Application for Approval of Research Protocol

Nanjing Hospital Affiliated to Nanjing University of Chinese Medicine

1. Title of the Proposed Project:

The changes of liver function and hepatic stellate cell (HSC) ferroptosis in advanced fibrotic patients with hepatocellular carcinoma receiving sorafenib monotherapy

2. Principal and Co-Investigators and Their Departments

Investigator(s)	Name	Departments
Principal Investigator(s)	Shizhong Zheng	Nanjing University of Chinese Medicine
Co-Investigator(s)	Zili Zhang	Nanjing University of Chinese Medicine
	Mei Guo	Southeast University
	Min Shen	Nanjing University of Chinese Medicine
	Zhenyi Wang	Nanjing University of Chinese Medicine
	Desong Kong	Nanjing University of Chinese Medicine
	Feng Zhang	Nanjing University of Chinese Medicine
	Jiangjuan Shao	Nanjing University of Chinese Medicine
	Shanzhong Tan	Nanjing Hospital Affiliated to Nanjing University of Chinese Medicine

3. Type of the Proposed Project

This is a retrospective cohort study, which will not interfere with the whole process of the patient's diagnosis and treatment.

4. Description of the Use of Human Biological Samples

The eligible blood samples and liver tissue samples from patients who underwent liver biopsy for accurate diagnosis of liver fibrosis and HCC before sorafenib treatment and patients who received curative hepatectomy for the treatment of HCC after sorafenib monotherapy will be carefully selected from our hospital preserved samples for the subsequent laboratory experiments.

5. Outline of the Proposed Project

The oral multikinase inhibitor sorafenib has been used to treat advanced fibrotic patients with hepatocellular carcinoma (HCC). However, the effect of sorafenib on HSC ferroptosis remains poorly understood. This study will be performed to evaluate the effect of sorafenib on liver function and HSC ferroptosis in advanced fibrotic patients with HCC receiving sorafenib monotherapy. Data on clinical and laboratory findings will be collected retrospectively from all patients by reviewing their electronic medical records. According to the inclusion criteria, the eligible blood samples and liver tissue samples from patients who underwent liver biopsy for accurate diagnosis of liver fibrosis and HCC without any treatment, and patients who received curative hepatectomy for the treatment of liver fibrosis and HCC after sorafenib monotherapy will be carefully selected from our hospital preserved samples for the subsequent laboratory experiments. Blood samples will be collected for the analyses of liver functions. Moreover, primary HSCs will be further isolated from the collected liver tissue samples for detection of ferroptosis markers. The investigation will be the first to reveal the effect of sorafenib on HSC ferroptosis in clinical.

6. Time of the Proposed Project:

The expected time span is from September 2014 to July 2019.

7. Place of the Proposed Project

Data on clinical and laboratory findings will be collected retrospectively from all patients by reviewing their electronic medical records in the Nanjing Hospital Affiliated to Nanjing University of Chinese Medicine. According to the inclusion criteria, we will further pick out the eligible samples from our hospital preserved samples for laboratory experiments. The subsequent laboratory experiments will be carried out at Nanjing University of Chinese Medicine.

8. Study procedures:

Objectives

This study will be performed to evaluate the effect of sorafenib on liver function and HSC ferroptosis in advanced fibrotic patients with HCC receiving sorafenib monotherapy.

Necessity of the study

Sorafenib is a multiple receptor tyrosine kinase inhibitor known to prolong overall survival in advanced fibrotic patients with HCC. However, the effect of sorafenib on HSC ferroptosis remains poorly understood. The current study will be performed to evaluate the effect of sorafenib on liver function and HSC ferroptosis in advanced fibrotic patients with HCC receiving sorafenib monotherapy. The investigation will be the first to reveal the effect of sorafenib on liver fibrosis in clinical.

Methods

We will retrospectively analyze consecutive advanced fibrotic patients with HCC who were treated with sorafenib monotherapy in the Nanjing Hospital

Affiliated to Nanjing University of Chinese Medicine between September 2014 to July 2019. The diagnosis of liver fibrosis and HCC was based on the criteria of the American Association for the Study of Liver Diseases. Data on clinical and laboratory findings will be collected retrospectively from all patients by reviewing their electronic medical records. Patient records will be scanned for the following information: Gender, Age, BMI, Child-Pugh grade, MELD score, ASA class, Serum ALT, AST, ALP, TBIL, GGTP, ALB and GLO levels, Etiology of liver cirrhosis, treatment and outcome data. According to the inclusion criteria, the eligible blood samples and liver tissue samples from patients who underwent liver biopsy for accurate diagnosis of liver fibrosis and HCC without any treatment and patients who received curative hepatectomy for the treatment of liver fibrosis and HCC after sorafenib monotherapy will be carefully selected from our hospital preserved samples for the subsequent laboratory experiments. Blood samples will be collected for the analyses of liver functions including ALT, AST, ALP, TBIL, GGTP, ALB and GLO. Primary HSCs will be isolated from liver tissue samples, and total RNAs will be extracted for western blot and real-time PCR. Liver fibrosis markers including α-SMA, collagen1α1 and fibronectin, and ferroptosis markers including BRD7, P53, and SLC25A28 will be determined by established molecular techniques.

Study population

We will investigate advanced fibrotic patients with HCC undergoing sorafenib monotherapy from September 2014 to July 2019 in Nanjing Hospital Affiliated to Nanjing University of Chinese Medicine. Based on previous studies, minimal clinical significance can be obtained by more than 20 patients.

Inclusion criteria

Patients included in the study should match the following criteria: (1) patients were more than 18 years old; (2) patients suffered from advanced liver fibrosis and HCC; (3) patients were treated with sorafenib monotherapy; (4) patients can read the symptom questionnaire and understand its meaning; (5) patients agreed with the clinical plan and voluntarily signed the IRB-approved

documents.

Exclusion criteria

Patients who meet the following conditions will be excluded from the study: (1) patients did not suffer from advanced liver fibrosis and HCC; (2) advanced fibrotic patients with HCC were treated with other treatment modality but not sorafenib; (3) advanced fibrotic patients with HCC were treated with sorafenib combined with other drug treatment; (4) advanced fibrotic patients with HCC were treated with post-liver transplantation usage of sorafenib; (5) advanced fibrotic patients with HCC were treated with adjuvant usage of sorafenib after curative surgery; (6) advanced fibrotic patients with HCC were lost from follow up; (7) advanced fibrotic patients with HCC did not agree with the clinical plan and did not sign the IRB-approved documents.

Data collection, access and management

The data of this study will be managed according to the standard work instructions of the Nanjing Hospital Affiliated to Nanjing University of Chinese Medicine. Other contents not specified in the protocol shall follow the standards of The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)-Guideline for Good Clinical Practice (GCP). The source document is recorded immediately when the data of case is collected. After the source document input is completed, it will be recorded in the case record form (CRF). All the documents will be kept safe, so that it can be verified by the relevant government agencies and IRB. Only research colleagues and others who have delegated the approval of the principle investigator have access to all data obtained from this study.

9. Confidentiality and Consent

All participants will be required to formally indicate their consent to participating in the research process. They will be given the opportunity to

withdraw from the research at any time prior to the publication of the research findings. The matter of how data will be collected and stored, with reference to the Data Protection legislation will be clarified for participants, with information being stored in locked cabinets or on IT hardware protected with the highest security software. The final thesis and possible significant elements of the project will be published and therefore openly accessible. However, no individual respondents will be identified.

Signature of applicant: Shi Zhong Zheng.

Date of signature: October 18, 2019.