


BMJ Open Tenofovir alafenamide fumarate therapy in subjects with positive HBV-DNA and normal levels of alanine transaminase: a study protocol for a randomised controlled trial

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To cite: Wang L, Wu L, Li X, *et al.* Tenofovir alafenamide fumarate therapy in subjects with positive HBV-DNA and normal levels of alanine transaminase: a study protocol for a randomised controlled trial. *BMJ Open* 2021;**11**:e048410. doi:10.1136/bmjopen-2020-048410

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2020-048410>).

LW, LW and XL are joint first authors.

Received 26 December 2020
Accepted 06 August 2021



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ABSTRACT

Introduction The current clinical guidelines do not recommend antiviral therapy for subjects with positive hepatitis B virus (HBV)-DNA and normal alanine transaminase (ALT). In this study, we will assess the safety and efficacy of tenofovir alafenamide fumarate (TAF) in the treatment of adults with positive HBV-DNA and normal ALT, including long-term prognosis.

Methods and analysis This study is a non-double-blind randomised controlled trial. Study participants will be randomised into the treatment group and the control group. In the treatment group, subjects will receive TAF monotherapy, while those in the control group will receive no antiviral treatment. Subjects will be followed up at the beginning of the study and every 12 or 24 weeks thereafter for review of laboratory findings and to record adverse events. The primary endpoint is the proportion of patients with serum hepatitis B surface antigen loss.

Ethics and dissemination This study protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University for Human Study (reference number [2019]02-599-01). The results of this study will be submitted for publication in a peer-reviewed journal.

Trial registration number NCT04231565.

INTRODUCTION

Hepatitis B virus (HBV) infection is still a serious public health problem that endangers human health. There are approximately 240 million chronic HBV carriers worldwide.¹ In 2016, the WHO proposed a strategy to ‘eliminate the threat of viral hepatitis by 2030’, but China is still far from achieving the goal at this stage.² There are approximately 70 million HBV-infected subjects in China, but only approximately 10% of patients receive antiviral treatment.³

Adults with positive HBV-DNA and normal alanine transaminase (ALT) refers to subjects who are hepatitis B surface antigen (HBsAg),

Strengths and limitations of this study

- This study is the first to investigate tenofovir alafenamide fumarate antiviral therapy for adults with positive hepatitis B virus-DNA and normal alanine transaminase in China.
- Both patients with positive hepatitis B e antigen (HBeAg) and negative HBeAg are to be included.
- This study will illustrate the safety and efficacy of antiviral treatment, including long-term prognosis.
- Hepatitis B surface antigen clearance is associated with good prognosis in patients with chronic hepatitis B.
- Because of the invasiveness, the study can not implement liver biopsy, which will be a likely limitation.

HBV-DNA positive for more than 6 months and ALT normal (continuous follow-up of more than three times within 1 year with at least a 3-month interval each time), including positive hepatitis B e antigen (HBeAg) and negative HBeAg patients. At present, clinical guidelines do not recommend antiviral therapy for this population.^{4 5} However, previous studies have found that viral replication is closely related to an increased risk of cirrhosis and hepatocellular carcinoma (HCC). With the increase in viral load, the risk of cirrhosis and HCC also increases even if the viral load is low.^{6 7} The Risk Evaluation of Viral Load Elevation and Associated Liver Disease (REVEAL) cohort study⁸ followed up HBV-DNA negative and HBV-DNA <2000 IU/mL HBV-infected subjects for 13 years and found that the cumulative incidence of cirrhosis and HCC in the HBV-DNA <2000 IU/mL group is significantly higher than that in the HBV-DNA negative group with statistically significant differences. The studies also confirmed that the ALT level is not a

reliable indicator of liver disease activity. In a group of 73 chronic hepatitis B patients with positive HBeAg and continuous normal ALT, liver biopsy found that 40% of the patients show significant liver fibrosis.⁹ A meta-analysis suggested that 19.7% of CHB patients with negative HBeAg and normal ALT show significant liver fibrosis by liver biopsy.¹⁰ In recent years, international research centres have performed studies on antiviral treatment for HBV-infected patients with normal ALT. A 192-week double-blind randomised controlled study conducted by the Chinese University of Hong Kong showed that in HBeAg-positive patients with chronic HBV infection and normal levels of ALT, therapy with the combination of Tenofovir disoproxil fumarate (TDF) and emtricitabine provides better viral suppression than TDF alone, but the rates of HBeAg seroconversion and HBsAg loss are low.¹¹ Feld *et al* showed that a lead-in strategy of 8 weeks of entecavir followed by combination peg-interferon (peg-IFN) and entecavir therapy for 40 weeks has limited efficacy in adults with positive HBeAg and continuous normal ALT of chronic HBV infection, indicating that this strategy cannot be recommended.¹² As for HBV-infected patients with negative HBeAg and normal ALT, Cao *et al* found that high rates of HBsAg clearance and seroconversion could be achieved by peg-IFN-2a based treatments and the treatments were relatively safe.¹³ While a 72-week double-blind randomised controlled study showed that in CHB patients with a low viral load and normal ALT, combination treatment (peg-IFN plus adefovir and peg-IFN plus tenofovir) did not result in significant HBsAg loss compared with no treatment, which does not support the use of combination treatment in this population of patients.¹⁴ Therefore, there are still controversies about the safety and efficacy of antiviral treatment in adults with positive HBV-DNA and normal ALT, which need to be further confirmed by multi-centre, large sample and long-term cohort studies.

HBsAg clearance has become the consensus for CHB treatment,^{15 16} which is associated with good prognosis and low risk of HCC in CHB patients.^{17 18} A systematic review including a total of 28 studies involving more than 105 411 CHB patients found that incidence of HCC was significantly lower among patients who experienced HBsAg seroclearance than among those who remained HBsAg-positive (1.86% vs 6.56%, $p < 0.001$). Thus, periodic surveillance is recommended, especially for male patients with cirrhosis and patients who experience HBsAg seroclearance when at least 50 years old.¹⁹

As the latest first-line nucleoside (acid) analogues, tenofovir alafenamide fumarate (TAF) has been widely recognised in terms of antiviral efficacy and the safety of bone and kidney function.²⁰ Currently, there are no studies on TAF in treating patients with positive HBV-DNA and normal levels of ALT in China and abroad.

METHODS AND ANALYSIS

Patient eligibility criteria

Patients will be divided into HBeAg-positive group and HBeAg-negative group according to the HBeAg status. All

the subjects have positive HBV-DNA with a detection limit of 20 IU/mL. But there are some different requirements on HBV-DNA level in two groups. The exclusion criteria are consistent.

Inclusion criteria

1. HBsAg positive >6 months.
2. HBeAg positive patients: HBV-DNA >20 IU/mL more than 6 months.
3. HBeAg negative patients: $20 < \text{HBV-DNA} < 2000$ IU/mL more than 6 months.
4. $\text{ALT} \leq 1 \times \text{ULN}$ (continuous follow-up of more than 3 times within 1 year with at least a 3-month interval each time).
5. Age between 18 and 65 years old.
6. No antiviral treatment with interferon or nucleoside (acid) analogues in the previous year.
7. FibroScan <5.8 kPa.

Exclusion criteria

1. Patients using immunosuppressive agents, such as chemotherapy and glucocorticoids.
2. Pregnant or lactating women.
3. Infection with A, C, D or E viral hepatitis.
4. Patients with liver fibrosis, cirrhosis or HCC.
5. Other causes of liver disease, including autoimmune liver disease, poisoning, drug-induced liver injury, alcoholic liver disease or genetic metabolic liver disease.
6. HIV infection or other immunodeficiency diseases.
7. Combined with diabetes, renal insufficiency, autoimmune diseases, thyroid diseases and other organ dysfunctions.
8. Cirrhosis or HCC family history.
9. Patients who are unable to comply with the arrangement of this study or sign the informed consent.
10. Failed to return to hospital regularly for follow-up according to the study plan.
11. Researchers determine other condition that does not fit into the study.

Study protocol

The study design is a non-double-blind randomised controlled trial.

HBeAg-positive and HBeAg-negative participants who meet the eligibility criteria will be randomised respectively into two groups as follows: (i) the TAF group and (ii) the control group (regular observation group). When subjects in the control group have an ALT level $> 1 \times \text{ULN}$ (continuous follow-up of more than 3 times within 1 year with at least a 3-month interval each time), they will receive TAF antiviral therapy (figure 1).

Recruitment

The subjects will be recruited through the Outpatient Department of Infectious Diseases of the Third Affiliated Hospital of Sun Yat-Sen University. Recruitment is expected to begin in December 2020 and end in April 2022.

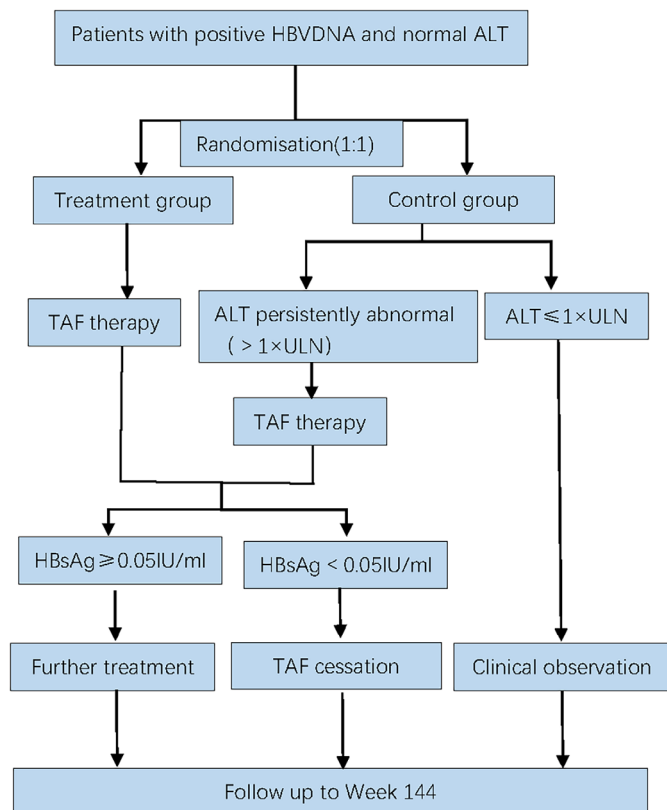


Figure 1 Study protocol. ALT, alanine transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; TAF, tenofovir alafenamide fumarate.

Randomisation and allocation concealment

The randomisation adopts a simple randomisation method, and random numbers will be generated by computer software (SPSS Statistics V.22).

The method of allocation concealment is envelope concealment. Random numbers will be put into uniform envelopes. Then, subjects will be given uniform envelopes according to the order of inclusion and will be assigned random numbers in the envelopes.

After participant eligibility has been confirmed and informed consent has been received, the participant will be randomised into the trial. A randomisation form will be provided to investigators and will be used to collate the necessary information prior to randomisation. Only when all eligibility criteria and baseline data items have been provided, a trial number will be allocated. Participants will be randomised at the level of the individual in a 1:1 ratio to either the treatment group or the control group. Both of these treatments will start on the same day as randomisation or as soon as possible.

Trial intervention

Tenofovir alafenamide fumarate

In the treatment group, participants will be prescribed 25 mg of oral TAF once a day, and they will be followed up at the beginning of the study and every 12 or 24 weeks thereafter for review of laboratory examinations and to record adverse reactions, such as weakness,

gastrointestinal side effects (including nausea), headache, rash and shortness of breath. Participants will be asked about adherence with their trial medication and adverse events, such as cirrhosis, HCC, liver transplantation and death at each follow-up visit. Their responses will be documented in the medical notes and subsequently transcribed onto the follow-up case report forms.

Evaluation

Laboratory examination will include the following: hepatitis B surface antigen quantification, HBeAg, hepatitis B e antibody (anti-HBe), HBV-DNA, HBV pregenomic RNA (HBV-pgRNA), total bilirubin, albumin, aspartate aminotransferase, ALT, cholinesterase, white cell count, platelet count, fasting blood glucose, creatinine, blood urea nitrogen, estimate glomerular filtration rate, prothrombin time, international normalised ratio, fibrinogen and alpha-fetoprotein.

Imaging tests will include the following: abdominal ultrasonography and liver hardness test (FibroScan, two-dimensional shear wave elastography (2D-SWE)).

Serum HBV-pgRNA examination

HBV-pgRNA in CHB patients is an ideal blood marker to reflect the activity of covalently closed circular DNA in liver tissue.²¹ The detection of HBV-pgRNA reflects the level of viral transcription replication, the response to antiviral therapy and also plays an important role in predicting the risk of drug withdrawal.^{22 23} In addition, Zhang WH *et al*²⁴ found that serum HBV-pgRNA levels are significantly correlated with liver inflammation and fibrosis degree. In this study, an HBV-pgRNA testing kit will be used to detect HBV-pgRNA content in serum samples at baseline and every 24 weeks thereafter. The detection method of serum HBV-pgRNA is real-time fluorescence quantitative PCR using the Hongshi SLAN-96 instrument.

Two-dimensional shear wave elastography

2D-SWE uses an ultrasonic probe to focus acoustic radiation force at multiple points in the deep tissue synchronously. It is able to obtain the tissue elasticity value in a large area at the same time and realise real-time quantitative measurement of tissue hardness.²⁴ The diagnostic value of 2D-SWE for grading liver fibrosis has been widely recognised. Large sample and multicentre studies have confirmed that 2D-SWE is more effective than transient elastography and has the advantages of high repeatability and a low failure rate.²⁵ 2D-SWE is used to monitor changes in liver hardness at baseline and every 24 weeks thereafter.

Study endpoints

The primary endpoint will be the proportion of patients with serum HBsAg loss at 144 weeks. HBsAg quantification will be tested by Roche COBAS HBsAg II-Q with a lower limit of detection of 0.05 IU/mL.

The secondary endpoints will include the following: the proportion of patients with HBV-DNA loss (quantified

Table 1 Schedule of assessments

Visit (weeks)	Baseline	12	24	36	48	72	96	120	144
Confirm eligibility	√								
Seek informed consent	√								
Randomisation	√								
Medical history*	√								
Medication review	√	√	√	√	√	√	√	√	√
Physical examination	√†	√	√†	√	√†	√†	√†	√†	√†
Laboratory examination	√‡	√	√‡	√	√‡	√‡	√‡	√‡	√‡
Adverse events evaluation	√	√	√	√	√	√	√	√	√
Adherence		√	√	√	√	√	√	√	√

*Including medical history (diabetes, ischaemic heart disease or pulmonary disease).

†Liver stiffness measurement using FibroScan or two-dimensional shear wave elastography.

‡Serum hepatitis B virus pregenomic RNA measurement using the fluorescent probe method.

by fluorescence quantitative PCR with a detection limit of 20 IU/mL); the rate of patients who develop serum HBeAg seroconversion (loss of HBeAg and presence of anti-HBe); adverse events; and long-term prognosis, including cirrhosis, HCC, liver transplantation and death.

Data collection

A study assistant will gather the following data elements from patient medical records:

Baseline data

1. Age and sex.
2. Baseline body mass index.
3. Years positive for HBV.
4. HBV genotype.
5. Drinking history and smoking history.
6. Previous treatments and medicine.
7. Comorbid conditions.
8. Baseline laboratory data.

Schedule of assessments is detailed in [table 1](#).

Statistical methods

Sample size estimation

There are some difficulties in calculating a proper sample size in this study which is initiated by the researcher. First, we found no similar studies on TAF in treating HBV infection patients. Compared with TAF monotherapy, previous studies mostly focus on combination treatments (peg-IFN plus nucleoside analogues) in HBV infection subjects with normal ALT. Second, the rate of HBsAg clearance is the primary outcome in the current study which is the key indicator for sample size calculation. Since the proportion of HBsAg loss is low, it is rarely used as the primary outcome in previous findings. Taken together, it is difficult to have a highly accurate sample size estimation. We referred to a study¹³ about inactive HBsAg carriers treated with peg-IFN combined with TDF, assuming a serum HBsAg loss rate of 30% for the treatment group and 1% for the control group. The sample size of 42 patients provided 80% power (two-sided significance level, 0.05) to show the statistical differences between the treatment

group and the control group. Assuming a 10% drop-out rate, 50 patients or more were needed in each group.

Analysis of outcome measures

In this study, a separate statistical analysis plan will be developed to analyse the data from the treatment group and the control group. All analyses will be based on the principle of intention-to-treat, that is, all participants will be analysed even though some subjects will fail to follow the original plan during the trial. This analysis method will better evaluate the intervention effect, reduce bias and be closer to the actual clinical situation.

Continuous variables will be expressed as the mean±SD, and categorical variables will be expressed as count and percentage. Continuous variables (including age, ALT level and HBV-DNA) will be compared between the two groups using Student's t-test or rank-sum test. Categorical variables (including the rate of serum HBsAg loss, the rate of serum HBeAg seroconversion, sex ratio and adverse event incidence) will be compared using Pearson χ^2 test or Fisher's exact test as applicable. The associations between variables as potential predictors of HBsAg loss or HBsAg decline were examined by multivariable logistic regression analysis. Receiver operator characteristic curves, which plot sensitivity by 1—specificity, and the area under the receiver operator characteristic curve will be used to evaluate the prognostic values of the quantitative HBsAg level and change at week 144 in predicting HBsAg clearance. The cut-off point, which will optimise the sensitivity and specificity, will be selected for the subsequent prediction. A two-sided p value <0.05 will be considered statistically significant. Analyses will be performed using the Statistical Package for the Social Sciences (SPSS, V.22) and STATA (V.14.1).

Missing data and sensitivity analyses

The research assistants are specifically responsible for follow-up to ensure that relatively complete data are obtained. Subjects with missing primary outcome data will not be included in the study from the beginning. To avoid

bias, sensitivity analyses will be used to assess the impact of missing values on outcomes, which include worst case assumptions and/or multiple imputation methods. If the results of the sensitivity analyses are constant and approximate efficacy evaluations can be obtained, the missing data can be guaranteed to have almost no effect on the overall conclusion of the study, confirming the robustness of the conclusion. Conversely, if the results of the sensitivity analysis are inconsistent, then the impact on the test results must be discussed.²⁶

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Strategies to maximise retention of patients

For a clinical study, it is crucial to improve patient adherence to the treatment, especially for a long-term study following up 144 weeks. We mainly take the following measures to maximise retention of patients. As shown below:

1. Strengthen patient education. Recruitment posters will be put up in the outpatient department, and science articles will be published on WeChat platform, so as to popularise the knowledge of HBV infection. Therefore, the subjects all have a strong will for diagnosis and treatment of the disease before enrolling this study. The subjective initiative of patients is the basis of ensuring adherence to the treatment.
2. Improve harmonious communication. The researchers will conduct a detailed interview with the subjects and fully explain the purpose and significance, as well as the benefits and potential risks of enrolling the study. At the same time, patients will be informed the method of taking antiviral drugs and follow-up in great detail. A harmonious communication environment is helpful to construct a good doctor–patient relationship, which is the important guarantee to improve patient compliance.
3. Establish professional follow-up. In this study, special CRC are responsible for long-term follow-up, including telephone follow-up and outpatient follow-up. Clinical data of patients will be uniformly preserved by the CRC. In addition, patients also maintain close contact with researchers. A professional long-term follow-up is the key link to enhance patient compliance.

Ethics and dissemination

Research ethics approval

The study protocol, informed consent form and other submitted documents were reviewed and approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University for Human Study (reference number [2019]02-599-01). No patient is registered at the submission of our manuscript.

Confidentiality

All data recorded on paper forms will be securely stored in the Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China, in accordance with data protection procedures. The collected data table will only be identified by the subject's ID. The informed consent form, name, contact information, ID and other documents of the subject will be saved separately from other data. All data will be stored in a file cabinet in the Infectious Diseases Follow-up office, and access rights will be set to ensure the safety and confidentiality of the study data.

Dissemination policy

The final data will be publicly disseminated. The results will be presented at relevant meetings and published in appropriate journals after the trial and analysis.

Contributors All authors contributed to the concept, design, patient recruitment and follow-up. LP and CX had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. LWang, LWu and XL contributed to acquisition, analysis and interpretation of data. LWang drafted the report. LP and CX obtained funding and took responsibility for the supervision. All authors read and approved the final report.

Funding This study was supported by grants from the National Major Science and Technology Project for the prevention and treatment of AIDS and viral hepatitis (2018ZX10302204-002, 2018ZX10302205-002), Natural Science Foundation of China (No. 81873572 and 82070611), Guangzhou Science and Technology Plan Projects (201904010442), Sun Yat-Sen University Clinical Research 5010 Program (2018009), Research Project on degree and postgraduate education reform in Guangdong province (2018JGXM04), Young Teacher Training Programme of Sun Yat-Sen university (16ykp40), the Five-Year Plan of Third Affiliated Hospital of Sun Yat-Sen University (K00006).

Competing interests None declared.

Patient consent for publication Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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