Randomized, double-blind, placebo-controlled, multicenter phase III clinical trial of Anlotinib Hydrochloride Capsules

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(The following ranking is in no particular order)

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Fujian Provincial Cancer Hospital  Beijing Union Medical College Hospital
First Affiliated Hospital of Guangzhou Medical University  Beijing Friendship Hospital of Capital Medical University
First Affiliated Hospital of Shantou University Medical College  Liaoning Province Cancer Hospital
Hunan Province Cancer Hospital  Affiliated Cancer Hospital, Harbin Medical University
Jiangxi Province Cancer Hospital  Shandong Province Cancer Hospital
Second Affiliated Hospital of Nanchang University  Qilu Hospital of Shandong University
Yunnan Province Cancer Hospital  Fourth Hospital of Hebei Medical University
Affiliated Hospital of Xuzhou Medical College  Linyi Cancer Hospital
Lanzhou Military General Hospital  Henan Province Cancer Hospital
Chongqing Cancer Hospital  Tangdu Hospital of Fourth Military Medical University
Affiliated Tumor Hospital, Xinjiang Medical University  First Affiliated Hospital of Xi’an Jiao Tong University
Qingdao University Hospital
General Hospital of Tianjin Medical University
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI</td>
<td>angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>ANC</td>
<td>neutrophils</td>
</tr>
<tr>
<td>APTT</td>
<td>activated partial thromboplastin time</td>
</tr>
<tr>
<td>ARB</td>
<td>angiotensin II receptor antagonist</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>bFGF</td>
<td>basic fibroblast growth factor</td>
</tr>
<tr>
<td>BSC</td>
<td>best supportive treatment</td>
</tr>
<tr>
<td>BUN</td>
<td>urea nitrogen</td>
</tr>
<tr>
<td>B ultrasound</td>
<td>B-type ultrasound examination</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
</tr>
<tr>
<td>Cl</td>
<td>chloride</td>
</tr>
<tr>
<td>Cr</td>
<td>creatinine</td>
</tr>
<tr>
<td>CR</td>
<td>complete remission</td>
</tr>
<tr>
<td>CRF</td>
<td>case report form</td>
</tr>
<tr>
<td>CT</td>
<td>computer X-ray tomography</td>
</tr>
<tr>
<td>DCR</td>
<td>disease control rate</td>
</tr>
<tr>
<td>DLT</td>
<td>dose-limited toxicity</td>
</tr>
<tr>
<td>DRQ</td>
<td>questions and answers</td>
</tr>
<tr>
<td>ECOG PS</td>
<td>physical status scoring criteria</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>GCP</td>
<td>code for quality management of drug clinical trials</td>
</tr>
<tr>
<td>FAS</td>
<td>full analysis set</td>
</tr>
<tr>
<td>FGFR</td>
<td>fibroblast growth factor receptor</td>
</tr>
<tr>
<td>Glu</td>
<td>glucose</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>HFS</td>
<td>hand, foot and skin reactions</td>
</tr>
<tr>
<td>ITT</td>
<td>Intentionality analysis</td>
</tr>
<tr>
<td>K</td>
<td>potassium</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>half lethal dose</td>
</tr>
<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>MRI</td>
<td>nuclear magnetic resonance imaging</td>
</tr>
</tbody>
</table>

**Note:**

- **Na:** sodium
- **NCCN:** National Cancer Comprehensive Network
- **NCI-CTC:** American Cancer Institute General Toxicity Standard
- **NSCLC:** non-small cell lung cancer
- **NYHA:** New York Heart Association
- **ORR:** objective remission rate
- **OS:** overall survival
- **PFS:** progression-free survival
- **PD:** progress of disease
- **PDGFR:** Platelet-derived growth factor receptor
- **PI:** principal Investigator
- **PLT:** platelets
- **PR:** partial relief
- **PRO:** protein
- **PPS:** per protocol set
- **PT:** prothrombin time
- **Qd:** once a day
- **QoL:** quality of life
- **RBC:** red blood cells
- **RECIST:** evaluation criteria for efficacy of solid tumors
- **γ-GT:** γ-glutamyltransferase
- **SAE:** serious adverse events
- **SAS:** security analysis set
- **SD:** steady
- **TT:** thrombin time
- **TTP:** time of tumor progression
- **ULN:** upper limit normal
- **UA:** uric acid
- **VEGFR:** vascular endothelial growth factor receptor
- **WBC:** white blood cells
- **WT:** wild-type
**Trial protocol Summary**

**Title**
Randomized, double-blinded, placebo-controlled, multicenter phase III clinical trial of treatment of advanced non-small cell lung cancer with Anlotinib Hydrochloride Capsules

**Program number**
ALTN-03-IIB

**Applicant**
CHIA TAI TIANQING PHARMACEUTICAL GROUP CO., LTD

**Subjects**
Patients with advanced non-small cell lung cancer

The efficacy and safety of Anlotinib Hydrochloride in the treatment of patients with advanced non-small cell lung cancer was evaluated in comparison to placebo.

**Research purposes**
Primary endpoint: Overall survival (OS)
Secondary endpoint: progression-free survival (PFS), objective remission rate (ORR = CR + PR), disease control rate (DCR = CR + PR + SD), quality of life score, biomarker evaluation, safety index (P < 0.05)

**Adverse Reaction Criteria**
NCI CTC AE V4.02 was used to evaluate the adverse drug reactions

**Study Design**
Multicenter, randomization, double-blind, placebo-controlled design

**Number of groups planned**
2:1, 300 in the test group and 150 in the control group

Qualified non-small cell lung cancer patients were treated with Anlotinib Hydrochloride capsules/placebo that were administered daily, each time 12mg/0 mg dose, continuously for 2 weeks and 1 week off. The therapeutic effect was assessed every 6 weeks (42 days). Those patients with disease control rate (CR + PR + SD) who can tolerate adverse reactions were given sustained medication. The study was terminated when the investigators judged that the patients are not suitable for continued medication or disease progression (PD).

Other anti-tumor treatments cannot be performed during treatment.

**Principal Investigator**
Professor Baohui Han, Professor Kai Li

**Inclusion Criteria:**
Those who fulfill the following criteria are eligible for this trial:

1) Patients who voluntarily participate in this study and sign the informed consent;
2) Pathologically diagnosed as late (IIIB/IV) non-small cell lung cancer with measurable lesions;
3) Patients who receive ≥2 chemotherapy regimens or are unable to tolerate the treatment;
4) Detection of genotype by providing detectable specimens (tissue or cancerous pleural effusion) prior to enrollment * patients with negative EGFR and ALK gene test results; or positive test results for resistance after targeted drug therapy or intolerable in patients;
5) 18–75-year-old; ECOG PS score: 0–1; expected survival time ≥3 months;
6) Major organ functions meet the following criteria within 7 days prior to treatment:
   (1) blood routine examination criteria (14 days without blood transfusion):
      a) hemoglobin (HB) ≥90g/L;
      b) neutrophil absolute (ANC) ≥1.5×10^9/L;
      c) platelet (PLT) ≥80×10^9/L
   (2) biochemical tests to meet the following criteria:
      a) total bilirubin (TBIL) ≤1.5-fold of the upper limit of normal (ULN);
      b) alanine aminotransferase (ALT) and aspartate aminotransferase AST ≤2.5×ULN; if the case of liver metastasis, ALT and AST ≤5×ULN;
      c) serum creatinine (Cr) ≤1.5×ULN, creatinine clearance (CCr) ≥60mL/min;
   (3) Doppler ultrasound evaluation: left ventricular ejection fraction (LVEF) ≥ normal low (50%).

Women of childbearing age should agree to the usage of contraceptive measures (such as intrauterine devices, birth control pills, or condoms) during the study period and for 6 months after the end of the study, the serum or urine test is negative within 7 days prior to the study, and should be non-lactating patients. Males should agree to the usage of contraceptives during the study and within 6 months after the end of the study period.

**Exclusion criteria:** Patients with any of the following criteria will not be enrolled in this study:

1) patients who had previously used Anlotinib hydrochloride capsules;
2) small cell lung cancer (including small cell carcinoma and non-small cell carcinoma mixed lung cancer);
3) patients who were tested positive for EGFR and ALK mutations but did not use the
relevant targeted drug;

4) central type, with empty lung squamous cell carcinoma or non-small cell lung cancer with hemoptysis (>50 mL/day);

5) other malignancies within 5 years or simultaneously, cured cervical carcinoma in situ, non-melanoma skin cancer, and superficial bladder tumor except [Ta (non-invasive tumor), Tis (carcinoma in situ), and T1 (tumor infiltration basement membrane)];

6) systemic anti-tumor therapy, including cytotoxic therapy, signal transduction inhibitors, immunotherapy (or 6 weeks prior to administration of the test drug) was planned within 4 weeks prior to or during this study. Intravenous radiotherapy (EF-RT) was performed 4 weeks prior to grouping or restricted radiotherapy for assessing tumor lesions within 2 weeks before grouping;

7) non-mitigated toxicity higher than CTC AE level 1, except hair loss due to any previous treatment;

8) patients have a variety of factors that affect oral medication (such as inability to swallow, chronic diarrhea, and intestinal obstruction;

9) patients with pleural effusion or ascites, causing respiratory syndrome (≥CTC AE level 2 dyspnea);

10) patients with brain metastasis symptoms or controlled symptoms for <2 months;

11) patients with any severe and/or uncontrolled disease, including:

   a) blood pressure that is not ideal (systolic blood pressure ≥150 mmHg, diastolic blood pressure ≥100 mmHg);

   b) myocardial ischemic or myocardial infarction, arrhythmia (including QTC ≥480 ms), and ≥2 levels of congestive heart failure (NYHA classification);

   c) active or uncontrollable severe infection (≥CTC AE level 2 infection);

   d) liver cirrhosis, decompensated liver disease, active hepatitis, or chronic hepatitis to be treated with antiretroviral therapy;

   e) renal failure requires hemodialysis or peritoneal dialysis;

   f) history of immunodeficiency, including HIV-positive or other acquired, congenital immune deficiency disease, or a history of organ transplantation;

   g) poor control of diabetes (fasting blood glucose (FBG) >10 mmol/L);

   h) routine urine examination indicates protein ≥ ++, and confirmed 24 h urine protein >1.0 g;
i) patients presenting seizure and need treatment;

12) received major surgical treatment within 28 days prior to grouping, with a biopsy or a significant traumatic injury;

13) imaging shows that the tumor has violated crucial vasculature or the researchers determine that the tumor is likely to invade major blood vessels and cause fatal bleeding during the follow-up study;

14) irrespective of severity, patients show any signs of bleeding or medical history; in the first 4 weeks before the group, patients have any bleeding or bleeding events (≥CTCAE 3) with non-healing wounds, ulcers, or fractures;

15) patients have active/venous thrombotic events, such as cerebrovascular accident (including temporary ischemic attack), deep vein thrombosis, and pulmonary embolism within 6 months;

16) patients have a history of mental illness and cannot quit or have mental disorders;

17) patients participated in other anti-tumor drug clinical trials within 4 weeks;

18) according to the researcher's judgment, patients have concomitant diseases, which might be detrimental to the patient's safety or affect the patient's ability to complete the study.
1 Research background

Protein tyrosine kinase (PTK) signaling pathway is associated with the proliferation, differentiation, and migration of tumor cells. Interference or blockade of tyrosine kinase pathway can be used to influence the growth of tumor cells. Thus, screening of PTK inhibitors could prove to be a novel approach for the development of anti-neoplastic drugs.

Anlotinib hydrochloride is a multi-target receptor tyrosine kinase inhibitor with specific inhibitory activity on vascular formation-related molecules such as VEGFR1/2/3, FGFR1/2/3, and other proliferation-associated kinases such as PDGFRα/β, c-Kit, and Ret. ① The inhibition of angiogenic kinase spectrum is broad (for example, Met, FGFR1/2/3). ② Some of the kinase targets, such as Aurora-B, c-FMS, and DDR1, can also be inhibited significantly. ③ The drug can also inhibit the activity of a variety of kinase mutants, such as PDGFRα, cKit, Met, and EGFR; the inhibitory activity to the mutant is more robust than the WT.

Chia Tai Tianqing Pharmaceutical Group Co., Ltd. developed the declaration of Anlotinib hydrochloride capsules, with independent intellectual property rights. On March 2011, the State Food and Drug Administration clinical research approval (SFDA: 2011L00661) was received that permitted the clinical research. From September 2013, Professor Han Baohui led the national team of experts to work together to complete the non-small cell lung cancer (NSCLC) phase II preliminary efficacy and safety studies at the Shanghai Jiao Tong University Affiliated Chest Hospital. In February 2012, the Shanghai Chest Hospital Ethics Committee approved the phase IIB clinical study. In March 2016, the company obtained the State Food and Drug Administration phase III clinical research approval (SFDA: 2016L03480/79/78). According to the national GCP requirements and drug registration management approach, new drug clinical research guidelines and other relevant laws or regulations, registration test program for advanced NSCLC is now developed. Clinical studies were conducted after approval by the Ethics Committee for approval.

1.1 Introduction to Drugs

Anlotinib hydrochloride is made in hard capsules. The structural formula of the main component, Anlotinib is as follows:

![Molecular structure of Anlotinib hydrochloride](image)

Molecular formula: C_{23}H_{22}FN_{3}O_{3}·2HCl; Molecular weight: 480.36
1.2 Overview of general preclinical pharmacology

In the pharmacological study of Anlotinib hydrochloride, mice were administered 2, 6, and 20 mg/kg for 24 h, and no significant effect was observed in the general behavior and spontaneous activity. 0.3, 0.9, and 3 mg/kg dose was administered, and no significant effect was observed on the systolic blood pressure, diastolic blood pressure, and mean arterial pressure in the anesthetized dogs during the 240-min observation period. No significant effect was noted on arrhythmia, heart rate, electrocardiogram PR, QRS, QTC, respiratory rate, and respiratory depth.

Overview of preclinical pharmacodynamics

Anlotinib significantly inhibited the growth of transplanted tumor in nude mice from colon cancer SW-620, ovarian cancer SK-OV-3, lung cancer Calu-3, hepatocellular carcinoma SMMC-7721, and led to partial shrinkage of the tumor. For renal cell carcinoma Caki-1 and glioma U87MG also exhibits an optimal effect. The effect on the above tumor model is equivalent to sunitinib; however, it was significantly stronger than sorafenib. Whether it is administered orally at a relatively low dose once a day, continuously for 21 days or a high dose once a day, for 10 consecutive days, Anlotinib hydrochloride exerted a significant effect, suggesting that the flexibility of clinical medication program. The tumor-bearing mice adequately tolerated Anlotinib hydrochloride; however, no significant reduction in body weight was observed. Anlotinib hydrochloride combined with 5-fluorouracil and oxaliplatin did not show any synergistic effect, at least in the colon cancer SW-620 model; no synergistic effect was noted between them and no obvious toxic superposition was observed.

1.4 Overview of preclinical toxicology

1.4.1 Animal acute toxicity test

ICR mice were administered Anlotinib once, and the LD50 was 1435.9 mg/kg for 14 days with 1365.5–5474.6 mg/kg for 95% confidential interval (CI). LD50 was 982.8 mg/kg for 22 days, with 95% CI 657.28–1180.3 mg/kg. The potential target organs included liver, gallbladder, small intestine (mainly duodenum), kidney, spleen, and testis. Beagle dogs were orally administered Anlotinib and observed for 14 days after treatment. The maximum tolerable dose was 20 mg/kg and the minimum lethal dose was 67.5 mg/kg. Drug-related toxic and side effects were similar to those reported in the literature for the same drug (P<0.05).

1.4.2 Long-term animal toxicity test.

SD rats were administered the drug in an intragastric manner for 13 weeks and then
discontinued drug for 6 weeks. The dose of NOAEL was 0.25 mg/kg and the toxic dose was ≥1 mg/kg. The toxicity was targeted to the organs such as teeth, liver, gallbladder system, duodenum, pancreas, adrenal gland, kidney, and blood system. Liver and gallbladder system, as well as, teeth and blood system lesions recovered significantly after stopping the treatment; the other organs were fully restored.

Beagle dogs were administered Anlotinib in an intragastric manner for 13 weeks and recovered for 4 weeks after the treatment. The main toxicity of 0.40 mg/kg (AUC_{0→8h}: 195 ng/h/mL) included gastrointestinal reaction, slight slowing of the heart rate slightly, and effects on the liver and kidney function. NOAEL was 0.12 mg/kg (51.7 ng/h/mL for AUC 0–8 h). After withdrawal, the recovery lasted for 4 weeks, and the toxicity recovered; no delayed toxicity was observed.

1.4.3 Mutagenic test and literature
Anlotinib did not induce (1) mutagenicity to *Salmonella typhimurium* in the *Salmonella typhimurium* response mutant test (Ames), (2) chromosomal aberrations in Chinese hamster lung fibroblasts (CHL) cells in chromosome aberration tests. It did not increase the micronucleus rate of the bone marrow polychromatic erythrocytes in mice.

1.4.4 Reproductive toxicity test and literature
SD pregnant rats were administered Anlotinib in an intragastric manner, and the no toxic reaction dose (NOAEL) of embryo development was <0.3 mg/kg.

1.5 Overview of preclinical pharmacokinetics
Pharmacokinetic parameters: Plasma pharmacokinetic studies in rats and dogs showed that after oral administration, Anlotinib hydrochloride was absorbed slowly in the gastrointestinal tract. Bioavailability: Approximately 34% in rats and 67% in dogs.

Plasma protein binding rate: Anlotinib hydrochloride has high protein binding rates with rat, dog, and human plasma protein, which are 97%, 96%, and 93%, respectively, which are independent of the drug concentration.

Tissue distribution: The concentration of drugs in the tissues was higher than that in the blood at the same time point, and the distribution of the Anlotinib did not differ significantly in various tissues. Among them, the peak concentration in the lung tissue was about 184- (male rats) and 331- (female) fold as the plasma concentration. The peak concentration in the spleen, adrenal gland, large intestine, small intestine, ovary, and kidney was 65–144-fold of the blood concentration; whereas, that in the uterus, heart, liver, stomach, bladder, bone marrow, and fat tissues was 20–47-fold. The peak concentration in the skeletal muscle, pancreas, testis, and brain was 1.7–13-fold as the plasma concentration.
Tissue distribution of the tumor-bearing mice: After oral administration, the concentration of Anlotinib in the tissue of nude mice was maximal at 4 h. The levels of AUC in the tissues were positively correlated with the dose; the AUC of the liver tissue was linearly related to the dose. The highest concentration of the drug was found in the lung and liver (about 10–14-fold as the plasma AUC), followed by kidney (about 5.9–8.6-fold as the plasma AUC). On the other hand, the AUC of the Anlotinib tumor was about 2.4–2.6-fold as that of the plasma. The concentration in colon and plasma are similar (about 0.8–1.0-fold as plasma AUC).

Excretion: The cumulative excrement (Cum.Ae) of Anlotinib hydrochloride excreted with urine (0–72 h), feces (0–72 h), and bile (0–24 h) was <5% dose of the injection (1.5 mg/kg), thereby suggesting that metabolic transformation is the primary elimination pathway for Anlotinib.

Metabolites: 23 metabolites were measured in the rat bile, 16 in rat urine, 12 in feces, and 8 in plasma. M16, M21, and M23 were the three most important plasma metabolites, and the cumulative excretion of M16 in urine and bile was the highest, while M18 was the highest in feces.

Metabolizing enzyme activity: Anlotinib had moderate and weak reversible inhibition of 7 CYP enzymes (8 substrates) in human liver microsomes with specific intensity (IC50)>100 μM (CYP1A2), 4.96 μM (CYP2B6), 3.71 μM (CYP2C8), 1.56 μM (CYP2C9), 1.67 μM (CYP2C19), 18.5 μM (CYP 2D6), 9.56 μM (CYP3A4-midazolam), and 2.09 μM (CYP3A4-testosterone). The in vitro effect of Anlotinib on the induction CYP1A2, CYP2B6, and CYP3A4, the 3 subtypes of CYP450 in human hepatocytes, suggested that the clinical application of Anlotinib did not exert distinct effects on CYP1A2, CYP2B6, and CYP3A4.

1.6 Phase I tolerance and preliminary outcome

Phase I tolerance and preliminary efficacy was examined in clearly diagnosed patients. The tolerance of the use of Anlotinib hydrochloride capsules was observed in patients with solid malignant tumors that failed with standard treatment or lack of standard treatment.

1.6.1 A drug tolerance with 2 weeks on treatment followed by 1 week off treatment

The initial study found that half-life to eliminate Anlotinib hydrochloride capsules in the body was extremely long, as after continuous medication, the drug accumulated in the patient’s body. Several adverse reactions occurred when the cycle of medication is 10 mg for 28 days. Considering the patient's tolerance, we explored the program of 3 weeks (21 days; 2 weeks medication and 1 week off) in order to study the tolerance over a minimum of 2 cycles (42 days). The results are as follows:
This study observed a total of 3 patients’ medication; adverse reactions in 2 cycles are as follows:

<table>
<thead>
<tr>
<th>Items</th>
<th>N = 3</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one adverse event</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Fat, amylase increased</td>
<td>1</td>
<td>Level III</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>Level II</td>
</tr>
<tr>
<td>Increased blood pressure</td>
<td>1</td>
<td>Level I</td>
</tr>
<tr>
<td>Diarrhea, abdominal pain</td>
<td>1</td>
<td>Level I</td>
</tr>
<tr>
<td>Voice hoarse</td>
<td>2</td>
<td>Level I</td>
</tr>
</tbody>
</table>

One patient with abnormal amylase showed an abnormal increase in lipase and amylase fluctuating in the range of level 1–3 after 5 cycles of treatment. The lipase increased to the highest level 4, and amylase increased to level 3. The patient self-administered anti-inflammatory gallbladder tablets until tumor progression (118 weeks) and was removed from the group.

After 2 cycles of medication, 1 patient had level 2 adverse reactions of diarrhea and leukopenia. On the other hand, level 1 adverse reactions cases included: 1 case of headache/dizziness, 1 case of hand and foot skin reaction, 1 case of leukopenia, proteinuria, and elevated transaminase each.

<table>
<thead>
<tr>
<th>Items</th>
<th>N = 3</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one adverse event</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Fatigue (DLT)</td>
<td>1</td>
<td>Level III</td>
</tr>
<tr>
<td>Elevated blood pressure (DLT)</td>
<td>1</td>
<td>Level III</td>
</tr>
<tr>
<td>hypothyroid</td>
<td>2</td>
<td>Level II</td>
</tr>
<tr>
<td>Elevation of ALT, AST</td>
<td>1</td>
<td>Level II</td>
</tr>
<tr>
<td>Bleeding</td>
<td>1</td>
<td>Level II</td>
</tr>
<tr>
<td>Diarrhea, abdominal pain</td>
<td>1</td>
<td>Level I</td>
</tr>
</tbody>
</table>
1 case presented the following adverse reactions after 2 cycles of treatment, including:

- level 2 adverse bleeding (dose was then decreased to 12 mg for continued observation for the effect of medication),
- increased bilirubin, high blood pressure, elevated transaminase,
- hypothyroidism, level 1 adverse reaction of proteinuria, hoarseness, diarrhea, toothache, and tinnitus.

12 mg qd program of 2 weeks on treatment followed by 1 week off treatment:

Taking into account the large qd range of 10 and 16 mg, the researchers further investigated the maximum tolerated dose that is 12mg qd dose. After observing the tolerance in 21 patients, the 12 mg qd program of continuous 2 weeks medication and 1-week withdrawal was recommended for the follow-up study. Therefore, 12 mg qd is the maximum tolerated dose.

Medication-related adverse reactions that occurred during the study process (as of July 1, 2014, 8 patients are still administering the medication), according to the previous two cycles and the entire process statistics, are shown in Table 1-3. In this dose group, 5 patients presented level 3 adverse reactions: specifically, the TG was higher in the 24th patient, the hypertensive condition and the hand and foot skin reactions were seen in the 29th patient, the lipase increased in the 31st patient, the bilirubin was elevated in the 32nd patients, and the 34th patient presented hypertension and elevated TGs.

Table 1-3. Adverse reactions in 2 cycles of 12 mg qd program
# Adverse reactions

<table>
<thead>
<tr>
<th>Adverse Reactions</th>
<th>Cases of Level I/II</th>
<th>Cases of Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First cycles</td>
<td>2 Full period of trial</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>2 (9.52%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Thyrotropin increased</td>
<td>2 (9.52%)</td>
<td>4 (19.05%)</td>
</tr>
<tr>
<td>ALT increased</td>
<td>6 (28.57%)</td>
<td>10 (47.62%)</td>
</tr>
<tr>
<td>AST increased</td>
<td>4 (19.05%)</td>
<td>9 (42.86%)</td>
</tr>
<tr>
<td>Creatinine increased</td>
<td>1 (4.76%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Total bilirubin increased</td>
<td>5 (23.81%)</td>
<td>8 (38.10%)</td>
</tr>
<tr>
<td>Direct bilirubin increased</td>
<td>3 (14.29%)</td>
<td>8 (38.10%)</td>
</tr>
<tr>
<td>Indirect bilirubin increased</td>
<td>4 (19.05%)</td>
<td>5 (23.81%)</td>
</tr>
<tr>
<td>Lipase</td>
<td>1 (4.76%)</td>
<td>5 (23.81%)</td>
</tr>
<tr>
<td>Blood Amylase</td>
<td>4 (19.05%)</td>
<td>9 (42.86%)</td>
</tr>
<tr>
<td>Myocardial zymogram abnormalities</td>
<td>2 (9.52%)</td>
<td>3 (14.29%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>3 (14.29%)</td>
<td>6 (28.57%)</td>
</tr>
<tr>
<td>Neutrophils decreased</td>
<td>0</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Platelet decreased</td>
<td>0</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0</td>
<td>1 (4.76%)</td>
</tr>
<tr>
<td>Urine occult blood</td>
<td>5 (23.81%)</td>
<td>8 (38.10%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5 (23.81%)</td>
<td>7 (33.33%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (28.57%)</td>
<td>7 (33.33%)</td>
</tr>
<tr>
<td>Voice hoarseness</td>
<td>3 (14.29%)</td>
<td>5 (23.81%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (14.29%)</td>
<td>3 (14.29%)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>1 (4.76%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Toothache</td>
<td>1 (4.76%)</td>
<td>4 (19.05%)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>1 (4.76%)</td>
<td>1 (4.76%)</td>
</tr>
<tr>
<td>Pain</td>
<td>4 (19.05%)</td>
<td>4 (19.05%)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>1 (4.76%)</td>
<td>4 (19.05%)</td>
</tr>
<tr>
<td>Dizziness/Headache</td>
<td>1 (4.76%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Fever</td>
<td>1 (4.76%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Oral mucositis</td>
<td>0</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>1 (4.76%)</td>
<td>1 (4.76%)</td>
</tr>
<tr>
<td>Early stroke</td>
<td>0</td>
<td>1 (4.76%)</td>
</tr>
</tbody>
</table>

1.7 Pharmacokinetic studies in human body

According to the Phase I clinical trial program, all the solid tumor subjects who
participated in the tolerance study were monitored for plasma concentration. The LC-MS/MS technique was used to estimate the pharmacokinetic parameters of Anlotinib hydrochloride capsules.

1.7.1 Pharmacokinetics of a single administration

Eligible Subjects orally administered an Anlotinib capsule for a single pharmacokinetic study. A total of 19 subjects in 3 dose groups (10, 16, and 12 mg) were tested for the plasma concentration. The average medication time curves for each dose group were shown in the left panel of Fig. 1. The pharmacokinetic parameters are shown in Table 1-4.

After a single oral administration of Anlotinib hydrochloride, the concentration of the drug in the plasma reached a high level at 4–8 h, with an extremely long half-life. The levels of Anlotinib [area under the curve (AUC) 0–168 h] were positively correlated with the administered dose; however, the linear correlation was uncertain at the dose range of 10, 12, and 16 mg/person.

After oral administration, the cumulative urinary excretion of Anlotinib accounts for less 4% of the oral dose.

Table 1-4. The pharmacokinetic study of Anlotinib hydrochloride capsules

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Mean ± SD (RSD%)</th>
<th>10 mg/person (n=4)</th>
<th>12 mg/person (n=11)</th>
<th>16 mg/person (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>5.78 ± 2.76 (47.7)</td>
<td>10.5 ± 2.9 (28.0)</td>
<td>15.8 ± 3.2 (20.1)</td>
<td></td>
</tr>
<tr>
<td>Body weight checked ( C_{\text{max}} )</td>
<td>0.08 ± 0.05 (58.2)</td>
<td>0.15 ± 0.05 (34.0)</td>
<td>0.28 ± 0.09 (32.0)</td>
<td></td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h; p.o)</td>
<td>6.0 ± 4.4 (73.3)</td>
<td>7.3 ± 3.3 (45.6)</td>
<td>11.0 ± 8.9 (80.6)</td>
<td></td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng/h/mL)</td>
<td>385 ± 175 (45.7)</td>
<td>875 ± 240 (27.5)</td>
<td>1290 ± 384 (29.7)</td>
<td></td>
</tr>
<tr>
<td>Body weight checked AUC</td>
<td>5.63 ± 3.37 (59.8)</td>
<td>12.8 ± 3.6 (28.1)</td>
<td>23.2 ± 10.1 (43.7)</td>
<td></td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng/h/mL)</td>
<td>562 ± 328 (58.3)</td>
<td>1066 ± 263 (24.6)</td>
<td>1585 ± 470 (29.6)</td>
<td></td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>95.3 ± 21.7 (23)</td>
<td>116 ± 47 (40.5)</td>
<td>97.9 ± 14.8 (15.1)</td>
<td></td>
</tr>
</tbody>
</table>

1.7.2 Pharmacokinetics of continuous administration

Eligible subjects were administered medication once for a single pharmacokinetic monitoring, followed by at least 7 days clearance to the start of the pharmacokinetic studies for continuous medication. A treatment cycle included continuous medication for 2 weeks and 1-week withdrawal, and continuous monitoring lasted for at least 2 cycles. 21 subjects in the three dose groups (10 mg, 16 mg, and 12 mg) received Anlotinib hydrochloride capsules, and
blood concentrations were monitored. The average medication time curve for each dose group is shown in Fig. 1 right panel. The pharmacokinetic parameters estimated here are shown in Table 2.

After continuous administration, due to a prolonged half-life of Anlotinib in the human body before eliminated, the drug concentration in the plasma increases with the number of administrations. In order to control the Anlotinib concentration in the plasma post-administration, the program of 2 weeks on treatment followed by 1 week off treatment was employed. With the "two continuous one withdrawal" mode of administration, the Anlotinib concentration in the plasma peaked on day 14 post-administration. At the dosage of 10 and 12 mg/person/day, the Anlotinib concentration in the plasma was controlled at ≤100 ng/mL.

Table 1-5. Estimated Anlotinib pharmacokinetic parameters with program of 2 weeks on treatment followed by 1 week off treatment for 2 cycles

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Mean ± SD (RSD%)</th>
<th>10 mg/person/day (n=3)</th>
<th>12 mg/person/day (n=15)</th>
<th>16 mg/person/day (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td></td>
<td>65.2 ± 28.9 (44.3)</td>
<td>61.6 ± 16.3 (27)</td>
<td>93.7 ± 27.8 (30.0)</td>
</tr>
<tr>
<td>Body weight checked C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1.09 ± 0.48 (44)</td>
<td>0.96 ± 0.31 (32)</td>
<td>1.84 ± 0.65 (35.4)</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-42&lt;/sub&gt; day (ng/h/mL)</td>
<td>1550 ± 864 (58)</td>
<td>1467 ± 395 (27)</td>
<td>2237 ± 814 (36.4)</td>
<td></td>
</tr>
<tr>
<td>Body weight checked AUC&lt;sub&gt;0-42&lt;/sub&gt; day</td>
<td>25.9 ± 14.3 (56)</td>
<td>22.7 ± 6.7 (31)</td>
<td>44.3 ± 18.1 (40.9)</td>
<td></td>
</tr>
</tbody>
</table>

Body weight checked C<sub>max</sub>: C<sub>max</sub>/body weight; Body weight checked AUC: AUC/body weight.
Figure 1-1. Medication time curves for a single medication and 2 weeks on treatment followed by 1 week off treatment mode.

1.7.3 Effects of different diet on pharmacokinetic parameters

Using a random, double-cycle, self-cross design, 12 healthy subjects aged 18–40 years were randomly divided into two groups (fasting group and high-fat diet group), 6 in each group (equal number of men and women). The subjects were administered 5 mg Anlotinib hydrochloride capsules with fasting for 10 hours or postprandial. Patients were then eluted for 28 days, and patients from each group were swapped to another group and were administered 5 mg Anlotinib hydrochloride capsules.

The pharmacokinetic parameters of fasting or postprandial medication are similar, as described in following chart. The results of the study showed that the peak time of Anlotinib in the human body after high-fat diet was prolonged and the degree of drug absorption was slightly lower (about 80% in empty stomach group) as compared to that in fasting group. Therefore, medication is recommended under the fasting condition.
Table 1-6. Effects of different diet on pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>High fat diet group</th>
<th>Fasting group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>3.35 ± 1.35</td>
<td>3.90 ± 1.60</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>10.5 ± 6.7</td>
<td>9.3 ± 5.1</td>
</tr>
<tr>
<td>$\text{AUC}<em>{\text{last}}/\text{AUC}</em>{0-1}$ (ng/h/mL)</td>
<td>308 ± 120</td>
<td>377 ± 134</td>
</tr>
<tr>
<td>$\text{AUC}<em>{\text{inf}}/\text{AUC}</em>{0-\infty}$ (ng/h/mL)</td>
<td>392 ± 145</td>
<td>486 ± 158</td>
</tr>
<tr>
<td>$\text{MRT}<em>{\text{last}}/\text{MRT}</em>{0-1}$ (h)</td>
<td>83.7 ± 6.8</td>
<td>86.0 ± 6.5</td>
</tr>
<tr>
<td>$\text{MRT}<em>{\text{inf}}/\text{MRT}</em>{0-\infty}$ (h)</td>
<td>153.2 ± 43.7</td>
<td>161.7 ± 41.3</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>107.2 ± 27.4</td>
<td>113.2 ± 30.3</td>
</tr>
</tbody>
</table>

1.8 Treatment status of non-small cell lung cancer

Among all cancers, both male and female lung cancer in Western countries has the highest mortality rate. The overall prognosis is yet poor, and only 15% of the patients can survive >5 years. According to the 2012 China Cancer Registration Annual Report, the national tumor registration area of malignant tumor incidence of lung cancer stated an incidence rate of 53.57/10 million. The incidence of Lung cancer rank the first in males, and the second in females among malignant tumors. Men are susceptible 1.94-fold that of women.
The city areas experience 37.41% more incidences than the rural areas; during the same period of cancer deaths in men, women ranked first in lung cancer with the rate of 45.57/10 million, the mortality of males is 2.05-fold than that in females.

NSCLC accounts for 80% of the lung cancer incidences. The three major histological subtypes of squamous cell carcinoma, adenocarcinoma (including bronchioloalveolar carcinoma subtype), and large cell lung cancer account for about 80–85% of all lung cancers (others are small cell lung cancer). A majority of the NSCLC patients were treated with locally advanced or metastatic disease with an extremely low cure rate.

Standard treatment of NSCLC includes surgery, radiotherapy, chemotherapy, or a combination of these treatments. Surgery remains the basic treatment for early NSCLC (phase I, II, and some of the selected phase IIIA). Neoadjuvant chemotherapy or preoperative chemotherapy for stage I-IIA NSCLC is yet an experimental treatment. For locally advanced, non-surgical resection of stage III disease, currently, concurrent chemotherapy and chest radiotherapy is the standard treatment. Two-drug chemotherapy is preferred for the physical condition of 0-1 points of metastatic disease (IV) patients with standard treatment. Patients with metastatic disease of 2 points or age >70 years also benefit from systemic therapy: monotherapy (gemcitabine, vinorelbine, paclitaxel) or targeted therapy, which is efficacious than palliative treatment.

In recent years, individualized targeted therapy under the guidance of molecular pathology of lung cancer has become a part of NSCLC clinical standard treatment. It is a new potential method with unmatched superiority as compared to the conventional chemotherapy. The treatment after diagnosis of lung cancer by molecular targeting has undergone major changes in the detection of late lung cancer biopsy identifying the mutations in the genes; this is gradually becoming an important basis for the optimal individualized treatment.

1.9 Phase II clinical trial results of Anlotinib hydrochloride treatment of NSCLC

From August 2013, 13 research centers in the country, using a multi-center, randomized, double-blind, placebo 1:1 control design, observed 117 patients with pathologically diagnosed advanced (IIIB/ IV) NSCLC who had been treated with ≥3 lines of therapy and cannot tolerate the treatment. The main efficacy indicator is PFS.
Figure 1-3. Median PFS of the two groups of patients after treatment

The above figure showed the median PFS (progression-free survival, months) of the two groups of patients after treatment; the Anlotinib (60 patients) and the placebo group (57 patients) showed 4.83 months (95% CI 3.47–6.40) and 1.2 months (95% CI 0.70–1.60), respectively of PFS (HR = 0.320 95% CI 0.200–0.511 P<0.0001).

The objective response rate was 10.00% in the Anlotinib group and 0 (P<0.0001) in the placebo group.

Figure 1-4. Median OS in the two groups

The median OS in the two groups of patients was 10.33 months (95% CI: 7.27-10.53), respectively, (HR = 0.656, 95% CI: 0.411–1.048, P=0.0776).

The incidence of adverse events in the Anlotinib and placebo groups was 91.67% and 70.18% (P=0.004), respectively. Compared to the placebo group, the adverse events,
including elevated blood pressure, increased TSH, hand-foot syndrome, rash, increased TG, increased TC, increased LDL, oral mucositis, sore throat, hoarseness, diarrhea, cough, and hemoptysis were statistically significant. No difference was observed in the presence of fatigue, proteinuria, and elevated transaminase in the placebo group.

Level 3/4 adverse reactions in the study are hypertension, elevated TGs, hand-foot syndrome, and fatigue.

2 Research purposes


The efficacy indicators include OS, PFS, ORR = CR + PR, DCR = CR + PR + SD, QoL score, biomarker assessment (P <0.05), and safety indicators.

3 Study population

Patients with advanced non-small cell lung cancer.

3.1 Selection criteria

1) patients voluntarily participate in this study and signed the informed consent;
2) pathologically diagnosed as late (IIIB/IV) NSCLC with measurable lesions;
3) Patients who receive ≥2 chemotherapy regimens or are unable to tolerate the treatment;
4) evaluation of the genotypes by providing detectable specimens (tissue or cancerous pleural effusion) prior to enrollment * patients with negative EGFR, ALK gene test results; or positive test results and resistance to targeted drug after treatment or cannot be tolerated by the patients #;

[*: EGFR and ALK gene mutations have been detected by the current detection method, and the original test report can be provided. The EGFR and ALK genotypes can be detected without confirmation by the investigator.
#: EGFR-positive patients are required to use at least one of the following drugs and monitored if they are resistant or intolerant: including erlotinib, alfortitine, gefitinib, iridinib, and AZD9291; ALK-positive patients are required to administer at least one of the following drugs and resistance or intolerance monitored: methazolidine and ceritinib].

5) age between 18–75 years; ECOG PS Scoring: 0-1 point, expected survival time ≥3 months;
6) major organ function within 7 days prior to treatment; the following criteria are fulfilled:
(1) blood routine examination criteria (without blood transfusion for 14 days):
   a) hemoglobin (Hb) ≥90 g/L;
   b) neutrophil absolute (ANC) ≥1.5×10⁹/L;
   c) platelet (PLT) ≥80×10⁹/L.

(2) biochemical tests meet the following criteria:
   a) total bilirubin (TBIL) ≤1.5-fold of the upper limit of normal (ULN);
   b) ALT and AST are ≤2.5×ULN; if liver metastasis occurred, ALT and AST are ≤5×ULN;
   c) serum creatinine (Cr) ≤1.5×ULN or creatinine clearance (CCr) ≥60 mL/min;

(3) doppler ultrasound evaluation: left ventricular ejection fraction (LVEF) ≥normal low (50%).

7) women of childbearing age should agree to the usage of contraceptive measures (such as intrauterine devices, birth control pills, or condoms) within the study period and up to 6 months after the end of the study. The serum or urine test is negative within 7 days prior to the study, and the patients must be non-lactating. Men should also agree to the usage of contraceptives during the study period and within 6 months after the end of the study period.

3.2 Exclusion criteria

Patients who present any of the following criteria will not be enrolled in this study:

1) patients who had previously used Anlotinib hydrochloride capsules;
2) small cell lung cancer (including small cell carcinoma and non-small cell carcinoma mixed lung cancer);
3) patients who were tested positive for EGFR and ALK mutations but did not use the relevant targeted drug;
4) central, empty lung SCC, or NSCLC with hemoptysis (>50 mL/day);
5) patients with other malignancies in the past 5 years or currently, except cured cervical cancer in situ, non-melanoma skin cancer, and superficial bladder tumor [Ta (non-invasive tumor), Tis (carcinoma in situ) and T1 (tumor infiltrating basement membrane)];
6) patients are planned to receive systemic anti-tumor therapy within 4 weeks prior to grouping or during the course of this study, including, cytotoxic therapy, signal transduction inhibitors, and immunotherapy (or mitomycin C 6 weeks prior to medication). Intravenous radiotherapy (EF-RT) was performed 4 weeks prior to grouping or restricted radiotherapy for assessing tumor lesions within 2 weeks prior
to grouping;

7) more than CTC AE level 1 unmitigated toxicity due to any previous treatment, not including hair loss and ≤2 level neurotoxicity caused by oxaliplatin;

8) patients have a variety of factors that affect oral medication (such as inability to swallow, chronic diarrhea, and intestinal obstruction);

9) pleural effusion or ascites, causing respiratory syndrome (≥CTC AE level 2 dyspnea);

10) patients with brain metastasis have symptoms or controlled symptoms for <2 months;

11) patients with any severe and/or uncontrolled disease, including:

a) blood pressure control that is not ideal (systolic blood pressure ≥150 mmHg, diastolic blood pressure ≥100 mmHg);

b) myocardial ischemia or myocardial infarction, arrhythmia (including QTc ≥480 ms) and ≥2 levels of congestive heart failure (NYHA classification);

c) active or uncontrollable severe infection (≥CTC AE level 2 infection);

d) liver cirrhosis, decompensated liver disease, active hepatitis or chronic hepatitis to be treated with antiretroviral therapy;

e) renal failure requiring hemodialysis or peritoneal dialysis;

f) history of immunodeficiency, including HIV-positive or other acquired, congenital immunodeficiency disease, or history of organ transplantation;

g) poor control of diabetes [fasting blood glucose (FBG) >10 mmol/L];

h) urine routine test protein ≥ ++, and confirmed 24-h urine protein >1.0 g;

i) patients experiencing seizure and need treatment;

12) received a major surgical treatment within 28 days prior to grouping, with a biopsy or a significant traumatic injury;

13) imaging shows that the tumor has violated important vascular weeks or the researchers determine that the tumor is likely to invade critical blood vessels by fatal bleeding during the follow-up study;

14) irrespective of severity, patients show any signs of bleeding or medical history; in the first 4 weeks before the group, patients have any bleeding or bleeding events (≥CTCAE 3) with non-healing wounds, ulcers, or fractures;

15) active/venous thrombotic events occurred within 6 months of cerebrovascular accident (including temporary ischemic attack), deep vein thrombosis, and pulmonary embolism;

16) patients with a history of mental illness or have mental disorders;
17) participated in other anti-tumor drug clinical trials within 4 weeks;
18) according to the researcher's judgment, there are adjoint diseases that may be
    hazardous to the patient's safety or affect the completion of the study.

3.3 Standards for termination of the subjects medication
1) determine the disease progression (PD) or clinically consider disease progression
    based on efficacy evaluation criteria;
2) the study physician had to necessarily terminate the treatment for the patient's
    benefit;
3) the occurrence of intolerable adverse reactions or serious adverse events confirmed
    by the researchers;
4) patients with poor compliance, and the use of drugs should range in 80–120% of the
    patients;
5) patient’s withdrawal of the informed consent;
6) use of other anti-tumor drug treatments (such as chemotherapy, targeted therapy, or
    biological agents) that affect the determination of efficacy;
7) accidental pregnancy;
8) death.

3.4 Elimination criteria
1) dosage and method error;
2) during the trial period, other chemotherapy, surgical treatment, or trial drug therapy
    other than the current program is used;
3) patient does not fulfill the standards and is included erroneously;
4) patient without medication.

4 Test drug
Test Drugs: Anlotinib hydrochloride capsules: Chia Tai Tianqing Pharmaceutical Group
    Co., Ltd. production
Specifications: 12 mg/tablet, 10 mg/tablet, 8 mg/tablet;
Placebo: Blank capsules substituting the Anlotinib hydrochloride Capsule: Chia Tai
    Tianqing Pharmaceutical Group Co., Ltd. production
Storage conditions: sealed, dark, room temperature, valid for 24 months.
According to the requirements of GCP, the research medication is governed by the
    hospital custody; the drug is released regularly and recycled. Both issuance and recycling
    requires a complete record. The recovered drug is submitted to the sponsor after completion
    of the trial. Inspectors regularly check the usage and record of the drugs, as well as, monitor
the recovery of the situation at any time.

5 experimental design

A randomized, double-blind, placebo-controlled, multicenter, phase III clinical trial was designed using an Anlotinib hydrochloride and placebo-controlled therapy in patients with advanced NSCLC.

5.1 Sample size estimation

According to the total survival time of NSCLC, in worldwide studies, combined with the total survival time of phase II clinical study of Anlotinib hydrochloride capsules, the sample size of the present study was estimated.

Assuming that the median OS was 11 months in the drug group and that in the placebo group was 7 months with a design risk ratio of 0.70, the median OS time in the Anlotinib group was 43% higher than that in the placebo group. Assuming $\alpha=0.05$, power=0.85, control ratio is 2:1. Furthermore, 291 cases of OS events occurred in the two groups, thereby revealing statistically significant difference in the two groups. Assuming 12 months for enrolling patients, 12 months follow-up, and 10% missing follow-up, the study planned to enroll 450 patients, including 300 in the trial group and 150 in the control group.

5.2 Interim analysis

In this study, an independent data monitoring committee (IDMC) was established to conduct the mid-term data analysis for assessing the patient's risk/benefit. The independent data oversight committee (IDOC) consists of four independent oncologists and an independent statistician.

During this study, a safety and validity analysis was conducted by the IDOC. The safety analysis was performed on approximately 150 patients who completed at least one treatment cycle in this study; whereas, the validity analysis was performed on approximately $2/3^{rd}$ of the total OS events. IDMC recommended the continuation or discontinuation of the study to the sponsor, and whether to summarize the data in advance or modify the program to continue the study according to the results of the analysis. The sponsor will take appropriate decision on the research project according to the recommendations of the IDMC, such as the safety or lack of validity to terminate the test or summarize the declaration in advance, and supplement the information after the end of the trial with respect to the drug.

In this study, the mid-term validity analysis was performed according to O'Brien-Fleming's method, $\alpha_1=0.005$ for mid-term analysis, and $\alpha_2=0.048$ for final analysis.

5.3 Random method

In this study, centralized randomized grouping method is used. Patients from centers
compete into the group. The centralized randomized grouping program utilize the centralized randomized grouping system provided by the Department of Biostatistics, Nanjing Medical University. The participants in the test center of this trial will log in to the random system after screening for every qualified subject, fill-in personal information, obtain the random number, and the corresponding drug number information. The appropriate research drugs were issued according to the random number and drug number.

During the study, if the patient need to adjust the dose; these reasons for the adjustment are recorded, and the specialized statistical staff is notified in writing. After confirmation of the same number, different doses of drugs will be issued to the research center.

In order to ensure the balance between the test and control groups, the following factors affecting the efficacy of indicators are controlled:

- histopathology classification: adenocarcinoma/squamous cell carcinoma or others;
- metastases sites: metastases ≤3/metastases >3;
- driving gene status: positive/negative.

5.4 Dosage regimen

(1) Administration method:

The initial medication: before breakfast, taking Anlotinib hydrochloride capsules/placebo, 1 tablet/once/day (12/0 mg). Continue the drug administration for 2 weeks and stop for 1 week; thus, 3 weeks (21 days) for a treatment cycle.

If there is missed medication and the interval from the next medication is <12 h, no medication is supplemented. Patients with disease control (CR + PR + SD) and who can tolerate adverse reactions continue the medication.

When the investigators confirm that the patient is unsuitable for continued medication or according to RECIST 1.1 standard evaluation for the PD, the treatment is ended.

(2) Principle of dose adjustment:

During the medication, the dose was adjusted by the investigator based on the degree of drug-related toxicity (according to NCI CTC AE 4.0) and the potential benefit to the patient. This study was designed in two levels of dose adjustment (10mg, 8mg). If more than two dose levels need to be reduced, the treatment should be terminated. For patients who have undergone such reduction two times (to 8/0 mg), the investigator determines that the disease is likely to progress, and the dose can be increased to 10/0 mg. Each patient can only be up-regulated to a specific dose.

Table 5-1. The levels of dose adjustment
Dose level | Usage | Specific amount of drugs
--- | --- | ---
1 Standard dose | 12 mg oral, once a day | 12 mg Anlotinib hydrochloride capsules, 1 pill, or placebo
2 Reduction 1 | 10 mg oral, once a day | 10 mg Anlotinib hydrochloride capsules, 1 pill, or placebo
3 Reduction 2 | 8 mg oral, once a day | 8 mg Anlotinib hydrochloride capsules, 1 pill, or placebo

Table 3-2 shows the delayed delivery time and/or change in the dosage regimen recommended when a drug-related toxic response occurs with the study drug. When the patient presents abnormal liver function, bleeding, proteinuria, decline in the platelet count, and other toxic reactions, medication and dose adjustment are implemented.

Table 5-2. Delayed administration and dose level adjustment criteria

<table>
<thead>
<tr>
<th>Adverse reaction level</th>
<th>NCI CTC Version AE4.0</th>
<th>Time of administration</th>
<th>Dose adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 0–2</td>
<td>on time</td>
<td>no change</td>
<td></td>
</tr>
<tr>
<td>Level 3</td>
<td>Delayed administration until the extent of recovery to &lt;2 #</td>
<td>Reduce one dose level</td>
<td></td>
</tr>
<tr>
<td>Level 4</td>
<td>Delayed administration until the extent of recovery to &lt;2 #</td>
<td>Reduce one dose level. The investigator can stop the treatment permanently judging by the treatment.</td>
<td></td>
</tr>
</tbody>
</table>

#: If the delay has not been restored after 2 weeks, the treatment should be terminated permanently.

Table 5-3. The delayed administration time and/or change in the dosage regimen in case a liver function abnormality occurs

<table>
<thead>
<tr>
<th>AE Levels</th>
<th>Dose adjustment program</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Maintain the original dose</td>
<td>Follow-up on the plan</td>
</tr>
<tr>
<td>Level 2 (normal baseline)</td>
<td>Delayed administration, restore to &lt;2 within 2 weeks, reduce a dose level</td>
<td>Active liver protection and strict monitoring of liver function, once a week</td>
</tr>
<tr>
<td>Level 2 (abnormal baseline)</td>
<td>Maintain the original dose</td>
<td>Active liver protection and strict monitoring of liver function, once a week</td>
</tr>
</tbody>
</table>
Level 3
Delayed administration, restore to <2 within 2 weeks, reduce a dose level
Active liver protection and strict monitoring of liver function, twice a week until the toxicity is restored to <2 or there is an explanation.

Level 4
Permanent termination of treatment
Active liver protection and strict monitoring of liver function, once or twice a week until the toxicity is restored to <2 or there is an explanation.

Table 5-4. The delayed administration time and/or change in the dosage procedure recommended for proteinuria

<table>
<thead>
<tr>
<th>AE Levels</th>
<th>Dose adjustment program</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1: Routine urine examination showed urine protein + or quantitative detection of 24 h urine protein &lt;1.0 g</td>
<td>Maintain the original dose</td>
<td>Follow up on the plan</td>
</tr>
<tr>
<td>Level 2: Routine urine examination showed urine protein + or quantitative detection of 24 h urine protein is 1.0–0 g (not included)</td>
<td>Maintain the original dose</td>
<td>Active treatment and urine monitoring (once a week); if necessary, consult renal clinicians</td>
</tr>
<tr>
<td>Level 2: Routine urine examination showed urine protein ++ or above, quantitative detection of 24 h urine protein is 2.0–3.5 g (excluding)</td>
<td>Delayed administration, restore to &lt;2 within 2 weeks, reduce a dose level</td>
<td>Active treatment and urine monitoring (once a week); if necessary, consult renal clinicians, terminate the treatment at the third occurrence</td>
</tr>
<tr>
<td>Level 3: Quantitative detection of 24 h urine protein is ≥3.5 g</td>
<td>Delayed administration, restore to &lt;2 within 2 weeks, reduce a dose level</td>
<td>Active treatment and urine monitoring (once a week); if necessary, consult renal clinicians, terminate the treatment at the third occurrence</td>
</tr>
</tbody>
</table>

Table 5-5. The delayed delivery time and / or dose level change procedure recommended when the platelet count is reduced

<table>
<thead>
<tr>
<th>AE Levels</th>
<th>Dose adjustment program</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1: Platelet count 100–75×10^9/L</td>
<td>Maintain the original dose</td>
<td>Follow-up on the plan</td>
</tr>
</tbody>
</table>
Table 5-6. The delayed delivery time and/or change in the dosage level recommended after a bleeding event

<table>
<thead>
<tr>
<th>AE Level</th>
<th>Dose adjustment program</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Maintain the original dose</td>
<td>Follow-up on the plan</td>
</tr>
<tr>
<td></td>
<td>Delayed administration, restore to &lt;2 within 2 weeks, maintain the original dose</td>
<td>Deal with it actively</td>
</tr>
<tr>
<td>Level 2</td>
<td>Restore to &lt;2 within 2 weeks, reduce a dose level</td>
<td>Emergency medical intervention</td>
</tr>
<tr>
<td>Level ≥3</td>
<td>Permanent termination of treatment</td>
<td></td>
</tr>
</tbody>
</table>

(4) Disease progression strategy

Patients who were evaluated for PD according to the RECIST 1.1 criteria were found to be associated with the cessation of tumor necrosis or density reduction and/or slow or local progression of the disease. Then, the investigator determined that if the persistence of the study drug may be clinically benefit, while the patients made a written application to continue the medication at the sub-center of the main researchers; at least one senior physician signed the approval, with the consent of the sponsor, in order to continue the usage of the original study drug until the researchers determined the clinical progress of the patient's disease.

When the patient's disease progresses, withdrawal from the study is essential.

Remarks [4]: Local progress: solitary extracranial progression or intracranial progression; symptom score ≤ 1;

Slow progress: Compared to the previous assessment, tumor load increased slightly (≤ 2 points); symptom score ≤ 1
Outbreaks progress: Tumor load increased rapidly (>2 points) compared to previous evaluations; symptom score =2

Symptom score: no symptoms scored 0; the symptoms that did not change the score is 1; the symptoms increased to score 2;

Tumor load score to non-target lesion score as the representative of the progress of the disease, the emergence of new chest lesions, new chest disease, malignant pleural effusion; four cases each 1 points with a total score of 4 points.

6 Efficacy evaluations

The main efficacy indicators: OS

Secondary efficacy indicators: (1) PFS
(2) ORR= CR + PR
(3) DCR = CR + PR + SD
(4) QoL score.

The objective efficacy index was evaluated according to the evaluation criteria of the efficacy of solid tumors (RECIST 1.1).

PFS is defined as the time from randomization to tumor progression or death.

DCR is defined as the percentage of patients with complete remission, partial remission, and disease stabilization and maintaining the number of evaluable patients over 4 weeks.

OS is defined as the time from randomization to death for any reason. The number of days, lost subjects, and usually the last follow-up time is used for calculating the as dead time.

For patients who discontinued the study without PD, follow-up to obtain the results of tumor evaluation should be recorded until the PD. Any of these anti-tumor treatments must also be recorded in the study. In the analysis, the censor time of these patients was set as the date of last follow-up visit. A long-term follow-up (including telephone follow-up) was performed on patients (including outpatients); in addition, they were followed up every 8 weeks to obtain OS, followed by anticancer treatment information and survival status during major follow-up.

7 Exploratory study

7.1 Biomarker exploration

To explore the mechanism of Anlotinib hydrochloride in the treatment of advanced NSCLC patients or the mechanism of potential resistance to follow-up more targeted treatment of patients with NSCLC, the following exploratory study was carried out. Patients were enrolled in this trial under informed and voluntary terms.

It is planned to use the tumor tissue specimens, cancerous pleural effusion, plasma, and/or serum. The source of the tumor tissue can be retained from the previous withdrawal, or
collected before the study. The analysis of plasma and/or serum samples can be obtained at the time of patient visits or when other parameters are inspected.

Patients provided informed consent to allow the usage of their specimens for gene and protein analysis including the detection of total expression and phosphorylation levels of specific cytokine receptors (such as VEGFR2), and the expression of relevant cytokines (for example, VEGF, PDGF, FGF) in an attempt to determine any correlation between the protein and its therapeutic effect. Detection of gene mutation status, assessment of specific genes such as EGFR, ALK, ROS1, BRAF, KRAS, and PIK3CA, and other mutations or related gene expression changes, and analysis of EGFR gene T790M mutation status in order to determine whether the genetic mutation and drug efficacy that might be related to drug resistance.

The results of all the analysis are shared by the sponsor and the clinical research unit, not for the purpose of scientific research, and the patient information should fulfill the relevant provisions of confidentiality.

The collection, handling, and transport of specimens are listed in Appendix V.

7.2 Studies on population pharmacokinetics

In order to further study the changes in plasma concentration in the body after long-term usage of the drug and explore the relationship between plasma concentration and efficacy as well as adverse reactions, some patients in conditional centers should be selected to carry out the group pharmacokinetic study.

The patient signed the informed consent for the pharmacokinetic study, according to the annex "salbutam hydrochloride system exposure level and efficacy and safety of the relevant research program" requirements for blood collection and transportation.

If the patients exited the trial, the medication was stopped and follow-up is no longer studied.

8 Observation items

8.1 Before the trial

The following criteria should be observed and evaluated 1 week before the start of the trial:

- collection of medical history and basic information, including patient ID, gender, age, mailing address, contact telephone;
- detailed history, history of treatment, QoL score, comprehensive physical examination: ECOG PS score, height, weight, vital signs, physical examination of the organs;
- collection of 5–10 tissue slices for related gene detection;
12 electrocardiogram (with special attention to QTc), echocardiography;
routine blood, urine, and stool analysis (including fecal occult blood);
liver function (TP, A, G, ALT, AST, LDH, ALP, TBil, DBil, IBil), renal function (BUN, Cr, UA), blood lipids (TC, TG, HDL, LDL), electrolyte (K+, Na+, CL-, Ca2+, Mg2+, P), lipase, amylase, fasting blood glucose analysis;
verify the HCG in women of childbearing age except in the case of pregnancy;
coagulation function test (PT, APTT, TT, Fbg, D-Dimer, INR), thyroid function (T3, T4, FT3, FT4, TSH); examination of serum carcinoembryonic antigen (CEA) or squamous cell carcinoma antigen (SCC), HIV-related indicators;
Imaging (CT/MRI) can be completed within 14 days before medication. PET examination is not evaluated as a conventional radiographic examination. Evaluation of the assessment site before medication must include chest, abdomen, and pelvic CT or MRI. All suspicious lesions should be examined radiographically. In case of suspected patients with brain metastasis or clinical symptoms, brain MRI examination should be performed.

8.2 Medication period
detect blood pressure and record the adverse reactions as well as other combined medication daily during the period of medication;
a comprehensive physical examination including weight, vital signs, physical examination of the organs is undertaken during every visit;
record the adverse reactions of the treatment, including nausea, vomiting, diarrhea, and abdominal distension. A variety of clinical manifestations were observed and documented;
check ECG (with particular attention to QTc) at every visit. In the event of chest pain, palpitations, and other symptoms, echocardiography, myocardial enzymes (CK, CK-MB) and troponin were assessed in addition.
initial efficacy (imaging) assessment of medication at 3 and 6 weeks to confirm the efficacy. Follow-up medication was evaluated two times per 2 cycles (CT/MRI).
ECOG PS score, QoL score, serum CEA or SCC examination time and imaging time were evaluated;
check blood routine every week in the first cycle, and check 1 time every 1–3 weeks in the follow-up cycle. After 8 cycles, check every 2 cycles (42 days). If the neutrophils were ≤1 × 10⁹/L or platelets ≤50 × 10⁹/L, the frequency of review should be increased (1/2-3 days). The blood routine should be rechecked every week if
hematologic events cause delayed toxicity or dose adjustment.

- check urine routine once every week. After 8 cycles, check every 2 cycles (42 days). If urinary protein in urine routine during the medication is ≥++ , the 24-h urine protein quantification is conducted in a week.

- check blood biochemistry on the 7th day of the first cycle and at the end of the first cycle, followed by checks at the end of each subsequent cycle. Then, the blood biochemistry is checked once every 2 cycles after 8 cycles. During the medication, before the discovery of blood biochemical abnormalities, if early symptoms such as liver damage (loss of appetite, nausea, vomiting, right upper abdominal discomfort, and fatigue) occur, blood biochemical should be tested immediately. If ALT or AST increased 3-fold of ULN or baseline values, total bilirubin increased by 2-fold of ULN or baseline values, the frequency of the test should be increased (recommended 1-2 times/week).

- blood coagulation function (PT, APTT, TT, Fbg, D-Dimer, INR), thyroid function (T3, T4, FT3, FT4, TSH), lipase, and amylase are verified every 2 cycles.

### 8.3 End of medication and follow-up

Follow-up period starts after the last administration of the trial drug. The treatment and follow-up of unadjusted adverse reactions are continued until recovery to NCI CTC AE v4.02 grade 1 or complete recovery.

- comprehensive physical examination: QoL score, PS score, weight, vital signs, physical examination of the organs;
- ECG, routine blood check, blood biochemistry, fasting blood glucose, urine routine, stool examination, electrolyte levels;
- coagulation function (PT, APTT, TT, Fbg, D-Dimer, INR); serum CEA or SCC examination;
- imaging (CT/MRI): the group of patients underwent tumor imaging assessment (from the last imaging assessment time <21 days).

The long-term follow-up of the patients was conducted every 8 weeks (including telephone follow-up). The treatment plan, number of cycles, and outcome are recorded if the patient was treated with other anticancer treatments before this follow-up visit. The follow-up was continued until the patient died; the cause of death and the specific time of OS are recorded.

### 9 Adjoint medication

#### 9.1 Support treatment
Patients may receive supportive care. This care can be combined with the following medications or related treatments: antibiotics, analgesics, hormones, transfusion, psychotherapy, palliative surgery, or any other essential symptomatic treatment used to provide the optimal supportive care. For other investigational antitumor drugs or anti-tumor chemotherapy/endocrine/immunotherapy is not defined within the scope of the supportive care.

If the investigators confirm that there is no impact on the end of the study, the application of unconventional treatment (for example, herbal or acupuncture) and vitamin/mineral supplement therapy is allowed. Patients during the treatment can receive bisphosphonates for bone metastases.

During the treatment, local progress of the disease is noted, i.e., increase or presenting 1-2 new non-target lesions, no symptoms or no change in the symptoms, and the non-target lesions can be considered in combination with local radiation therapy.

If systemic pain or local analgesia cannot be used to effectively control the pain of bone metastases, a palliative small area (radiotherapy area must be <5% of the bone marrow region) radiotherapy should be used, provided that the target lesion does not include radiation field.

During treatment, the granulocyte colony stimulating factor (G-CSF) and other hematopoietic growth factors can be employed if the clinical presentation suggests or if the investigator determines the need to treat acute toxicity such as neutropenic fever. Then, the long-term usage of erythropoietin is permitted.

9.2 Drugs banned or cautiously used during the trial

- Caution with anticoagulant and antithrombotic drugs

The anticoagulant and antithrombotic drugs should be cautiously used during the treatment in order to avoid the increased risk of potential bleeding including but not limited to the following list of several types of drugs: salicylic acid derivatives (aspirin), heparin anticoagulant drugs (low molecular weight heparin, enoxaparin, heparin, and arabinavalin), prophylactic anticoagulant drugs (clopidogrel and ticagrelor) after cardiovascular events.

- Drugs that interfere with liver P450 enzymes

During the treatment, caution should be exerted while using CYP3A inducers (carbamidazole, rifampicin, and phenobarbital) and inhibitors (ketoconazole, itraconazole, erythromycin, and clarithromycin), CYP3A4 substrate (simvastatin, cyclosporine, and pimidine), drugs metabolized by CYP3A4 [benzodiazepines, dihydropyrididine calcium antagonists (for hypertension that cannot be controlled by ACEI), selected calcium antagonists), and HMG-COA reductase inhibitors] (Diclofenac, phenytoin, piroxacin,
Drugs that cause prolonged QT interval

As the dipyridine drugs in the clinical extension of the QT interval cause toxic side effects, caution should be exerted during the trial period while using the categories of drugs including but not limited to the following list:

- Antimicrobials (clarithromycin, azithromycin, erythromycin, roxithromycin, metronidazole, moxifloxacin);
- Antiarrhythmic drugs (quinidine, sotalol, amiodarone, propiramine, procainamide);
- Antipsychotics (risperidone, fluphenazine, droperidol, haloperidol, thioridazine, pimozide, olanzapine, clozapine);
- Antifungals (fluconazole, ketoconazole);
- Antimalariais (mefloquine, chloroquine);
- Antidepressants (amitriptyline, imipramine, clomipramine, thiazepine, doxepin).

Chinese medicine and immunizing agents with anti-cancer effect

During the trial period, it is forbidden to use the CFDA approved modern Chinese medicine preparations and immunomodulators (such as Lentinan, Eddie, compound Sophora, cinobufagin, Kanglaite, Ginseng polysaccharide, Xiaoganping, Shenqi Fuzheng, Brucea javanica Oil emulsion, Kang Ai, thymosin, interferon, IL-2, BCG, transfer factor, and levamisole).

Citrus, carambola, grapefruit, and grapefruit juice can affect the cytochrome P450 activity and thus, should be avoided.

10 Safety assessment

10.1 Adverse events

Adverse events are adverse medical events that occur after a patient is admitted to a clinical trial. This period designates from when the patient signed informed consent and accept the test drug treatment to 1 month after the ending of treatment. Any adverse medical events were recorded, irrespective of the causal relationship with the test drug.

The researcher should record in detail any adverse events that occur in the patient. These include description of adverse events and all related symptoms, time of occurrence, severity, duration, measures taken, and outcome.

10.2 Evaluation of adverse events

The criteria for assessing the nature and severity of adverse events were in accordance with the National Cancer Institute's Common Toxicity Criteria [CTC AE v4.02].
10.3 Criteria for judging the relevance of drugs to adverse events

Researchers should assess the potential association between adverse events and test drugs. Specific criteria can be determined by referring to the 5 criteria and using Table 10-1 to decide. Five criteria were: 1) adverse events that occur during the medication; 2) adverse events and the drug-related adverse reactions; 3) adverse events that cannot be explained by other reasons; 4) adverse events disappeared after stopping the medication; 5) adverse events reproduced after administration of the drug. The results determined to be affirmative might indicate that the relevant three items were adverse reactions, according to the calculation of the incidences.

Table 10-1. The relevance of drugs to adverse events evaluation form

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed correlation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Likely correlation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Possible correlation</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>?</td>
</tr>
<tr>
<td>Possibly unrelated</td>
<td>+</td>
<td>--</td>
<td>±</td>
<td>±</td>
<td>?</td>
</tr>
<tr>
<td>Unrelated</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: + affirmative, - negative, ± difficult to affirm or negative, indicating that the situation is unknown

10.4 Serious adverse events (SAEs)

SAEs are classified as those according to one or more of the following criteria: death, life risk (for example, immediate danger of death), hospitalization or prolonged hospitalization, permanent or severe disability, congenital malformations, or defects. Furthermore, there is no medical event that causes death, life risk, or hospitalization, and is considered a SAE by a physician who speculates it as detrimental to the patient or that the medication or surgical treatment should be avoided.

Death due to progression of the disease is reported as SAE, and during the study period, the patient must report to the sponsor when a pregnancy event occurs in the patient or his/her partner. Although pregnancy is not a SAE, it is still reported in the form recording SAEs.

10.5 SAE handling

Any SAEs in the clinical trial must immediately call the Ethics Committee and the sponsor (below). The investigator should complete and submit the report of SAEs within 24 h to the Ethics Committee, upstream authorities, and the sponsor. The contents of the written
report include the time of SAEs, the severity, the duration, the measures and the outcome. The researchers and the main investigators decide whether the patient with SAE should be unblinded. The blind codes should be kept by staff of Nanjing Medical University Department of Biostatistics. If unblinding is necessary, the project manager of sponsor and inspectors from each center should be informed. Once unblinded, the case should be removed from the trial.

10.2 Reporting unit

<table>
<thead>
<tr>
<th>Reporting unit</th>
<th>Contact person</th>
<th>contact number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chia Tai Tianqing Pharmaceutical Group Co., Ltd</td>
<td>Miao Yadong</td>
<td>18551674600</td>
</tr>
<tr>
<td>Shanghai Jiao Tong University Affiliated Chest Hospital</td>
<td>Ethics Committee</td>
<td>021-22200000*3115</td>
</tr>
<tr>
<td>Tianjin Cancer Hospital</td>
<td></td>
<td>022-23340123*6417</td>
</tr>
<tr>
<td>National Food and Drug Administration Registration</td>
<td>Research Oversight</td>
<td>010-68586295</td>
</tr>
<tr>
<td></td>
<td></td>
<td>010-88330728</td>
</tr>
</tbody>
</table>

10.6 Suggestions for symptomatic treatment of common adverse reactions

10.6.1 Palmar-plantar erythrodysesthesia syndrome

Hand-foot syndrome is defined by a slow feeling in the palms and foot or acral red, which is obviously uncomfortable, with swelling, tingling, compression or force in the regional performance. Tumor patients may be presented with such symptoms in the course of chemotherapy or molecular targeted therapy.

Grade 1 is characterized by painless, slight skin change or dermatitis (erythema, edema, swelling, hyperkeratosis); grade 2 is characterized by painful skin changes (such as peeling, blisters, bleeding, swelling, hyperkeratosis); instrumental daily activities are affected; grade 2 is characterized by severe skin changes (peeling, blisters, bleeding, edema, hyperkeratosis) with pain, thereby affecting the personal daily activities.

For patients with level 1 toxicity, support treatment is not required. Level 2 or more toxic patients consider the following symptomatic supportive care including strengthening skin care, keeping the skin clean, avoiding secondary infection, avoiding stress or friction, using moisturizer or lubrication agents, topical use of urea and corticosteroid ingredients of the emulsion or lubricant, and the use of local anti-fungal or antibiotic treatment is necessary.

10.6.2 Hypertension

At the beginning of this study, the first 6 weeks of medication should be monitored daily. In the case of an increase in blood pressure, it needs to actively communicated with the doctor. In the event of increased blood pressure, conventional antihypertensive therapy can be controlled. If blood pressure was difficult to control, the target drug dose could be reduced or
withdrawn.

Hypertension staging and routine treatment recommendations:

Hypertension refers to pathological blood pressure, repeated measurement of blood pressure, >140/90 mmHg.

Severity Rating:

1: high blood pressure: (systolic blood pressure 120–139, diastolic blood pressure 80–89 mmHg) without the use of antihypertensive drugs indications, only to monitor the blood pressure;

Level 2: first stage of hypertension (systolic blood pressure 140–159mmHg, diastolic blood pressure 90–99mmHg), need medical intervention, repeated or lasting (>24 h), symptomatic systolic blood pressure increased by more than 20 mmHg or past the normal range >140/90 mmHg; monotherapy is required to monitor the blood pressure simultaneously; thiazide diuretics are used maximally; however, the use of angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB) receptor blockers, and calcium channel blockers are also considered.

Level 3: stage 2 hypertension (systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥100 mmHg); requires medical intervention; requires multiple medications, usually thiazide diuretics with ACEI or β-blockers or calcium channel blockers;

Level 4: life-threatening (such as malignant hypertension, transient or persistent nerve damage, high blood pressure crisis) conditions require emergency treatment. Presently, there is no uniform classification worldwide; however, based on the recent perspective of clinical treatment, hypertension can be divided into two types:

(1) Hypertension emergencies, diastolic blood pressure >120 mmHg, with acute or progressive target organ damage, such as cerebral infarction, intracranial or subarachnoid hemorrhage, and hypertensive encephalopathy, of which, chronic is approximately 40–50%;

(2) Hypertension emergency (hypertension urgencies), diastolic blood pressure >120 mmHg is not associated with or exhibits only minor organ damage.

Sodium nitroprusside or nifedipine are used to rapidly decrease blood pressure. Diazepam and phenobarbital are used to stop convulsions. Furosemide and mannitol are used to reduce the intracranial pressure.

Once the patient has a high blood pressure crisis, the medication should be terminated and the patient should quit the clinical study.

10.6.3 Proteinuria

During the entire treatment period, all patients were closely monitored for proteinuria,
especially those with a history of hypertension, and 24 h urinary protein was measured for 2 consecutive urine proteins ≥++. After the appearance of proteinuria, the principle of dose adjustment is followed based on the relevant information in the dosing regimen.

10.6.4 Diarrhea

The presence of 1–2 diarrhea can be given supportive care, such as the use of loradine in the earliest episode (for example, initial oral administration of 4mg and 2 mg orally until diarrhea is relieved).

10.6.5 Hyperlipidemia

The treatment of hyperlipidemia should consider the patient's pre-treatment state and eating habits. In addition to dietary control, high levels of hypercholesterolemia (≥7.75 mmol/L) at grade 2 or higher, or hypertriglyceridemia at level 2 or higher (≥ 2.5-fold of normal upper limit), HMG-CoA reductase inhibitors (atorvastatin) or appropriate lipid lowering drugs are administered.

10.6.6 Gastrointestinal bleeding

In the case of gastrointestinal bleeding, including fecal occult blood (+++) or more, hematemesis or blood, symptomatic treatment should be administered. In the case of upper gastrointestinal bleeding, hemostasis, fasting, acid suppression, blood transfusion, and supportive treatment are administered. If necessary, octreotide could be used. In the case of lower gastrointestinal bleeding, hemostasis, blood transfusion, and supportive care are given. If the bleeding cannot be controlled, surgery is essential.

10.6.7 Hypothyroidism

Thyroid function is closely monitored in all patients throughout the treatment period. When TSH ≥20 mU/L or any value of T3, T4, FT3, FT4 is lower than normal, excellent treatment should be used.

10.6.8 Pleural effusion

Pleural effusion refers to the increase in the liquid inside the chest, leading to shortness of breath, cough, and chest discomfort. The presence of malignant pleural effusion indicates that the tumor has spread or has progressed to a later stage, and that the patient's life expectancy will be significantly shorter.

Most patients with malignant pleural effusion (MPE) present clinical symptoms, while about 25% of the patients are asymptomatic; physical examination or X-ray examination found accidental MPE. Breathing difficulties are the most common symptoms. Once the diagnosis of MPE is clear, palliative care should be considered at the earliest. The patient's symptoms, general situation, and the expected survival time are assessed comprehensively to
develop a treatment program in order to reduce the symptoms of dyspnea. Pleural effusion severity is rated according to the following criteria:

- Level 1: asymptomatic; only clinical examination or diagnosis; no intervention required.
- Level 2: symptoms; need treatment (diuretics or thoracic puncture).
- Level 3: respiratory distress and hypoxia symptoms; surgery includes intubation or pleural fixation.
- Level 4: life-threatening breathing disorders or hemodynamic disorders; need intubation or emergency treatment.

Specific recommendations include [6]:

- Clinical observation: primary tumor has been clear; however, asymptomatic MPE patients do not require any treatment intervention; the symptomatic MPE patients need to consult the respiratory specialist and decide whether just to take a simple observation.

- Treatment of thoracic puncture: 1 month after chest puncture, MPE recurrence rate is higher, and thus, life expectancy is >1 month in patients is not recommended. Repeated treatment of thoracic puncture can temporarily alleviate the breathing difficulties; as a consequence, partial expected survival time is short, and poor physical condition patients avoid hospitalization for physical weakness, as well as, end stage patients.

- Intercostal catheter drainage and pleural fixation: life expectancy is extremely short; patients are not recommended repeated thoracic puncture surgery. They can be placed in the intercostal small diameter drainage tube for pleural effusion to ease the symptoms of dyspnea. If there is no obvious collapse of the lungs, intercostal catheter drainage should be conducted for pleural fixation (injection of hardening agent) to prevent the recurrence of MPE. Patients with simple intercostal catheter drainage without the implementation of pleural fixation revealed a high rate of MPE recurrence; thus, simple intercostal catheter drainage should be avoided. It is recommended to insert a small diameter intercostal drainage tube under thoracic effusion and pleural fixation under ultrasound guidance.

- Outpatient long-term retention of the chest drainage tube: retention of the chest drainage tube is an effective way to control recurrent MPE, especially for lung collapse or for shortening the hospital stay. At regular intervals, the catheter is connected to the vacuum bottle for drainage that can promote pulmonary re-expansion and thoracic atresia. Most drainage tubes can be removed after short-term retention.

10.6.9 Pneumothorax

Pneumothorax refers to the chest experiencing abnormal gas, resulting in lung compression. The condition is sudden onset with typical symptoms of sudden chest pain,
followed by chest tightness or difficulty in breathing and may have irritating dry cough. A part
of the disease is slow, even without symptoms.

Clinical signs: depending on the amount of gas. A small amount of pneumothorax might
not show obvious signs of gas when the patient exhibits chest fullness. As a result, the
respiratory movement weakened, tactile chatter is weakened or disappeared, percussion drum
sound is heard, and auscultation breath sounds are weakened or disappeared. Although both
sides of the breath sounds are weakened in emphysema and pneumothorax patients, the
weakening is rather obvious, even if there is little change in the amount of pneumothorax;
thus, the percussion and auscultation should be concerned about the contrast and up and down
comparison. A large number of pneumothorax is seen to the contralateral shift. On the right
side of the large number of pneumothorax when the liver system is down, the left side of the
pneumothorax or mediastinal emphysema in the left sternal edge is used to hear the heartbeat
that is consistent with the sound or high-profile metal tone (Ham-levy). When patients present
cyanosis, sweating, severe shortness of breath, tachycardia, and hypotension tension
pneumothorax should be considered.

X-ray examination is a major approach for the diagnosis of pneumothorax. If the clinical
degree of suspicion of pneumothorax and chest position before the normal is high, lateral
chest radiograph or lateral position chest X-ray examination should be conducted. Pneumatic
chest X-ray shows a clear line of the pneumothorax, while that of the atrophy of lung tissue
and pleural cavity gas junction line showed a convex line on the film. Outside the
pneumothorax line was observed for the non-lung texture of the light transmission area, and
the line for the compressed lung tissue was also noted. A large number of pneumothorax
visible mediastinum and the heart to the contralateral shift were observed when combined
with pleural effusion visible gas-liquid surface. When the localized pneumothorax in the
anteroposterior X-ray examination is missed, lateral chest radiograph can help diagnosis,
X-ray can also be found under the rotation position. If there is a light band around the edge of
the heart, it should be considered as mediastinal emphysema. X-ray is the most commonly
used method for the diagnosis of pneumothorax, CT for a small amount of pneumothorax,
localized pneumothorax and bullae than X-ray diagnosis that might be accurate. The basic CT
manifestations of pneumothorax comprise of extremely low-density gas in the pleural cavity,
accompanied by varying degrees of compression and collapse of the lung tissue. Notably,
pneumothorax is different from the following: pulmonary bullae, acute myocardial infarction,
pulmonary embolism, chronic obstructive pulmonary disease, and bronchial asthma.
Pneumatic severity and treatment recommendations are as follows:
Level 1: Asymptomatic; only clinical examination or diagnosis found; no intervention required;
Level 2: Symptoms; need intervention (for example, placement of catheter, no hardening agent);
Level 3: Hardening agent treatment and/or surgical treatment; need hospitalization;
Level 4: Life-threatening; need emergency treatment.

11 Data management and statistical analysis

11.1 Electronic case report form
Researchers or their authorized personnel use the Electronic Input System (EDC) within a specified period of time to complete the relevant information in the electronic case report Table. The contents of the entry are ensured to be consistent with that of the study of medical records along with the patient's privacy (usage of code instead of the patient's name). After completing the study, the electronic case report form is maintained by the sponsor and the research unit.

11.2 Database
Statistical experts specify the data administrator in advance to carry out the electronic case entries and the data query data; the system prompts the researcher or the authorized personnel to verify the changes. The database is audited and the data locked by key investigators, data managers, statisticians, and auditors. In order to ensure data security, unrelated personnel cannot enter and modify the data. Electronic case report data must also be backed up. Any changes in data require the principal investigator, statistician, and data administrator to sign the consent form before proceeding.

11.3 Data lock
After all the concerns are resolved and the database is confirmed, the data management audit report is written by the data manager and the analysis dataset is determined by the presence of the principal investigator, sponsor, statistical analyst, and data manager simultaneously. Then, the data files are locked in principle and no changes are permitted.

11.4 Choice of statistical analysis data
● Full Analysis Set: Analyze all cases received at least one dose in accordance with the intentional analysis (ITT) principle.
● Per-protocol Set: All treatments are completed for more than 6 weeks (including 6 weeks), the test plan fulfilled, compliance is good, the trial did not use the banned medication,
and the case report form is completed to fill the contents of the case. No missing data is not filled. The efficacy of the drug is determined at the same time by FAS and PPS statistical analysis.

- Safety Analysis Set: All patients enrolled in the study receive at least one trial medication, and all patients with safety records were classified as safety analysis. This dataset is used for security analysis.

11.5 Statistical analysis plan

According to the above sample size estimates, when there are 291 OS events, the statistical data are reported as follows:

All statistical analyses will be conducted using SAS 9.2 statistical analysis software. All statistical tests were conducted on both sides of the test, P-value ≤0.05 will be considered as the statistical significance with 95% confidence interval.

The baseline data are analyzed by the whole analysis set. All the validity indexes are analyzed according to full analysis set and per-protocol set. The safety analysis is based on the safety analysis set.

The measurement data of each treatment group in different treatment groups will be described by mean ± standard deviation or median (minimum, maximum). Compared to the screening period, the paired t-test was used to compare the differences between the two groups. Changes were made before and after treatment in each group using ANOVA or rank sum test. The enumeration data of each treatment group were statistically described by frequency (composition ratio). Changes in each group before and after treatment were evaluated by \( \chi^2 \) test accuracy or nonparametric test.

- Shedding analysis: descriptive analysis, if necessary, was estimated for the two groups based on the total rate of shedding and loss due to AE by \( \chi^2 \) test or the Fisher’s exact probability.

- Quantitative analysis of basal values: t-test or \( \chi^2 \) test compared the demographic data and other baseline values to estimate the balance between the two groups.

- The results were as follows: 25%, 50% (median), and 75% OS were calculated according to the actual situation of the two groups. The log-rank test was used to compare the two groups. For the secondary indicators of ORR and DCR comparison, the central stratification of the CMH-\( \chi^2 \) test as used to compare the efficacy of the two groups, and calculate the 95% CI between the groups. For the index PFS, the multiplication limit was used and 25%, 50% (median), 75% PFS and PFS at different time points after the start of treatment were calculated according to the actual situation of the data; the two groups were compared.
by log rank test.

- Safety analysis: mainly descriptive statistical analysis, was carried out on AEs occurring in this study; if necessary, Fisher’s exact probability method compared the incidence of AEs in each group. The laboratory test results describe the abnormal post-trial conditions and the relationship between the medications and abnormal changes.

(See Statistical Analysis Plan Book)

12 Applicant and investigator's responsibilities

12.1 Applicant

1) provides information and other support to the researchers, explain the program to the researchers, and fill out a variety of information before starting the clinical trial;

2) sends a clinical inspector, visit regularly; inspectors ensure the use of telephone, fax, e-mail, at any time to maintain contact with the researchers;

3) inspectors supervise the investigators to follow the approved program to carry out clinical studies, check the release and recovery of drugs in accordance with the relevant provisions, and ensure the test records of clinical trial are consistent with the original report data.

12.2 Investigator

1) After the GCP and the training of this trial program, the investigators have sufficient time to carry out the test according to the research program.

2) Patients should be informed of the relevant information before recruitment and sign the informed consent.

3) The investigator is obliged to take the necessary measures to ensure the safety of the patient. In the event of adverse reactions, the investigator immediately followed the relevant regulations, reported to the primary investigator, and followed up the serious adverse reactions.

4) Actively cooperate with the clinical inspector for the regular visit;

5) Maintain all the laboratory records, clinical records, and the patient's original medical records;

6) In order to ensure the evaluation and supervision of clinical trials by the State Food and Drug Administration and the sponsor, the research hospitals shall maintain all the research materials uniformly, including confirmation of all patients (for effective verification of the different records), effective signature of the informed consent, and detailed distribution of the drug records. The preservation period is 5 years. The ownership of all the information in this clinical trial belongs to the sponsor. The
investigators cannot provide any information to a third party without the written consent of the sponsor in addition to the national drug regulatory authorities.

13 Ethical norms and informed consent

This clinical trial must follow the Helsinki Declaration (2013) and the relevant Chinese clinical trials research norms and regulations. Before the start of the clinical trial, the test plan shall be formulated by the researcher and the sponsor, and shall be submitted to the Hospital Ethics Committee for examination and approval. During the actual implementation of the trial, if the program needs to be revised, the revised trial will be submitted to the Ethics Committee for approval. If critical new information concerning the test drug is found, informed consent form must be revised and approved by the Ethics Committee, followed by the patient’s agreement.

Before the start of the clinical trial, the investigator must provide the patient with detailed information about the clinical trial, including the nature of the trial, the purpose of the trial, the possible benefits and risks, and the patient’s rights and obligations. Clinical trials cannot start unless the patient is fully aware of the consent and has signed the form of informed consent.

14 Summary of trial

The person in charge of the trial will summarize the results of the statistical analysis objectively and in detail, and actively complete the summary report of the clinical trial to meet the CFDA standard review of the clinical new drugs, while the participating hospitals will complete the sub-unit summary.

15 Clinical trial progress

Proposed start time: January 2015 (after the approval of the Ethics Committee); proposed end date: December 2016.

16 References

1) Hao Jie, China Cancer Registration Annual Report 2012
2) Baxter Chemotherapy Program 2012 Chinese Edition
3) NCCN non-small cell lung cancer clinical practice guidelines
Appendix one: Clinical trial flow-chart of Anlotinib hydrochloride capsules

<table>
<thead>
<tr>
<th>Items</th>
<th>Screening Group</th>
<th>First cycle (day)</th>
<th>Second–n\textsuperscript{th} cycles</th>
<th>Out of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History, past history of treatment</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor biomarker, HIV check</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid function</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase, amylase</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulation function</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QoL, PS score</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood biochemistry</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine, stool routine test</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrocardiogram, blood routine#</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echocardiography</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging examination</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical signs physical examination</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Release the recovered drug</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recorded adjoint medication, adverse events</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- The visit window period for the first cycle is ± 2 days, follow-up visit window period is ± 3 days, OS follow-up window period is ± 7 days when off the group.
- Initial efficacy (imaging) was assessed after 3 weeks of medication and the efficacy was confirmed after 6 weeks. Follow-up medication was evaluated once per 2 cycles (CT/MRI). PS QoL score and tumor biomarker were evaluated the same time points as the imaging time.
- Routine blood test was conducted once a week in the first cycle, then once every 1–3 weeks case-by-case in the follow-up cycles. After 8 cycles, it was performed every 2 cycles (42 days). If the neutrophil count was \( \leq 1 \times 10^9/L \) or platelets \( \leq 50 \times 10^9/L \), the frequency of review needs to be increased (once every 2-3 days). If the medication is delayed or dose adjusted due to hematologic toxicity, patients’ blood routine should be reexamined. Urine is routinely checked once per week. After 8 cycles, it is checked every 2 cycles (42 days). If the urine protein increases to ++ or above during the medication, 24-h urine protein quantification is needed in a week.
- Routine blood biochemistry is conducted at the end of the first 7-day cycle, and repeatedly checked at the end of each cycle. After 8 cycles, the biochemistry is conducted every 2 cycles. During the medication, the blood biochemical tests of patients should be immediately examined if they present early symptoms such as liver damage (loss of appetite, nausea, vomiting, right upper abdominal discomfort, and fatigue) before the discovery of blood biochemical abnormalities. If ALT or AST increased 3-fold as the ULN or baseline values and total bilirubin increased 2-fold as ULN or baseline values, the frequency of the test should to be increased (recommended 1-2 times/week).
- The ECG (with special attention to QTc) is checked during every visit. Echocardiography and levels of myocardial enzymes are verified (CK, CK-MB); troponin should be added in the examinations if chest pain, palpitations, and other symptoms are noted.
- check coagulation function, thyroid function, lipase, amylase every 2 weeks.
Appendix Two: QoL assessment ECOG PS score criteria (ZPS 5 points method)

0  normal activity
1  mild symptoms, self-care, can engage in light physical activity
2  can tolerate the symptoms of the tumor, take care of themselves, but during the day bedtime does not exceed 50%
   tumor symptoms are serious, bedtime time more than 50% during the day, but can take care of themselves partially.
3  serious illness in bed
4  death

Karnofsky functional state scoring criteria (KPS Percentage)

<table>
<thead>
<tr>
<th>Physical condition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, asymptomatic, and signs</td>
<td>100</td>
</tr>
<tr>
<td>Can carry out normal activities, with mild symptoms and signs</td>
<td>90</td>
</tr>
<tr>
<td>Reluctant to carry out normal activities, there are some symptoms or signs</td>
<td>80</td>
</tr>
<tr>
<td>Can take care of themselves, but cannot maintain normal life and work</td>
<td>70</td>
</tr>
<tr>
<td>Can be largely self-caring, but occasionally need someone else to help</td>
<td>60</td>
</tr>
<tr>
<td>Often need people to take care of them</td>
<td>50</td>
</tr>
<tr>
<td>Cannot take care of themselves, need special care and help</td>
<td>40</td>
</tr>
<tr>
<td>Serious life changes, cannot take care of themselves</td>
<td>30</td>
</tr>
<tr>
<td>Sick, requiring hospitalization, and active supportive care</td>
<td>20</td>
</tr>
<tr>
<td>Heavy danger, near death</td>
<td>10</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
</tr>
</tbody>
</table>

Appendix Three: New York Heart Association (NYHA) cardiac function classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>New York Heart Association (NYHA) Heart function classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Physical activity is not limited, daily activities do not cause excessive fatigue, difficulty breathing or palpitations; cardiac function is compensatory period.</td>
</tr>
<tr>
<td>Class II</td>
<td>Physical activity is slightly limited, rest is asymptomatic, daily activities can cause fatigue, palpitations, difficulty breathing or angina; also known as degree I or mild heart failure.</td>
</tr>
<tr>
<td>Class III</td>
<td>Physical activity was limited; rest is asymptomatic, lighter than the daily activities can cause the above symptoms; also known as degree II or moderate heart failure.</td>
</tr>
<tr>
<td>Class IV</td>
<td>Patient cannot engage in any physical activity, patient at rest also has congestive heart failure or angina symptoms, any physical activity will worsen the condition; also known as degree III or severe heart failure.</td>
</tr>
</tbody>
</table>

Heart function is divided into four degrees, heart failure is divided into three degrees (slightly added by NYHA classification).
Appendix Four: TNM staging standards of International Association of Lung Cancer Research (IASLC), seventh edition

1. Definition of T, N, M

T: Primary tumor

T0: No evidence of primary tumor

Tis: Carcinoma in situ

T1: Tumor 3 cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e., not in the main bronchus)

T1a: Tumor ≤2 cm in greatest dimension

T1b: Tumor >2 cm but ≤3 cm in greatest dimension

T2: Tumor >3 cm but ≤7 cm or tumor with any of the following features:

- Involves main bronchus, ≥2 cm distal to the carina
- Invades visceral pleura
- Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung

T2a: Tumor >3 cm but ≤5 cm in greatest dimension

T2b: Tumor >5 cm but ≤7 cm in greatest dimension

T3: Tumor >7 cm or one that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium; or tumor in the main bronchus <2 cm distal to the carina but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe

T4: Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, carina; separate tumor nodule(s) in a different ipsilateral lobe

N: Regional lymph nodes

Nx: Regional lymph nodes cannot be assessed

N0: No regional lymph node metastasis

N1: Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension

N2: Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)

N3: Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

M: Distant Metastasis

Mx: Distant metastasis cannot be assessed

M0: No distant metastasis

M1: Distant metastasis

M1a: Separate tumor nodule(s) in a contralateral lobe; tumor with pleural nodules or malignant pleural (or pericardial) effusion

M1b: Distant metastasis
2. Histological staging and prognosis (NCCN 2013 V2)

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>TNM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invisible lung cancer</td>
<td>Tx N_0 M_0</td>
</tr>
<tr>
<td>0</td>
<td>T_is N_0 M_0</td>
</tr>
<tr>
<td>IA</td>
<td>T_1a N_0 M_0</td>
</tr>
<tr>
<td></td>
<td>T_1b N_0 M_0</td>
</tr>
<tr>
<td>IB</td>
<td>T_2a N_0 M_0</td>
</tr>
<tr>
<td>IIA</td>
<td>T_2b N_0 M_0</td>
</tr>
<tr>
<td></td>
<td>T_1a N_1 M_0</td>
</tr>
<tr>
<td></td>
<td>T_1b N_1 M_0</td>
</tr>
<tr>
<td></td>
<td>T_2a N_1 M_0</td>
</tr>
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<td>IIB</td>
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</tr>
<tr>
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<td>T_1a N_2 M_0</td>
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<td>T_1b N_2 M_0</td>
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<tr>
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<td></td>
<td>T_4 N_3 M_0</td>
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<td>IV</td>
<td>Any T Any N M_1a</td>
</tr>
<tr>
<td></td>
<td>Any T Any N M_1b</td>
</tr>
</tbody>
</table>
Appendix Five: Biological sample collection SOP

Part 1: EGFR gene and ALK gene screening

Investigation of EGFR gene and ALK gene screening and other lung cancer related genes.

Patient screening, EGFR and ALK gene detection requirements and flow chart can be seen in "EGFR and ALK gene screening record table."

Patients who were unable to provide approved the EGFR and ALK gene test reports before enrollment were required to provide the pathological or biopsy tissue (1 or more puncture, 1 cm, tumor cell ratio ≥50%, necrotic tissue area <10%=gene screening). In the qualified hospital, patients who provide biopsy before the group underwent biopsy tissue retrieval at PD for screening EGFR, ALK, and other gene mutations before and after treatment. Such patients do not need to provide pathological examination at screening and are recommended for the collection of 5–20 pieces of pathological tissue slices at 5–10-µm thickness (area >1 cm × 1 cm) after the group biopsy.
Part II: Retrospective study of tumor biomarkers

Objective: To detect the expression of related cytokines in blood.

Number of acquisitions: This study was conducted in qualified hospitals for three times. The first time was before medication (within the first 3 days), the second time for the second cycle at the end of the drug administration (imaging assessment day, day 42\textsuperscript{nd}), the third time for the emergence of disease progression (within 3 days after imaging assessment).

Collection volume: 3 mL whole blood was collected in the blood tubes each time (without anticoagulants, red blood tubes), room temperature, natural coagulation for approximately 1 h, followed by centrifugation at 2000 \times g for 20 min. The upper serum was withdrawn (do not absorb the lower layer of precipitation) into two 1.5 mL tubes to centrifuge tube, about 0.5 mL/tube.

Storage shipping: -20 °C or lower temperature preservation to avoid repeated freezing and thawing. Use dry ice to transport to the company in stages.

Number Principle: N-Drug No. -X1-CHJM-Collection Date (For example: N034X1-CHJM20150305), N represents NSCLN cancer research, X, Y, Z said that the first 1, 2, 3 times of serum collection, followed by 1, 2 indicates tube 1 or 2 different tubes. (If the patient has no drug number, the sample number is underlined, as N__X1-CHJM-20150305, followed by CRA supplement).

Schematic:
- Take peripheral blood 3 mL
- Store at room temperature for about 1 h
- 2000 \times g centrifugation for 20 min
- Take the upper serum
- Splitting in tubes, labeling, and freezing
- Plasma

OBJECTIVE: To extract tumor DNA from plasma and detect the gene mutation status.

Number of acquisitions: Sample from each patient was collected twice; first before the medication (within the first 3 days) and second during the disease progression.

Collection volume: Use the custom Streck blood collection tube of the company provided specifically, each blood sample not less than 8 mL, mixed thoroughly (gentle action, mild).

Storage shipping: Shanghai Shengsheng staff is appointed. Before transport, the collection tube is stored at room temperature. Shanghai Shengsheng staff will fill out the express delivery forms. CRA will fill the blood bank bar code, and stick the bar code on the blood collection tube, fill in the checklist, and send them together.

Numbering Principle: N-drug number -P1- Pinyin abbreviation - Sample date; for example (N034P1-CHJM-20150305), N for non-small cell lung cancer study, P1 for the first acquisition,
P2 for the second acquisition. (If the patient has no drug number, the sample number is underlined, such as N_P1-CHJM-20150305, followed by mail to lhy@amoydx.com as a supplement).

Schematic:

**Appendix six: Pharmacokinetics of blood sample collection criteria**

Another article attached to "The program of the relevance between Anlotinib hydrochloride capsule system exposure level and efficacy and safety".

**Appendix seven: Screening of related gene pathways**