was to systematically evaluate and compare the treatment effects of regorafenib and sorafenib in HCC using multimodality molecular imaging, which may provide a comprehensive experimental basis for the clinical HCC targeted treatment with regorafenib and sorafenib.

2. Materials and methods

The details of the materials and methods are available in the Supporting information.

3. Results

3.1. Effects of regorafenib and sorafenib on cell viability in in vitro

The survival rate was examined using the MTT assay after SMMC-7721-fLuc cells and HUVECs were treated with regorafenib and sorafenib *in vitro*. The results are shown in Fig. 1. The half-maximal inhibitory concentrations (IC_{50}) of regorafenib for SMMC-7721 cells and HUVECs were 0.0229 ± 0.0117 μM and 0.0491 ± 0.0238 μM, respectively, and the IC_{50} values of sorafenib were 0.1222 ± 0.0634 μM

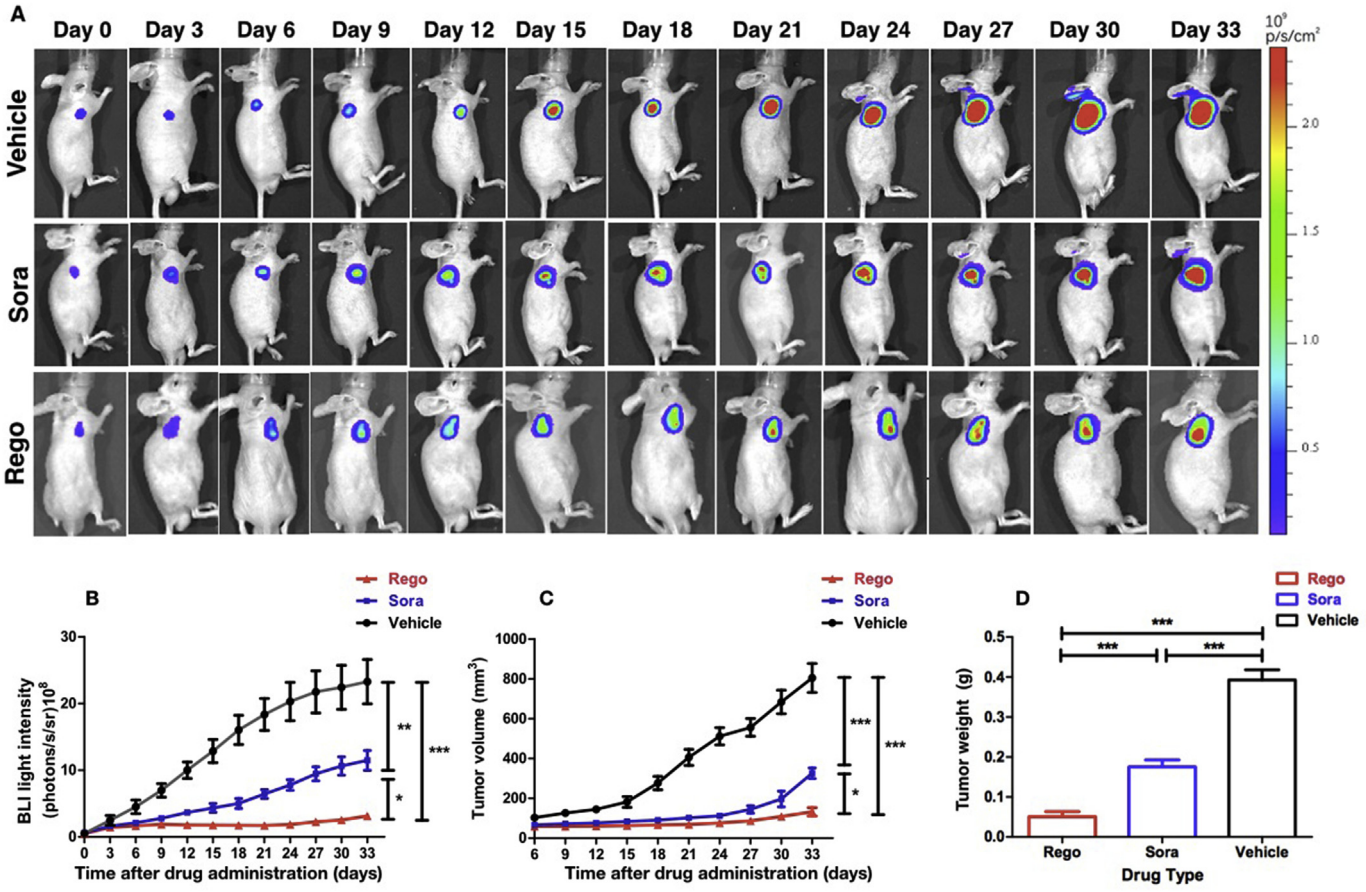

Fig. 2. Evaluation of therapeutic effects of the two drugs in subcutaneous HCC model mice treated with regorafenib (Rego), sorafenib (Sora), and the vehicle (n = 12 per group). (A) Continuous BLI observation of subcutaneous tumors from day 0 to day 33. (B) BLI light intensity measurement and calculation. (C) Tumor volume measurement and calculation. (D) Tumor weights were measured at the end of drug treatment. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

and 0.2871 ± 0.0858 μM respectively. The data indicated that regorafenib and sorafenib could both decrease the survival rate of HCC cells and inhibit the angiogenesis. Moreover, the inhibition potency of regorafenib was higher than that of sorafenib for both HCC and endothelial cells *in vitro*.

3.2. Therapeutic effects of regorafenib and sorafenib on tumor progression in vivo

The SMMC-7721-fLuc tumor xenograft growth was initially monitored using BLI and tumor volume measurement during the treatment course, and the data are shown in Fig. 2A, where day 0 is the observation time before treatment and day 1 is the first day of drug administration. We observed the treatment effects for more than 1 month and found that the BLI intensity in the vehicle group increased at a power exponent over time. The BLI intensity for sorafenib treatment also steadily increased during the treatment period. But it was relatively lower than that in the vehicle group, suggesting the antitumor therapeutic effects of sorafenib. Moreover, we found that the BLI intensity of regorafenib-treated tumors was the lowest among three groups at each corresponding time point, suggesting that regorafenib was more effective than sorafenib as an anti-HCC treatment.

The calculated BLI light intensity results, shown in Fig. 2B, are consistent with the *in vivo* BLI observation, suggesting the stronger antitumor treatment effects of regorafenib compared with those of sorafenib. Significant differences in the BLI intensity between regorafenib and sorafenib treatment groups could be noticed as early as 15 days of drug administration.

In addition, the tumor volumes were measured dynamically during the drug treatment course and the results are shown in Fig. 2C. The tumor volumes increased in the vehicle group at a power exponent over time. The tumor volumes also increased in the sorafenib group, but at a lower rate compared with that in the vehicle group. Meanwhile, the tumor volumes were the lowest in the regorafenib group compared with those in the other groups. A significant difference in the tumor volumes was observed between regorafenib and sorafenib treatments after 33 days of treatment. At the end of the *in vivo* observation, the tumors were dissected out and weighed as well, and the results were consistent with those of the *in vivo* BLI observation and tumor volume measurement (Fig. 2D).

Overall, the above data indicated that regorafenib exhibited stronger antitumor effects than sorafenib did both *in vitro* and *in vivo*. Moreover, in terms of evaluating the drug treatment efficacy, BLI was more sensitive than the traditional tumor volume measurement. Thus, it can help predict the treatment outcome at an earlier stage.

3.3. Evaluation of the drug treatment efficacy in the orthotopic HCC model

To mimic a HCC tumor growth environment, we established an orthotopic HCC nude mouse model. During the drug treatment, BLI was performed every 4 days to monitor the tumor growth, and representative BLI images of treated mice in each group are shown in Fig. 3A. In addition, we quantified the BLI light intensity in Fig. 3B. The changes in BLI light intensity after treatment with regorafenib and sorafenib were similar to those in the subcutaneous HCC model. Regorafenib was a more effective antitumor treatment than sorafenib in both orthotopic and xenograft HCC nude mouse models.

Because BLI can only provide 2D images of tumors, we further used

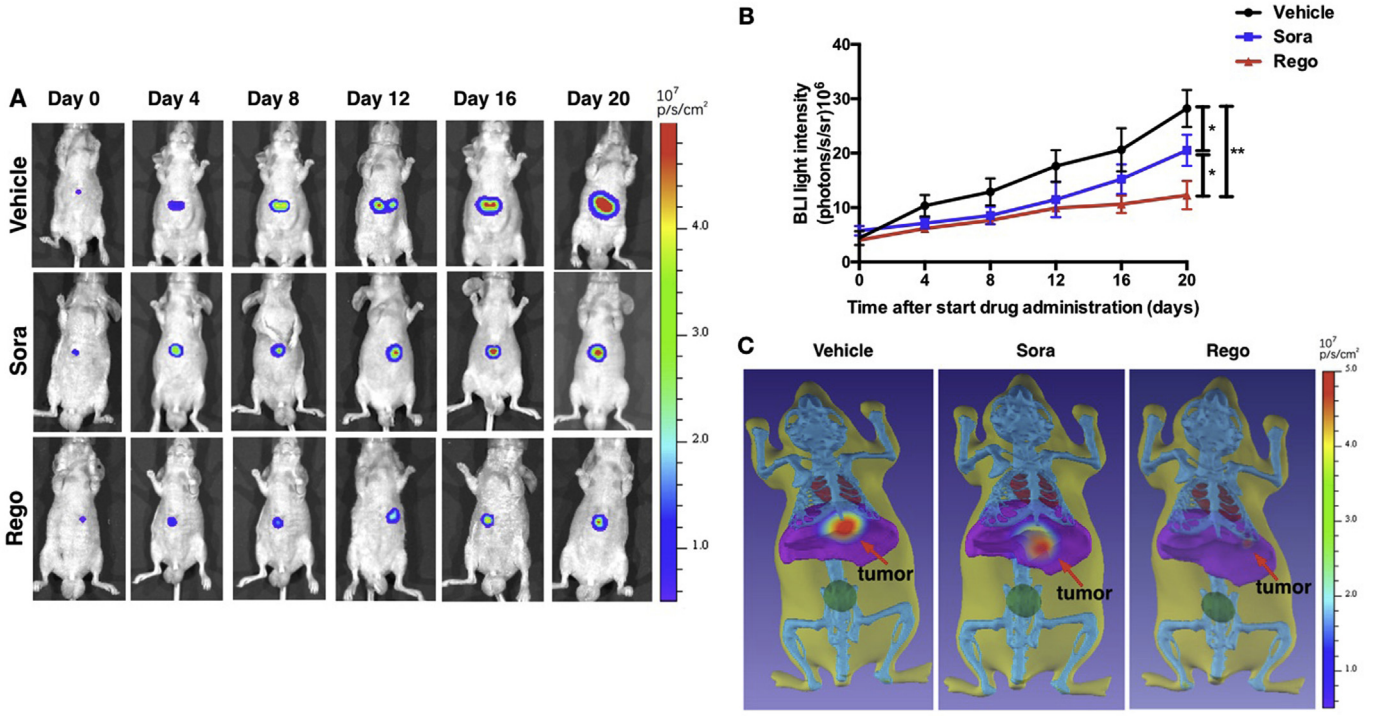

Fig. 3. Dynamic BLI and BLT in the orthotopic HCC tumor model during drug treatment. (A) Continuous BLI light intensity observation from day 0 to day 20. (B) the average BLI light intensity calculation. (C) 3D BLT images after different treatments. Red arrows indicate the tumor location. (*$P < 0.05$; **$P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

bioluminescence tomography (BLT) imaging, which can provide full information on the tumor growth, in terms of the tumor location and volume, in a 3D mode. In Fig. 3C, it can be observed that the tumor in the vehicle group exhibited a larger volume than sorafenib treatment group, while regorafenib treatment led to the smallest tumor volume among the three groups. Hence, the data further confirmed that regorafenib exerted a better therapeutic effect on HCC compared with sorafenib treatment.

3.4. *In vivo* FMI of angiogenesis after drug treatment

Since the major functional mechanism of regorafenib and sorafenib is inhibition of tumor angiogenesis, FMI was performed with the IntegriSense 750 probe, which has been applied in many aspects as the visualization and quantification of integrin αvβ3 expression *in vivo*, the expression of tumor cells and neovascularization, and monitoring tumor angiogenesis (add reference). The *in vivo* antiangiogenic effects were examined during the different treatments (Fig. 4A). We also quantified the FMI intensity in Fig. 4B.

We found that IntegriSense 750 could specifically target orthotopic HCC tumors in all three groups. Moreover, the FMI intensity in the tumor regions was the highest in the vehicle group, lower in the sorafenib group, and the lowest in the regorafenib group (Fig. 4B, red circles).

Tumor-bearing livers were dissected out 48 h after *in vivo* observation for further *in vitro* FMI (Fig. 4C), which could clearly discern the tumor angiogenesis signal in the liver lobe. The FMI signal was also the lowest in the regorafenib group among three groups, consistent with the *in vivo* observation. The BLI was used to indicate the tumor location in the liver lobe (Fig. 4C), and co-localization of angiogenesis was confirmed by FMI. We also found that the FMI and BLI region is indeed the tumor area confirmed by HE staining shown in Fig. 4D (red arrows).

3.5. *CTA of tumor vascularity*

Because FMI can only provide 2D images, we further performed CTA 3D imaging of tumor vascularity using the blood pool contrast agent Fenestra VC to reveal vascularity in exquisite details. The 3D portrayal of angioarchitecture conveyed the status of angiogenesis. Besides, it offered a quantitative evidence to assess the tumor growth in the process. We seized some details of vasculature changes, even when ripe tumor vessels were not clearly visualized (Fig. 5). We combined a CT slice with the tumor area and a 3D CT reconstruction model. The 2D slice image was replaced with its original location in the 3D model to label the position of vessels. The data showed that there were obviously fewer vessels close to the tumor in the regorafenib group than in the vehicle and sorafenib groups.

Although CTA cannot visualize morphological changes of tumor capillaries as small as those that can be observed by the electron microscopy, CTA is a noninvasive method for continuous and dynamic observations of tumor capillaries in a 3D mode, which is important for drug evaluation.

3.6. *Tumor immunohistochemistry*

To further confirm the *in vivo* observations, tumors were dissected out at the end of treatment and subjected to immunohistochemical staining for CD31, which is a marker of angiogenesis, and for Ki-67, a marker of cell proliferation, while the TUNEL assay was used for apoptosis detection. CD31 is mainly used to evaluate the tumor angiogenesis, and Fig. 6A shows that there were many CD31 positively stained blood vessels in the vehicle group and relatively fewer blood vessels in the sorafenib group, whereas we could hardly find any in the regorafenib group (Fig. 6B and C). The data suggested that regorafenib and sorafenib could both inhibit neovascularization, but regorafenib exhibited stronger inhibition of angiogenesis.

As a cell proliferation antigen marker, Ki-67 is indispensable in cell proliferation studies and is commonly used for immunohistochemical

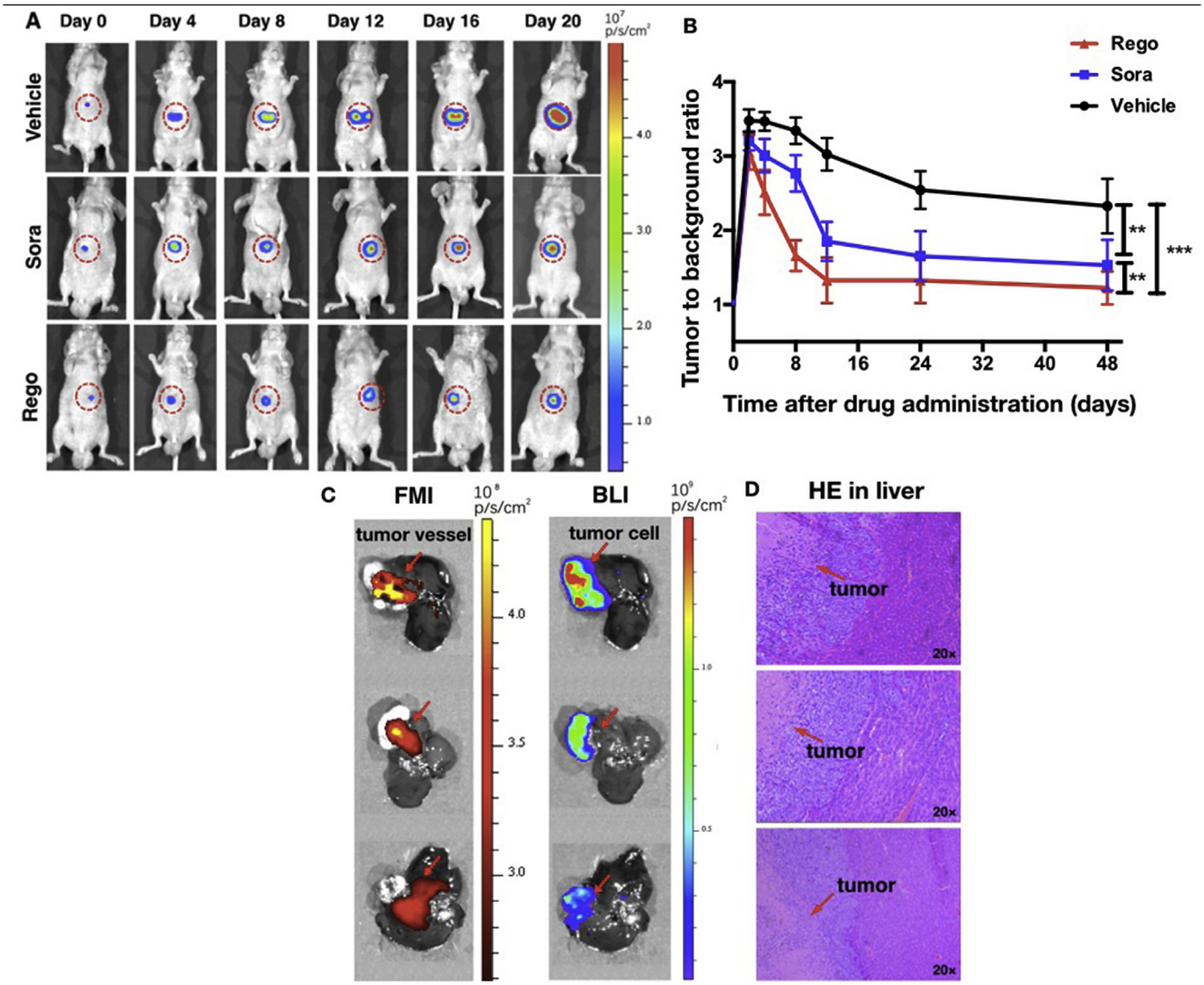

Fig. 4. *In vivo* FMI of tumor angiogenesis after different drug treatments. (A) Biodistribution of IntegriSense 750 was monitored *in vivo* by fluorescence molecular tomography (FMT), and the red circles indicate the tumor regions. (B) Quantified FMI light intensity. (C) The livers with tumors were dissected out and processed by *in vitro* FMI and *in vitro* BLI of HCC tumors. (D) H&E staining of liver tissues. Red arrows indicate the tumor areas. (**$P < 0.01$; ***$P < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

staining. In this study, there were substantial regions stained for Ki-67 in the vehicle group (Fig. 6D), but relatively less Ki-67 expression was observed in the sorafenib group (Fig. 6E). There was almost no positive staining in the regorafenib group (Fig. 6F). The data suggested that both drugs could effectively inhibit the tumor cell proliferation, but regorafenib was more effective than sorafenib.

TUNEL is a common staining assay for apoptosis detection. Our data revealed that cells grew well, and there was no obvious apoptosis phenomenon in the vehicle group (Fig. 6G). Regorafenib and sorafenib treatments led to the apoptosis of SMMC-7721-fLuc cells, and apoptosis was more severe after regorafenib treatment (Fig. 6H and I).

In conclusion, our data suggested that both regorafenib and sorafenib could inhibit angiogenesis and cell proliferation and promote the apoptosis in HCC. Moreover, the inhibitory effect of regorafenib was stronger than that of sorafenib.

3.7. Survival analysis

The data of survival analysis, used to compare the treatment effects of two drugs on HCC in the orthotopic HCC model, are shown in Fig. 7. The median survival time in the regorafenib group was 40.5 days, which was longer than that in the sorafenib group (35 days) and in the vehicle group (30 days). The differences in survival among the three groups were significant (***$P < 0.001$), including that between the regorafenib and sorafenib groups. The data suggested that regorafenib was more effective than sorafenib for HCC treatment.

3.8. Analysis of the side effects of drug treatments

Although molecularly targeted drugs are non-cytotoxic and their toxicity profiles and clinical manifestations are quite different from commonly used chemotherapeutic drugs, there are still toxicities in clinical applications. Therefore, paying attention to the side effects of a treatment is as important as paying attention to the therapeutic efficacy during the course of drug treatment.

The body weight changes during the treatment course are shown in Fig. 8A. The data showed that the body weight in the vehicle group steadily increased, but that in the regorafenib and sorafenib groups first increased and then decreased. The body weight of the sorafenib-treated tumor-bearing mice decreased more significantly than that in the

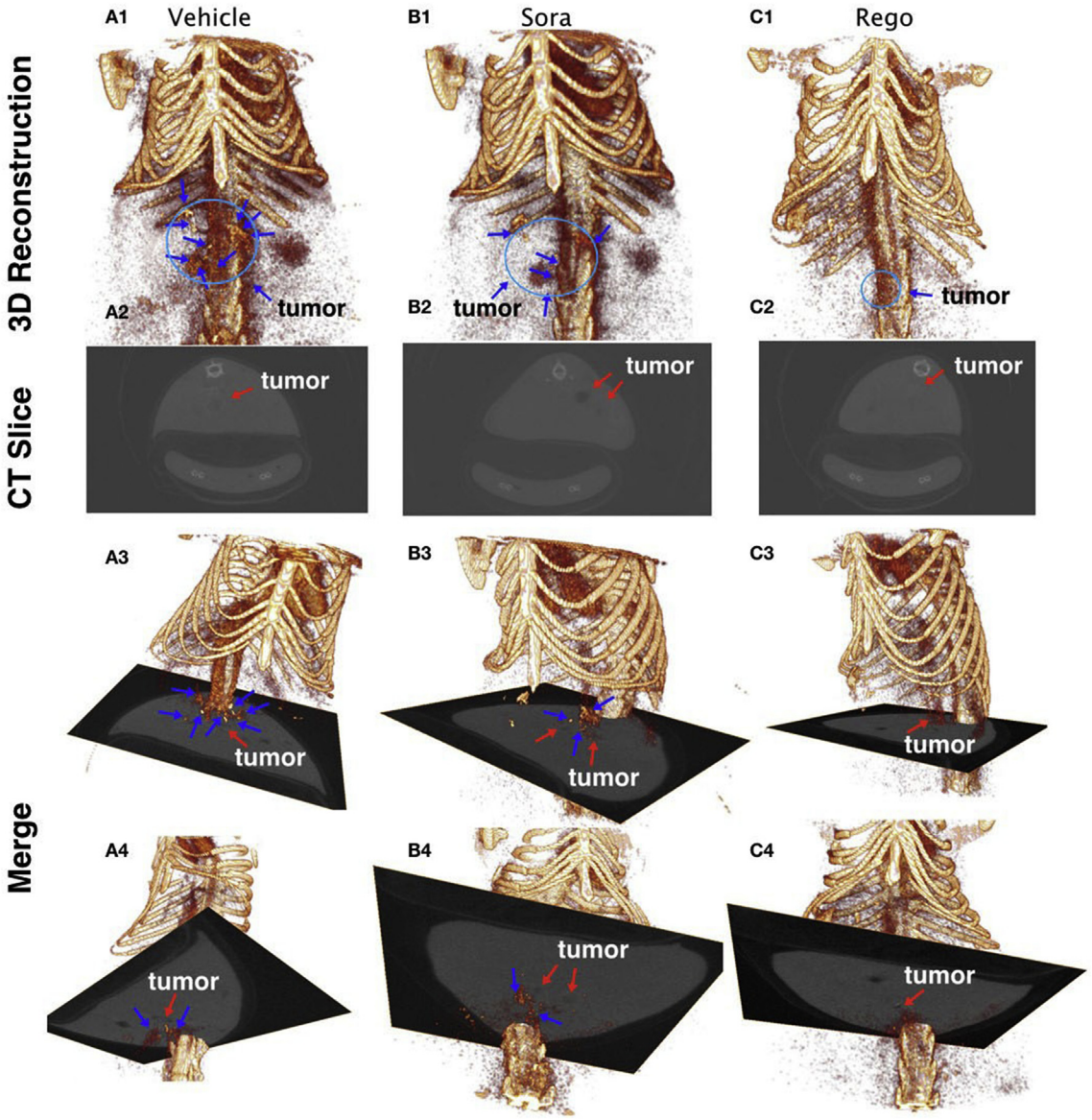

Fig. 5. CTA imaging of orthotopic HCC tumor model after different drug treatments. Images acquired after vehicle, sorafenib (Sora), and regorafenib (Rego) treatments are presented in columns A, B, and C, respectively. (A1–C1) 3D reconstruction results of CT images, in which the yellow part represents the bone, and the brown area is the location of the contrast agent Fenestra VC; the location of tumors is indicated by blue circles, and blood vessels are indicated by blue arrows. (A2–C2) Original CT images, indicating the tumor location (red arrows) and layers in which the tumor is located. (A3–C3) Reconstruction of the location above the tumor. (A4–C4) Reconstruction of the location below the tumor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

regorafenib-treated mice (*$P < 0.05$).

We also examined changes in systolic blood pressure (Fig. 8B) and HR (Fig. 8C) during the treatment course. The data showed that the blood pressure in all mice was basically identical before treatment. After the drug treatment, the blood pressure increased in both drug-treated groups and was significantly higher in the regorafenib than in the sorafenib group. The changes in HR were similar in the regorafenib and the vehicle group, but HR was slightly lower in the sorafenib group, although there were no statistically significant differences among the groups.

In addition, we evaluated the liver and renal functions after drug treatment, and the data are shown in Fig. 8D–H. We found that sorafenib caused a marked increase in ALT and AST (**$P < 0.01$), suggesting liver dysfunction. In addition, a decrease in BUN was observed for regorafenib treatment compared with that in the sorafenib and vehicle treatment groups (*$P < 0.05$).

At the end of treatment, major organs, including the heart, liver, spleen, and kidneys, were dissected out, and tissues were subject to H&E staining (Fig. 8I). We found that the histological structure of the liver was damaged in the sorafenib group, but no abnormalities were found in the other organs and in the other groups.

Based on the above data, sorafenib treatment may lead to a serious weight loss and liver dysfunction. Regorafenib, on the other hand, may cause an increase in blood pressure instead of in HR. It should be noted that other side effects that have been previously reported for these two drugs, such as the hand–foot reaction, diarrhea, and fatigue [31], were also observed in this study.

4. Discussion

In this study, we systematically evaluated the therapeutic effects of regorafenib and sorafenib against HCC using multimodality molecular

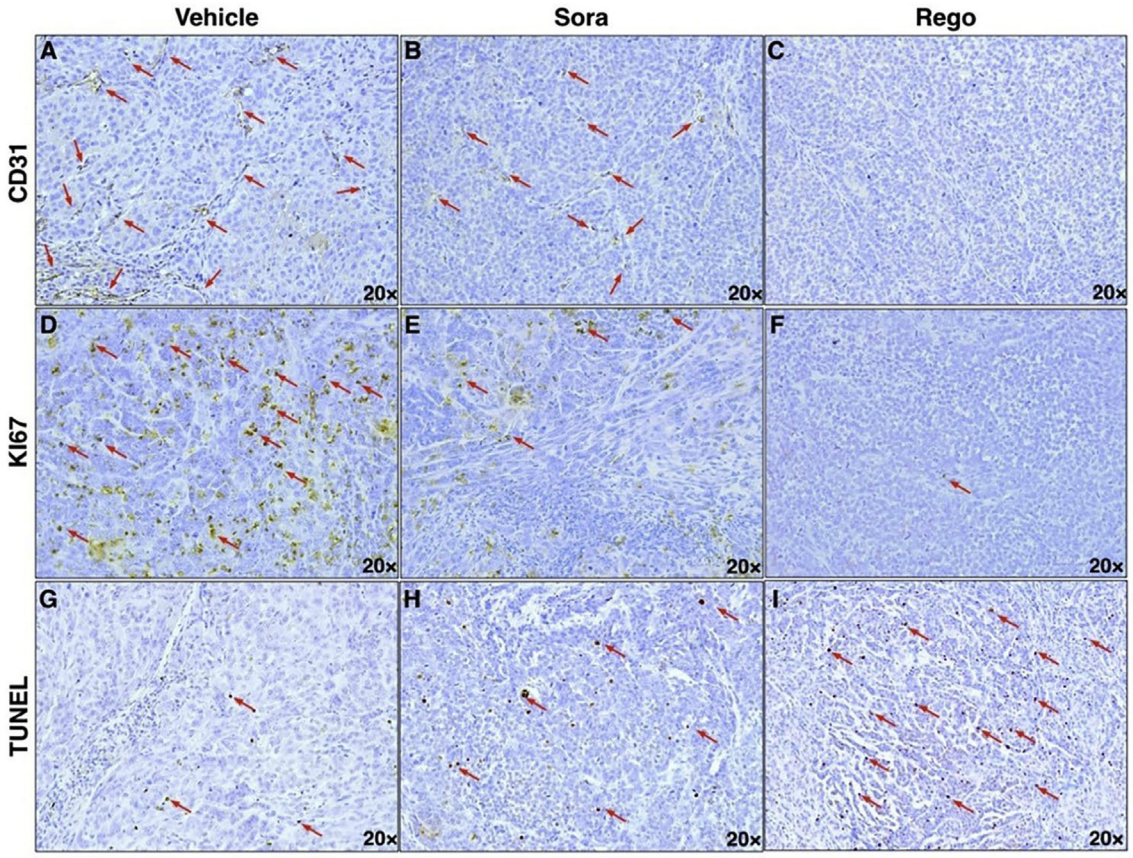

Fig. 6. CD31, Ki-67, and TUNEL apoptosis staining of tumor sections after treatments. (A–C) CD31 expression, showing tumor vessels stained for CD31 as filaments (red arrows). (D–F) Ki-67 expression in the cell nuclei is shown by a brown color (red arrows). (G–I) Apoptotic cells were stained using the TUNEL assay and are displayed as dark brown dots (red arrows). Scale bar = 500 μm (20 ×). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

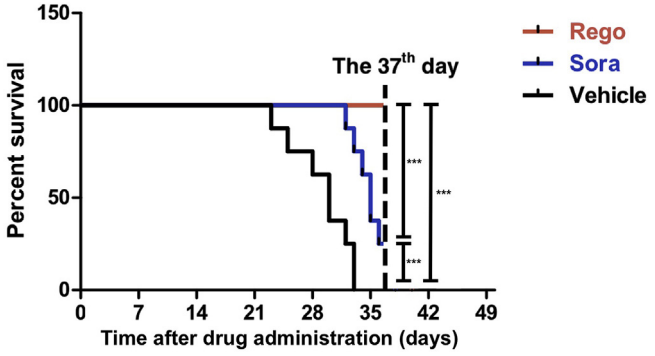

Fig. 7. Survival analysis after treatment of orthotopic HCC model mice with regorafenib (Rego), sorafenib (Sora), and the vehicle. The survival status was recorded for 12 mice per group from the start of treatment. (***$P < 0.001$).

imaging and comprehensively assessed side effects in a preclinical setting. The results showed that regorafenib could more effectively inhibit HCC growth and tumor angiogenesis than sorafenib. The highlight of the study is that it demonstrates the possibility of evaluating the drug treatment efficacy using multimodality molecular imaging, which allows monitoring, in a real-time and noninvasive manner, of the cancer progression and the drug treatment efficacy at molecular and cellular levels and before the appearance of the anatomical changes. Moreover, we collected 3D BLT data for tumors and CTA data for tumor blood vessels. 3D models were mapped via 2D imaging to overcome the limitation of the light penetration depth for optical imaging. In addition, the side effects of the drug treatments were comprehensively evaluated, which can provide some preclinical information to guide patients in drug selection. Overall, this study provides critical and effective information for the clinical application of regorafenib and for guiding treatment based on the patient's tolerance of adverse reactions. It may also help develop more scientifically based approaches to the application of drug treatment.

One of the highlights of our study is comparison of the antitumor effects of regorafenib and sorafenib using multimodality molecular imaging. Currently, CT/MRI and tumor volume measurement are often used to monitor the tumor progression and to evaluate the drug treatment efficacy in preclinical and clinical settings [32]. The disadvantage of these methods is that they can only reveal the tumor differences when there are anatomical changes. To evaluate the therapeutic effect and the tumor growth trend at an earlier stage, we performed optical BLI. The BLI data showed that significant differences between drug treatment effects can be revealed much earlier than tumor volume measurement allows, suggesting that the application of BLI is more sensitive and accurate for investigating living tumor cells and can be used as a promising approach for the evaluation and prediction of drug treatment effects. Because BLI allows 2D imaging only, 3D BLT imaging was then performed in this study, which can provide more detailed information on the tumor location and volume. Our BLI data showed that regorafenib was more effective for anti-HCC treatment than sorafenib was, and a significant difference could be found as early as after 15 days of treatment. The tumor volume measurement data also confirmed a better therapeutic effect of regorafenib than that of sorafenib; however, the earliest time when the difference could be detected was 30 days after treatment. These data confirmed the possibility of early

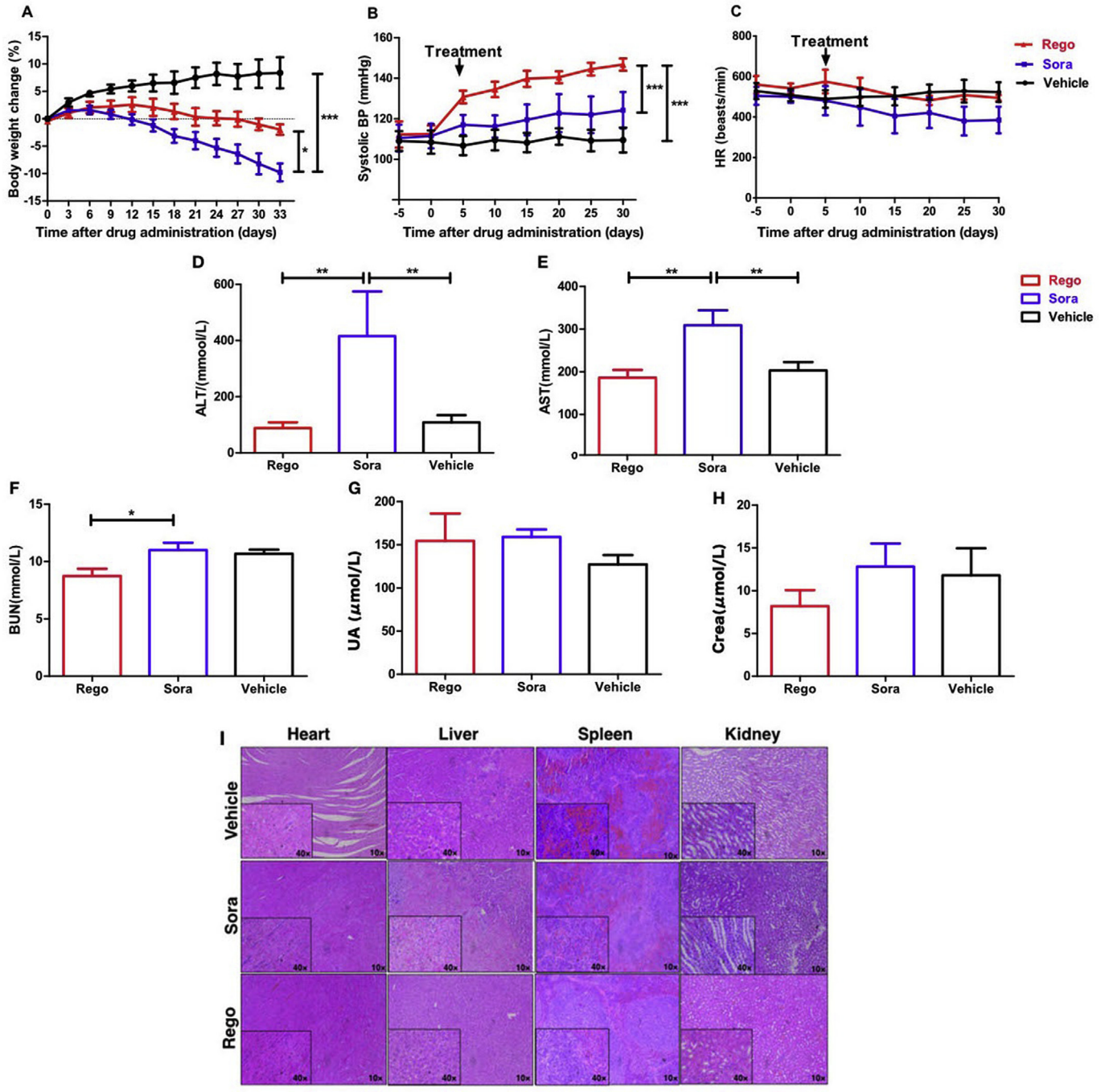

Fig. 8. Assessment of side effects in the drug-treated and vehicle groups. (A) The body weight was measured every 3 days, and the percentage of weight loss during treatment was calculated relative to baseline. (B) Changes in systolic blood pressure (BP). (C) Changes in the heart rate (HR). Each mouse was measured five times at different time points. Liver function was assessed by measuring (D) ALT and (E) AST. Renal function was assessed by measuring (F) blood urea nitrogen (BUN), (G) uric acid (UA), and (H) serum creatinine (Scr). (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (I) Detection of toxicity in major organs. Hematoxylin and eosin (H&E) staining of major organs, such as the heart, liver, spleen, and kidney, was performed after regorafenib (Rego), sorafenib (Sora), and vehicle treatment (magnification: 10 × and 40 ×).

and sensitive evaluation of drug treatment by using molecular imaging. Moreover, we found that compared to sorafenib, regorafenib induces less drug resistance according to the BLI data. The results of previous studies [33–35] are in agreement with our results. Tai et al. [36] have found that regorafenib exhibited significant tumor inhibition *in vivo* in a subcutaneous HCC model; however, the data were only compared with the vehicle group but not with sorafenib treatment.

To examine the underlying antiangiogenic effects of regorafenib and sorafenib, 2D FMI with the IntegriSense 750 probe and 3D CTA imaging were performed in this study. After the administration of the IntegriSense 750 probe to orthotopic HCC tumor-bearing mice, the angiogenesis condition of tumors can be directly visualized using 2D FMI. To avoid the high scattering of fluorescence in biological tissues, CTA imaging was further performed with the angiographic agent Fenestra VC. Small animal micro-CT equipment was used to acquire 2D CT images, and then 3D reconstruction was performed to evaluate angiogenesis in a 3D format. The results demonstrated that both regorafenib and sorafenib could effectively inhibit the angiogenesis in HCC,

but regorafenib exhibited better effects. It has been reported that regorafenib is more effective than sorafenib in terms of inhibition of angiogenesis. It has been verified to inhibit a series of receptor tyrosine kinases, including VEGF1–3, PDGFR-β, FGFR1, TIE2, KIT, RET, etc [37,38].

Moreover, side effects of regorafenib and sorafenib treatment were systematically and quantitatively evaluated, including effects on the body weight, liver and kidney functions, and hypertension, as well as other side effects such as diarrhea, hand–foot syndrome (HFSR), fatigue, etc. The overall conclusions are as follows: 1) regorafenib caused less body weight loss and liver and kidney dysfunction than did sorafenib; 2) regorafenib caused a larger increase in blood pressure than did sorafenib treatment; 3) side effects such as the hand–foot reaction, diarrhea, and fatigue occurred with both treatments, but more severe symptoms were found in mice treated with sorafenib. Our results are consistent with existing literature data. Thus, in the third phase of the RESORCE study on regorafenib, completed in 2017, tumor progression was investigated after previous treatment with sorafenib. The most common clinical grade 3/4 adverse events after treatment with regorafenib included hypertension, HFSR, fatigue, and diarrhea. In this study, the safety of these two drugs was examined during HCC treatment, and no other safety problems were observed. In general, the body weight loss, diarrhea, and other adverse reactions were more severe for sorafenib treatment than for regorafenib treatment. This may be one of the factors explaining why regorafenib treatment resulted in longer survival than did sorafenib treatment.

Our future work direction is to further improve multimodality molecular imaging techniques and reconstruction algorithms, which can provide early and detailed information for clinical drug evaluation at the molecular and cellular levels and provide more information for the patient's drug administration and treatment [39–41]. In addition, we aim at strengthening the link between benchwork and the clinic for future clinical translation and patient care.

Declaration of interests

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.03.037.

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