Evaluation of AKR1B10 as a New Marker for interventional therapy of Hepatocellular Carcinoma

Research Plan

Plan Number: AKR-TACE-001

Plan Date: 12/1/2022

Sponsor: Yuemin Nan

Responsible department: Integrated Traditional Chinese and Western Medicine Hepatology Department

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1. Research background

Primary liver cancer is currently the fourth most common malignant tumor and the second leading cause of tumor mortality in China, posing a serious threat to the lives and health of the Chinese people [1]. Primary liver cancer mainly includes three pathological types: hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and mixed type, with HCC accounting for 85% to 90% [2]. According to the Diagnosis and Treatment Guidelines for Primary Liver Cancer (2022 Edition) [3], after comprehensive evaluation of various indicators of patients, surgery is considered the preferred treatment method for radical liver cancer treatment. However, most patients have varying degrees of liver cirrhosis, and some patients cannot tolerate surgical treatment. In this case, non-surgical treatment methods are often used [4], such as radiofrequency ablation (RFA), including microwave ablation (MWA), anhydrous ethanol injection (PEI), etc., Transcatheter arterial chemoembolization (TACE), radiation therapy, and systemic anti-tumor therapy [5] which also known as systemic therapy, mainly refers to anti-tumor therapy, including molecular targeted drug therapy, immunotherapy, chemotherapy, and traditional Chinese medicine treatment; in addition, it also includes treatment for basic liver cancer diseases, such as antiviral therapy, liver and gallbladder protection, and supportive symptomatic treatment.

At present, whether it is surgical treatment or non-surgical treatment, commonly used liver cancer related biomarkers in clinical practice during the evaluation of treatment efficacy or regular follow-up of patients include AFP, AFP-L3%, DCP, etc. [6,7], but there are no reports on whether AKR1B10 can be used for the efficacy evaluation of these treatment methods.

In the early stage, a research team detected the levels of AKR1B10 in serum samples from 477 normal individuals, 107 benign liver tumors, 32 patients with chronic hepatitis B, 78 patients with liver cirrhosis, and 482 patients with liver cancer, and evaluated its early diagnostic value in liver cancer. It was found that AKR1B10 protein, as a serum marker for liver cancer [8], had significantly better performance than AFP, mainly reflected in three aspects: first, it is more sensitive, The normal background level (reference range) of AKR1B10 is significantly lower than AFP, which means that during clinical testing, changes in serum concentration are more pronounced, making it easier to identify asymptomatic early liver cancer patients; Secondly, it is more specific and has a higher detection rate for liver cancer, with lower false positive and false negative rates. In addition, more than 70% of liver cancer patients who test negative for AFP will be tested positive for AKR1B10, resulting in lower rates of missed diagnosis and misdiagnosis; Thirdly, the time to reflect changes in the condition is 6-7 times faster than AFP. The half-life of AKR1B10 is 23 hours, while AFP has a half-life of 6-7 days. Postoperative AKR1B10 testing can help liver cancer patients determine surgical outcomes earlier, providing scientific basis for doctors to develop reasonable postoperative treatment plans. Therefore, this project aims to explore the clinical value of AKR1B10 in evaluating the efficacy of liver cancer treatment.

2. Research purpose

2.1 Main purpose

Evaluate the clinical value of serum AKR1B10 as a marker of the interventional therapy in patients with primary liver cancer (N=200).

2.2 Secondary objectives

Evaluate the clinical value of serum AKR1B10 in combination with AFP and DCP in the interventional therapy in patients with primary liver cancer.

3. Research Methods and Content

3.1 Research design

This study aims to detect the dynamic changes of AKR1B10 in the treatment process of primary liver cancer patients and evaluate the clinical value of this tumor indicator in monitoring the therapeutic efficacy of liver cancer treatment.

3.2 Study population

All participants will sign an informed consent form, agreeing that their samples and related information can be used for this medical studies. The relevant standards are as follows:

3.2.1 Inclusion Criteria

(1) age between 18 and 80

(2) diagnosis of HCC according the AASLD criteria

(3) TACE is planned

(4) resection is impossible

(5) No significant underlying medical illness affecting patient's survival

(6) Patients available for regular follow-up according to the study protocol

3.2.2 Exclusion criteria:

(1) Previous history of other tumors;
(2) Combined with other tumors;
(3) Patients who have received blood transfusions within one month;
(4) The patient was unable to participate in this study for other reasons.

3.2.3 Midway exit criteria

(1) The patient or their guardian has decided to withdraw from the study at any time without any adverse circumstances affecting them and without the need to provide a reason.
(2) If the patient's physician deems it no longer appropriates for them to continue participating in the study, they may withdraw from the study at any time.

3.3 Research contents

This project collects serum samples of primary liver cancer patients (N=200) who have received initial diagnosis and are planning to receive treatment in our hospital. The serum will be

collected in 3 day before treatment and then a serum will be collected 3-5 days again after treatment. Thereafter, serum samples will be collected in the monthly regular follow-ups of the patients. The specific implementation plan is as follows:

(1) Sample preparation and requirements: Approximately 2-5 ml venous blood will be collected and then centrifuged at 4 °C, 4000g for 10 minutes. This study will use the discarded procoagulant serum.

(2) The supernatant serum will be transferred into a 1.5mL EP tube and stored at -80 °C for use.

(3) Serum levels of AKR1B10, AFP, and DCP will be measured. The decline rates, magnitudes, and lowest levels of AKR1B10, AFP, and DCP after treatment will be analyzed. Dynamic curves of serum levels of AKR1B10, AFP, and DCP before and after treatment will be drawn to construct a dynamic change model. The clinical data of patients, such as gender, age, HBsAg, liver function, imaging data, and other clinical information will be dynamically queried and recorded.

(4) Dynamic curves of AKR1B10, AFP, and DCP in serum levels before and after treatment will be drawn.

(5) The relationship between the dynamic changes of AKR1B10, AFP, and DCP alone or in combination with imaging data, histological types, and tumor size and number (tumor burden) will be analyzed.

(6) Pay attention to the phenomenon of temporary elevation of AKR1B10 after treatment as the death of cancer cells and non-specific release of intracellular proteins, including AKR1B10, as well as the potentials unable to decrease to normal levels and an elevation after reduction of these markers. Combination analysis with other clinical phenotypes will be conducted to solve the potential issues.

4. Research related ethics

4.1 Ethics Committee Review

This protocol, written informed consent form, and materials directly related to the subjects must be submitted to the ethics committee for written approval before the study can be officially conducted. Researchers must submit their annual research report to the ethics committee at least annually (if applicable). When the study is terminated and/or completed, the researcher must notify the ethics committee in writing; Researchers must promptly report all changes that have occurred in their research work to the ethics committee (such as revisions to protocols and/or informed consent numbers), and these changes must not be implemented without approval from the ethics committee, unless they are made to eliminate obvious and direct risks to the subjects. When such situations occur, the ethics committee will be notified.

4.2 Informed consent

The procedure for obtaining informed consent: The researcher must provide the subject or their legal representative with an easily understandable and approved informed consent form by the ethics committee, and give the subject or their legal representative sufficient time to consider the study. Before obtaining a signed written informed consent form from the subject, the subject is

not allowed to participate in the study. During the participant period, all updated versions of the informed consent form and written information will be provided to the participants. The informed consent form should be kept as an important document for clinical trials for future reference.

Note: In this project, a dedicated person will be arranged to obtain the patient's informed consent form.

4.3 Confidentiality measures

The results of this project may be published in medical journals, but we will keep patient information confidential in accordance with legal requirements, and only display codes externally. Unless required by relevant laws, patient personal and clinical information will not be disclosed. When necessary, government management departments, hospital ethics committees, and their relevant personnel may access patient information in accordance with regulations.

4.4 Regarding research expenses and related compensation

The testing fees in this project will be paid by our project team, and you do not need to pay any fees. The results of this study can provide a basis for personalized and precise diagnosis and treatment of liver cancer patients, enabling them to benefit the most from this clinical study. In addition, participating in this study will not incur any additional costs related to diagnosis and treatment of your illness.

5. Risks and Benefits

5.1 Risks associated with participating in this study

This study will use the discarded procoagulant serum that has been used for clinical diagnosis purposes and has no impact on the relevant diagnosis and treatment activities of patients.

5.1 Project benefits

The results of this study can evaluate the changes of AKR1B10 in the treatment stage of liver cancer patients, and also help to evaluate the therapeutic effect of liver cancer treatment from a new perspective, providing a theoretical basis for achieving personalized and accurate diagnosis and treatment of liver cancer patients.

6. Annual Implementation Plan

From January to June 2024, serum levels of AKR1B10, AFP, and DCP will be detected in patients with primary liver cancer before and after interventional treatment.

Establish a study queue for primary liver cancer patient follow-up studies.

- January - December 2024: Enrollment and maintenance of the study cohort for patients with primary liver cancer, measurement of serum AKR1B10, AFP, and DCP levels, and combining analysis of AKR1B10, AFP, and DCP data alone or in combination with imaging results and other clinical data.
- January - June 2025: Clinical cohort study will be concluded.

  Continue to follow up with enrolled liver cancer patients, record their diagnosis and treatment history, recurrence, and survival status, and then comprehensively analyze clinical research

data and AKR1B10, AFP, and DCP for evaluation of therapeutic efficacy, making a conclusion.

7. Reference

[1]. Zhou, M., et al., Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet, 2019. 394(10204): p. 1145-1158.

[2]. Bray, F., et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018. 68(6): p. 394-424.

[3]. 原发性肝癌诊疗指南(2022 年版). 临床肝胆病杂志, 2022. 38(02): 第 288-303 页.

[4]. Zhu, X.D., et al., Downstaging and Resection of Initially Unresectable Hepatocellular Carcinoma with Tyrosine Kinase Inhibitor and Anti-PD-1 Antibody Combinations. Liver Cancer, 2021. 10(4): p. 320-329.

[5]. Wang, Z., et al., Adjuvant Transarterial Chemoembolization for HBV-Related Hepatocellular Carcinoma After Resection: A Randomized Controlled Study. Clin Cancer Res, 2018. 24(9): p. 2074-2081.

[6]. Tateishi, R., et al., Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology, 2006. 44(6): p. 1518-27.

[7]. Yoo, J., et al., Evaluation of a serum tumour marker-based recurrence prediction model after radiofrequency ablation for hepatocellular carcinoma. Liver Int, 2020. 40(5): p. 1189-1200.

[8]. Ye, X., et al., A Large-Scale Multicenter Study Validates Aldo-Keto Reductase Family 1 Member B10 as a Prevalent Serum Marker for Detection of Hepatocellular Carcinoma. Hepatology, 2019. 69(6): p. 2489-2501.

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Review and Approval

Plan Number: AKR-TACE-001

Plan Date: 12/1/2022

Sponsor: Yuemin Nan

Responsible department: Integrated Traditional Chinese and Western Medicine Hepatology Department

Review and approval documents from the Ethics Committee of the Third Hospital of Hebei Medical University

<table>
<tr><td>Approval Number</td><td colspan="5">K2019–014–2</td></tr>
<tr><td>Project Name</td><td colspan="5">Evaluation of AKR1B10 as a New Marker for interventional therapy of Hepatocellular Carcinoma</td></tr>
<tr><td>Plan Number</td><td colspan="5">AKR-TACE-001</td></tr>
<tr><td>Sponsor</td><td>Yuemin Nan</td><td>Responsible department</td><td colspan="3">Integrated Traditional Chinese and Western Medicine Hepatology Department</td></tr>
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<tr><td>Review category</td><td colspan="5">☑ Initial review
□ Tracking review
□ Annual/Regular Tracking Review
□ Amendment review
Review of reports on serious adverse events and unexpected events
□ Review of non–compliance/violation of plan events
Suspend or terminate approved research review
Closing review
Complaints from subjects
site visit
Review</td></tr>
<tr><td>Submission documents</td><td colspan="5">Research protocol and informed consent form</td></tr>
<tr><td>Review Committee Member</td><td colspan="5">See the attendance form of the committee members</td></tr>
<tr><td rowspan="3">Review opinions</td><td>Agree</td><td>Agree after making necessary revisions</td><td>Review after making necessary corrections</td><td>Disagree</td><td>Termination or Suspension</td></tr>
<tr><td>☑</td><td>□</td><td>□</td><td>□</td><td>□</td></tr>
<tr><td colspan="5">After review by this ethics committee, it has been agreed to conduct this study in accordance with the agreed clinical research protocol and informed consent form.
Attention:
1. Please follow the GCP principles and follow the protocol agreed upon by the ethics committee to conduct clinical research and protect the health and rights of subjects.
2. Please submit an amendment application for any modifications to</td></tr>
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	the research protocol, informed consent form, recruitment materials, etc. 3. If SAE occurs, please submit a serious adverse event report in a timely manner. 4. If there is any non-compliance/violation of the plan, please submit a violation report in a timely manner. 5. Please submit research progress reports in a timely manner based on the frequency of annual/regular tracking reviews. 6. Please submit a report on the suspension or termination of clinical research in a timely manner. 7. Please submit a final report after completing the clinical study.				
Annual regular/tracking review frequency	3 months □	6 months □	9 months □	12 months ☑	
contacts	Pei Guo		Phone Number	17835683894	
Signature of Chairman: Date: 12/25/2023			Ethics Committee of the Third Hospital of Hebei Medical University (Seal): Date:12/25/2023		

Evaluation of AKR1B10 as a New Marker for interventional therapy of Hepatocellular Carcinoma

Informed Consent

Plan Number: AKR-TACE-001

Plan Date: 12/1/2022

Sponsor: Yuemin Nan

Responsible department: Integrated Traditional Chinese and Western Medicine Hepatology Department

Informed Consent

Dear friend:

You will be invited to participate in the study of the clinical application value of aldosterone reductase 1B10 in liver cancer. Please read the following instructions carefully before deciding whether to participate in this study. If you have any questions, please do not hesitate to ask, and the researchers will answer them for you.

Your participation in this study is voluntary. This study has been reviewed by the ethics review committee of this research institution.

1. Research Background

Research Project Name: Evaluation of AKR1B10 as a New Marker for interventional therapy of Hepatocellular Carcinoma
Research Unit Name: The Third Hospital of Hebei Medical University
Research Sponsor: Hunan Light of Life Biotechnology Co., Ltd
Project Leader and Contact Number: Nan Yuemin 18533112266

At present, there is an auxiliary diagnostic kit for liver cancer with aldo-keto reductase 1B10 (AKR1B10) protein as a tumor marker, namely the aldo-keto reductase 1B10 measurement kit (magnetic particle chemiluminescence). A multicenter clinical study has detected serum samples from 477 healthy people, 107 cases of benign liver tumors, 32 cases of chronic hepatitis B patients, 78 cases of liver cirrhosis patients and 482 cases of liver cancer patients. The results showed that AKR1B10 protein as a serum marker of liver cancer has significantly better performance than AFP, mainly reflected in three aspects: First, it is more sensitive. The normal baseline level (reference range) of AKR1B10 is much lower than that of AFP, which means that in the clinical detection process, the change of serum concentration is more obvious, and it is easier to identify asymptomatic early liver cancer patients. Second, it is more specific, with a higher detection rate of liver cancer, lower false positive and false negative rates, and more than 70% of liver cancer patients with negative AFP detection will be positive for AKR1B10 detection, thus bringing lower missed diagnosis rate and misdiagnosis rate; Third, the time to reflect the change of the disease is 6-7 times faster than that of AFP. The half-life of AKR1B10 is only 23 hours, while the half-life of AFP is 6-7 days. Doctors can know the surgical effect earlier through AKR1B10 detection after surgery, providing a scientific basis for doctors to make a reasonable postoperative treatment plan. In addition, this method has been tested in three clinical trial institutions: Peking Union Medical College Hospital (leading unit), Hunan Cancer Hospital, and Hunan Provincial People's Hospital. The applicability and accuracy of this detection kit in clinical application have been preliminarily completed. In order to further evaluate the value of AKR1B10 as a marker of liver cancer treatment, it is planned to carry out this clinical verification work in The Third Hospital of Hebei Medical University.

2. Research Objectives

With the clue that the serum marker AKR1B10 we found earlier can be used as an effective indicator for detecting HCC, we will conduct an in-depth study on AKR1B10 as a new tumor

marker for TACE efficacy evaluation, so as to provide detailed clinical research data and scientific basis for improving the survival rate of liver cancer patients.

If you agree to participate in this study, we will number each subject and establish a research file. During the study, we need to collect some of your samples. Professionals will take samples from you, using only the blood or pathological tissue wax remaining in the routine treatment or routine diagnostic process, or extracting 4 ml venous blood from your arm. Your samples will only be used for the study, will not affect the results of routine treatment or diagnosis, and will not be used for any commercial activities.

3. Risks and benefits

Your sample collection will be operated in strict accordance with aseptic requirements. There may be some very small risks in blood sample collection, including transient pain, local bruising, mild dizziness in a small number of people, or extremely rare needle infection; or only the blood left in the routine treatment or routine diagnostic process, without additional risks. The detection of your samples will help make a diagnosis of the disease, provide necessary advice for your treatment, or provide useful information for the study of the disease.

4. Privacy issues

If you decide to participate in this study, your participation in the test and personal information in the test will be kept confidential. Your blood sample will be identified by the study number rather than your name. Information that can identify you will not be disclosed to members outside the research group unless you give permission. All research members are required to keep your information confidential. When the results of this study are published, no personal information will be disclosed.

5. Free Withdrawal

You may choose not to participate in this study, or withdraw from the study by notifying the investigator at any time. Your data will not be included in the study results, and your rights and interests will not be affected.

6. Contact Information

You can always get information and progress related to this study. If you have any questions about this study, or about the rights and interests of participants in this study, you can contact Han Fang at the project team at the phone number 13933847150.

I have read this informed consent. I have had the opportunity to ask questions and all questions have been answered. I understand that participation in this study is voluntary. I may choose not to participate in this study, or withdraw after notifying the investigator at any time without discrimination or retaliation, and my rights and interests will not be affected. If I fail to comply with the study plan, or any other reasons related to the study occur, the study leader may terminate my participation in the study.

I will receive a copy of the signed informed consent.

Name of Subject:
Signature of Subject:
(If a proxy is involved, the proxy's signature is required)
Date:

I have accurately informed the subject of this document, he/she has accurately read this informed consent, and I certify that the subject has had the opportunity to ask questions. I certify that he/she has given voluntary consent.

Name of the study leader:
Signature of the study leader:
Date:

(Note: If the subject is illiterate, a witness is required to sign; if the subject is incapacitated, an agent is required to sign)