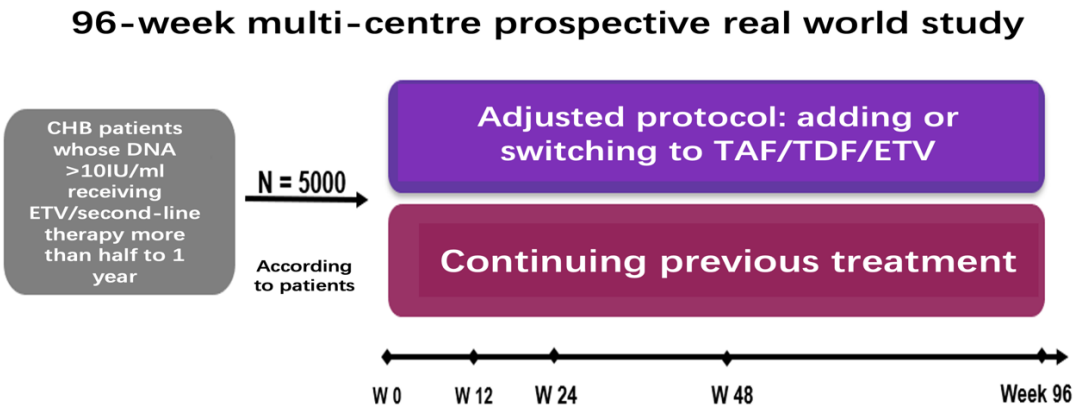
Study Protocol and Statistical Analysis

A. Study Protocol

1. Research subjects

The research subjects were patients with CHB receiving ETV or second-line NA(LAM/ADV/LdT) who were admitted to The Second Affiliated Hospital of Chongqing Medical University and other centers.

2. Research design



3. Research methods

In our hospital, about 150 patients are screened for HBV-DNA every day. Therefore, 54 million patients will be tested for HBV-DNA within one year, of which 30% are estimated to be HBV-DNA ⩾10 IU. These patients will be informed to the Department of Infectious Diseases of the Second Affiliated Hospital of Chongqing Medical University for follow-up, and will be randomly divided into three groups according to 1:1. Patients in all three groups will be educated about hepatitis B virus infection and antiviral treatment, and the treatment regimen will be adjusted according to whether their HBV DNA is ⩾10 IU/ml or not. Patients in group 1: patients with persistant low level HBV DNA (<10 IU/ml). Patients in group 2: HBV-DNA⩾10 IU/ml, receiving HBV-related education and being advised by the doctor to change or to add another NA. Patients in group 2: patients with persistant HBV DNA (>10 IU/ml) but refuse to change the regimen. Patients in group 3: patients with persistant HBV DNA (>10 IU/ml) and agree to change the regimen. Educational methods include videos, including an introduction to hepatitis B virus (disease profile, infection, outcome, HBV infection, vertical transmission and other risk factors) for 5 minutes, brochures with relevant information and consultations with physicians and nurses.

All patients with CHB receiving ETV or second-line NA(LAM/ADV/LdT) treatment for more than six months to one year will receive HBV-DNA

detection, and patients with HBV-DNA≥10 IU/ml will be informed and recommended to adjust the treatment regimen so that we could obtain the actual prevalence of HBV-DNA load < 10 IU/ml in Chongqing HBV cohort. We estimated that 30% of the patients had HBV-DNA≥ 10 IU/ml, so there were about 16,200 patients had HBV-DNA≥ 10 IU/ml among 54,000 patients a year. These patients will be diagnosed with LLV and will undergo a treatment regimen adjustment, with a recommendation to switch to or use a different type of NA for viral treatment.

4. Outcome indexes

(i) The main index:

The proportion of patients who received a complete virologic response (HBV DNA<10 IU/ml) at 24 weeks after therapy adjustment.

(ii)The secondary index:

1.The proportion of patients with complete virologic response (HBV DNA<10 IU/ml) at 12 weeks, 48 weeks and 96 weeks after therapy adjustment.

2.The proportion of patients with normal ALT at baseline and at each follow-up time point.

3.Changes of bone and kidney indicators (eGFR, SCr, BMD, etc.) compared with baseline at each follow-up time point.

B. Statistical Analysis

1. Statistical description

. Whether the measurement data conforms to normal distribution: the statistical method is modified or the data is converted when the measurement data does not conform to normal distribution, then it is managed as normal distribution when the mean and median are close due to the large sample size because of the opening test. Outliers: Statistical and professional analysis is performed to determine whether to keep outliers. Management of missing value in major efficacy indicators data: when a major efficacy data is missing in individual subjects, the method of filling is determined from a statistical and professional perspective. If the case is lost, data from the previous measurement shall be transferred.

. Case analysis of unfinished tests: the reason of lost cases should be analyzed one by one.

. Descriptive statistics: indicating the mean, the standard deviation, the maximum, the minimum, the median, the upper and lower quartile (Q1 and Q3), the confidence interval, the frequency (composition ratio), etc.

2. Statistical inference method

. The measurement data: T test, paired T test, rank sum test, paired

rank sum test, correlation analysis was used. The counting data: Chi-square test, Fisher exact test, etc. The grade data were tested by CMH method.

3. Statistical expression

. The report is mainly expressed in tables, which are self-explanatory, that is, with headings, notes and main statistical indicators.

. The results of repeated measurements are presented in tables with statistical charts for readability. F value and P value of an OVA of repeated measurement data are added.

. Bilateral tests are generally used for statistical tests, and P less than or equal to 0.05 is considered to be statistically significant.

4. Statistical software

SPSS22.0 software will be used for analysis.