Supplementary Online Content

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Supplement. Trial Protocol of the Substudy

This supplementary material has been provided by the authors to give readers additional information about their work.

A prospective, randomized, controlled, multi-center study to compare the efficacy of prophylactic entecavir and lamivudine in preventing hepatitis B reactivation in HBsAg-positive patients with untreated diffuse large B-cell lymphoma under R-CHOP chemotherapy

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Principal institution: Sun Yat-Sen University Cancer Center

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Remark: this study is a branch study of the primary study which has been detailed in previous section. The subject population of this study comes from the primary study. The primary version of this protocol was in Chinese. We have translated it into English.

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List Of Abbreviations

ADCC	**Antibody-Dependent Cell-mediated Cytotoxicity**
AE	**Adverse Event**
ALT	**Alanine aminotransferase**
AST	**Aspartate aminotransferase**
CDCC	**Complement-Dependent Cytotoxicity**
CHOP	**Cyclophosphamide, Doxorubicin, Vincristine, Prednisone**
CRF	**Case report form**
CTCAE	**Common Terminology Criteria for Adverse Events**
DLBCL	**Diffuse Large B-cell Lymphoma**
HAV	**Hepatitis A Virus**
HBV	**Hepatitis B Virus**
HBcAg	**Hepatitis B c-Antibody**
HBeAb	**Hepatitis B e-Antibody**
HBeAg	**Hepatitis B e-Antigen**
HBsAb	**Hepatitis B Surface Antibody**
HBsAg	**Hepatitis B Surface Antigen**
HCV	**Hepatitis C Virus**
HDV	**Hepatitis D Virus**
HEV	**Hepatitis E Virus**
HIV	**Human Immunodeficiency Virus**
NHL	**Non-Hodgkin Lymphoma**
R-CHOP	**Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone**
SAE	**Serious Adverse Event**

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Protocol Summary

applicant	Sun Yat-Sen University Cancer Center
phase	Phase III
indication	HBsAg-positive subjects with untreated DLBCL under R-CHOP chemotherapy
objectives	compare the incidence of hepatitis B reactivation in HBsAg-positive patients with untreated DLBCL under R-CHOP chemotherapy between prophylactic entecavir and lamivudine group; compare the incidence of HBV reactivation, chemotherapy disruption due to hepatitis in HBsAg-positive patients with untreated DLBCL under R-CHOP chemotherapy between prophylactic entecavir and lamivudine group
study design	prospective, multi-center, controlled, open-label, randomized phase Ⅲ study
required subject	108 subjects
Study sites	10 study sites (Sun Yat-Sen University Cancer Center, The First Affiliated Hospital of Sun Yat-sen University, The Third Affiliated Hospital of Sun Yat-sen University, The Fifth Affiliated Hospital of Sun Yat-sen University, Beijing Cancer Hospital, Southern Hospital of Southern Medical University, The First People's Hospital of Foshan, People's Hospital of Zhongshan, Shantou University Medical College and Kiang Wu Hospital)
Screening Criteria	HBsAg-positive subjects with untreated DLBCL without treatment contraindication
Control Group	Lamivudine (from 1 week before the initiation of R-CHOP chemotherapy to 6 months after the completion of chemotherapy)
Experimental Group	Entecavir (from 1 week before the initiation of R-CHOP chemotherapy to 6 months after the completion of chemotherapy)
Primary Endpoint	Incidence of hepatitis B reactivation
Secondary Endpoints	Incidence of HBV reactivation and chemotherapy disruption due to hepatitis
Study procedures	This is a prospective, multi-center, controlled, open-lable, randomized phase Ⅲ study. A total of 108 HBsAg-positive subjects with untreated DLBCL meeting the inclusion criteria is required. After giving written informed consent, all subjects will be randomized to receive prophylactic entecavir or lamivudine, which will be initiated 1 week before R-CHOP chemotherapy and withdrawn 6 months after the completion of chemotherapy. HBV DNA copies and liver function will be performed at the end of every cycle of chemotherapy, every month after the cessation of chemotherapy, and every 3 to 6 months after the withdrawal of antiviral prophylaxis. Viral markers of HAV, HBV, HCV, HDV, HEV, and HIV will be evaluated when hepatitis occurrs.

Statistical analysis	A total of 108 HBsAg-positive patients with untreated DLBCL will be randomized to two prophylactic antiviral treatment group at a 1:1 scheme. The incidence of hepatitis B reactivation, HBV reactivation and chemotherapy disruption due to hepatitis will be compared between the two groups. All adverse events related to antiviral drugs will be analyzed.

1. Background

Hepatitis B virus (HBV) infection is a worldwide health problem and more than 350 million individuals worldwide are infected with HBV. In endemic areas such as China, the prevalence of positive hepatitis B surface antigen (HBsAg) is about 7%. For several decades, it has been considered that there is no relationship between HBV infection and tumors deriving from non-hepatobiliary system due to the hepatotrophic characteristic of HBV. However, numerous fundamental researches have indicated that HBV is lymphotrophic. It was proposed by Heimann R in 1970s that HBV infection might stimulate lymphatic system persistently, which would lead to the development of malignant clone of lymphocytes. Several epidemiologic studies abroad have demonstrated that there is a high prevalence of HBV infection in lymphoma, suggesting HBV may be related to the pathogenesis of lymphoma. A case-control study in our center demonstrated that there was a higher prevalence of HBV infection in patients with B-cell NHL (30.2%) than in patients with other cancers; however, there was no statistical difference in HBV prevalence between T-cell NHL and other cancers.

Cytotoxic drugs could increase the risk of hepatitis flare in HBV carriers with normal liver function before chemotherapy. The reported incidence of HBV reactivation in patients with malignancies is as high as 44% during chemotherapy. Young age, male, positive hepatitis B e-antigen (HBeAg) and lymphoma are demonstrated as risk factors of HBV reactivation.

As the most frequent subtype of non-Hodgkin lymphoma (NHL), diffuse large B-cell lymphoma (DLBCL) accounts for more than one fourth of all cases in Asia. For more than 25 years, the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen has been standard care for DLBCL. As the addition of rituximab to traditional chemotherapy dramatically improves the outcome of DLBCL, rituximab combined with CHOP regimen (R-CHOP) has become the standard first-line treatment for DLBCL. Rituximab, a human-mouse chimeric monoclonal antibody that binds specifically to the CD20 antigen located on B lymphocytes, can induce CD20-positive B-cell depletion and secondary immunosuppression as a result of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDCC). With the extensive use of rituximab-contained chemotherapy, the risk of HBV reactivation has greatly increased in HBV carriers.

Available antiviral drugs for HBV infection are mainly nucleoside analogues including lamivudine and entecavir. Lamivudine is the first approved nucleoside analogue and is well-tolerated with minimal toxicity. But the high incidence of lamivudine-resistant mutations limits its efficacy, which is as high as 70% after using for 5 years. For patients with concomitant HIV and HBV infection, the incidence of drug-resistance is about 90% after using for 4 years. Entecavir, the third generation of nucleoside analogues, displays superior liver histological improvement and stronger inhibition of HBV DNA compared to lamivudine in both HBeAg-positive and HBeAg-negative HBV carriers in two large randomized phase III studies. Several guidelines on the management of chronic hepatitis B have recommended entecavir as the first-line treatment for chronic HBV infection.

Prophylactic antiviral treatment with lamivudine in HBsAg-positive patients with malignancies receiving chemotherapy can reduce the incidence of HBV reactivation, HBV-related hepatitis and mortality in pre-rituximab era, which has been confirmed in several prospective studies. However, in rituximab era, there are still cases who develop HBV reactivation despite lamivudine prophylaxis. The optimal preventive antiviral protocol, including drugs and treatment duration, is undetermined. We initiate this randomized study to compare the efficacy of prophylactic lamivudine and entecavir in preventing HBV reactivation in HBV carriers with DLBCL and investigate the optimal duration of antiviral treatment.

2. Objectives

2.1. Primary Objective

The primary objective of this study is to compare the incidence of hepatitis B reactivation following prophylactic antiviral treatment with entecavir versus lamivudine in seropositive HBsAg subjects with untreated CD20-positive DLBCL under R-CHOP chemotherapy.

2.2. Secondary Objective

The secondary objective is to compare the incidence of HBV reactivation and chemotherapy disruption due to hepatitis and safety following prophylactic antiviral treatment with entecavir versus lamivudine in seropositive HBsAg subjects with untreated CD20-positive DLBCL under R-CHOP chemotherapy.

3. Study Design

This is a prospective, multi-center, controlled, open-label, randomized (1:1) phase Ⅲ study of prophylactic antiviral treatment with entecavir compared to lamivudine in seropositive HBsAg subjects with untreated CD20-positive DLBCL under R-CHOP chemotherapy. Prophylactic antiviral treatment will be initiated 1 week before R-CHOP chemotherapy and withdrawn 6 months after the completion of chemotherapy.

Screening:

The subjects or their legally acceptable representative will provide written informed consent. Besides all examinations detailed in the primary study, coagulation function, hepatitis B surface-antigen/antibody (HBsAg/HBsAb), hepatitis B e-antigen/antibody (HBeAg/HBeAb), hepatitis B core antibody (HBcAb), HBV DNA level, hepatitis A virus (HAV) antibody, hepatitis C virus (HCV) antibody, hepatitis D virus (HDV) antibody, hepatitis E virus (HEV) antibody and human immunodeficiency virus (HIV) antibody will be performed to determine baseline virus status and study eligibility, all of which must be performed ≤14 days prior to randomization.

Treatment:

Based upon their assignment, subjects will receive prophylactic entecavir (0.5 mg/day) or lamivudine (100 mg/day), which will be initiated 1 week before chemotherapy and withdrawn 6 months after the completion of chemotherapy. Antiviral treatment will be modified or resumed at the investigator's discretion when HBV reactivation or HBV-related hepatitis occurs.

4.Subject Selection Criteria

4.1. Subject Selection Criteria

4.1.1. Number Of Subjects

A total of 108 subjects will be randomized to entecavir group or lamivudine group.

4.1.2 Inclusion Criteria

Besides inclusion criteria detailed in the primary study, subjects eligible for enrolment in this study must meet all of the following criteria at the same time:

1. Normal liver function, including normal alanine aminotransferase(ALT), aspartate aminotransferase(AST) and bilirubin

2. Seropositive HBsAg

3. Serum HBV DNA levels $<10^3$copies/ml

4. No prior antiviral therapy

4.1.3 Exclusion Criteria

Besides exclusion criteria detailed in the primary study, subjects meeting any of the following criteria are ineligible for this study:

1. Hepatic insufficiency for any reason

2. Positive viral markers for HAV, HCV, HDV, HEV, or HIV

4.2. Removal Criteria

Subjects who have participated in the study will be removed from statistical analysis for any of the following:

1. Subjects who are ineligible for this study

2. Continuous study antiviral treatment less than 28 days

3. Deviation(s) from the protocol

4.3. Withdrawal Criteria

Subjects will be withdrawn from study antiviral treatment for any of the following:

1. Inability to fully comply with the study protocol

2. Occurrence of hepatitis B reactivation or HBV reactivation

3. Initiation of alternative antiviral treatment

4. Unacceptable toxicity

5. Best interest of the subject based upon the investigator's discretion

6. At the request of the study subject at any time for any reason

Subjects will be followed up unless the informed consent is withdrawn. The reason for withdrawal from study participation and the date must be documented in the CRF. The investigator must complete the last visit, including vital signs, physical examination, laboratory tests including hematology, liver function, HBV DNA levels, viral markers of HAV, HCV, HDV, HEV, HIV, and AE assessment, all of which must be documented in the CRF.

5. Study Procedures

5.1. Antiviral Treatment Assignment

Subjects will be identified by a unique subject number that will remain consistent for the duration of the study. Upon completion of all the required screening assessments, eligible subjects will be centrally randomized using a randomization schedule generated by Sun Yat-sen University Cancer Center statistical department, which will assign subjects in a 1:1 ratio to entecavir 0.5mg daily or lamivudine 100mg daily.

5.2. Study Antiviral Treatment

Study antiviral treatment will be modified at the investigator's discretion when HBV reactivation or HBV-related hepatitis occurs.

5.2.1. Entecavir Group

drug	dose	frequency	route	time
entecavir	0.5 mg	Once daily	oral	From 1 week before R-CHOP chemotherapy to 6 months after the completion of chemotherapy

5.2.2. Lamivudine Group

drug	dose	frequency	route	time
lamivudine	100 mg	Once daily	oral	From 1 week before R-CHOP chemotherapy to 6 months after the completion of chemotherapy

6. Efficacy Endpoints

6.1. Defination

- Hepatitis, defined as a 3-fold or greater increase in the serum ALT level that exceeded the reference range (>58 U/L) or as an absolute increase in ALT of >100 U/L compared with the baseline level.
- HBV reactivation, defined as an increase in HBV DNA levels ≥10-fold or an absolute increase ≥10^5 copies/mL when compared with the baseline value.
- HBV-related hepatitis, defined as HBV reactivation preceding or accompanying hepatitis during and after chemotherapy in the absence of clinical or laboratory features of acute infection with other hepatitis viruses or systemic disease

- Chemotherapy disruption, defined as either premature termination or a delay of at least 7 days between chemotherapy cycles
- Delayed HBV-related hepatitis, defined as hepatitis related to HBV reactivation (an increase in the DNA level of ≥10-fold or an absolute increase of ≥10^5 copies/mL when compared with the baseline value) more than 6 months after the initiation of chemotherapy

6.2. Primary Efficacy Endpoint

Incidence of HBV-related hepatitis, defined as the percent of subjects who develop HBV-related hepatitis during and after completion of chemotherapy

6.3. Secondary Efficacy Endpoints

- Incidence of HBV reactivation, defined as the percent of subjects who develop HBV reactivation during and after completion of chemotherapy
- Incidence of chemotherapy disruption, defined as the percent of subjects whose chemotherapy is prematurely terminated or delayed at least 7 days between two continuous chemotherapy cycles
- Incidence of delayed HBV-related hepatitis, defined as the percent of subjects who develop HBV-related hepatitis more than 6 months after the initiation of chemotherapy

6.4. Schedule Of Efficacy Assessments

HBV DNA levels and liver function test including ALT, AST and bilirubin will be performed before randomization, at the end of every cycle of R-CHOP chemotherapy, every month after the cessation of chemotherapy for 6 months, and every 3 to 6 months after the withdrawal of antiviral prophylaxis. Viral markers of HAV, HBV, HCV, HDV, HEV, and HIV will be evaluated when hepatitis occurs.

6.5. Methods of Efficacy Assessments

HBV DNA assay, measured by real-time viral polymerase chain reaction (PCR) assays using the kit made in Da An gene detection company of Sun Yat-Sen University. The lower limit of quantification was 100 copies/mL.

7. Safety (the same as the primary study)

8. Adverse Events (the same as the primary study)

9. Rules Of Withdrawal (the same as the primary study)

10. Rules Of Follow-Up

10.1. Follow-up Period

Starting from randomization.

10.2. Visit Scheduling

At the end of every cycle of R-CHOP chemotherapy, every month after completion of chemotherapy, every three months for one and a half year after withdrawal of antiviral prophylaxis, and then then every six months until the primary study is completed. (After withdrawal of antiviral prophylaxis, the visit scheduling of this study is the same as the primary study.)

10.3. Contents Of Follow-Up

Besides of the contents detailed in the primary study, HBV DNA levels must be performed. All of the results must be documented in the original medical record. The results of liver function test and HBV DNA levels must be documented in the CRF.

11. Data Analysis And Statistical Considerations

11.1. Hypotheses

The primary endpoint is the incidence of HBV-related hepatitis. The null and alternative hypotheses are designed with the goal of demonstrating the superiority of entecavir over lamivudine. Superiority will be determined using the following hypothesis:

H0: incidences of HBV-related hepatitis for entecavir and lamivudine are the same.

H1: incidences of HBV-related hepatitis for entecavir and lamivudine are not the same.

11.2. Study Design Considerations

This prospective, multi-center, controlled, open-label, randomized (1:1) study compares the efficacy of prophylactic entecavir and lamivudine for prevention hepatitis B reactivation in seropositive HBsAg subjects with untreated CD20-positive DLBCL under R-CHOP chemotherapy. The primary outcome is the incidence of HBV-related hepatitis, and the study is designed to determine if entecavir is superior to lamivudine in the study population. Based on the reported

incidence of HBV-related hepatitis of 25% for lamivudine in subjects with NHL under CHOP chemotherapy, a clinically meaningful reduction in HBV-related hepatitis rate would be 20% for entecavir.

11.2.1. Sample Size Assumptions

The sample size calculation is based on the primary endpoint, incidence of HBV-related hepatitis, with the following assumptions:

- Incidence of HBV-related hepatitis for entecavir group: 5%
- incidence of HBV-related hepatitis for lamivudine group: 25%
- a 1:1 randomization scheme
- a 5% two-sided risk of erroneously claiming a difference in the presence of no true underlying difference by Chi-square test or Fisher's exact test
- a 80% chance of successfully declaring a difference in the presence of a true underlying difference (power)
- 10% percent of cases drop

Under the above assumptions, a total sample size of 108 subjects is required.

11.2.2. Primary Efficacy Endpoint

The primary efficacy endpoint of this study is the incidence of HBV-related hepatitis.

11.2.3. Secondary Efficacy Endpoints

The secondary efficacy endpoints of this study include incidence of HBV reactivation, incidence of chemotherapy disruption due to hepatitis, incidence of delayed HBV-related hepatitis and safety.

11.3. Data Analysis Considerations

11.3.1. Analysis Data Sets

The primary data set for efficacy will be based on the incidence of HBV-related hepatitis and incidence of HBV reactivation. The primary data set for safety will be the adverse events and the laboratory data sets.

The efficacy will be assessed by the investigators and by the independent reviewers. Data will be summarized for investigators assessed efficacy as well as for independent reviewers assessed efficacy. Statistical inference for efficacy claims will be based on the incidence of

HBV-related hepatitis and incidence of HBV reactivation data assessed by the independent reviewers.

11.3.2. Analysis Plan

11.3.2.1. Baseline Data

Baseline characteristics will be summarized and described in a frequency list.

11.3.2.2. Analysis of Efficacy

The definition of efficacy endpoints has been detailed in previous section. Incidences of HBV-related hepatitis, HBV reactivation, chemotherapy disruption and delayed HBV-related hepatitis will be tested based on a two-sided test, with a significance level of 0.05.

The number and proportion of subjects who develop hepatitis, HBV-related hepatitis , HBV reactivation, chemotherapy disruption, delayed HBV-related hepatitis will be provided. Pearson chi-square test or Fisher's exact test will be used to compare the proportion of subjects with HBV-related hepatitis , HBV reactivation, chemotherapy disruption, delayed HBV-related hepatitis for entecavir vs. lamivudine.

11.3.2.3. Analysis of Safety

Values of laboratory tests, vital signs and results of physical examination before antiviral treatment, during antiviral treatment and after antiviral treatment must be documented in original medical record and the CRF. The definition of AEs and SAEs has been detailed in the primary study. Data of clinical symptoms, signs and laboratory tests will be summarized according to NCI CTCAE grade (version 3.0).

Categorical data such as AEs and SAEs will be summarized by frequency and proportion of total subjects, which will be compared using Pearson chi-square test or Fisher's exact test between entecavir group and lamivudine group. Quantitative data such as laboratory tests will be described using arithmetic average or median for central tendency and standard deviation or interquartile range for distribution range, which will be compared using t test or non-parametric test between the two groups.

12. Materials For the Study (the same as the primary study)

13. Ethical Considerations (the same as the primary study)

14. Administrative Requirements (the same as the primary study)

15. Quality Control Of Data (the same as the primary study)