Brief Title: Effectiveness of TAF in Reducing Clinical Events in CHB Patients Beyond Treatment Indications by Current Guidelines (ATTENTION)

ClinicalTrials.gov ID (NCT Number): NCT03753074

(https://clinicaltrials.gov/study/NCT03753074)

Unique Protocol Id: IN-KR-320-5358

Statistical Analysis Plan

Version 1.1

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Roles and responsibilities

Name	Affiliation	Role
Professor Young-Suk Lim	Department of Gastroenterology, Asan Medical Center	Chief Investigator
Professor Jong-Gi Choi	Department of Gastroenterology, Asan Medical Center	Clinical Reviewer
Professor Seung-Bong Han	Department of Medical Statistics, Korea University	Statistician

Statement of Compliance

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive explanation of the pre-planned final analysis for the study. Version 1.1 was agreed upon before any data was reviewed, and the statistical analysis will be conducted by a statistician. All analyses will be performed using standard statistical software (SAS v9.4 or later and R v4.2.2 or later). The final analysis dataset, programs, and outputs will be stored in accordance with Good Clinical Practice (GCP) guidelines.

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1 Background

1.1 Purpose

To determine whether or not TAF treatment in the patients beyond treatment indications by current guidelines who have high risk of HCC, deaths and events associated with hepatic diseases reduces the incidence of HCC, deaths and events associated with hepatic diseases.

1.2 Primary objective

To provide an appropriate estimate of the hazard ratio for event(Composite endpoint: HCC, death, liver transplantation, or decompensated liver diseases [Child-Pugh score ≥7], development of portal hypertensive complications [ascites, gastroesophageal varices],)) occurrence between the Treatment Arm and Observation Arm.

1.3 Secondary objective

To estimate the cumulative incidence rates of a composite endpoint (death, liver transplantation, development of decompensated liver function [Child-Pugh score ≥7], complications of portal hypertension [ascites, esophagogastric varices], or diagnosis of hepatocellular carcinoma) in the Treatment Arm and Observation Arm. Additionally, to estimate the cumulative incidence rates of hepatocellular carcinoma, death, liver transplantation, development of decompensated liver function (Child-Pugh score ≥7), complications of portal hypertension (ascites, esophagogastric varices), achievement of viral response (HBV <15 IU/mL), ALT normalization, and HBeAg seroconversion (for initially HBeAg-positive patients). Furthermore, the Fibroscan values, APRI index, and FIB-4 index values will be calculated using specified formulas, and the changes in these values will be observed during the follow-up period.

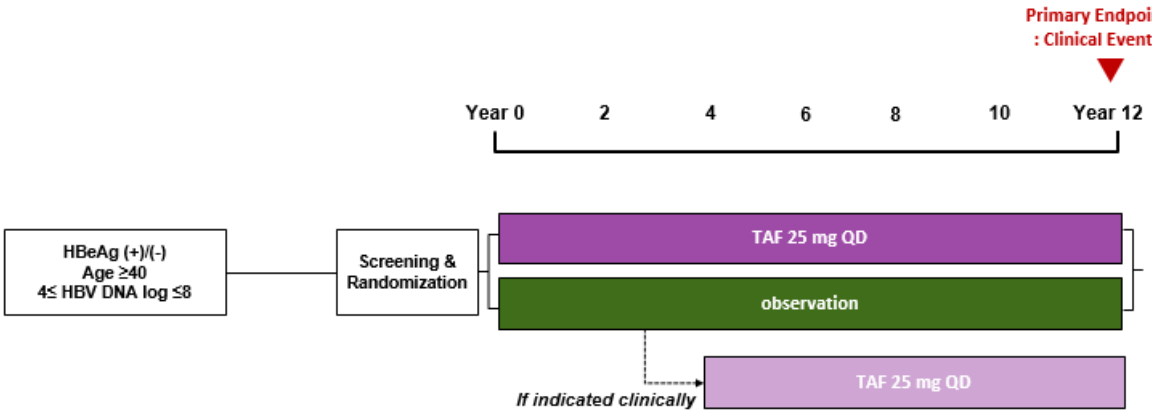
$$\text{APRI} = \frac{\dfrac{AST\ \left(\frac{IU}{L}\right)}{AST\ \left(upper\ limit\ of\ normal, \frac{IU}{L}\right)}}{Platelet\ Count\ \left(\frac{10^9}{L}\right)} * 100$$

$$\text{FIB} - 4 = \frac{Age\ (years) * AST\ (IU/L)}{Platelet\ Count\ \left(\frac{10^9}{L}\right) * \sqrt{ALT\ \left(\frac{IU}{L}\right)}}$$

Moreover, the changes in health-related quality of life (EQ-5D) will be tracked according to treatment, treatment response, disease state (hepatitis, cirrhosis, hepatocellular carcinoma), and duration of illness. Subgroup analyses will be conducted for HBeAg-positive and HBeAg-negative patients, as well as for patients with normal and elevated ALT levels.

1.4 Trial design and Treatments

This is a multicenter, open-label, randomized, Two Arm controlled Clinical Trial.
This study will be conducted over a total of 12 years after the first participant enrollment, with interim analyses planned at 4 and 8 years. If the interim analysis shows significant efficacy of the Treatment Arm, the study will be stopped, and the significant differences compared to the Observation Arm will be reported.



1.5 Randomization

An independent statistician will generate the code to randomly allocate participants to either Treatment Arm or Observation Arm in 1:1 ratio by stratified block randomization. Centralized stratified block randomization will be utilized in which participants will be stratified by HBeAg status (positive or negative) and, in each stratum, randomly allocated to either Treatment or Observation arm in 1:1 ratio. The ratio of HBeAg(+) to HBeAg(-) in each arm may be variable. Study sites will then randomly allocate the eligible patients who provide the informed consent to each study arm. Interactive Web Response System (IWRS) will be utilized to randomly allocate the participants to either Treatment Arm or Observation Arm in 1:1 ratio. Both investigators and patients will be aware of the allocation as this study is the open-label trial.

1.6 Projected Duration of the Study

Until December 31, 2031., from the date of IRB approval.

The study's end is defined as the last date of information collection for the primary and secondary outcome variable analyses for the first subject.

1.7 Study Population

The study population consists of chronic hepatitis B male and female adults, without cirrhosis who are not currently receiving treatment for hepatitis B and are beyond treatment indications by current guidelines

2 Study Subjects Criteria (Inclusion/Exclusion)

2.1 Inclusion Criteria

1) Willing and able to provide written informed consent prior to study entry.

2) Age ≥40 years and ≤80 years at the time of screening.

3) Chronic hepatitis B infection defined as HBsAg (+) or HBV DNA (+) for at least 6 months prior to the Screening visit, or the subject is not regarded to have acute hepatitis B according to the clinical assessment of the investigator.

4) Either HBeAg (+) or HBeAg (-)

5) Subject must be documented as non-cirrhotic (Platelet ≥ 100,000/ mm3)

6) Serum HBV DNA levels ≥1.0 x 104 IU/mL and ≤1.0 x 108 IU/mL (4.00 log10 IU/mL ≤ Serum HBV DNA levels ≤ 8.00 log10 IU/mL)

7) Serum ALT levels<70 IU/L (males) or <50 IU/L (females)

8) Estimated creatinine clearance ≥ 30 ml/min (CrCl or CKD-EPI)

9) Ability to comply with all study requirements

2.2 Exclusion Criteria

1) Confirmed known co-infection with HCV, HIV, or HDV

2) Current alcohol (60g/day) or substance abuse judged by the investigator that will potentially interfere with subject compliance

3) History or current evidence of clinically hepatic decompensation (e.g., ascites, encephalopathy, variceal hemorrhage) 1 year prior to Screening, or a Child-Pugh grade 7 (with the exception of Gilbert syndrome) at the time of Screening.

4-1) Evidence of liver cirrhosis defined as meeting any of the following criteria:

a) Platelet count <100,000/mm3

b) Clinically significant portal hypertension
c) Presence of esophageal or gastric varices by endoscopy in 2 years before the time of screening
d) Fibroscan ≥ 12.0 kPa (If the test was done in 3 months before the time of screening.) and confirmed to have liver cirrhosis by an investigator
4-2) 40≤ALT levels<70 IU/L (males) or 40≤ ALT levels<50 IU/L (females) with evidence of significant fibrosis(F2; ≥7.2 kPa) as measured by either liver biopsy, Fibroscan or MR Elastography performed within 3 months.
5) Currently on or have received therapy with Interferon or immunosuppressant (including systemic chemotherapy) within 12 months prior to the screening
6) Requirement for chronic use of systemic immunosuppressant including, but not limited to, corticosteroid (prednisone equivalent of >40 mg/day for >2 weeks), azathioprine, or monoclonal antibodies
7) Received solid organ or bone marrow transplant
8) History of severe, life-threatening or other significant sensitivity to any excipients of the study drugs
9) Any other clinical conditions (cardiovascular, respiratory, neurologic, or renal conditions) or prior therapy that, in the opinion of the investigator, would make the subject unsuitable for the study or unable to comply with dosing requirements.
10) Currently on or have received antiviral treatment for ≥ 2 weeks within 6 months prior to the screening
11) History or current evidence of HCC, or high α-fetoprotein (AFP) > 20 ng/mL. (Patients with AFP>20ng/mL can be enrolled, however if imaging investigations, such as dynamic CT or MRI, provide no evidence of HCC within 4 months prior to Screening)
12) Malignancy other than HCC within 5 years prior to the screening, with the exception of specific cancers that are cured by surgical resection (within 2 years prior to screening with confirmation of no evidence of disease). Subjects under evaluation for possible malignancy are not eligible.
13) Concurrent enrollment in another clinical study for other type of antiviral treatment for CHB or immune modulatory drug within 3 months prior to randomization,, Participation to an observational (non-interventional) clinical studies or interventional studies not using anti-HBV or immune modulatory drugs, or during the follow-up period of an interventional study are not exclusion criteria.
14) Pregnant women, women who are breastfeeding or who believe they may wish to become pregnant during the course of the study

3 Efficacy Evaluation

3.1 Primary outcome

The primary efficacy endpoint of this study is the occurrence of composite events during follow-up observation including HCC, death, liver transplantation, or decompensated liver diseases (e.g., development of portal hypertensive complications including ascites, gastroesophageal varices, or Child-Pugh score ≥7), in the full analysis set.

3.2 Secondary clinical outcomes

Defined by the incidence rates of hepatocellular carcinoma, death, liver transplantation, development of decompensated liver function, complications of portal hypertension, achievement of viral response, ALT normalization, and HBeAg seroconversion.

3.3 Subsidiary clinical outcomes

Defined by the changes in Fibroscan values, APRI index, and FIB-4 index values, as well as the changes in health-related quality of life (EQ-5D) according to treatment, treatment response, disease state (hepatitis, cirrhosis, hepatocellular carcinoma), and duration of illness.

3.4 Hypothesis Setting

For each primary, secondary, and subsidiary outcome variable, the null hypothesis is "There is no actual difference in effect between the treatment groups," and the alternative hypothesis is "There is a difference in effect between the treatment groups," tested using a two-sided test.

4 Data Collection Plan

Patient's basic information and outcome variable data will be collected via eCRF and entered into a web-based IT system by hospital or research staff members. Written consent, baseline information, and medical history will be obtained from all participants within 28 days prior to thebaseline. At this point, eligibility criteria will be reviewed, and information on physical examination, vital signs, and blood tests will be collected. Additionally, data on US, CT, or MRI (It can be replaced if relevant imaging tests were done within 4 months prior to the Screening

), and liver fibrosis test (Fibroscan, It can be replaced if relevant imaging tests were done within 3 months prior to the Screening), will be gathered. Every 6 months until the end of the study, data on physical examination, vital signs, blood tests, US, CT, or MRI, bone density tests, and liver fibrosis test results will be collected, and adverse events, study drug dispensation and compliance in the treatment group, and concomitant medications will be monitored.

5 Data Monitoring Committee (DMC)

During the study, all data will be provided to an independent DMC under strict confidentiality. The DMC will request these data for independent review and evaluation. The DMC will primarily review potential risks to participant safety and recommend necessary actions. The investigator will provide data to the DMC for interim analyses of the efficacy of the treatment group at 4 and 8 years after the start of the study. Based on the interim analysis results, the DMC will recommend whether there is a need to modify or terminate the trial.

The DMC will include at least two independent experts unrelated to this clinical trial, including a statistician. If necessary, additional members such as the principal investigator, sponsor, CRO project manager, monitors, and the sponsor's medical advisor may attend to provide relevant information. The overall operations, including detailed membership, review items, and meeting frequency, will follow a separate charter.

6 Analysis Population

The primary analysis for assessing treatment efficacy and safety will be conducted using the Full Analysis Set, including all randomly assigned patients (Modified Intention-to-Treat analysis). Secondary analyses will involve on-treatment analysis, which includes patients participating in the study at the analysis points. For the interim analysis, baseline data will be reported for all participants with available data, and outcome data will record whether events occurred and when they occurred.

7 Statistical Analyses

7.1 Participant Flowchart

The flow of clinical trial participants will be explained using the CONSORT diagram, including the number of participants randomly assigned to each treatment group, those

who withdrew consent, and those included in the modified ITT analysis population.

7.2 Survival Curve Estimation

The cumulative incidence of primary outcome events in the two groups will be estimated using the Kaplan-Meier method

7.3 Comparison of baseline statistics for two groups

Continuous or categorical variables between the two groups will be compared using appropriate tests such as Student's t-test, Chi-square test, or Fisher's exact test. The statistical significance level will be set at 5%

- Physical examination (Lower leg edema, Ascites), Vital signs (blood pressure, pulse rate, body weight, height)
- Laboratory assessments (Hematology, Chemistry, Prothrombin Time, HBV DNA, HBsAg, HBeAg/HBeAb, AFP, Serum for storage)
- Abdominal US, CT, or MRI,
- Fibroscan
- Bone mineral density
- Review concomitant drugs
- EQ-5D)

7.4 Comparison of follow-up test results for the two groups

Differences in health-related quality of life utility weights (EQ-5D and EQ-VAS) based on treatment, treatment response, disease status (hepatitis, liver cirrhosis, HCC), and duration of illness will be analyzed using Generalized Estimation Equation (GEE). Adjustments will be made for age, gender, and clinical baseline variables. Before performing the GEE analysis, exploratory analyses will compare trends in utility weight changes for each variable using paired t-tests.

7.5 Completeness of follow-up

To minimize follow-up loss, regular meetings will be held to reduce the follow-up loss rate. Participant numbers and rates will be reported for each group after the first 28 days and every 6 months thereafter for follow-up information review. Clinical trials may be discontinued or participants excluded based on the investigator's judgment if any of the following conditions are met.

- Development of a toxicity or adverse event which warrants drug discontinuation
- The lack of efficacy of the medication or the exacerbation of signs or symptoms determined by the investigators, which warrant drug discontinuation
- The subjects are revealed to be ineligible to participate in the clinical trial regarding the safety of the participants
- The investigators determine that it is inappropriate to continue the clinical trial
- Unexpected pregnancy during the trial
- The subjects withdraw the consent to participate in the clinical trial

Treatment after discontinuation or withdrawal will be determined by the investigator. In case of discontinuation or withdrawal due to adverse events or safety issue, subjects should be followed until full recovery and the events should be recorded in CRFs.

7.6 Adherence to treatment

Subjects should bring all the study drug to the study drug compliance at every follow-up visits including premature discontinuation. The site will record the number of tablets returned at these visits and determine compliance to the medication

$$\textbf{Compliance (\%)} = \frac{\text{The amount of drug ingested}}{\text{The amount the subject should have ingested}} \times 100$$

7.7 Primary endpoint

The primary analysis for assessing treatment efficacy and safety will be conducted using the Full Analysis Set, including all randomly assigned patients (Modified Intention-to-Treat analysis). Patients who discontinue the clinical trial during the 12-year study period will be considered as having early termination for all endpoints from the discontinuation point onwards. The cumulative incidence of primary outcome events in the two groups will be estimated using the Kaplan-Meier method, and comparison will be made using the log-rank test statistics (Zk), where k represents the analysis time point. The first interim analysis

will be conducted at 4-year from the study initiation. If the null hypothesis is not rejected, the second interim analysis will be performed at 8-year from the study initiation. If the null hypothesis is not also rejected in the second interim analysis, the final analysis will be conducted at the end of the study. The stopping boundaries for the log-rank test statistics, determined using the O'Brien-Fleming method, are as follows: if the absolute value of the test statistics is less than 4.17084 (first interim analysis) or 2.8458 (second interim analysis), the trial will be continued. However, if the absolute value of the test statistics exceeds 4.17084 (first interim analysis) or 2.8458 (second interim analysis), the null hypothesis is rejected, and the efficacy of the treatment group is declared, leading to the termination of the study.

Details when Spending = O'Brien-Fleming, E = 115, S1 = 0.801, S2 = 0.889

Look	Time	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	4	-4.17084	4.17084	0.00003	0.00003	0.00003	0.01160	0.01160
2	8	-2.84580	2.84580	0.00443	0.00441	0.00444	0.42583	0.43743
3	12	-2.26370	2.26370	0.02359	0.02056	0.02500	0.41284	0.85027

Additionally, a Cox proportional hazard model adjusted for baseline variables will be performed. Adjustments will be made for variables such as age, gender, HBeAg positivity, HBV DNA level, ALT level, platelet count, and more. The statistical significance level will be set at 5%.

7.8 Secondary Outcome Variable Analysis

Analysis of secondary outcome variables will follow the on-treatment analysis principle, targeting only participants currently in the study. The analysis will be performed at interim or final analysis points without adjustments for multiple comparisons. Log-rank tests will be performed on survival data, and a multivariable Cox proportional hazards model will be fitted, considering age, gender, HBeAg positivity, HBV DNA level, ALT level, and platelet count as adjustment factors, similar to the primary outcome variable analysis. Additionally, changes in secondary outcome variables such as viral response, liver function changes, and liver fibrosis changes will be observed. These changes will be analyzed using GEE, and the data will be utilized for the development of markers for predicting long-term clinical outcomes. To calculate the quality-adjusted life year (QALY) during the economic evaluation, differences in changes in treatment, treatment response, disease state (hepatitis, cirrhosis, hepatocellular carcinoma), and quality of life utility weights (EQ-5D and EQ-VAS) will be analyzed using GEE. Results adjusted for age, gender, and clinical baseline variables will

also be presented.

7.9 Economic Evaluation

The strategy of early drug treatment for patients at high risk of major clinical indicators such as hepatocellular carcinoma and liver disease-related death, although not currently eligible for antiviral drug treatment under chronic hepatitis B treatment guidelines, will be compared for cost-effectiveness against the current observation strategy (drug treatment after ALT elevation).

7.9.1 Inputting Results Collected from Clinical Research into the Economic Evaluation Model

Chronic hepatitis B progresses over a long period to complications such as cirrhosis and liver cancer, significantly impacting the patient's quality of life and medical expenses. Therefore, a model reflecting the natural history of this disease will be established (see analysis model draft, Figure 2). Primary outcome variables measured in the Treatment Arm and Observation Arm of the ATTENTION study (such as the occurrence of hepatocellular carcinoma and liver disease-related deaths) will be applied as input variables in the model. Major effect values can be calculated as incidence rates for each group or using hazard ratios. If it is assumed that certain unobtainable items are replaced with values of calculable variables, standardization reflecting the population structure will be used for adjustment.

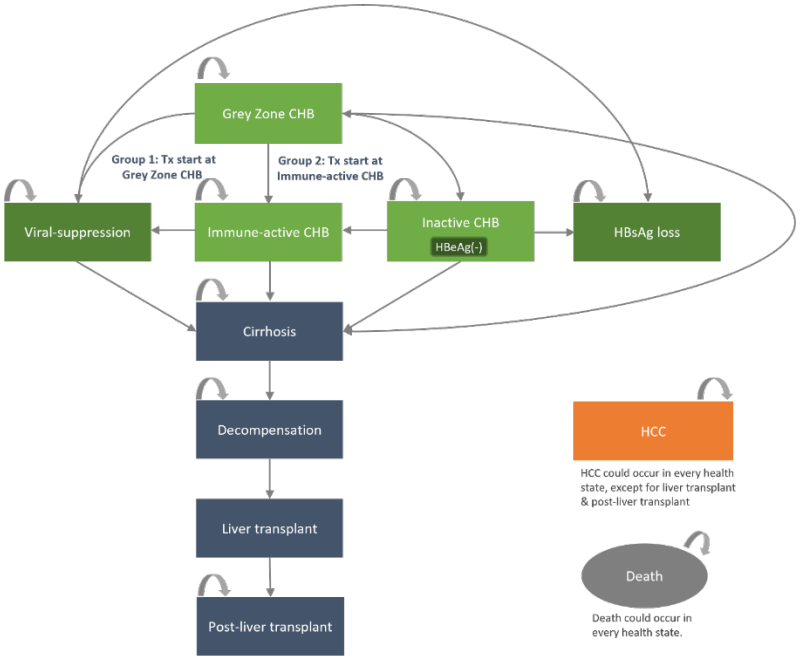


Figure 2. analysis model draft

7.9.2 Basic Analysis

The economic evaluation result indicators will be presented as the incremental cost-effectiveness ratio (ICER), representing the cost and performance difference between alternatives.

$$ICER = \frac{Cost_{Intervention} - Cost_{Comparator}}{Effectiveness_{Intervention} - Effectiveness_{Comparator}}$$

If the calculated ICER is below the threshold of 30.5 million KRW (approximately $25,000) per QALY, as presented in the National Evidence-Based Healthcare Collaborating Agency (NECA) report (An et al., 2012), early drug treatment for high-risk patients, who are not currently eligible for treatment under the current guidelines but are at high risk for hepatocellular carcinoma and liver disease-related death, will be considered cost-effective.

7.9.3 Sensitivity Analysis/Scenario Analysis

- Deterministic sensitivity analysis will be conducted using upper and lower bounds for various clinical indicators to confirm the stability of the basic analysis results. Furthermore, probabilistic sensitivity analysis will be performed using probability distributions (beta distribution for transition probabilities and utility weight variables, gamma distribution for cost variables) to comprehensively assess the uncertainties associated with changes in various input variables applied to the model.
- The incidence rates according to major evaluation variables, such as age group (30s, 40s, 50s, 60s and above), HBV DNA level range (4-5, 5-6, 6-7, 7-8 log), HBeAg positivity, and medication adherence, will be input into the analysis model. The ICER for each subgroup (or scenario) will be calculated to identify specific patient groups with optimal cost-effectiveness.
- If EQ-5D values by health status are stably derived, an analysis will be conducted applying values derived from this clinical study instead of utility weights extracted from preceding literature.

7.10 Subgroup Analysis

The primary outcome variables for pre-specified subgroups will be analyzed in relation to secondary outcome variables. Adjustments for multiple comparisons will not be additionally considered. The analysis results will be presented as hazard ratios and corresponding 95% confidence intervals through a forest plot. The subgroups considered

are as follows.

- HBeAg-positive or HBeAg-negative patients
- Patients with normal or elevated ALT levels

7.11 Adverse Event Analysis

Safety data will be summarized using descriptive statistical methods, and if appropriate, 95% confidence intervals will be calculated to supplement the analysis. Patients with protocol violations, adverse events, and missing data will be identified, and additional descriptive analyses, including the relationship to treatment, will be performed.

7.12 Statistical Analysis Software

All statistical analyses will be performed using SPSS (SPSS, Chicago, IL, USA) and R (http://cran.r-project.org/).

8 Reference

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