TMF-Informed Consent Form and Statistical Analysis Plan

Project Title	**An Experimental Study on the Effect of Tenofovir Amibufenamide on Blood Lipid During Anti-HBV Treatment**
Scheme number	**TMF3.0**
Version number	**V3.0**
Version date	**2021.10.15**
Principal Investigator	**Jin Ye**
Department	**Division of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology**

Dear Madam/Sir:

You will be invited to participate in "an experimental study on the effect of tenofovir on blood lipid levels during anti-HBV treatment", the research is initiated by division of gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The following items describe the research background, purpose, methods, benefits and possible risks or inconveniences as well as your rights and interests during the research process of this clinical study. Please read it carefully before participating in the clinical study. The information provided to you in this informed consent form can help you decide whether to participate in this clinical study. If you have any questions, please ask the investigator in charge of the study to ensure that you fully understand the relevant content. Your participation in this study is voluntary. If you agree to participate in this clinical study, please sign the statement of informed consent.

This study has been approved by the Ethics Committee of the Medical Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.

1. Research Background

In China, it is estimated about 70 million people are infected with chronic HBV, of which about 20-30 million are CHB patients. Moreover, the percentage of liver cirrhosis and hepatocellular carcinoma caused by HBV infection in our country was 77% and 84%, respectively. Chronic HBV infection bring severe burden to people's life and health as well as the national health care system. It is possible to reduce the incidence of cirrhosis and liver cancer by inhibiting hepatitis B virus replication, reducing viral load and improving liver function and other measures. Currently, domestic and foreign drugs for the treatment of chronic hepatitis B (CHB) are mainly divided into two categories: interferons and nucleosides (acids). According to the recommendations of the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 Edition)", the first choice for treatment is strong, low-resistance nucleoside (acid) analogs and peginterferon.

After the emergence of nucleoside (acid) drugs, the treatment of CHB had a revolutionary change. The main drugs are lamivudine, adefovir, entecavir, tenofovir, etc. However, lamivudine and adefovir has gradually relegated to the second-line due to their efficacy and safety. And now the first-line recommended drugs are entecavir and tenofovir. Entecavir has stricter medication requirements and should be taken between meals, which is considered inconvenient by some patients. In addition, for patients who have been treated with other nucleoside (acid) analogs, and then switch to entecavir, drug resistance is prone to occur. Moreover, some patients are still unable to achieve a complete virological response after switching the entecavir for about a year. As a first-line antiviral drug, tenofovir works well, whose side effects are mainly renal function damage and bone metabolism disorder.

In June 2021, the China National Medical Products Administration approved the launch of a self-developed anti-HBV drug, tenofovir amibufenamide (TMF). TMF is the phosphoramidite precursor of tenofovir, a nucleoside reverse transcriptase inhibitor which has higher cell membrane penetration rate, to achieve liver targeted therapy. At the same time, it can effectively improve the stability of drug concentration in plasma and reduce systemic TFV exposure which make long-term treatment safer. From the existing data, the antiviral efficacy of TMF and tenofovir are basically similar to tenofovir. In addition, It is suitable for a wide range of CHB patients, especially those people with older age, at risk for bone and kidney impairment. Previous studies have shown that tenofovir disoproxil (TDF), the first-generation

drug of tenofovir, has the effect of lowering blood lipids and patients who switch to the second-generation drug tenofovir alafenamide (TAF) have blood lipids elevation. However, as a new generation of tenofovir, the effect of TMF on blood lipids is still unclear.

2. Research purpose

This study intends to collect patients with chronic hepatitis B who are clinically treated with TMF antiviral therapy to determine whether TMF in the antiviral process will cause dyslipidemia in hepatitis B patients with normal blood lipids; ; Whether combined application of lipid-lowering drugs can reduce the degree of dyslipidemia while maintaining its antiviral efficacy.

3. Who can participate in the study

Diagnostic criteria: patients with CHB who meet the guidelines for the prevention and treatment of chronic hepatitis B (2019 edition)

HBsAg positive, HBV DNA detectable

Inclusion criteria:

1. age 18-70 years old;
2. meets the CHB diagnostic criteria of "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 Edition)"
3. HBV-DNA can be detected (>20IU/mL);
4. with or without liver cirrhosis caused by CHB;
5. The treatment plan is TMF antiviral treatment, and no other antiviral drugs have been used for 1 year before TMF;
6. The clinical data are relatively complete, and the follow-up time reaches 24 weeks (6 months).

Exclusion criteria:

1. patients with primary liver cancer or liver metastases;
2. patients with hepatitis A virus, hepatitis C virus, hepatitis D virus, hepatitis E virus and human immunodeficiency virus infection;
3. combined with alcoholic liver disease, drug-induced liver disease, autoimmune liver disease and other causes of liver disease;
4. history of dysglycemia and dyslipidemia treatment;
5. lactose intolerance patients;
6. pregnant and breastfeeding women;
7. patients with severe systemic disease.

4. Research Introduction

A prospective cohort, about 150 patients, who meet the diagnostic and inclusion criteria and treated with TMF will be included. The patients will be divided into normal blood lipid groups (group A, about 50 patients) and dyslipidemia group according to their baseline blood lipids. The dyslipidemia group will be divided into subgroup B1(about 50 patients) without lipid-lowering drugs intervention and subgroup B2 (about 50 patients) with lipid-lowering drugs treatment. Investigators will follow up those patients for HBV-DNA, blood lipids, blood routine, liver and kidney function, hepatitis B markers, ultrasound of liver or abdominal CT, fibrotouch test and other indicators at 1, 3, 6, and 12 months respectively.

SPSS software was used for statistical analysis, and quantitative data were analyzed by mean ± standard

deviation. Three groups of quantitative and enumeration data were involved, the statistical analysis method used chi-square test, receiver operating curve and logistic regression analysis, etc. to explore the effect of TMF on blood lipids during the antiviral process.

In this study, follow-up will be conducted on the 1st, 3rd, 6th, 12th and every six months after enrollment. During the follow-up process, the clinical routine examination data of the patients will be collected, including blood routine, liver and kidney function, blood sugar and blood lipids, blood calcium, blood phosphorus, hepatitis B virus biomarkers, HBV DNA, liver transient elastography, hepatobiliary ultrasound or CT and other indicators (the above inspections are routine monitoring items in antiviral treatment for patients with CHB), patients can get equal access by use medical card. In order to better analyze the results, an additional tube of blood is required for genetic testing when the study is enrolled, and the results of this test will be provided to patients free of charge.

5. Obligations of Subjects

1. sign the informed consent form;
2. take medicines on time every day according to the needs of diagnosis and treatment;
3. if there are adverse reactions related to taking TMF during the research, please contact investigators in time;
4. at the time of successful enrollment and the 1st, 3rd, 6th, and 12th months after enrollment, patients should follow-up on time, cooperate with researchers to complete blood and urine routine, liver and kidney function， AFP, coagulation function, glycosylated hemoglobin, hepatitis B virus biomarkers, HBV- DNA, Fibrotouch, hepatobiliary ultrasound/abdominal CT and other related tests.

6. Detection, preservation and destruction of biological samples

This study intends to collect whole blood samples for genotyping, which will be stored in the Department of Gastroenterology, Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, until the end of the study. Once investigators finish the study, those samples will be packed in special containers or bags according to the Regulations on Medical Waste Management , and then be sent to the designated disinfection site by a special staff, and finally be incinerated by a special organization.

7. Possible research risks of participating in this research

1. Risks associated with this drug:

a) ALT, AST increased, parathyroid hormone increased, hypophosphatemia;

b) Dyslipidemia: hypertriglyceridemia, hyperlipidemia, elevated low-density lipoprotein;

c) Lactic acidosis or significant hepatotoxicity (may include hepatomegaly and steatosis).

2. Risks related to the research procedure:

Blood collection, Fibrotouch, ultrasound of liver or CT will be used in this study, which are mature technologies in our hospital. If you experience any special discomfort during the examination, you can apply for suspension of relevant examinations.

3. There are very limited data on pregnancy of TMF (only 2 cases of pregnancy and childbirth), and no neonatal malformations related to TMF have occurred. The possible toxicity of TMF to the fetus is currently unknown. Only when the expected benefit outweighs the potential risk to the fetus, TMF can be used during pregnancy. It must be informed clearly of the possible harm to the fetus when TMF is

used during pregnancy. Women who are at childbearing age are advised to take effective contraceptive measures while taking TMF.
4. Unknown risks: When any other risks occur during the research process, subjects can apply to withdraw from the research at any time and receive corresponding treatment.
5. Risk control measures: This study does not collect pregnant and lactating patients, and patients with other serious systemic diseases. It is recommended to regularly monitor liver and kidney functions, blood lipids and other indicators during medication.

8. Possible benefits of participating in this clinical study

1. Clinical benefits: For the vast majority of patients, the replication of hepatitis B virus can be inhibited effectively by taking TMF, so that the level of hepatitis B virus in CHB patients can be reduced to control the outbreak of hepatitis, to reduce infectivity, and to effectively prevent the occurrence of hepatitis B cirrhosis and hepatitis B liver cancer;
2. The collection of patients' information will be helpful for evaluating the treatment effect of the disease and provide necessary suggestions for your treatment;
3. Gain knowledge related to your own health;
4. Provide more useful clinical data for the use of TMF.

9. If I do not participate in this study, is there any other alternative treatment for me?

If you do not participate in this study, you can choose other drugs such as entecavir, tenofovir disoproxil (TDF), tenofovir fumarate (TAF), etc. as antiviral therapy, which should be carried out under the guidance of experienced physicians.

10. How to deal with research-related injuries?

When subjects experience any drug-related damage during the use of TMF, they will be assessed whether to change the drug or stop the drug according to the clinical situation.

11. How much do I need to pay?

The drugs used in this project are marketed drugs for conventional anti-HBV treatment. The investigators collect the clinical examination data of patients during routine follow-up for analysis. Therefore, the subjects need to pay for the drugs, tests and examinations related to the treatment, which is not increased in this study. There is no additional fees for patients participating in this study.

12. How will new clinical research information be handled?

When there is new information that may affect the subject's continued participation in the trial, the procedures for informing the subjects or their guardians in a clear and timely manner.

13. Under what circumstances may the clinical study be terminated?

1. emerge a large number of cases with serious adverse reaction during the research;
2. the case enrollment is not ideal;
3.excessive loss of follow-up data.

14. How long is participation in this study likely to last?

one year

15. How many people will be involved in this study?

About 150 patients will be collected in this study.

16. Privacy and Confidentiality

If you decide to participate in this study, your participation in the trial and your personal data in the trial will be kept confidential. For you, all information will be kept confidential. Information that could identify you will not be disclosed to members outside the research team unless you have given your permission. All study members and study sponsors are required to keep your identity confidential. Your information is only stored in the Department of Gastroenterology of Union Hospital and is only accessible to researchers. To ensure that research is conducted in accordance with regulations, when necessary, and without violating the principle of confidentiality and relevant regulations, supervisors, auditors, ethics committees and inspectors of drug regulatory authorities can access your original medical records to verify clinical trials, process and data. When research results are published, your personal data will also be kept confidential.

17. The right to voluntarily choose to participate in and withdraw from research

You may choose not to participate in this study, or withdraw from the study at any time after notifying the investigator without discrimination or retaliation, and any of your medical treatment and rights will not be affected. The investigator may terminate your continued participation in this study if you require additional diagnosis/treatment, or if you do not comply with the study plan, or for any other reasonable reason.

18. How to get help in research?

When there are questions about trial information, research progress and rights of subjects, as well as any discomfort and damage related to the trial, you can contact researcher or contact the ethics committee of our center.

Subject Statement

I have read this informed consent form carefully, I have opportunity to ask questions and all questions have been answered. I understand that participation in this study is voluntary, and I can choose not to participate in this study, or withdraw at any time after notifying the investigator without discrimination or retaliation, and my medical treatment and rights will not be affected by this.

The investigator may terminate my continued participation in this clinical study if I require additional diagnosis/treatment, or if I do not comply with the study plan, or for other reasonable reasons.

I voluntarily agree to participate in this clinical study and I will receive a signed copy of the Informed Consent Form.

Subject's Name (Print):	Contact Number:
Subject Signature:	Date:

If the subject cannot sign informed consent due to incapacity or other reasons, or if the subject is a minor, the guardian shall sign it.

Guardian Name (Print):	Contact Number:
Guardian's Signature:	Date:
Relationship with the subject:	
Reasons why subjects cannot sign informed consent:	

Signed by an impartial witness when the subject or his guardian is incapable of reading.

Name of impartial witness (print):	Contact number:
Signature of an impartial witness:	Date:

Investigator Statement

I have accurately informed the subject of the informed consent form and answered the subject's questions, and the subject voluntarily participated in this clinical study.

Investigator's name (print):	Contact number:
Investigator Signature:	Date: