

BMJ Open Prospective, multicentre, randomised controlled trial comparing the seroclearance of HBsAg between combination therapy of peg-interferon alpha and tenofovir with tenofovir monotherapy in nucleos(t)ide analogue-experienced patients with HBV-related liver fibrosis: a study protocol

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To cite: Zhu S, Wu L, Mei Y, *et al.* Prospective, multicentre, randomised controlled trial comparing the seroclearance of HBsAg between combination therapy of peg-interferon alpha and tenofovir with tenofovir monotherapy in nucleos(t)ide analogue-experienced patients with HBV-related liver fibrosis: a study protocol. *BMJ Open* 2021;**11**:e049104. doi:10.1136/bmjopen-2021-049104

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-049104>).

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Received 16 January 2021
Accepted 27 September 2021



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ABSTRACT

Introduction Combination antiviral therapy of nucleos(t)ide analogue (NA) and pegylated interferon alpha (peg-IFN alpha) decrease hepatitis B virus (HBV) surface antigen (HBsAg) levels to achieve functional cure and improve long-term prognosis in chronic hepatitis B patients. However, for hepatitis B-related liver fibrosis, studies on combination of these two medicines are limited. This study was designed to compare the efficacy between peg-IFN alpha combined with tenofovir (TDF) and TDF monotherapy for the clearance of HBsAg in NA-experienced patients with HBV-related liver fibrosis.

Methods and analysis This study was designed to be a prospective, multicentre, open, randomised controlled study. A total of 272 patients with HBV-related liver fibrosis will be randomised into the combination therapy group or the monotherapy group at a 1:1 ratio. Participants in the combination group will receive subcutaneous injections of peg-IFN alpha 180 µg per week for 48 weeks combined with oral TDF 300 mg daily. Participants in the monotherapy group will receive 300 mg oral TDF daily alone. All participants will undergo long-term treatment with TDF and will be followed up at the outpatient department for 144 weeks after randomisation. Clinical symptoms, laboratory tests and examination indicators will be collected at each follow-up time point, and adverse events will be recorded. The primary endpoint is serological clearance rate of HBsAg at 48 weeks.

Ethics and dissemination The ethics committee of the Third Affiliated Hospital at Sun Yat-sen University approved this study (Approval Number: (2020)02-183-01). The results of the study will be presented at relevant meetings and published in an appropriate journal after the completion of the trial and the analysis of the data.

Trial registration number NCT04640129.

Strengths and limitation of this study

- This is a prospective, multicentre, randomised controlled trial which focus on the functional cure of nucleos(t)ide analogue-experienced patients with hepatitis B virus (HBV)-related liver fibrosis which lies on the former stage of cirrhosis.
- We dynamically monitor the quantification of hepatitis B surface antigen and HBV DNA of participants and measure the serum pg-RNA concentration at multiple check points. Quantitative detection of covalently closed circular DNA in liver tissue will be performed when liver biopsy is conducted.
- This study will provide data confirming the effectiveness and safety of combination therapy of pegylated interferon alpha and tenofovir in liver fibrosis population so that more patients will have the chance to achieve the goal of functional cure.
- A significant limitation of this trial is that both participants and investigators will be informed of the treatment plan which may result in bias.
- This study does not investigate the modality, dosage and duration of the combination therapy. Further studies are needed.

BACKGROUND

Hepatitis B virus-related liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in chronic hepatitis B (CHB) progression. Advanced liver fibrosis leads to cirrhosis and even hepatocellular carcinoma (HCC). Currently, approximately 296 million people have been infected with chronic hepatitis B

virus (HBV) globally. Cirrhosis and HCC caused by HBV infection account for 30% and 45% of all cirrhosis and liver cancer cases worldwide, respectively; in China, these percentages reach 60% and 80%, respectively.¹ Inhibiting HBV replication effectively improves liver fibrosis and delays or prevents the progression from compensated cirrhosis to decompensated cirrhosis. Further risk of deterioration in decompensated patients as well as the incidence of related complications would be reduced. Thus, the survival period is prolonged.²

In the past, the treatment goal of CHB was mainly to maintain the virological response (HBV DNA remains negative).³ With the in-depth understanding of HBV, the current treatment goal is to achieve functional cure which is defined as sustained, undetectable serum hepatitis B surface antigen (HBsAg) and HBV DNA with or without seroconversion to anti-HBs after a finite course of therapy.⁴ HBsAg clearance is not only an indicator of functional cure but also a predictor of reducing the occurrence of cirrhosis and HCC.⁵ However, the annual cumulative rate of HBsAg serological clearance was low. A systematic review and meta-analysis indicated that the cumulative serological clearance rate of HBsAg was 4.03% in 5 years, and there was no significant difference in HBsAg seroclearance between treated and untreated patients.⁶ Entecavir, tenofovir (TDF) and TDF alafenamide, as first-line nucleos(t)ide analogue (NAs) recommended by guidelines, can inhibit HBV replication effectively and efficiently.^{7–9} Among current antiviral treatment regimens, mathematical models suggested that it would take nearly 52 years for HBsAg clearance relying on NA,¹⁰ so it is almost impossible to achieve functional cure with NA therapy alone. Pegylated interferon (peg-IFN) alpha has both antiviral and immunoregulatory effects and it has a sustained response after drug withdrawal, which enables patients to have a higher serological clearance and even higher conversion rate of HBsAg.

The OSST (Optimising HBeAg Seroconversion in HBeAg-positive CHB patients with combination and Sequential Treatment of PegIFN alfa-2a and ETV) study and New Switch (HBsAg Loss with Peg-interferon Alfa-2a in hepatitis B patients with Partial Response to Nucleos(t)ide Analog) study both suggested that sequential therapy with peg-IFN alpha could improve the serological clearance rate of HBsAg in patients.^{11 12} A study conducted by Hamad S suggested that the combined application of peg-IFN and TDF for 1 year can increase the degree of HBsAg decline.¹³ A domestic study suggested that for CHB patients with HBsAg ≤ 1500 IU/mL, combination therapy including peg-IFN can improve the serological clearance rate of HBsAg compared with monotherapy with NA.¹⁴ The combination therapy of peg-IFN alpha with NA has achieved promising results in CHB patients, but its application in patients with hepatitis B virus-related liver fibrosis remains to be explored.

In this study, a prospective, multicentre, open-label, randomised controlled clinical trial was designed to determine the efficacy and safety of peg-IFN alpha

combined with TDF for the elimination of HBsAg in NA-experienced patients with HBV-related liver fibrosis and to compare this treatment with TDF monotherapy. At the same time, the improvement of liver fibrosis and the occurrences of long-term cirrhosis, liver cancer and other important indicators were also considered. The aim of this study is to determine a more effective and safer antiviral regimen for patients with hepatitis B-related liver fibrosis than those recommended in the current guidelines and to guide clinical decision making.

METHODS AND ANALYSIS

Overall study design

This study is a prospective, multicentre, open-label, randomised controlled clinical trial developed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) list (online supplemental file 1).¹⁵ The Third Affiliated Hospital of Sun Yat-sen University, as the chief research centre, together with Shenzhen Third People's Hospital, Nanfang Hospital and Guangzhou Eighth People's Hospital, will be coded from 1 to 4 at random. All participants will be randomly assigned to the combination therapy group or the monotherapy group at a 1:1 ratio according to block randomisation. The allocation sequence is generated by statistics professionals from the School of Public Health, Sun Yat-sen University. The envelope method will be used for allocation and concealment.

Recruitment

All participants will be recruited from the four centres mentioned above. None of the them have reached the goal of HBsAg loss or have met the criteria of NA cessation according to the guidelines for the prevention and treatment for CHB (2019 version).¹⁶

Screening procedure and inclusion criteria

Participants' medical and medication history, physical examination findings, laboratory test results and imaging findings will be collected to identify eligible participants.

Inclusion criteria: Patients are eligible to participate in the study if their

- ▶ HBsAg are positive whether hepatitis B envelope antigen (HBeAg) are positive or not.
- ▶ Duration of HBV infection was longer than 6 months before antiviral treatment.
- ▶ Duration of antiviral treatment was longer than 1 year.
- ▶ Ages are 18–55.
- ▶ Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin maintained lower than the upper limit of normal.
- ▶ Serum HBV DNA quantitation was less than 100 IU/mL or not detected.
- ▶ Liver biopsy indicated F1 ~F3 (Metavir scoring system), or transient elastography (Fibroscan) indicated liver stiffness measurement (LSM) of 6.0–12.0 kPa.

- ▶ Ultrasound examinations of the liver were normal or showed echo thickening, and the portal vein diameters were smaller than 12 mm.

Participants must meet all the above criteria to be included in the study.

Exclusion criteria

Participants will be excluded if they

- ▶ Were IFN-experienced or have contraindications to IFN.
- ▶ Were diagnosed with cirrhosis of the liver or HCC complicated with other tumours.
- ▶ Are pregnant or planning to become pregnant within one and a half years or are lactating.
- ▶ Had liver diseases caused by other causes (hepatitis A, C, D and E, autoimmune liver disease, drug-induced liver injury, alcoholic liver disease, genetic metabolic liver disease, etc).
- ▶ Also have HIV infection or other immunodeficiency diseases.
- ▶ Also have diabetes, autoimmune diseases or other organ dysfunction or failure or other serious complications (infection, hepatic encephalopathy, gastrointestinal bleeding, hepatorenal syndrome, etc).
- ▶ Cannot comply with the study arrangement or sign the informed consent form.
- ▶ Are not able to complete follow-up visits according to the study plan.
- ▶ Are thought to be patients who are unsuitable for inclusion as determined by the investigator.

Exit criteria

Participants are allowed to exit the study if they

- ▶ Request to withdraw.
- ▶ Cannot continue the study because of adverse events (AEs) (such as side effects).
- ▶ Have poor compliance or fail to use drugs according to the protocol, which affects the efficacy.
- ▶ Male's female partner becomes pregnant.

Sample size

A one-sided test was used to estimate the sample size in this superiority trial. The significant level (α) was 0.025. The power of test ($1-\beta$) was 0.8. The number of participants in the two groups was distributed at a 1:1 ratio. According to relevant research, the serological clearance rate of HBsAg was 0–2.8%^{11 17 18} after 48 weeks of TDF treatment in patients with CHB, while the values changed to 12.1%–31.3%^{19 20} after adding IFN for 48 weeks in NA-experienced patients. To minimise errors, 2.8% and 12.1% were chosen as the serological clearance rates of HBsAg in the monotherapy group and the combination therapy group, respectively. Taking a 10% drop-out rate into consideration, at least 134 participants should be included in each group according to the tests of two independent proportions. 94, 94, 40 and 40 participants from the Third Affiliated Hospital of Sun Yat-sen University, Shenzhen Third People's Hospital, Nanfang Hospital

and Guangzhou Eighth People's Hospital will be enrolled in proportions of 35%, 35%, 15% and 15%, respectively. Considering that the number of cases in each centre should be an integer multiple of the block length, which was designed as 4, the numbers were adjusted to be 96, 96, 40 and 40. In conclusion, a total of 272 participants will be included in this study, with 136 participants in each group.

Intervention therapy

In this study, peg-IFN alpha is the experimental intervention factor. Participants allocated to the combination therapy group will receive 180 µg peg-IFN alpha subcutaneously once a week for 48 weeks and 300 mg oral TDF daily. Participants allocated to the monotherapy group will receive 300 mg oral TDF daily. Peg-IFN alpha is manufactured by Shanghai Roche Pharmaceutical. TDF is manufactured by GlaxoSmithKline (Tianjin).

Efficacy and safety evaluation

- ▶ The primary efficacy endpoint is the seroclearance rate of HBsAg at 48 weeks.
- ▶ The secondary efficacy endpoints include the seroclearance rate of HBsAg, and the improvement rate of liver fibrosis degree in 96 weeks, which is defined as the ratio of participants with fibrosis degree improvement to the total number of participants in each group.

LSM and Metavir scoring system will be used to evaluate the degree of fibrosis. Each participant will undergo liver transient elastography (TE) (Fibroscan: Echosens, France) measuring the liver stiffness to determine the degree of liver fibrosis. LSM <6 kPa indicates a low likelihood of liver fibrosis, 6–9 kPa indicates nonadvanced liver fibrosis, 9–12 kPa indicates advanced liver fibrosis and ≥12 kPa indicates a high likelihood of cirrhosis.²¹ Liver biopsy will be performed with patient's consent. All sections will be independently evaluated by two pathologists who are not aware of the patient's condition. When the results of the two pathologists are inconsistent, they will re-evaluate and analyse the differences and reach a consensus. F0 in the Metavir scoring system is considered fibrosis absent. F1 shows fibrosis in the portal area but no fibrous septa. F2 shows portal fibrosis with a small amount of fibrous septum. F3 shows a large amount of fibrous septal formation (septal fibrosis). F4 is considered as cirrhosis.²²

The improvement of liver fibrosis degree refers to the reduction in the liver fibrosis score from F3 to F2 and below, F2 to F1 and below, F1 to F0 according to Metavir scoring system or from 9 kPa ≤ LSM < 12 kPa to 6 ≤ LSM < 9 kPa and below, and from 9 kPa ≤ LSM < 12 kPa to LSM ≤ 6 kPa according to TE.

- ▶ Safety evaluation includes the incidence of cirrhosis, liver cancer and liver failure; the rate of virology breakthrough; and AEs such as influenza-like syndrome and manifestations of symptoms or abnormalities of the digestive, urinary, nervous, circulatory, cardiovascular,

and bone and joint systems, as well as mental abnormalities, autoimmune phenomena, fundus lesions, etc.

The above indexes will be evaluated by routine examination of blood, urine and stool; biochemical and immunological examinations; tests of coagulation function and alpha-fetoprotein; liver imaging examination; and comprehensive evaluations of clinical symptoms and signs.

Study process

In this trial, the combination therapy group will be treated with peg-IFN alpha for 48 weeks, while TDF will be used for long periods in both groups. All participants will be followed up to 144 weeks after randomisation. Basic information, medical and medication history and general clinical manifestations will be collected in screening as baseline data. Leucocyte counts, neutrophil count, platelet count, ALT, AST, albumin, total bilirubin, lactic dehydrogenase, creatine phosphokinase, creatinine, estimated glomerular filtration rate, calcium, inorganic phosphorus, and fasting blood-glucose will be measured at 4 and 8 weeks after intervention. Quantitative of serum HBsAg, HBeAg, hepatitis B envelope antibody, HBV DNA concentration, alpha fetoprotein, abdominal ultrasound and LSM as well as tests conducted at 4 and 8 weeks will be assessed in screening and every 3 months. Anxiety and depression scales and autoimmune and fundus examination tests will be completed at 24 and 48 weeks. Quantitative detection of HBV pregenomic RNA (pg-RNA) will be carried out before randomisation and at 48, 96 and 144 weeks after randomisation. Liver biopsy will be performed before and at 96 and 144 weeks after randomisation with patients' consents. Once the biopsy performs, the expression of viral markers (HBsAg, HBeAg, HBcAg) of hepatic tissue, the level of intrahepatic covalently closed circular DNA and histological scores for staging (fibrosis) and grading (inflammation) will be evaluated.

Adverse events

The most common AEs of peg-IFN alpha include fever, fatigue, arthralgia, myalgia, headache, dizziness, decreased appetite, nausea, alopecia, decreased neutrophil count, gum bleeding, fever and cold intolerance, and elevated ALT. IFN-induced influenza-like syndrome can usually be treated with an antipyretic analgesic. During the treatment of peg-IFN alpha, routine blood tests will be performed to detect possible neutropenia and thrombocytopenia. The dosage of peg-IFN alpha will be adjusted over time. For AEs, appropriate treatment will be recommended. If severe AEs are encountered, even if the incidence is extremely low, they will be reported to the principal investigator, the ethics committee and the state supervision institutions within 24 hours of occurrence. All AEs will be coded in accordance with WHO Adverse Reaction Terminology. At the end of the clinical trial, the severity of every AE and its relationship to the study intervention will be determined.

Data collection and management

All study documents from the four centres will be uniformly stored in locked filing cabinets with restricted access. Data on each paper case report form (CRF) will be collected and managed with a three-letter pseudonym. In the office with restricted access, two independent trial coordinators will work together to check the integrity and consistency of the CRFs. Unreasonable or missing data will be identified and supplemented by searching the original data query forms after the consultation with the investigator under the supervision of independent trial inspectors. To ensure the accuracy of the data, all correct data from paper CRFs will be digitised and stored in the database twice by two subject-blinded staff members. When data revision is necessary, the corrected data will be entered by independent trial coordinators under the supervision of trial investigators and inspectors. The principal investigator and biostatistician can log into the database and access information only with the permission from the head of this study. Trial coordinators will be responsible for data backups and paper CRF archiving regularly. Data transfer between the centres will be encrypted and any information that identifies individuals will be deleted if necessary.

Test quality assurance

To ensure that the study is carried out in accordance with the current trial protocol with high quality and that the relevant personnel is able to manage the project effectively, all research staff including investigators, research assistants and outcome assessors will be trained before the study begins. Unless any SAE occurs during its implementation, the study will be conducted strictly according to the current trial protocol. Once the trial begins, an independent trial inspector will visit every timing of the follow-up, reviewing contents including the compliance with inclusion criteria and interventions, the compliance with national regulations and overall progress of the study. The trial inspector may make recommendations to the principal investigator, who will make any final decisions on the modification, continuation or termination of the clinical trial.

Statistical analysis

The analysis populations were defined a priori as follows: (1) modified intention-to-treat (mITT) population, consisting of all randomised participants who received at least one dose of the study treatment and who had at least one postbaseline assessment of the main variable; (2) per-protocol population, consisting of all participants in the mITT population who accomplished 80% of the study treatment with required data; (3) safety population, made up of all randomised participants who received at least one dose of the study treatment. For baseline features, continuous variables will be expressed as the mean \pm SD, while classification data will be expressed as frequencies. The independent t-test, χ^2 test or χ^2 test correction formula and Fisher's exact test will used for continuous

and classified variables, respectively. All statistical analyses will be performed by SPSS (Statistical Product and Service Solutions V.22.0, IBM). A $p < 0.05$ will be considered statistically significant.

ETHICS AND DISSEMINATION

The ethics and research plan of this study were approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (Approval Number: (2020)02-183-01) (online supplemental file 2). All participants will be fully informed about the potential benefits and disadvantages of all existing treatments, including peg-IFN alpha and TDF and sign an informed consent (online supplemental file 3) form approved by the ethics committee prior to intervention. This study was registered with ClinicalTrials.gov on 17 November 2020 (NCT04640129). On request, a summary of the study results will be submitted to ClinicalTrials.gov. Any significant protocol modifications (such as changes in primary endpoint, outcome and analysis) will be reported to ClinicalTrials.gov and the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. The results of the study will be presented at the relevant meeting and published in the appropriate journal after the completion of the trial and the analysis of the data. This clinical trial follows the principles of the Declaration of Helsinki of the World Medical Association and all its amendments, as well as Good Clinical Practice for drug clinical trials.

DISCUSSION

The current goal of antiviral treatment is no longer just virological response, but functional cure characterised by seroclearance of HBsAg and HBV DNA. Neither NA nor peg-IFN alpha monotherapy could achieve the goal easily. In recent years, clinical studies on combination therapy with IFN and NA have got promising results in HBsAg reduction and clearance among CHB population.^{23–25} However, combination therapy seldom applied to advanced fibrosis population because of the concerns raised about the safety of IFN and that it might precipitate hepatitis flares and subsequent hepatic decompensation. Nevertheless, previous study has confirmed the safety of IFN in patients with advanced liver fibrosis.²⁶ Combination therapy of peg-IFN alpha with NA for patients with HBV related liver fibrosis might be the chance in pursuit of functional cure before cirrhosis especially decompensated cirrhosis.

Compared with previous studies, this study focuses on patients with hepatitis B-related liver fibrosis under virological suppression status, evaluating the efficacy of HBsAg clearance and the improvement of liver fibrosis, as well as the long-term prognosis. The safety related to the haematological, immune and other systems will be assessed as well. Once the efficacy and safety of combination therapy are confirmed, more patients will have chances to achieve the goal of functional cure, and we will

carry out further clinical studies to verify the combination method, dose and course to create greater possibility of functional cure and better prognosis for patients with liver fibrosis.

The research phase

This study was registered with ClinicalTrials.gov on 17 November 2020 (NCT04640129). After submission, patient recruitment for this study started at the end of November 2020, and enrolment is expected to be completed at the end of November 2022.

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Acknowledgements We thank all the participants who took part in this research study.

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Funding This study was supported by grants from the Sun Yat-Sen University Clinical Research 5010 Programme (recipient: LP; Financial number: 2020007) and grants from the Third Affiliated Hospital of Sun Yat-Sen University, Clinical Research Programme (recipient: SZ; Financial number: QJHJ201903).

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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