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Afatinib in combination with GEMOX chemotherapy as the adjuvant treatment in patients with ErbB pathway mutated, resectable gallbladder cancer: study protocol for a ctDNA-based, multicentre, open-label, randomised, controlled, phase II trial

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ABSTRACT

Introduction Gallbladder cancer (GBC) is an aggressive type of digestive system cancer with a dismal outcome. Given the lack of effective treatment options, the disease rapidly reoccurs and 5-year survival rate is <5%. Our team previously found that a significant percentage of GBC tissues harboured mutations of the ErbB-related pathway. Afatinib is a chemically synthesised drug specifically targeting the ErbB pathway mutations. However, its efficacy in the treatment of patients with GBC remains unknown. Circulating tumour DNA (ctDNA) refers to a proportion of cell-free DNA in the blood which is released by apoptotic and necrotic cells from tumours in situ, metastatic foci or circulating tumour cells. ctDNA-based liquid biopsy is a non-invasive pathological detection method that offers additional value to evaluate the therapeutic efficacy of antitumour drugs.

Methods and analysis We conduct a multicentre and randomised study on afatinib combined with gemcitabine and oxaliplatin (GEMOX) in patients with ErbB pathway mutated GBC. Clinical and biological evaluation involving ErbB pathway ctDNA detection will be made during the 3-year follow-up after participation. The primary objective of this clinical trial is to evaluate the clinical efficacy of afatinib. Disease-free survival is the primary end point and will be correlated with plasma ctDNA of patients in the treatment with afatinib. In addition, we will evaluate the sensitivity and specificity of plasma ctDNA for monitoring tumour recurrence and progression. Finally, we will assess the safety of afatinib by keeping an eye on the safety indicators.

Ethics and dissemination The study was approved by the medical-ethical review committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. The clinical trials results, even inconclusive, will be published in peer-reviewed journals.

Trial registration number NCT04183712.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first clinical trial to evaluate the efficacy of afatinib as an adjuvant therapy regimen in patients with gallbladder cancer.
⇒ This is a prospective multicentre trial with a randomised, controlled design to ensure the reliability of the data.
⇒ Only patients with ErbB pathway mutations are enrolled in this study, as these mutations are the target of afatinib.
⇒ After surgery, both image and circulating tumour DNA detection are used to monitor tumour recurrence and evaluate the efficacy of afatinib.
⇒ There is a lack of sample size due to the low incidence of gallbladder cancer with ErbB pathway mutations.

INTRODUCTION

Gallbladder cancer (GBC) is one of the most common and lethal tumours of the biliary tract system with 5% of 5-year survival rate.[1 2] GBC lacks typical symptoms at early stages but rapidly undergoes cancer malignant transformation that is characterised by rigorous tumour infiltration and metastasis.[3] To date, while no powerful means are available for curing GBC, surgical treatment is the mainstay of this intractable malignancy. Unfortunately, previous studies from our group reported that the resection rate of GBC in China is only 44.7% after diagnosis and the radical resection rate is even less than half (18.84%–21.59%).[4 5] Thereof, a large population of cancers fall into non-surgical therapy including radiotherapy, chemotherapy and

targeted therapy.[6] We recently have completed a clinical trial that comparing efficacy of gemcitabine combined with oxaliplatin (GEMOX) versus modified fluorouracil, leucovorin, irinotecan and oxaliplatin (mFOLFIRINOX) in the prognosis of patients with unresectable locally advanced or metastatic GBC. The result is still unsatisfactory as the median survival time of patients treated with GEMOX is 7 months versus mFOLFIRINOX 9 months.[7] Therefore, it is critically important to find alternative therapeutic strategies for GBC.

Targeted therapy is emerging as more effective interventions than traditional radiotherapy and chemotherapy. A number of clinical trials have demonstrated the efficacy of targeted therapy in haematological tumours, lung cancer as well as biliary tract cancer (BTC). For example, phase II clinical trials with cetuximab[8 9] and panitumumab,[10] targeted to EGFR and KRAS, respectively, can improve the prognosis of patients with biliary tract cancer. However, other phase II clinical trials of multitargeted tyrosine kinase inhibitors (TKI), sorafenib and EGFR-targeted TKI, erlotinib, did not yield significant improvement in the prognosis of patients with BTC.[11 12] These outcomes may be attributed to the insufficient sample size from a single cancer centre or lack of subgroup settings based on their genetic features such as ERBB family somatic gene mutations.[6] Our team previously found that high-frequency somatic mutations in the ErbB pathway (including EGFR, ERBB2, ERBB3, ERBB4 and their downstream genes) up to 36.8% accounted for the occurrence and development of GBC.[13] These patients with GBC were associated with tumour proliferation, invasion, immune escape and poor prognosis.[13 14] At present, afatinib, a targeted drug for the ErbB pathway, has been approved for clinical treatment in EGFR-positive lung cancer and also engaged for clinical research on cholangiocarcinoma.[15 16] Preclinical studies have discovered that afatinib can inhibit the invasiveness of GBC cell lines and reduce the tumour size of GBC xenografts.[17] Given these evidences, here, we set up a clinical trial to test the hypothesis that afatinib may help improve the prognosis of patients with ErbB pathway mutated GBC.

Liquid biopsy is a non-invasive pathological detection method which was first reported by Sorrells in 1974 in the diagnosis of synovitis from synovial fluid.[18] The advantage of this new technology such as easy accessibility and non-invasive approach offers significant value in the cancer research including cancer screening, early diagnosis, finding treatment targets and monitoring disease progress. As the result, this innovation was listed as one of the top 10 technological breakthroughs in 2015 and considered to be of great clinical significance and application prospect.[19] Liquid biopsy mainly includes circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), circulating tumour RNA, tumour-associated platelets and exosomes, among which ctDNA and CTCs are the most well-studied and have been approved by the US Food and Drug Administration for clinical application.[19–21] ctDNA, first reported in 1948, refers to cell-free DNA (cfDNA) released into the blood by apoptotic and necrotic cells from tumours in situ, metastatic foci or CTCs.[22] Currently, based on the rapid development of advanced detection technology, such as digital PCR and the next-generation sequencing (NGS), ctDNA-based liquid biopsy has received remarkable attention to monitor tumour burden and response to therapy.[23 24] Several studies in gastrointestinal cancers reported that ctDNA dynamic changes may be predictive markers to monitor therapy efficacy.[25–27] However, the application of ctDNA in evaluation of GBC diagnostic and therapeutic studies remains to be established. Therefore, we add ctDNA detection of participants to monitor disease progression and evaluate the therapeutic effects of afatinib on the recurrence and metastasis of GBC.

Aim of the study

The aim of this study is to evaluate the clinical efficacy and safety of afatinib in combination with GEMOX as an adjuvant therapy in patients with resectable GBC with ErbB pathway mutation by monitoring the dynamic changes of ctDNA.

METHODS AND ANALYSIS

Study design

The study is designed as a randomised, open-label and multicentre clinical trial with a combined regimen of afatinib and chemotherapy drugs compared with chemotherapy drugs alone in patients with GBC with ErbB pathway mutation who underwent surgical removal (see figure 1 for an overview of the study design). A minimum of 102 patients will be enrolled from national four top-ranked hospitals in Shanghai, China (Renji Hospital, Ruijin Hospital and Xinhua Hospital, all affiliated to Shanghai Jiao Tong University School of Medicine, and Zhongshan Hospital Affiliated to Fudan University). The study has started on 1 June 2020 and the recruitment is expected to last 36 months. Medical records and biological samples including ctDNA detection will be collected and evaluated during the 3-year follow-up after diagnosis (see online supplemental appendix 1 for a detailed time schedule of study). Disease-free survival (DFS) and overall survival (OS) will also be evaluated.

Objectives

Primary objective

To assess the efficacy of afatinib combined with GEMOX chemotherapy. Three-year DFS and 3-year OS will be used as the primary and secondary end points, respectively.

Secondary objectives

To assess the correlation between plasma ctDNA level and OS and DFS of patients.

To evaluate the sensitivity and specificity of plasma ctDNA for monitoring tumour recurrence and progression.

To assess the safety of afatinib in this study population.

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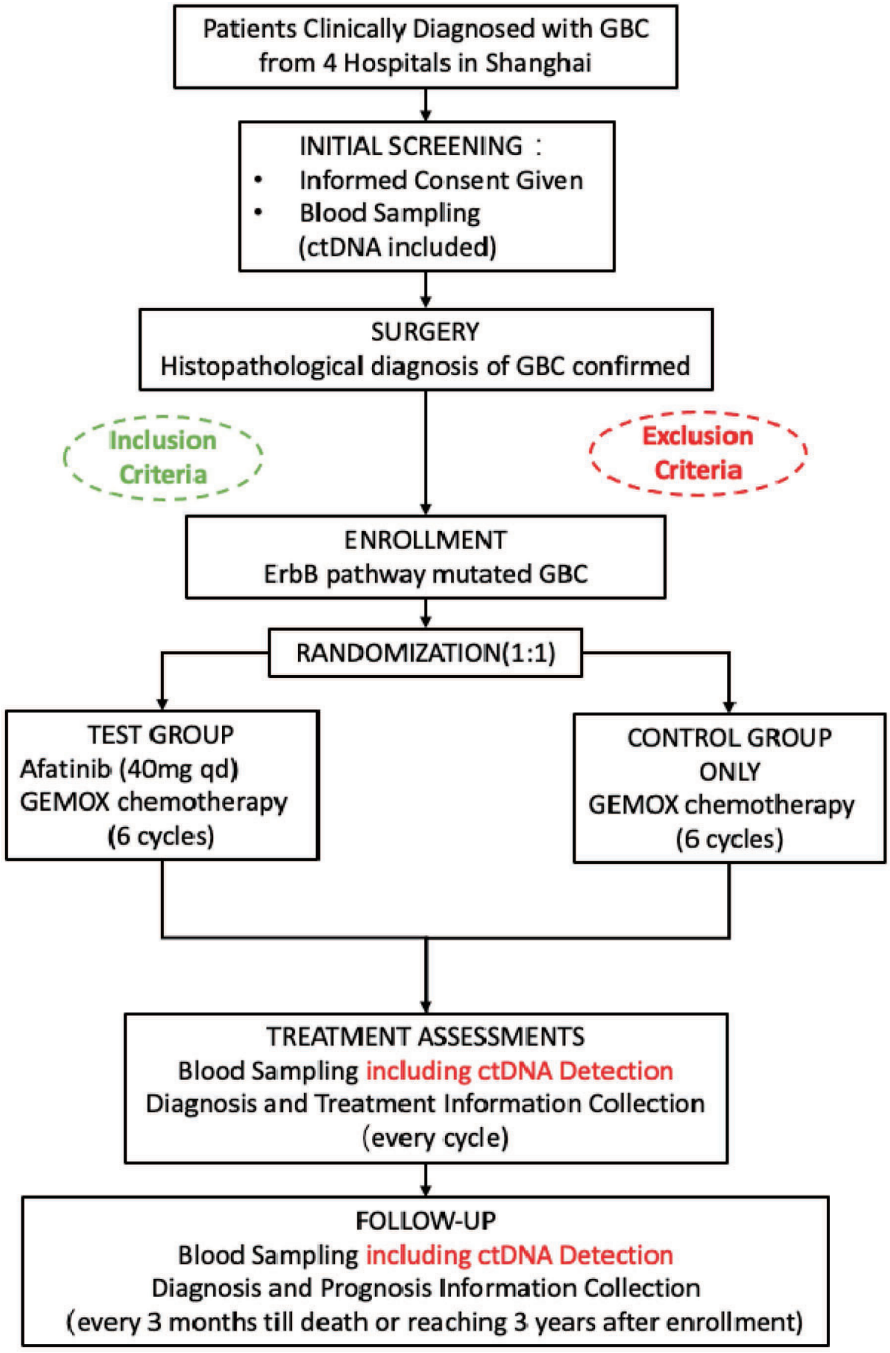

Figure 1 A clinical trial flow diagram. The study was designed as a randomised, open-label, multicentre clinical trial with a combined regimen of afatinib and chemotherapy drugs compared with chemotherapy drugs alone in patients with GBC with ErbB pathway mutations. ctDNA, circulating tumour DNA; GBC, gallbladder cancer; GEMOX, gemcitabine and oxaliplatin.

Study population

Inclusion criteria

Participants must:

- be pathologically diagnosed with GBC that is resectable;
- have ErbB pathway mutations (EGFR, ERBB2, ERBB3, ERBB4) both on surgical tumour tissue samples and preoperative blood samples based on NGS;
- sign written informed consent (if the participant is unable to read or sign, the legal representative shall sign the informed consent form. For participants who are incapable of expressing consent, their legal representative shall be told the introduction and explanation above, and sign the informed consent);
- age: 18–80 years old;
- have stable vital signs and an Eastern Cooperative Oncology Group performance status ≤1;
- show pathologically at least stage T2 or positive lymph nodes or R1 resection, according to the 8th American Joint Committee on Cancer tumour, node, metastases staging system, and have an evaluation of survival >18 weeks;

Table 1 Summary of drugs

Gemcitabine	Intravenous	1000 mg/m^2	Day 1, day 8
Oxaliplatin	Intravenous	100 mg/m^2	Day 1
Afatinib	Oral	40 mg	Once daily, from day 1 to day 21

- have important organs to be functional including bone marrow, kidney and liver: leucocytes >3000/μL, with an absolute neutrophil count >1500/μL, platelets >75 000/μL, haemoglobin ≥90 g/L, total bilirubin ≤3.0×institutional upper limit of the normal (ULN), aspartate aminotransferase/alanine transaminase levels ≤5×institutional ULN, creatinine clearance ≥30 mL/min;
- agree to use adequate contraception prior to and during the study specific for women bearing child and men.

Exclusion criteria

Participants with any of the following conditions or characteristics are excluded:

- All without presence of ErbB pathway mutations either in tumour tissue samples or in blood samples.
- Have targeted therapy or chemotherapy before enrolment. Have experienced radiotherapy but have not progressed prior to this study.
- Participate in other therapeutic/interventional clinical trials.
- Have not been disease-free for at least 5 years of other cancers prior to registration, except for curatively treated cervical cancer in situ and non-melanoma skin cancer.
- Have uncontrolled concurrent illness including but not limited to: uncontrolled congestive heart failure (New York Heart Association class ≥3), unstable angina pectoris, uncontrolled cardiac arrhythmia, uncontrolled hypertension (defined by systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg despite optimal medical management).
- Are ongoing or active infection.
- Have uncontrolled diabetes.
- Have active autoimmune system diseases requiring long-term use of steroids.
- Have any history of organ allograft.
- Experience substance abuse, medical, psychological or social conditions that may interfere with the patient's ability to understand informed consent and participation in the study or evaluation of the study results.
- Keep any serious illness or medical conditions that are not suitable for the study.

Withdrawal criteria

- Participants or their legal representative (such as a parent or legal guardian) withdraw the informed consent.
- Participants loss to follow-up.
- The sponsor suspends the study.

Termination criteria

Termination of study does not mean withdrawal from the study. Participants who terminate the study must continue to complete the remaining follow-up as required by the protocol. Participants must stop receiving any treatment from the study when any of the following conditions occurs:

- Participants withdraw from the trial on their own: consciously think the effect is not as good as prospect; cannot tolerate some adverse events (AEs).
- GBC relapse or metastasis during the treatment cycles.
- Participants whose condition changes after inclusion and do not meet the inclusion criteria anymore.
- Unexpected and intolerable AEs happen after the doctor's judgement.
- If an uncontrollable factor affects the trial process and/or the interpretation of the trial results significantly, the researcher must suspend the treatment.

Study contents

Sample size calculation

We expect that the 3-year DFS rate for patients in the experimental group can rise to about 52% given that the study indicates that the 3-year DFS rate for patients with GBC in the control group is approximately 21%.[13 14 28] The trial is designed with a two-sided significance alpha level of 0.05 and an estimated 90% power. Calculated by PASS 11, this trial requires 46 patients to be enrolled in each group. Considering a 10% drop-off rate, a total sample size of 102 is required in order to have a 90% probability of drawing a conclusive conclusion about the difference in the 3-year DFS rate between two groups.

The annual average number of visits for resectable GBC is about 40 in each hospital and the frequency of ErbB pathway mutations is roughly 36.8%. About 59 patients from 4 hospitals are expected to have GBC with ErbB pathway mutations each year. Within 3 years, it is anticipated that at least 102 patients will have signed up for the study, taking into account factors for all potential reasons for non-participation, such as patient refusal or abrupt termination.

Randomisation

Patients are randomly assigned in a ratio of 1:1 to either the control or intervention arm in a double-blinded manner. Briefly, administrators at each centre assign a random number in an envelope. When the patient is enrolled and given an envelope, the commissioner will provide the participant's medical record required for the study to a new file with corresponding number. Researchers will be only accessible to this new file with assigned number throughout the study.

Intervention to be measured

The treatment involves up to six cycles of a 21-day cycle. All participants will receive GEMOX, as conventional chemotherapy. For participants in experimental group, they will

also receive afatinib, as defined in table 1. When the clinician observes that the participant shows the indication of terminating chemotherapy and has completed for more than four cycles, the participant is deemed to finish the trial. If the participant during the course requests to end the test, the case will be terminated at any time.

Sample collection

In our study, both blood and tissue samples are collected to detect ErbB pathway mutations (EGFR, ERBB2, ERBB3, ERBB4) through NGS.

Blood samples

An amount of 10 mL of venous blood samples is collected from patients with GBC at scheduled timepoints (see online supplemental appendix 1 for a detailed time schedule of study) in Cell-Free DNA Blood Collection Tubes (Streck, USA). Samples are centrifuged to extract cfDNA to detect mutations.

Tissue samples

Tumour and paracancerous tissue samples are collected from resectable patients during surgery, cryopreserved in liquid nitrogen and stored in the biobank of Renji Hospital, Shanghai Jiao Tong University School of Medicine. Formalin-fixed and paraffin-embedded tumour sections are collected as well for further study.

Study end points

Primary end point

The primary end point was 3-year DFS rate. The DFS will be reached when GBC relapses indicated by contrast-enhanced MRI/CT in the enrolled cases. And the 3-year DFS rate was considered as the proportion of patients without recurrence in 3-year follow-up.

Secondary end points

Secondary end points included 3-year OS rate, safety and exploratory translational end points of ctDNA prognostic value. After tumour recurrence, the patients will continue to be followed up every 3 months till death or reaching 3 years after enrolment (see online supplemental appendix 1 for a detailed time schedule of study for participants) to assess 3-year OS rate. Safety was evaluated according to AEs, serious AEs (SAEs) and adverse drug reactions (ADR) rate.

Statistical analysis

Effectiveness analysis

The 3-year DFS rate and the 3-year OS rate of the two groups will be calculated, respectively. The log-rank test and the Kaplan-Meier curve of the DFS and OS are used for comparison between the two groups. Stratification factors and other important data related to clinical prognosis can be transformed into categorical variables for univariate Cox regression analysis; variables with clinical and statistical significance are incorporated as covariates into the multivariate Cox proportional hazard model to calculate HR and 95% CI.

Sensitivity, specificity, kappa value and correlation coefficient will be used to evaluate the prognostic value of plasma ctDNA test for patients with GBC treated with afatinib.

Safety analysis

Descriptive study will be mainly performed to analyse AEs, SAEs and ADR according to the Common Terminology Criteria for Adverse Events V.5.0. Definitions of different types of AEs and ADR are listed in online supplemental appendix 2. The incidence of AEs and ADR and their 95% CIs will be calculated. And every AE, SAE and ADR case will be listed in detail.

Data management

Source data from the trial are locally stored in electronic case report forms (eCRF) via Oracle Clinical/Remote Data Capture (OC/RDC) system by clinical researchers. The eCRF is activated when the patient is enrolled. After all subjects complete the trial and the medical records load into the system, the principal investigator, sponsors, statistical analysts and data administrator will review the data and confirm that the database is accurate and complete. Finally, the data are stored in a computer that needs authorised personnel to access once providing a code number.

During the study, the clinical monitors appointed by the sponsor regularly perform on-site audit to ensure the authenticity of the documents and the protocol to be strictly followed.

Ethics and dissemination

Ethical considerations

The principal investigator ensures that this study conforms to the Declaration of Helsinki or the laws and regulations of the country, whichever provides the greater protection to the patient. Ethical approval has been obtained from both Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (XHEC-C-2019-023-2) and Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (RA-2021-641). Participants are required to provide written informed consent (see an example of informed consent form in online supplemental materials.

Dissemination

The protocol and the trial results, even inconclusive, will be presented at national and international scientific meetings, and published in peer-reviewed journals. Genomic data will be made available in public open data sets.

Patient and public involvement

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

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Contributors YBL is the principal investigator steering the study, and responsible for funding acquisition and supervision of the study. MY, YZ and YSL wrote and revised the manuscript. MY, YZ, YSL and XC are responsible for data collection and coordination. FL is responsible for data analysis. MY, YSL, WW, X-AW, ML and YL are responsible for the study design. All authors reviewed the manuscript for intellectual content and approved the final version of the report.

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Competing interests None declared.

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