Clinical Study Protocol

Project Title: A Multicenter Clinical Study Evaluating the Efficacy and Safety of Sacituzumab Tirumotecan Combined With Anlotinib as Second-line Therapy in Patients With Driver Gene-negative Locally Advanced or Metastatic Non-squamous Non-small Cell Lung Cancer

Protocol Version No./Date: V1.0 / August 13, 2025

Leading Site/Principal Investigator: Nanfang Hospital, Southern Medical University / Qiang Zuo

Address of Leading Site: Nanfang Hospital, Southern Medical University / 1838 Guangzhou Avenue North, Guangzhou

Study Department/Principal Investigator: Department of Medical Oncology / Qiang Zuo

Sponsor/Address: Nanfang Hospital, Southern Medical University / 1838 Guangzhou Avenue North, Guangzhou

Confidentiality Statement:

The information contained in this study protocol is provided only for review by the investigators, the ethics committee, and relevant institutions involved in this project. The copyright belongs to Nanfang Hospital, Southern Medical University. Without approval from Nanfang Hospital, Southern Medical University, disclosure of any information to third parties unrelated to this study is strictly prohibited.

Table of Contents

Protocol Synopsis 4
Expected Timeline 11
List of Abbreviations and Definitions of Terms 12
Schedule of Assessments 13
1. Study Background 19
2. Study Objectives 22
    2.1 Primary Objective 22
    2.2 Secondary Objectives 22
    2.3 Exploratory Objective 22
3. Study Endpoints: 23
    3.1 Analysis of Secondary Study Endpoints 23
    3.2 Safety Analysis 23
    3.3 Adverse Event Analysis 24
4. Trial Design 24
    4.1 Overall Design 24
    4.2 Definition of Study Completion 25
5. Study Population 25
    5.1 Inclusion Criteria 25
    5.2 Exclusion Criteria 26
    5.3 Withdrawal Criteria 28
    5.4 Termination Criteria 29
6. Study Drugs 29
    6.1 Investigational Products: 29
    6.2 Dose and Administration 30
    6.3 Dose Adjustment 32
    6.4 Dose Adjustment and Toxicity Management for Adverse Events Related to Sacituzumab Tirumotecan 32
    6.5 Dose Adjustment and Precautions for Adverse Events Related to Anlotinib 42
    6.6 Guidelines for Management of Overlapping Toxicities 47
    6.7 Management Guidelines for Infusion-Related Reactions Associated With Study Treatment 48
    6.8 Guidelines for Management of Overlapping Toxicities 51
7. Drug Management 51
    7.1 Drug Packaging and Labeling 51
    7.2 Drug Preparation 52
    sacituzumab tirumotecan 52
    7.3 Concomitant Medications and Concomitant Therapy 53
    7.4 Drug Accountability 55
8. Study Methods and Procedures 55
    8.1 Study Procedures 55
    8.2 Biological Sample Collection and Testing 59
9. Evaluation Measures 62
    9.1 Efficacy evaluation and follow-up 62
    9.2 Safety Evaluation 64
    9.3 Evaluation of Other Study Objectives 64
10. Safety Monitoring, Reporting, and Medical Management 64
    10.1 Adverse Events 64
    10.2 Adverse Drug Reactions 65

10.3 Serious Adverse Events 65
10.4 Hospitalization 66
10.5 Disease Progression and Death 66
10.6 Collection, Recording, and Description of Adverse Events/Serious Adverse Events 67
10.7 Pregnancy Reporting 79
11. Criteria for Trial Termination/Suspension 75
11.1 Sponsor's Right to Terminate/Suspend This Trial 75
11.2 Termination/Suspension Requested by the Ethics Committee 75
12. Data Management 75
12.1 Data Management 75
12.2 Data Recording and Document Retention 75
12.3 Access to Source Data/Documents 76
12.4 Protocol Amendments 76

13. Statistical Analysis 76
13.1 Sample Size Determination 76
13.2 Definition and Selection of Analysis Sets 76
13.3 Statistical Methods 77
14. Trial Management 77
14.1 Compliance With GCP Requirements 77
14.2 Protection of Subject Privacy 78
14.3 Problems Occurring During the Trial and Management Measures 78
14.4 Quality Control and Quality Assurance 79
14.5 Expected Clinical Trial Progress and Completion Date 79
14.6 Sponsor Responsibilities 79

15. Trial-Related Ethics 80
15.1 Ethics Committee 80
15.2 Informed Consent 81
15.3 Others 81

Protocol Synopsis

Protocol Title	A Multicenter Clinical Study Evaluating the Efficacy and Safety of Sacituzumab Tirumotecan Combined With Anlotinib as Second-line Therapy in Patients With Driver Gene-negative Locally Advanced or Metastatic Non-squamous Non-small Cell Lung Cancer
Sponsor	Nanfang Hospital, Southern Medical University
Principal Investigator	Qiang Zuo
Leading Site	Nanfang Hospital, Southern Medical University
Study Population	Non-squamous non-small cell lung cancer
Study Objectives	• To evaluate the efficacy and safety of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC. • Primary objective: To evaluate the objective response rate (ORR), as assessed by the investigator according to RECIST v1.1. • Secondary objectives: To evaluate the progression-free survival (PFS) of sacituzumab tirumotecan combined with anlotinib as second -line treatment for driver gene-negative locally advanced or metastatic non-squamous non-small cell lung cancer (nsq-NSCLC), as assessed by investigators according to RECIST v1.1;To evaluate the disease control rate (DCR), duration of response (DOR), and time to response (TTR) of sacituzumab tirumotecan combined with anlotinib as second-line treatment for driver gene-negative locally advanced or metastatic nsq-NSCLC, as assessed by investigators according to RECIST v1.1;To evaluate the overall survival (OS) of sacituzumab tirumotecan combined with anlotinib as second-line treatment for driver gene-negative locally advanced or metastatic nsq -NSCLC;To evaluate the safety and tolerability of sacituzumab tirumotecan combined with anlotinib as second-line treatment for driver gene-negative locally advanced or metastatic nsq-NSCLC. • Exploratory objective: To evaluate the correlation between plasma metabolome, proteome and ctDNA genome and the efficacy of the combination therapy of luconasertumab and crizotinib with anlotinib in the second-line treatment of patients with driver gene-negative locally advanced or metastatic nsq-NSCLC.

Study Design	This study is an investigator-initiated, single-arm, multicenter, prospective clinical study designed to evaluate the safety and efficacy of sacituzumab tirumotecan in combination with anlotinib as second-line treatment in patients with driver gene-negative locally advanced or metastatic non-squamous non-small cell lung cancer (nsq-NSCLC). The study plans to enroll 38 eligible subjects. The primary objective of this phase of the study is to evaluate the efficacy and safety of sacituzumab tirumotecan in combination with anlotinib as second-line treatment in patients with driver gene-negative locally advanced or metastatic nsq-NSCLC. After signing the informed consent form, subjects will undergo screening assessments within 28 days prior to the first dose to determine whether they meet the study eligibility criteria. Subjects who meet the inclusion and exclusion criteria will receive treatment with sacituzumab tirumotecan and anlotinib. The treatment regimen is as follows: 1) Sacituzumab tirumotecan 4 mg/kg, administered by intravenous infusion (IV) once every 2 weeks. 2) Anlotinib 10 mg, orally administered (PO) once daily (QD) before breakfast, taken continuously for 2 weeks followed by 1 week off treatment, with each 3-week period defined as one treatment cycle. Subjects will continue treatment until radiographic disease progression confirmed by the investigator, intolerable toxicity, the subject's request to discontinue treatment, or other protocol-defined treatment discontinuation criteria, whichever occurs first. Eligible subjects will undergo regular tumor assessments based on RECIST v1.1. Within 48 weeks after treatment initiation, imaging examinations for tumor assessment will be performed every 6 weeks (±7 days). After 48 weeks, tumor assessments will be performed every 12 weeks (±7 days) until radiographic disease progression, initiation of new anti-tumor therapy, withdrawal of informed consent, loss to follow-up, death, or termination of the study by the sponsor, whichever occurs first. Imaging examinations will be performed according to the original schedule regardless of any delay or adjustment in treatment administration. After a patient first achieves complete response (CR) or partial response (PR), confirmation of the observed response is required at least 4 weeks (28 days) later. After the end of treatment, safety follow-up will be completed for all subjects regardless of whether new anti-tumor therapy has been initiated. Survival follow-up visits will then be conducted by telephone every 3 months (±14 days) after the last dose of study treatment to collect survival information and information on new anti-tumor therapies, until the subject decides to withdraw from the study, is lost to follow-up, dies, or the study ends, whichever occurs first.

Study Duration	2 years
Sample Size	38 subjects
Inclusion Criteria	1) Age ≥18 years at signing informed consent, regardless of sex; 2) Histologically or cytologically confirmed locally advanced (stage IIIB/IIIC) or metastatic (stage IV) non-squamous NSCLC, with disease progression after prior anti-PD-1/L1 monoclonal antibody plus platinum-based doublet chemotherapy; 3) No known driver gene alterations such as EGFR, ALK, ROS1, NTRK, BRAF, MET, KRAS, HER2, or RET; 4) At least one measurable target lesion not previously treated with radiotherapy according to RECIST v1.1; 5) ECOG performance status score of 0 or 1; 6) Asymptomatic brain metastases or brain metastases with stable symptoms after local therapy are allowed; 7) Expected survival ≥12 weeks; 8) Adequate organ and bone marrow function, defined as follows: a) Hematology: neutrophil count (NEUT) ≥ 1.5 × 10^9/L; platelet count (PLT) ≥ 100 × 10^9/L; hemoglobin ≥ 90 g/L; b) Liver function: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 × ULN; for subjects with liver metastases at baseline, ALT and AST ≤ 5 × ULN; albumin ≥ 30 g/L; total bilirubin (TBIL) ≤ 1.5 × ULN; c) Renal function: creatinine clearance ≥ 50 mL/min, calculated using the standard Cockcroft-Gault formula, or serum creatinine ≤ 1.5 × ULN. 9) Subjects of childbearing potential must agree to effective medical contraception from informed consent until 6 months after the last dose; 10) Voluntary participation, signed informed consent, and ability to comply with protocol visits and procedures.
Efficacy Analysis	1. Efficacy evaluation: 1) ORR: proportion of subjects with CR or PR, assessed by the investigator according to RECIST v1.1; 2) PFS: time from first dose to first radiographic disease progression or death, whichever occurs first, assessed according to RECIST v1.1; 3) DCR: proportion of subjects with CR, PR, or SD, assessed according to RECIST v1.1; 4) DOR: interval from first documented response to disease progression or death, whichever occurs first, assessed according to RECIST v1.1; 5) OS: time from first dose to death from any cause.

Safety Analysis	During the study and follow-up, adverse event severity will be graded according to NCI CTCAE version 5.0. Safety endpoints include: 1) incidence, severity, and relationship to study drugs of all AEs, TEAEs, and SAEs; 2) number and proportion of subjects with dose reduction or treatment discontinuation due to AEs; 3) changes in vital signs, physical examination findings, and laboratory results before, during, and after study treatment.
Statistical Analysis	Statistical analyses will be performed using the full analysis set, per-protocol set, and safety analysis set. Full Analysis Set (FAS): includes all randomized subjects who have received at least one dose of the study drug and have measurable baseline tumors. Per-Protocol Set (PPS): includes subjects in the FAS population who have at least one post-treatment tumor imaging assessment, have good compliance, and have no major protocol violations or deviations. Safety Set (SS): includes subjects who have received at least one dose of study treatment and have undergone subsequent safety assessments. Sample Size Determination: 1. Statistical hypothesis and sample size calculation This is a single-arm study. The objective response rate (ORR) of docetaxel monotherapy is 13.3%; therefore, 13% is selected as the historical control rate (p0 = 13%). The ORR of lukang sacituzumab combined with anlotinib is expected to reach 33% (p1 = 33%). Simon's optimal two-stage design will be adopted, with a one-sided type I error rate of 0.05 and a power of 80%. Under these assumptions, 9 subjects will be enrolled in the first stage. If ≤1 subject achieves CR/PR, the study will be terminated; if >1 subject achieves CR/PR, the study will proceed to the second stage, with a total sample size of 34 subjects. Considering an approximately 10% dropout rate in the actual study, a total of 38 subjects will ultimately be enrolled. 2. General methods for statistical analysis

	All statistical analyses will be performed using PASS statistical analysis software, version 15 or above. Unless otherwise specified, continuous variables will be summarized using mean ± standard deviation or median (minimum, maximum). Categorical variables will be summarized using frequencies and percentages. Survival time will be analyzed using the Kaplan–Meier method to estimate median PFS/OS and the corresponding 95% confidence intervals (95% CIs), and survival curves will be plotted. ORR/DCR will be summarized based on the number and percentage of responders, and the corresponding 95% CIs will be calculated using the Clopper–Pearson method.

	1) Histology or cytology showing small cell lung cancer, squamous cell carcinoma, neuroendocrine carcinoma, or carcinosarcoma components; 2) Previous TROP2-targeted therapy or any therapy containing a topoisomerase I-targeting agent, including ADC therapy; 3) Known meningeal, brainstem, spinal cord metastasis/compression, or active CNS metastasis; 4) Other malignancy within 3 years before dosing, except locally cured tumors such as basal cell carcinoma, squamous cell carcinoma of the skin, or cervical carcinoma in situ; 5) Presence of any of the following cardiovascular or cerebrovascular diseases or risk factors: a) Myocardial infarction, unstable angina, acute or persistent myocardial ischemia, Grade 3 or 4 heart failure according to the New York Heart Association (NYHA) classification, symptomatic or poorly controlled severe arrhythmia, cerebrovascular accident, transient ischemic attack, or other serious cardiovascular or cerebrovascular diseases occurring within 6 months prior to administration; b) History of myocardial diseases such as myocarditis, primary cardiomyopathy, or specific cardiomyopathy; d) Major vascular diseases that may be life-threatening or require surgery within 6 months prior to administration, such as aortic aneurysm or aortic dissecting aneurysm. 6) Poorly controlled hypertension; 7) History or presence of ILD/noninfectious pneumonitis requiring steroid therapy, or suspected ILD/noninfectious pneumonitis not excluded by imaging; 8) Severe dry eye syndrome, severe meibomian gland disease/blepharitis, or corneal disease affecting healing; 9) Active chronic inflammatory bowel disease, gastrointestinal

Exclusion Criteria	obstruction, severe ulcer, gastrointestinal perforation, abdominal abscess, or acute gastrointestinal bleeding; 10) Risk of esophagotracheal or esophagopleural fistula; 11) Tumor invasion of major vessels or high risk of fatal bleeding; 12) Bleeding symptoms of any severity, or any ≥CTCAE grade 3 bleeding event within 1 month before first dose with unhealed wound, ulcer, or fracture; 13) Active tuberculosis; 14) History of allogeneic organ transplantation or allogeneic hematopoietic stem cell transplantation; 15) HIV positivity, AIDS history, or active syphilis infection; 16) Major surgery within 4 weeks before dosing or expected need for major surgery during the study; 17) Use of medicinal products or herbal supplements known to be strong cytochrome P450 (CYP) 3A4 inducers at present, or inability to discontinue such agents at least 3 weeks prior to the first administration of study treatment. All subjects shall avoid concomitant use of any medicinal products, herbal supplements known to induce CYP3A4 and/or intake of corresponding foods to the greatest extent possible; 18) Subjects who have received live vaccines within 30 days before administration, or intend to receive live vaccines during the study; 19) Pregnant or lactating women; 20) Any condition that, in the opinion of the investigator, may interfere with the evaluation of the study drug, subject safety, or the interpretation of study results, or any other condition judged by the investigator to render the subject unsuitable for participation in this study.
Efficacy Analysis	1. Efficacy evaluation: 1) ORR: proportion of subjects with CR or PR, assessed by the investigator according to RECIST v1.1; 2) PFS: time from first dose to first radiographic disease progression or death, whichever occurs first, assessed according to RECIST v1.1; 3) DCR: proportion of subjects with CR, PR, or SD, assessed according to RECIST v1.1; 4) DOR: interval from first documented response to disease progression or death, whichever occurs first, assessed according to RECIST v1.1; 5) OS: time from first dose to death from any cause.
Safety	During the study and follow-up, adverse event severity will be graded according to NCI CTCAE version 5.0. Safety endpoints include: 1) incidence, severity, and relationship to study drugs of all AEs, TEAEs, and SAEs; 2) number and proportion of subjects with

Analysis	dose reduction or treatment discontinuation due to AEs; 3) changes in vital signs, physical examination findings, and laboratory results before, during, and after study treatment.
Statistical Analysis	Statistical analyses will be performed using the full analysis set, per-protocol set, and safety analysis set. Full Analysis Set (FAS): includes all randomized subjects who have received at least one dose of the study drug and have measurable baseline tumors. Per-Protocol Set (PPS): includes subjects in the FAS population who have at least one post-treatment tumor imaging assessment, have good compliance, and have no major protocol violations or deviations. Safety Set (SS): includes subjects who have received at least one dose of study treatment and have undergone subsequent safety assessments. Sample Size Determination: 1. Statistical hypothesis and sample size calculation This is a single-arm study. The objective response rate (ORR) of docetaxel monotherapy is 13.3%; therefore, 13% is selected as the historical control rate (p0 = 13%). The ORR of lukang sacituzumab combined with anlotinib is expected to reach 33% (p1 = 33%). Simon's optimal two-stage design will be adopted, with a one-sided type I error rate of 0.05 and a power of 80%. Under these assumptions, 9 subjects will be enrolled in the first stage. If ≤1 subject achieves CR/PR, the study will be terminated; if >1 subject achieves CR/PR, the study will proceed to the second stage, with a total sample size of 34 subjects. Considering an approximately 10% dropout rate in the actual study, a total of 38 subjects will ultimately be enrolled. 2. General methods for statistical analysis All statistical analyses will be performed using PASS statistical analysis software, version 15 or above. Unless otherwise specified, continuous variables will be summarized using mean ± standard deviation or median (minimum, maximum). Categorical variables

	will be summarized using frequencies and percentages. Survival time will be analyzed using the Kaplan–Meier method to estimate median PFS/OS and the corresponding 95% confidence intervals (95% CIs), and survival curves will be plotted. ORR/DCR will be summarized based on the number and percentage of responders, and the corresponding 95% CIs will be calculated using the Clopper–Pearson method.
Follow-up	Follow-up duration: 12 months.
Statistical Methods	All statistical analyses will be performed using PASS statistical analysis software, version 15 or above. Unless otherwise specified, continuous variables will be described using mean ± standard deviation or median (minimum, maximum). Categorical variables will be described using frequency and percentage. Survival time will be estimated using the Kaplan–Meier method to determine the median PFS/OS and its 95% confidence interval (95% CI), and survival curves will be plotted. ORR/DCR will be summarized based on the number and percentage of responders, and the corresponding 95% CI will be calculated using the Clopper–Pearson method.
Expected Timeline	The estimated enrollment period is 18 months, with 6 months of follow-up. The estimated study start time is September 2025, and the estimated study completion time is September 2027.

List of Abbreviations and Definitions of Terms

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
RECIST	Response Evaluation Criteria in Solid Tumors
GCP	Good Clinical Practice for drug clinical trials
EGFR	Human epidermal growth factor receptor
ICF	Informed consent form
OS	Overall survival
ORR	Objective response rate
PFS	Progression-free survival
CR	Complete response
PR	Partial response
SD	Stable disease
PD	Disease progression
SAE	Serious adverse event
TROP2	Human trophoblast cell-surface glycoprotein antigen 2

Schedule of Assessments

Item	Screening Period		Sacituzumab tirumotecan Treatment Period（Q2W）	Anlotinib Treatment Period（Q3W）	End-of-Treatment (EOT）Visit [a]	Survival Follow-up Visit
Time	-D28~-D1	-D7~-D1	±3 days	±3 days	Within 30 days after the investigator determines discontinuation ofstudy treatment	Approximately every 3 months
Informed consent form [b]	X					
Inclusion/Exclusion Criteria	X					
Demographic data [c]	X					
Medical history [d]	X					
Genetic test results [e]	X					
Tumor tissue/plasma sample collection [f]	X					
Pregnancy test (women of childbearing potential only) [g]	X					
Vital signs [h]		X	According to clinical routine practice	According to clinical routine practice	X	
Physical examination [i]		X	According to clinical routine practice	According to clinical routine practice	X	
Height and body weight [j]		X	According to clinical routine practice	According to clinical routine practice		
ECOG score [k]		X	According to clinical routine practice	According to clinical routine practice		
Complete blood count [m]		X	According to clinical routine practice	According to clinical routine practice	X	

Blood biochemistry [n]		X	According to clinical routine practice	According to clinical routine practice	X	
Urinalysis [o]		X	According to clinical routine practice	According to clinical routine practice	X	
Coagulation function [p]		X	According to clinical routine practice	According to clinical routine practice	X	
12-lead ECG [q]	X		According to clinical routine practice	According to clinical routine practice	X	
Oral care plan [r]			X	X		
Adverse events [s]	X		X	X	X	
Concomitant medications [t]	X		X	X	X	X
Sacituzumab tirumotecan [u]			X			X
Anlotinib [v]				X		
Imaging examinations [w]	X		last dose after 48 weeks within every 6 weeks time (±7 days), 48 weeks after every 12 weeks time (±7 days)		X	
Survival follow-up [x]						X
New antitumor therapy						X

Note: Carry out various inspections and test procedures according to the test process table. Minor deviations are allowed during the inspection window periods due to holidays or other management reasons, but they should be as close as possible to the originally scheduled visit window periods. In addition, apart from the inspection items and points listed in the table, the researcher may increase the number of inspections or conduct other inspection items as necessary.

a)EOT visit should at investigator defined termination study treatment after 30 days within progress. if in treatment visit before 7 days within progress test examination, no must serious progress examination. for at disease progression outside of reason treatment or investigator evaluate disease progression treatment of subject, time imaging examination major at 4 weeks of should in EOT visit time progress, after continued of imaging examination should dose stage progress, untilinitiation of new antitumor therapy, subject withdrawal of informed consent, loss to follow-up, death or study, whichever occurs first. should may in initiation of new antitumor therapy before investigator of disease progression.

b)informed consent form: sign the informed consent form(ICF) should in study process before progress, before the first dose 28 days within has of tumor imaging examination, heart Figure examination and disease toxicity test and before the first dose 42 days within has of bone scan, tumor group tissue active test, genetic testing outside.

c) Demographic Data: including date of birth, gender, ethnicity, age, etc.

d) Medical History: including prior tumor diagnosis records, history of anti-tumor therapies (together with treatment responses), History of Present Illness (non-oncologic), past medical history, surgical history, allergy history, smoking history, alcohol abuse history, substance abuse history, etc.

e)Genetic testing results: Subjects must be able to provide a previous tissue-based driver gene testing report to confirm the absence of known driver gene alterations, such as EGFR, ALK, ROS1, NTRK, BRAF, MET, KRAS, HER2, RET, etc. If such a report cannot be provided, subjects must undergo tumor tissue-based testing during the screening period for the evaluation of driver gene alterations.
Prior to the first dose, subjects are required to provide tumor tissue samples on a voluntary basis, but must agree to the collection of plasma samples.

f) in before the first dose, subject must consent tumor group tissue sample, blood sample of collection.

g) pregnancy examination: for female subjects of childbearing potential, stage progress blood /urine pregnancy examination.

h)Vital Signs: including blood pressure, pulse, respiratory rate and body temperature.

i)physical examination: stage should progress adjust of physical examination. after continued by investigator according to clinical clinical progression for property of physical examination.

j) Height: Measured only during the screening period. Weight: Body weight shall be measured prior to each administration of sacituzumab govitecan-hziy, and the measured weight will be used to calculate the administration dose of sacituzumab govitecan-hziy. If the subject's body weight changes by more than ±10% compared with the weight recorded before the first dose, the dose of sacituzumab govitecan-hziy shall be recalculated, and the weight used for recalculation will serve as the new baseline for dose calculation.

k)ECOG score: in stage, treatment stage, EOT for has patient progress ECOG score.

l)laboratory tests: withdraw has clinical significance of examination, may according to patient condition examination rate.

m)Routine Blood Test: including red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and lymphocyte count. Testing shall be performed per routine clinical practice; test results from external hospitals may be acceptable under special circumstances.

n)Serum Biochemistry: including but not limited to alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, blood urea nitrogen/urea, creatinine, uric acid, lactate dehydrogenase, total protein, albumin, blood glucose, calcium, phosphorus, potassium, sodium, chloride and magnesium. Testing shall be performed in accordance with routine clinical practice; test reports from external hospitals may be accepted under special circumstances.

o) Urinalysis: including urine protein, urine leukocytes, urine glucose, urine ketone bodies, urine bilirubin, urobilinogen, urine pH, urine occult blood and urine specific gravity. Testing shall be conducted in accordance with routine clinical practice; test results from external hospitals may be accepted under special circumstances.

p) Coagulation Function Tests: including prothrombin time, activated partial thromboplastin time, and international normalized ratio. Tests shall be performed in accordance with routine clinical practice; test results from external hospitals may be accepted under special circumstances.

q)12-Lead Electrocardiogram: Testing shall be performed in accordance with routine clinical practice; test results from external hospitals may be accepted under special circumstances.

r) Oral Care Plan: Subjects are strongly recommended to brush their teeth with a soft-bristled toothbrush after meals and before bedtime every day, and receive prophylactic treatment with steroid-containing mouthwash (formula: 50 mL normal saline plus 5 mg dexamethasone). The mouthwash shall be used for gargling 4 times daily, 1 to 2 minutes each time, then spit out. If steroid-containing mouthwash is unavailable, inert alcohol-free mouthwash may be used as a substitute; however, steroid-containing mouthwash is still the preferred option.Dental floss may be used for cleaning after meals, with caution taken to avoid oral pain and gingival bleeding.

s) adverse event: sign the informed consent form to before the first dose record and study process related of adverse event. last dose to last dose after 30 days must combined record all adverse events. follow visit occurs of adverse event to investigator of, has after syndrome, symptom status stable defined, test examination of to, subject loss to follow-up, death or for event other relief.

t) concomitant use drug: sign the informed consent form to last dose after 30 days must record concomitant use drug. last dose 30 days after, must record use at treatment and study drug related AE of concomitant use drug.

u)Sacituzumab tirumotecan will be administered at a dose of 5 mg/kg by intravenous infusion once every 3 weeks. Premedication before administration of sacituzumab tirumotecan: For the first 4 doses, premedication should include diphenhydramine 40 mg, or another antihistamine H1 receptor blocker at an equivalent dose; acetaminophen, immediate-release 0.5 g or sustained-release 0.65 g, or another nonsteroidal antipyretic analgesic at an equivalent dose; dexamethasone 10 mg administered intravenously; and an antihistamine H2 receptor blocker, such as famotidine 20 mg or another available drug at an equivalent dose. If no infusion-related reaction/hypersensitivity reaction occurs during the first 4 doses of this product, starting from the 5th dose, including the 5th dose, premedication should include diphenhydramine 40 mg, or another antihistamine H1 receptor blocker at an equivalent dose, and acetaminophen, immediate-release 0.5 g or sustained-release 0.65 g, or another nonsteroidal antipyretic analgesic at an equivalent dose. Corticosteroids and antihistamine H2 receptor blockers may be administered as prophylactic medications at

the investigator's discretion. The investigator may also decide, at their discretion, to administer a higher dose of dexamethasone, i.e., > the recommended dose of 10 mg. Prophylactic medications administered intravenously or intramuscularly should be given 30–60 minutes before administration of sacituzumab tirumotecan, and oral prophylactic medications should be given 60 ± 10 minutes before administration of sacituzumab tirumotecan.

v)Anlotinib 10 mg shall be administered orally once daily before breakfast for 2 consecutive weeks, followed by a 1-week treatment break, with a 3-week cycle as one treatment cycle.w)Imaging examinations: CT and/or MRI examinations shall be performed within 28 days before administration of study treatment as the baseline assessment. If there are no contraindications to contrast agents, contrast-enhanced CT and/or contrast-enhanced MRI should be preferred. The same examination method should be used throughout the study. The examination site and method shall be determined by the investigator based on clinical indications. Subjects will undergo imaging-based tumor assessment every 6 weeks (±7 days) within 48 weeks after the first dose, and every 12 weeks (±7 days) thereafter, until disease progression, initiation of new antitumor therapy, withdrawal of informed consent by the subject, loss to follow-up, death, or study completion, whichever occurs first. Imaging examinations shall be performed as scheduled regardless of any delay or adjustment in dosing.

Bone scan: During the screening period, radionuclide bone scan (ECT) shall be performed. Examination results obtained within 42 days before the first dose are acceptable, and positron emission tomography-computed tomography (PET-CT) is also acceptable. Subsequent bone imaging shall be performed at the investigator's discretion based on clinical indications. However, bone imaging must be performed when the investigator's efficacy assessment is complete response (CR) or when progression of bone lesions is suspected.

x) survival follow-up visit: 3 month follow visit time, collect subject of survival information and after continued of tumor treatment.

1. Study Background

According to the World Health Organization (WHO), there were approximately 2.481 million new lung cancer cases and 1.817 million lung cancer deaths worldwide in 2022, ranking first among all cancers in both incidence and mortality, accounting for 12.4% and 18.7%[1], respectively. In China, there were approximately 1.061 million new lung cancer cases and 733, 000 lung cancer deaths in 2022, also ranking first among all cancers, accounting for 22.0% and 28.5%[2], respectively. Lung cancer is classified into two major histopathological types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is the most common type, accounting for approximately 85%[3] of all lung cancers. According to pathological subtype, NSCLC is divided into non-squamous carcinoma and squamous cell carcinoma, with adenocarcinoma being the main component of non-squamous carcinoma. According to molecular classification, NSCLC may be classified as driver-gene-negative or driver-gene-positive.

Systemic treatment for patients with advanced NSCLC is generally selected based on pathological subtype (non-squamous or squamous carcinoma) and driver gene mutation status (driver-gene-positive or driver-gene-negative). According to the 2024 Chinese Society of Clinical Oncology (CSCO) Guidelines for NSCLC, immunotherapy combined with platinum-based doublet chemotherapy has become the standard first-line treatment for driver-gene-negative locally advanced or metastatic non-squamous NSCLC, with a median PFS of approximately 5.5–11.3 months; however, resistance is ultimately inevitable[4]. After disease progression following first-line immunotherapy plus chemotherapy in patients with driver-gene-negative advanced NSCLC, single-agent chemotherapy, mainly docetaxel, remains the standard regimen recommended by the CSCO guidelines. However, its benefit is limited, with a median OS of 10.5 –12.0 months, median PFS of 4.0–4.2 months, and an objective response rate (ORR) of approximately 13.3%, indicating a substantial unmet clinical need[4-6].

Minimizing damage to normal cells is an important direction in the development of cancer therapy. Antibody-drug conjugates (ADCs) have become a major research focus in oncology in recent years. They combine the targeting specificity of antibodies with the high cytotoxicity of toxin molecules and achieve efficient and precise tumor cell killing through targeted payload release while improving drug safety. Therefore, in terms of efficacy and safety, ADCs are expected to be more potent than targeted therapies and better tolerated than chemotherapy. Previous studies have shown that topoisomerase I inhibitors may enhance

immune system responses and increase PD-L1 expression. Accordingly, ADCs carrying topoisomerase I inhibitors may further improve antitumor efficacy when combined with anti-PD-1/PD-L1 immunotherapy.

At present, ADCs targeting trophoblast cell-surface antigen 2 (TROP2) have become an active area of drug development in oncology. Human trophoblast cell-surface antigen 2 (TROP2) is a transmembrane glycoprotein expressed on human trophoblast cells. It participates in tumor cell proliferation, invasion, and metastasis mainly through calcium signaling pathways, cyclin expression, and reduced fibronectin-mediated adhesion. TROP2 is expressed at low levels in normal epithelial tissues but is highly expressed in epithelial cancers. For example, the expression rate of TROP2 is approximately 64% in lung adenocarcinoma and approximately 75% in lung squamous cell carcinoma.Studies have also found that high TROP2 expression is closely associated with more aggressive disease, shorter survival, and poor prognosis[9]. In addition, TROP2 shows strong internalization and lysosomal targeting properties, and its internalization is stronger in tumor cells than in normal cells[10]. Based on these characteristics, TROP2 is a potential therapeutic target in epithelial cancers with high TROP2 expression and has important clinical significance. Notably, high TROP2 expression has also been reported to be associated with primary resistance to immunotherapy in advanced NSCLC[11].

Sacituzumab tirumotecan for injection is a TROP2-targeting ADC. It contains a novel moderately toxic small molecule payload (a belotecan derivative), an innovative linker and conjugation structure, and a high drug-to-antibody ratio. It can selectively release the cytotoxic payload inside tumor cells, thereby efficiently killing tumor cells, inhibiting tumor cell proliferation, inducing tumor cell apoptosis, and ultimately exerting antitumor effects. Its design strengthens the linkage between the antibody and linker, which may prolong the serum half-life of the ADC molecule. While further improving drug targeting, it reduces systemic safety risks and improves the therapeutic index[12].

Among 21 patients with EGFR-negative advanced NSCLC who had failed prior immunotherapy and chemotherapy and received sacituzumab tirumotecan 5 mg/kg monotherapy (data cut-off: November 22, 2023), the median follow-up was 17.2 months, the ORR was 26.3% (5/19), the median DOR was 9.6 months, the median PFS was 5.3 months, and the median OS was 14.1 months[13]. These data suggest that the efficacy of sacituzumab tirumotecan monotherapy still needs to be improved.

Although multiple clinical trials of sacituzumab tirumotecan have been conducted, little is known about predictors of efficacy and mechanisms of primary or acquired resistance. Studies suggest that molecular alterations in the antibody-binding target TROP2 and the

payload target topoisomerase I (TOP1) may mediate resistance to sacituzumab tirumotecan. Therefore, we performed genomic sequencing of blood samples collected before treatment and after progression from patients with driver-gene-negative advanced non-squamous NSCLC treated with sacituzumab tirumotecan, aiming to further understand predictors of efficacy and mechanisms of resistance.

Previous studies have shown that combining anti-angiogenic agents with standard docetaxel therapy as second-line treatment for driver-gene-negative advanced NSCLC can improve efficacy.Theoretically, anti-angiogenic agents can normalize tumor vasculature and promote the delivery and distribution of ADC drugs within tumors. Therefore, combining anti-angiogenic agents represents a potential TROP2-ADC combination strategy with a synergistic mechanism. Whether adding an anti-angiogenic agent to sacituzumab tirumotecan can further improve efficacy warrants exploration.

Anlotinib is a small-molecule multi-target tyrosine kinase inhibitor that blocks signaling pathways including VEGFR1–3, PDGFR α/β, FGFR1–4, and c-Kit, thereby inhibiting tumor angiogenesis[22]. The results of a multicenter, randomized, double-blind, placebo-controlled phase III clinical trial (ALTER0303) showed that, in patients with advanced/metastatic NSCLC who had failed at least two prior systemic chemotherapy regimens, anlotinib significantly prolonged PFS compared with placebo (median: 5.4 months vs. 1.4 months; HR 0.25) and OS (median: 9.5 months vs. 6.4 months; HR 0.70)[23]. Based on these results, the NMPA approved anlotinib on May 9, 2018 for patients with locally advanced or metastatic NSCLC who had progressed or relapsed after at least two prior systemic chemotherapy regimens. Therefore, anlotinib is selected for this study.

Based on the above rationale, this study will conduct a prospective, multicenter clinical study of sacituzumab tirumotecan combined with anlotinib in patients with driver-gene-negative locally advanced or metastatic nsq-NSCLC who have progressed after immunotherapy plus chemotherapy. The study aims to evaluate the safety and efficacy of this combination regimen and preliminarily explore biomarkers that may predict the population benefiting from this regimen, thereby providing a new second-line treatment option after resistance to immunotherapy plus chemotherapy.

2. Study Objectives

To evaluate the efficacy and safety of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC.

2.1 Primary Objective

To evaluate the objective response rate (ORR) of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC, as assessed by the investigator according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1).

2.2 Secondary Objectives

To evaluate progression-free survival (PFS) of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC, as assessed by the investigator according to RECIST v1.1;

To evaluate the disease control rate (DCR), duration of response (DOR), and time to response (TTR) of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC, as assessed by the investigator according to RECIST v1.1;

To evaluate overall survival (OS) of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC;

To evaluate the safety and tolerability of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC.

2.3 Exploratory Objective

To evaluate the correlations of plasma metabolomics, proteomics, and ctDNA genomics with the efficacy of sacituzumab tirumotecan combined with anlotinib as second-line therapy for driver-gene-negative locally advanced or metastatic nsq-NSCLC.

3. Study Endpoints:

The analysis of ORR will be based on the FAS. ORR is defined as the proportion of subjects in the analysis population who achieve complete response (CR) or partial response (PR), as determined by the investigator according to RECIST 1.1. The 95% confidence interval for ORR will be estimated using the Clopper–Pearson method. The determination of CR and PR should be based on the best overall tumor response assessment confirmed by a second evaluation during the study.

3.1 Analysis of Secondary Study Endpoints

The analysis of progression-free survival (PFS), as assessed by the investigator, will be based on the FAS. PFS is defined as the time from the start of treatment to the first radiographic disease progression or death, whichever occurs first. Disease progression will be determined by the investigator according to RECIST v1.1. Subjects without disease progression or death at the time of analysis will be censored at the date of the last tumor assessment. Median PFS and its 95% CI will be estimated using the Kaplan–Meier method, and survival curves will be plotted.

The analysis of overall survival (OS) will be based on the FAS. OS is defined as the time from the start of treatment to death from any cause. If a subject is still alive at the end of the study, the subject will be censored at the known “last date alive”. Median OS and its 95% CI will be estimated using the Kaplan–Meier method, and survival curves will be plotted.

The analysis of disease control rate (DCR) will be based on the FAS. DCR is defined as the proportion of subjects with complete response (CR), partial response (PR), or stable disease (SD) among all subjects, as assessed by the investigator according to RECIST v1.1. The 95% confidence interval for DCR will be estimated using the Clopper–Pearson method.

The analysis of duration of response (DOR) will be based on the FAS. DOR is defined as the interval from the first documented response to disease progression or death, whichever occurs first, as assessed by the investigator according to RECIST v1.1. For patients without progression or death, DOR will be censored at the date of the last valid imaging assessment.

Median DOR will be estimated using the Kaplan–Meier method, and survival curves will be plotted.

3.2 Safety Analysis

The safety analysis will be performed using the Safety Set (SS). Safety variables include adverse events and safety data such as laboratory tests, vital signs, and electrocardiograms.

3.3 Adverse Event Analysis

Adverse events, adverse reactions, serious adverse events/adverse reactions, adverse events/adverse reactions leading to dose reduction or treatment interruption, adverse events/adverse reactions leading to withdrawal from the trial, and adverse events/adverse reactions leading to death will be summarized. The severity of adverse events and adverse reactions will be graded according to NCI CTCAE version 5.0.

4. Trial Design

4.1 Overall Design

This is a prospective, multicenter, single-arm clinical study of sacituzumab tirumotecan combined with anlotinib as second-line therapy in patients with driver-gene-negative locally advanced or metastatic nsq-NSCLC. The study plans to enroll 38 eligible subjects.

After signing the informed consent form, subjects will undergo screening assessments within 28 days before the first dose to determine whether they are eligible for the study. Subjects who meet the inclusion and exclusion criteria will receive sacituzumab tirumotecan and anlotinib. The treatment regimen is as follows: 1) sacituzumab tirumotecan 4 mg/kg, intravenous infusion (IV), once every 2 weeks. 2) Anlotinib 10 mg orally before breakfast (po), once daily (qd), taken continuously for 2 weeks followed by 1 week off, with 3 weeks constituting one cycle. Subjects will continue treatment until radiographic disease progression confirmed by the investigator, unacceptable toxicity, the subject's request to discontinue treatment, or other protocol-specified treatment discontinuation criteria, whichever occurs first.

Eligible subjects will undergo regular tumor assessments according to RECIST v1.1. Within 48 weeks after dosing, imaging examinations for tumor assessment will be performed every 6 weeks (±7 days); after 48 weeks, tumor assessment will be performed every 12 weeks (±7 days) until radiographic disease progression, initiation of new antitumor therapy, withdrawal of informed consent, loss to follow-up, death, or study termination by the sponsor, whichever occurs first. Imaging examinations will be performed as originally scheduled regardless of dose delay or adjustment.

After a patient first achieves complete response (CR) or partial response (PR), the observed response must be confirmed at least 4 weeks (28 days) later.

After treatment completion, safety follow-up will be completed regardless of whether new antitumor therapy is initiated. Survival follow-up will then be conducted by telephone every 3 months (±14 days) after the last dose of study treatment to collect survival

information and information on new antitumor therapy until the subject decides to withdraw from the study, is lost to follow-up, dies, or the study ends, whichever occurs first.

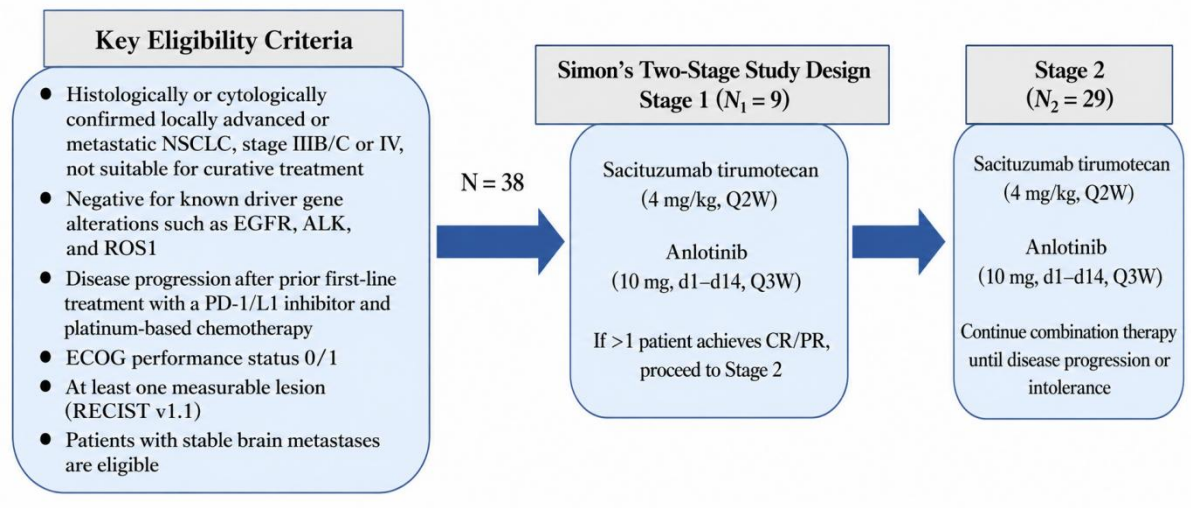

Figure 1. Schematic diagram of study design

4.2 Definition of Study Completion

Study completion is defined as 12 months after the last subject is enrolled, or when 80% of enrolled subjects have experienced PFS events, whichever occurs first.

5. Study Population

5.1 Inclusion Criteria

Subjects eligible for enrollment in this study must meet all of the following criteria:

1) Age ≥18 years at the time of signing the informed consent form, regardless of sex;

2) Histologically or cytologically confirmed locally advanced (stage IIIB/IIIC) or metastatic (stage IV) non-squamous NSCLC, with disease progression after prior anti-PD-1/L1 monoclonal antibody combined with platinum-based doublet chemotherapy;

3) No known driver gene alterations, such as EGFR, ALK, ROS1, NTRK, BRAF, MET, KRAS, HER2, RET, etc.;

4) At least one measurable target lesion not previously treated with radiotherapy, as assessed by the investigator according to RECIST v1.1;

5) Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1;

6) Subjects with asymptomatic brain metastases or brain metastases with stable symptoms after local therapy are allowed to enroll;

7) Expected survival ≥12 weeks;

8) Adequate organ and bone marrow function, defined as follows:

a) Complete blood count: neutrophil count (NEUT) ≥1.5×10^9/L; platelets (PLT) ≥100×10^9/L; hemoglobin ≥90 g/L;

b) Liver function: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5×ULN; For subjects with liver metastases at baseline, ALT and AST ≤5×ULN; albumin ≥30 g/L; total bilirubin (TBIL) ≤1.5×ULN;

c) Renal function: creatinine clearance ≥50 mL/min (calculated using the standard Cockcroft–Gault formula) or serum creatinine ≤1.5×ULN;

9) Female subjects of childbearing potential and male subjects with partners of childbearing potential must agree to use effective medical contraception from signing the informed consent form until 6 months after the last dose;

10) The subject voluntarily participates in this study, signs the informed consent form, and is able to comply with protocol-specified visits and related procedures.

5.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded:

1) Tumor histology or cytology confirms the presence of small cell lung cancer, squamous cell carcinoma, neuroendocrine carcinoma, or carcinosarcoma components;

2) Previous treatment with any of the following therapies (including in the adjuvant or neoadjuvant setting):

a)TROP2-targeted therapy;

b) Any topoisomerase I-targeting drug therapy, including antibody-drug conjugate (ADC) therapy;

3) Known meningeal metastasis, brainstem metastasis, spinal cord metastasis and/or compression, or active central nervous system (CNS) metastasis;

4) Other malignancies within 3 years before dosing, except tumors cured by local treatment, such as basal cell carcinoma of the skin, squamous cell carcinoma of the skin, and cervical carcinoma in situ;

5) Any of the following cardiovascular or cerebrovascular diseases or cardiovascular/cerebrovascular risk factors:

a) Myocardial infarction, unstable angina, acute or persistent myocardial ischemia, grade 3 or 4 heart failure [according to New York Heart Association (NYHA) classification], symptomatic or poorly controlled severe arrhythmia, cerebrovascular accident, transient ischemic attack, or other serious cardiovascular or cerebrovascular disease within 6 months before dosing;
b) History of myocardial diseases such as myocarditis, primary cardiomyopathy, or specific cardiomyopathy;
c) There are major vascular diseases such as aortic aneurysm, aortic dissection aneurysm, etc. which may pose a threat to life or require surgery within 6 months before administration.
6)Poorly controlled hypertension(systolic blood pressure> 160 mmHg and / or diastolic blood pressure> 100 mmHg);
7) Store in must main steroid therapy of (property)interstitial lung disease(ILD) or noninfectious pneumonitis disease, objective before has ILD or noninfectious pneumonitis, or at screening store in cannot be excluded by imaging examination of may ILD or noninfectious pneumonitis;
8) Subjects with documented severe dry eye syndrome, severe meibomian gland disease and/or blepharitis, or a history of corneal disorders that hinder or delay corneal healing;
9) Subjects with active chronic inflammatory bowel disease, gastrointestinal obstruction, severe ulcers, gastrointestinal perforation, intra-abdominal abscess or acute gastrointestinal bleeding;
10) At risk of developing tracheoesophageal fistula or esophagopleural fistula;
11) Subjects with imaging evidence of tumor invasion into major blood vessels, or those judged to have a high likelihood of tumor infiltration into critical blood vessels leading to fatal massive hemorrhage during the study period;
12) Subjects presenting any bleeding symptoms regardless of severity; subjects who experienced any Grade ≥3 bleeding event per CTCAE within 1 month prior to the first dose, accompanied by unhealed wounds, ulcers or fractures;
13) Confirmed active pulmonary tuberculosis. Subjects with suspected active pulmonary tuberculosis shall undergo clinical examinations to rule out the condition;
14) Known history of allogeneic solid organ transplantation and allogeneic hematopoietic stem cell transplantation;
15) Positive human immunodeficiency virus (HIV) test result or documented history of acquired immunodeficiency syndrome (AIDS); confirmed active syphilis infection;

16) Subjects who have undergone major surgery within 4 weeks prior to dosing or are scheduled to receive major surgery during the study period;
17) Current use of medicinal products or herbal supplements known as strong cytochrome P450 (CYP) 3A4 inducers (discontinuation for at least 3 weeks prior to the first study treatment dose is required if such agents cannot be stopped). All subjects shall avoid concomitant use of any drugs, herbal supplements and/or intake of foods with known CYP3A4 induction effects to the greatest extent possible;
18) Received live vaccines within 30 days prior to dosing, or plan to receive live vaccines during the study;
19) Pregnant or lactating women;
20) Any medical condition that, in the investigator's opinion, may interfere with the evaluation of the study drug, subject safety, or interpretation of study results, or any other condition deemed inappropriate for study participation by the investigator.

5.3 Withdrawal Criteria

Stopping the treatment does not mean withdrawing from the study. Since the data of certain clinical events after stopping the treatment may be very important for the study, these information must be collected until the last planned visit of the subject, even if the subject has stopped the treatment.

The subject can stop the treatment at any time for any reason, or the investigator can decide whether to stop the treatment when any adverse event occurs. Additionally, if the subject is not suitable for the treatment, violates the study protocol, or for management or other safety reasons, the investigator can stop the subject's treatment.

For any of the following reasons, the subject must stop the treatment but can continue to be monitored in the study:• The subject or the subject's legal representative requested to stop the treatment.

• An adverse event as stipulated in the plan occurs, requiring the cessation of treatment.

• Another malignant tumor requiring active treatment occurs.

• Complicated diseases that hinder further treatment occur.

• The researcher decides to have the subject withdraw from the study.

• The subject's pregnancy test result is positive.

• The subject has poor compliance.

• The researcher believes that, based on the subject's disease condition or personal circumstances, if the study drug is continued to be administered, it will place the subject at unnecessary risks.

For the subjects who have stopped the treatment but continue to receive follow-up visits during the study, all the visits and procedures should be completed.

5.4 Termination Criteria

If the following criteria are met, the research will be terminated in advance:

• The quality and quantity of data records are inaccurate or incomplete.

• The compliance with the research plan and regulatory requirements is poor.

• The incidence or severity of adverse drug reactions in this study or other studies suggests that the health of the subjects may be at risk.

• Changes or cessation of the development of the study drug are planned.

If it is decided not to provide the research drugs any longer, adequate notice will be given to ensure appropriate adjustments can be made to the treatment of the subjects.

6. Study Drugs

6.1 Investigational Products:

Sacituzumab govitecan-hziy

Product Name: Sacituzumab Govitecan-hziy for Injection

Dosage Form: Injection

Specification: 200 mg per vial

Ingredient: Sacituzumab Govitecan-hziy

Storage Conditions: Store at 2~8°C, protected from light.

Developer and Manufacturer: Sichuan Kelun-Biotech Biopharmaceutical Co., Ltd.

Anlotinib

Generic Name: Anlotinib Hydrochloride Capsules

Dosage Form: Capsule

Specification: Calculated as anlotinib (C23H22FN3O3): (1) 12 mg; (2) 10 mg; (3) 8 mg

Ingredient: Anlotinib Hydrochloride

Storage Conditions: Protect from light, seal tightly, and store below 25°C.

6.2 Dose and Administration

1)Lukosatuzumab is administered at a dose of 4 mg/kg via intravenous infusion once every two weeks on Day 1 of each cycle. For provisions on dose interruption due to AEs, please refer to Section 6.1.3 Dose Modifications.

2)The administered dose of anlotinib is 10 mg taken orally once daily before breakfast.Medication is administered for 2 consecutive weeks followed by a 1-week drug holiday; one treatment cycle consists of 3 weeks (21 days).Doses should be separated by approximately 24 hours, and patients should take the drug at roughly the same time each day. Missed doses should be avoided.If a dose is missed during treatment and the time remaining until the next scheduled dose is less than 12 hours, the missed dose shall not be made up.For provisions on treatment interruption due to adverse events (AEs), please refer to Section 6.1.3 Dose Modification.

Infusion time: Lukosatuzumab is for intravenous infusion only; intravenous bolus injection or rapid intravenous injection is prohibited. The infusion duration for the first 4 administrations shall be 90 ± 15 minutes, and may be extended to more than 105 minutes at the investigator's discretion if necessary. If no infusion-related reactions/hypersensitivity reactions occur, the infusion duration may be shortened at the physician's discretion starting from the 5th administration (inclusive), but shall not be less than 60 minutes.

After completion of the first 4 infusions of this product, monitor patients for any infusion-related reactions/hypersensitivity reactions for a minimum of 90 minutes.For subsequent administrations, monitor patients for at least 60 minutes following completion of the infusion of this product. If an infusion-related reaction/hypersensitivity reaction occurs, extended observation shall be performed after infusion completion based on clinical indications.

Oral Care: Subjects shall establish daily oral care routines in accordance with the daily oral care regimen prior to the first administration of sacituzumab govitecan-hziy and throughout the study treatment period. Subjects are strongly recommended to brush teeth with a soft-bristled toothbrush after meals and before bedtime every day, and receive prophylactic treatment with steroid-containing mouthwash (formula: 50 mL normal saline plus 5 mg dexamethasone). Gargle 4 times per day for 1 to 2 minutes each time, then spit out the mouthwash. If steroid-containing mouthwash is unavailable, inert alcohol-free mouthwash may be used as an alternative; nevertheless, steroid-containing mouthwash remains the preferred option. Dental floss can be used for cleaning after meals, with care taken to avoid oral pain and gingival bleeding.

premedication:

1)Sacituzumab Govitecan-hziy: Prophylactic premedication is required prior to each administration of this product to prevent infusion-related reactions/hypersensitivity reactions.For the first 4 administrations of this product, premedication shall consist of diphenhydramine 40 mg (or alternative H1 antihistamine receptor antagonists at equivalent doses), immediate-release paracetamol 0.5 g or extended-release paracetamol 0.65 g (or other nonsteroidal antipyretic analgesics at equivalent doses), intravenous dexamethasone 10 mg, and an H2 antihistamine receptor antagonist (e.g., famotidine 20 mg or other available agents at equivalent doses).If no infusion-related reactions or hypersensitivity reactions occur during the first 4 administrations of this product, starting from the 5th administration (inclusive), premedication shall consist of diphenhydramine 40 mg (or alternative H1 antihistamine receptor antagonists at equivalent doses) plus immediate-release paracetamol 0.5 g or extended-release paracetamol 0.65 g (or other nonsteroidal antipyretic analgesics at equivalent doses). The use of corticosteroids for prophylaxis is at the physician’s discretion.For patients assessed by physicians to be at higher risk (e.g., those with a history of hypersensitivity to other medicinal products or previous symptoms suggestive of allergy), additional prophylactic treatment with an H2 antihistamine receptor antagonist (e.g., famotidine 20 mg or other available agents at equivalent doses) is recommended, and higher doses of dexamethasone (> the recommended dose of 10 mg) may be administered at the physician’s discretion.

Intravenous or intramuscular prophylactic medications shall be administered 30 to 60 minutes before infusion of this product. Oral prophylactic medications shall be administered approximately 60 minutes prior to administration of this product.

Note: Emergency rescue medications (including epinephrine) and appropriate resuscitation equipment shall be available at the bedside during administration, and a physician shall be readily accessible at all times.

6.3 Dose Adjustment

Toxicities shall be graded in accordance with NCI CTCAE Version 5.0. All subjects shall be closely monitored for toxic reactions throughout treatment, and symptomatic treatment shall be administered promptly as needed. Guidelines for recommended dose modifications of lukosatuzumab are shown in Table 6-1.

6.4 Dose Adjustment and Toxicity Management for Adverse Events Related to Sacituzumab Tirumotecan

6.4.1 Dose Adjustment of Sacituzumab Tirumotecan

If subjects experience sacituzumab govitecan-hziy-related AEs requiring dose reduction, the dose may be decreased by 1 mg/kg (Table 6-1), with one dose reduction permitted (i.e., from 4 mg/kg to 3 mg/kg).

Once the dose of sacituzumab govitecan-hziy is reduced due to toxicity, all subsequent cycles shall be administered at the reduced dose level. Dose escalation back to the original level is prohibited after dose reduction. If deemed necessary by the investigator, subjects shall receive appropriate supportive care.

Table 6- 1 sacituzumab tirumotecan related adverse event of Dose adjustment and toxicity management guideline

<table>
<tr><td colspan="3">Hematologic toxicity</td></tr>
<tr><td colspan="2">NCI-CTCAE</td><td>Dose adjustment</td></tr>
<tr><td colspan="2">1~2 grade</td><td>No dose change</td></tr>
<tr><td>3 grade</td><td>Without fever 3 grade neutropenia</td><td>First occurrence:
• Hold administration until toxicity resolves to Grade ≤1, then resume treatment at the original dose.
• Prophylactic administration of G-CSF shall be given in subsequent treatment cycles (in accordance with guidelines or local standard practice).
Re-occurrence:
• Hold treatment until toxicity recovers to Grade ≤ 1. The investigator shall decide to resume treatment at the original dose or reduce the dose by one level based on the subject’s condition.</td></tr>
</table>

		• Prophylactic administration of G-CSF may be administered in subsequent treatment cycles at the investigator's discretion (in accordance with guidelines or local standard practice).
	with fever 3 grade neutropenia	First occurrence: Hold treatment until toxicity resolves to Grade ≤ 1, then resume therapy at one dose level reduction. Prophylactic G-CSF should be administered in subsequent treatment cycles (per guidelines or local standard practice). Re-occurrence: Hold treatment until toxicity resolves to Grade ≤ 1, then resume therapy with one dose level reduction (if prophylactic G-CSF has been administered). Prophylactic G-CSF may be given in subsequent treatment cycles at the investigator's discretion (in accordance with guidelines or local standard practice).
	3 grade thrombocytopenia	First occurrence: • Hold administration until toxicity improves to Grade ≤ 2, then resume treatment at one dose level lower. Re-occurrence: • Hold treatment until toxicity resolves to Grade ≤ 2, followed by a one-dose-level reduction, except in the following circumstances: • Thrombocytopenia with spontaneous bleeding in vital organs (discontinue protocol treatment if this occurs)
	3 grade anemia	The investigator shall determine whether to resume study treatment at the original dose or decrease the dose by one dose level based on the subject's clinical status.

4 grade	4 grade neutropenia	First occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1. If toxicity resolves to Grade ≤ 1 within 7 days from the onset date, resume treatment at the original dose. If toxicity fails to recover to Grade ≤ 1 within 7 days from the onset date, resume treatment with one dose level reduction. Prophylactic G-CSF shall be administered in subsequent treatment cycles (in accordance with guidelines or local standard practice). Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume therapy with one dose level reduction (if prophylactic G-CSF has been administered). Prophylactic G-CSF may be administered in subsequent treatment cycles at the investigator's discretion (in accordance with relevant guidelines or local standard clinical practice).
	4 grade other Hematologic toxicity(Excluding lymphopenia)	First occurrence: Hold study treatment until toxicity recovers to Grade ≤ 2, then resume treatment with one dose level reduction; Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 2, then resume treatment with one dose level reduction, except for the following circumstances: Thrombocytopenia accompanied by spontaneous bleeding in vital organs (discontinue study treatment if this event occurs).

Non-hematologic toxicity		
NCI-CTCAE **(Unless otherwise specified)**		**Dose adjustment**
oral mucositis	1 grade	No dose change
	2 grade	First occurrence: The investigator shall decide whether to administer the original scheduled dose or resume dosing only after toxicity recovers to Grade ≤ 1 based on the subject's clinical status. Re-occurrence: If the time required for toxicity to recover to Grade ≤ 1 is ≤ 14 days, resume treatment at the original dose after toxicity resolves to Grade ≤ 1. If the time taken for toxicity to recover to Grade ≤ 1 exceeds 14 days, after the toxicity resolves to Grade ≤ 1, the investigator shall determine whether to administer the originally scheduled dose or reduce the dose by one dose level based on the subject's condition (provided adequate prophylactic and supportive medications have been administered).
	3-4 grade	First occurrence: Hold study treatment until toxicity resolves to Grade ≤ 1, then resume dosing with a one-dose-level reduction. Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing with one dose level reduction (provided adequate prophylactic and supportive medications have been administered).
rash	1~2 grade	No dose change

	3 grade	First occurrence: Hold study treatment until toxicity recovers to Grade ≤ 2, then resume treatment at the originally scheduled dose without dose modification. If adequate prophylactic and supportive medications have been administered, a one dose level reduction may be considered. Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 2, then resume dosing with one dose level reduction.
	4 grade	First occurrence: The investigator shall discuss with the sponsor, and either hold study treatment until toxicity recovers to Grade ≤ 2 followed by resumption at one dose level lower, or permanently discontinue study treatment. Re-occurrence: Permanently discontinue study treatment
keratitis/corneal disorder	For keratitis/corneal disorders, dose adjustments shall be performed with reference to the following grading criteria for corneal toxicity severity.	
	1 grade non-confluent superficial keratitis	No dose change
	2 grade Confluent superficial keratitis, corneal defect, or a decrease of ≥3 lines in best	First occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing at the original scheduled dose without any dose reduction. Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing with a one-dose-level reduction.

	corrected visual acuity	
	3 grade Corneal ulcer, stromal opacification, or best corrected distant visual acuity ≤ 20/200	First occurrence: Hold study treatment until toxicity resolves to Grade ≤ 1, then resume dosing with a one-dose-level reduction. Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing with a one-dose-level reduction or permanently discontinue study treatment.
	4 grade Corneal perforation	permanent discontinuation
other ocular surface toxicity(dry eye, conjunctivitis etc.)	1 grade	No dose change
	2 grade	dose interruption toxicity to <= 1 grade or stable defined, drug, No dose change
	3 grade	First occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing with one dose level reduction. Re-occurrence: Hold study treatment until toxicity recovers to Grade ≤ 1, then resume dosing with one dose level reduction.
	4 grade	permanent discontinuation
noninfectious pneumonitis/ILD	1 grade	No dose change
	2 grade	First occurrence: Hold treatment until toxicity resolves to Grade ≤ 1, then resume dosing with one dose level reduction. Re-occurrence:

		·permanent discontinuation
	3~4 grade	permanent discontinuation
other non-hematologic toxicity	1~2 grade	No dose change
	3 grade (Excluding isolated laboratory abnormalities without significant clinical significance)	First occurrence: Hold treatment until toxicity recovers to Grade ≤ 1. The investigator shall decide whether to resume dosing at the original dose or with a one-dose-level reduction based on the subject's condition (if the investigator deems the toxicity clinically significant or adequate prophylactic and supportive medications have been administered). Re-occurrence: Withhold treatment until toxicity improves to Grade ≤ 1, then resume administration with one dose level reduction.
	4 grade Excluding isolated laboratory abnormalities without significant clinical significance)	First occurrence: The investigator shall discuss with the sponsor, withhold treatment until toxicity recovers to Grade ≤ 1, followed by resumption at one dose level reduction or permanent discontinuation of treatment. Re-occurrence: ·Permanent discontinuation

6.4.2 Management of Adverse Events Related to Sacituzumab Tirumotecan

It is recommended that specific sacituzumab tirumotecan-related adverse events be managed in accordance with Table 6-2. In addition, investigators shall follow clinical practice guidelines, which shall prevail in the event of any inconsistency.

Table 6-2 sacituzumab tirumotecan related adverse event management

Event name	**Recommended treatment**
Oral mucositis	Oral care, steroid-containing mouthwash is strongly recommended. Meanwhile, investigators may administer mucosal protective agents (e.g., Episil, Lempin), oral care solutions (e.g., benzydamine oral rinse, chlorhexidine mouthwash), anti-infective treatments and other interventions at their discretion based on the subject's condition. See Section 6.1.3 for details.
Rash	Symptomatic treatment with topical corticosteroids and antibiotics may be administered, and oral antihistamines should be considered to treat pruritic rashes. If a fungal infection is diagnosed, topical antifungal agents shall be used. In cases where topical treatment fails to produce improvement, oral antibiotics (e.g., minocycline) and systemic corticosteroids may be considered.
Nausea	Antiemetics
Vomiting	Antiemetics
Neutropenia	The investigator may decide to administer antibiotics, granulocyte colony-stimulating factor (G-CSF), or granulocyte-macrophage colony -stimulating factor (GM-CSF) for prophylactic or symptomatic management.
Anemia	Transfuse red blood cells or administer erythropoietin; blood transfusion may be considered as appropriate.

Decreased white blood cell count	Consider the use of leukopoietic agents such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) for treatment.
Decreased platelet count	Interleukin-11 (IL-11) and thrombopoietin (TPO) may be considered, along with platelet transfusion. Closely monitor bleeding manifestations and repeat platelet level testing.
Ocular surface toxicity	Pay close attention to participants' ocular disorders, particularly dry eye disease, epiphora, conjunctivitis and keratitis. Given the high natural incidence of ocular surface disorders including dry eye and conjunctivitis in the general population, investigators shall comprehensively take participants' medical history, predisposing factors (such as local infection, seasonal changes and allergen exposure, etc.) and ophthalmic assessment findings into consideration, and implement appropriate dose modifications and treatments to manage ocular surface toxicity associated with sacituzumab govitecan. If participants develop ocular-related symptoms, preservative-free artificial eye drops (e.g., sodium hyaluronate eye drops, administered four times daily, with frequency increased up to eight times daily as needed) are recommended, and contact lens wear shall be avoided. If participants experience ocular adverse events (AEs) of CTCAE Grade ≥2, an ophthalmology consultation shall be arranged to confirm the ophthalmic diagnosis. For CTCAE Grade 1 ocular AEs, ophthalmology consultation may be considered at the investigator's discretion when necessary (e.g., visual changes occur or no improvement is observed within 2 weeks). During the ophthalmic visit, the ophthalmologist is recommended to perform visual acuity assessment (uncorrected and best-corrected visual acuity), slit-lamp examination, corneal fluorescein staining (including anterior segment photography), tear film break-up time measurement, fundoscopy, and intraocular pressure measurement; additional examinations may be performed as clinically indicated. For corneal lesions, investigators shall refer to ophthalmic

	assessment findings documented by ophthalmologists, including best-corrected visual acuity, the severity of corneal epithelial lesions (superficial punctate epithelial erosions, confluent erosions, or corneal ulcers), and medication recommendations to conduct severity grading, dose modification, and treatment. On top of artificial tears, additional therapies such as topical antibiotics and deproteinized calf blood extract eye drops may be administered.
non-infectious pneumonitis/ILD	1 grade: Monitor participants for signs and symptoms of noninfectious pneumonitis/interstitial lung disease (ILD). 2 grade: Participants with suspected noninfectious pneumonitis/ILD shall undergo imaging evaluation, and corticosteroid therapy shall be initiated at an initial prednisone dose of 1~2 mg/kg or equivalent agents, followed by gradual tapering. Prophylactic antibiotics may be considered to prevent opportunistic infections. Meanwhile, a pulmonology consultation should be arranged, and examinations such as bronchoscopy and pulmonary function tests shall be performed. 3~4grade:Participants suspected of noninfectious pneumonitis/ILD shall receive imaging evaluation and start corticosteroid therapy with an initial prednisone dose of 1~2 mg/kg or equivalent agents, followed by gradual tapering. Prophylactic antibiotics shall be administered to prevent opportunistic infections. Meanwhile, pulmonology consultation should be considered, and examinations including bronchoscopy and pulmonary function tests shall be conducted.

Guidelines for the Management of Mucositis Events Associated with Sacituzumab Govitecan

Sacituzumab govitecan may cause oral mucositis. Subjects shall perform daily oral care prior to the initiation of study treatment and throughout the treatment period. Subjects are strongly advised to brush their teeth with a soft-bristled toothbrush after each meal and before bedtime, and use steroid-containing mouthwash for prophylaxis (formulation: 50 mL normal saline plus 5 mg dexamethasone; gargle 4 times daily for 1 to 2 minutes each time, then spit out). If steroid-containing mouthwash is unavailable, bland alcohol-free mouthwash may be used as a substitute; nevertheless, steroid-containing mouthwash is preferred. Dental flossing may be performed after meals, with caution to avoid oral pain and gingival bleeding.

Subjects shall also consider following additional preventive measures:

If the subject is identified as being at high risk of developing oral mucositis, an early dental evaluation should be considered for proactive preventive interventions (e.g., caries treatment and extraction of damaged teeth).

Ice chip therapy: subjects shall hold ice chips in their oral cavity for 30 minutes before and during sacituzumab govitecan infusion.

Once a subject develops symptoms related to oral mucositis, corticosteroid-containing mouthwash shall be used, with topical anesthetics added as needed (formula: 50 mL normal saline + 10 mg dexamethasone + 0.2 g lidocaine hydrochloride injection). Mucosal protective agents (e.g., Episil, L-Mesitran Oral), oral care solutions (e.g., benzydamine mouthwash, chlorhexidine mouth rinse), anti-infective treatments and other therapies may also be administered as appropriate.

For Grade 3 oral mucositis, medications not utilized for Grade 2 oral mucositis may be added in addition to the above recommended treatments. For subjects with oral mucositis accompanied by Grade ≥3 oral pain, adequate pain control is recommended. A 0.2% morphine mouthwash may be considered, and systemic strong opioids including morphine, oxycodone hydrochloride sustained-release tablets and fentanyl transdermal patches can be administered when necessary; anxiolytics may also be used. If necessary and clinically appropriate, the frequency of bland alcohol-free mouthwash use may be increased to once per hour.

6.5 Dose Adjustment and Precautions for Adverse Events Related to Anlotinib

The recommended dose adjustment principles for anlotinib are shown in Table 6-3 and Table 6-4.

If a subject receiving anlotinib 10 mg develops anlotinib-related adverse events (AEs) requiring dose reduction, one dose reduction is permitted: from 10 mg to 8 mg. Permanent discontinuation is required if the 8 mg dose remains intolerable.When the 8 mg dose is administered, the dose may be re-escalated back to 10 mg as needed based on adverse event status.

Table 6-3 General Principles for Anlotinib Dose Adjustment According to Adverse Reaction Grades

adverse reaction grade(NCI CTCAE4.0)	**Administration timing**	**Dose adjustment principles**
3 grade	Withhold dosing until the adverse reaction recovers to Grade < 2.	Continue treatment with one dose level reduced. If the adverse reaction fails to resolve within 2 weeks, permanent discontinuation shall be considered.
4 grade	Withhold dosing until the adverse reaction recovers to Grade < 2.	Continue treatment with one dose level reduced. If the adverse reaction fails to resolve within 2 weeks, permanent discontinuation shall be considered, or permanent discontinuation may be implemented at the investigator’s discretion.

NCI CTCAE Version 4.0: Common Terminology Criteria for Adverse Events Version 4.0 of the National Cancer Institute (NCI)

Table 6-4 Dose adjustment principles when anlotinib-related adverse blood reactions occur

bleeding event*	**Dose adjustment principles**
2 grade	Withhold the study drug and administer active symptomatic supportive treatment. If the adverse reaction recovers to Grade < 2 within 2 weeks, resume administration at one reduced dose level.
≥3 grade	Permanently discontinue the study drug and implement urgent medical intervention.

* Hemorrhagic adverse reactions include hemoptysis, gastrointestinal hemorrhage, epistaxis, bronchial hemorrhage, gingival bleeding, gross hematuria, fecal occult blood, cerebral hemorrhage, etc.

Precautions:

Bleeding:

VEGFR inhibitors may elevate the risk of bleeding. Bleeding events, including severe bleeding and fatal outcomes, have been reported in patients receiving anlotinib treatment. Patients should be closely monitored for bleeding-related signs and symptoms throughout the treatment period. Caution should be exercised when administering anlotinib to patients with bleeding risks or abnormal coagulation function. Close monitoring of platelet count and prothrombin time is required during medication.

For patients with Grade 2 hemorrhagic events, anlotinib treatment shall be withheld. If the event recovers to Grade < 2 within 2 weeks, treatment shall be resumed at one reduced dose level. Permanent discontinuation shall be implemented in case of recurrent hemorrhage. Any Grade 3 or higher hemorrhagic event requires permanent discontinuation of the study drug (see Table 6-4).

Hypertension

Hypertension has been reported in patients treated with anlotinib, which mostly occurs within 2 weeks after drug administration and does not fluctuate with intermittent drug interruption.

Blood pressure shall be monitored daily during the first 6 weeks of treatment. Subsequently, blood pressure shall be monitored 2 to 3 times per week throughout the treatment period. Patients with hypertension, headache or dizziness shall actively communicate with physicians and receive antihypertensive medication, temporary suspension of anlotinib hydrochloride or dose adjustment under medical guidance.

In the event of Grade 3/4 hypertension (systolic blood pressure ≥180 mmHg or diastolic blood pressure ≥110 mmHg, dosing shall be withheld. If Grade 3/4 hypertension recurs after treatment resumption, treatment shall be continued at one reduced dose level (see Table 6-3). Discontinuation is recommended if Grade 3/4 hypertension persists.

Patients presenting with hypertensive emergency shall immediately discontinue anlotinib and seek consultation in the department of cardiology.

Pneumothorax

Pneumothorax has been reported in patients treated with anlotinib. Tumor patients are at risk of spontaneous pneumothorax due to regression of pulmonary and subpleural lesions. Patients experiencing sudden chest pain, dyspnea or other related symptoms after anlotinib administration shall seek immediate medical care. Patients with confirmed pneumothorax shall receive closed thoracic drainage or other medical interventions in hospital.

Diarrhea

Diarrhea has been reported in patients treated with anlotinib.

During treatment, patients shall be assessed for dehydration or electrolyte imbalance. Intravenous fluid supplementation may be administered when necessary, together with loperamide, probiotics and smectite powder for symptomatic management. Prophylactic antibiotics combined with somatostatin may be considered for severe cases.

For patients with severe, persistent diarrhea or concurrent dehydration, if other etiologies (such as intestinal flora disturbance, immunodeficiency, carcinoid syndrome, etc.) are ruled out or differentiated, dose management measures including treatment withholding, one-level dose reduction up to permanent discontinuation may be implemented (see Table 6-3).

Gingival and oral swelling/pain

Oral pain, stomatitis and toothache have been reported in patients receiving anlotinib therapy.

For gingival and oral swelling and pain, oral hygiene shall be maintained, pain shall be controlled, multiple infections shall be reduced, and further deterioration of stomatitis shall be prevented. Mouth rinses or corresponding coatings containing lidocaine, sodium bicarbonate or chlorhexidine may be recommended for symptomatic relief and wound healing promotion.

Balanced nutrition and adequate water intake shall be ensured with individualized diet; hot and spicy food shall be avoided, alcohol and tobacco shall be prohibited, and alcohol-containing mouth rinses shall not be used. Consultation with a department of stomatology is recommended if needed.

In case of gingival and oral swelling and pain, interventions including treatment withholding, one-level dose reduction up to permanent discontinuation may be adopted (see Table 6-3).

Hand-foot syndrome

Hand-foot syndrome has been reported in patients treated with anlotinib. It mostly develops within 2 weeks after administration, manifested as a combination of swelling, desquamation, blistering, cracking, bleeding or erythema on the palms and soles, often accompanied by pain.

Patients with Grade 2 hand-foot syndrome shall receive symptomatic treatment, including intensified skin care, maintenance of clean skin, prevention of secondary infection, and avoidance of pressure and friction; topical application of lotions or lubricants containing urea and corticosteroids; topical antifungal or antibiotic agents shall be administered in case of infection, preferably under the guidance of a dermatologist. If Grade ≥3 hand-foot syndrome occurs, treatment shall be continued at one reduced dose level (see Table 6-3). Discontinuation shall be implemented if the adverse reaction persists.

QT interval prolongation

QT interval prolongation has been reported in patients receiving anlotinib treatment. Prolonged QTc interval may raise the risk of ventricular tachyarrhythmias (such as torsades de pointes) or sudden cardiac death. Electrocardiogram (ECG) shall be routinely monitored every 6 to 8 weeks during treatment.

This product is contraindicated in patients with congenital long QT syndrome. Regular monitoring of ECG and serum electrolytes (every 3–6 weeks) shall be performed for patients with congestive heart failure, electrolyte abnormalities, or concomitant use of medications known to prolong the QTc interval. Dosing shall be withheld in patients with QTc interval >500 ms on two consecutive independent ECG assessments until the QTc interval decreases to ≤480 ms or returns to baseline (if baseline QTc interval >480 ms), after which treatment shall be resumed at one reduced dose level.

Permanent discontinuation of this product is required for patients with any grade of QTc interval prolongation (≥450 ms) accompanied by any of the following conditions: torsades de pointes, polymorphic ventricular tachycardia, or signs and symptoms of severe arrhythmia. Such patients shall receive prompt consultation at the department of cardiology.

Proteinuria

Proteinuria has been reported in patients treated with anlotinib. Anlotinib shall be used with caution and under close monitoring by physicians in patients with pre-existing renal insufficiency. Urinalysis is recommended every 6 to 8 weeks. A 24-hour urinary protein quantification test shall be conducted for patients with ≥++ proteinuria on two consecutive urinalyses. Interventions including treatment withholding, dose adjustment and permanent discontinuation shall be implemented according to the grade of adverse reaction (see Table 6-3).

Hyperlipidemia

Hyperlipidemia has been reported in patients treated with anlotinib. A low-fat diet is recommended for patients with hyperlipidemia. Lipid-lowering agents such as hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors shall be administered for patients with Grade 2 or higher hypercholesterolemia (≥7.75 mmol/L) or Grade 2 or higher hypertriglyceridemia (≥2.5 times the upper limit of normal).

Seizure

Seizures have been reported in patients treated with anlotinib. It remains unclear whether this product can induce seizures or increase seizure risk, so caution shall be exercised in patients with a prior history of seizures.

Reversible posterior leukoencephalopathy syndrome (RPLS)

Reversible Posterior Leukoencephalopathy Syndrome (RPLS) has been reported in tumor patients receiving VEGFR inhibitor therapy and may be fatal. RPLS is a neurological disorder that may be accompanied by headache, seizure, somnolence, confusion, blindness and other visual and neurological dysfunctions, and may be associated with mild to severe hypertension. Magnetic resonance imaging (MRI) is the optimal diagnostic method for confirmed RPLS. No such events have been reported in anlotinib clinical studies. Relevant signs and symptoms shall be closely monitored during clinical practice. Permanent discontinuation of anlotinib shall be implemented immediately once RPLS is identified in patients.

6.6 Guidelines for Management of Overlapping Toxicities

The respective prescribing information of sacituzumab govitecan and anlotinib describes common treatment-related adverse events (AEs) as well as less frequent severe outcomes associated with each agent. The combination of sacituzumab govitecan and anlotinib carries potential overlapping toxicities, and such AEs may not be readily attributable to a specific

study treatment. Toxicity management interventions (i.e., dose reduction, treatment interruption, or permanent discontinuation) with uncertain causality shall apply to both sacituzumab govitecan and anlotinib simultaneously. Investigators shall follow the most conservative dose adjustment guidelines outlined in Sections 6.1.3.1 and 6.1.3.2. Investigators shall consider permanent discontinuation of the subject's study treatment if the toxicity fails to resolve.

6.7 Management Guidelines for Infusion-Related Reactions Associated With Study Treatment

Sacituzumab govitecan may cause severe or life-threatening infusion-related reactions, including severe hypersensitivity or anaphylactic reactions. Relevant signs and symptoms generally occur during or shortly after drug infusion and usually resolve completely within 24 hours upon completion of infusion. Dose modification and management guidelines for infusion-related reactions associated with sacituzumab govitecan are provided in Table 6-5.

Premedication measures for infusion-related reactions of sacituzumab govitecan are specified in Section 6.1.2.

During and after infusion, investigators shall closely monitor subjects for signs and symptoms of infusion-related reactions (such as fever and/or chills, flushing and/or pruritus, changes in heart rate and blood pressure, dyspnea or chest discomfort, rash, etc.) and severe systemic hypersensitivity reactions (such as generalized urticaria, angioedema, asthma, hypotension, tachycardia, etc.). If a subject develops signs or symptoms of severe systemic hypersensitivity during administration, appropriate medical treatment shall be administered: epinephrine shall be given immediately, followed by corticosteroids, antihistamines, bronchodilators, oxygen inhalation and other supportive treatments. All study sites shall ensure that appropriate medical equipment and medications are readily available at all times.

Management of infusion reactions:

Table 6-5 Dose Adjustment and Management Guidelines for Infusion-Related Reactions or Hypersensitivity Reactions of Sacituzumab Govitecan

NCI CTCAE v5.0 grade	Treatment and adjustment	Premedication and infusion time for subsequent dosing

1 grade Mild reaction No indication to interrupt infusion No intervention indicated	Monitoring of vital signs shall be intensified according to clinical indications until the investigator determines that the subject is clinically stable.	the subject should receive the following premedication: •Diphenhydramine 40 mg (or other H1 antihistamine blockers at equivalent doses) •Paracetamol (immediate-release) 0.5 g or (extended-release) 0.65 g (or other nonsteroidal antipyretic analgesics at equivalent doses) •Dexamethasone 10 mg administered intravenously •H2 antihistamine receptor blocker (e.g., famotidine 20 mg or other available agents at equivalent doses). Intravenous or intramuscular premedication shall be administered 30 to 60 minutes prior to sacituzumab govitecan administration, and oral premedication shall be administered 60 ± 10 minutes before sacituzumab govitecan infusion. The infusion
2 grade Symptoms resolve rapidly with symptomatic treatments (e.g., antihistamines, nonsteroidal anti-inflammatory drugs, anesthetics, intravenous fluids) following drug administration or temporary infusion interruption; premedication shall be completed within 24 hours.	Stop infusion Other use of drug treatment may including not at: - Intravenous fluid -Antihistamine drug -NSAIDs -Paracetamol -Anesthetics -Corticosteroids Monitoring of vital signs shall be intensified according to clinical indications until the investigator determines that the subject is clinically stable. If symptoms resolve within 1 hour after discontinuation of study drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/h to 50 mL/h). Otherwise, administration shall be withheld until symptoms resolve, and the subject shall receive premedication prior to the next scheduled dose. If a subject presents with Grade 2 immediate	

	-type hypersensitivity reactions or recurrent infusion-related reactions despite adequate premedication, study treatment shall be permanently discontinued.	duration of sacituzumab govitecan shall be 90 (±15) minutes. Infusion-related adverse events shall be monitored throughout all subsequent treatment cycles. The infusion duration may be extended to more than 105 minutes at the investigator's discretion.

3 grade or 4 grade **3 grade** Prolonged duration (e.g., lack of rapid response to symptomatic medications and/or temporary infusion interruption)Recurrence of symptoms after initial improvementHospitalization required due to other clinical sequelae (e.g., renal	Discontinue the infusion. Other applicable medicinal treatments include, but are not limited to: • Epinephrine (to be administered immediately upon onset of immediate hypersensitivity reactions) • Intravenous fluids • Antihistamines • Nonsteroidal anti-inflammatory drugs (NSAIDs) • Paracetamol • Anesthetics • Oxygen • Vasopressors • Corticosteroids Monitoring of vital signs shall be intensified according to clinical indications until the investigator confirms that the	no subsequent dosing

insufficiency, pulmonary infiltrates) **4 grade** Life-threatening Requirement for vasopressor or ventilatory support	subject is clinically stable. Hospitalization may be required. Permanent discontinuation of study treatment for the subject.	
AEs= adverse event; CTCAE v5.0= adverse event use criteria 5.0; h= small; IV = Intravenous; min= analysis; NCI= cancer syndrome study; NSAIDs=NSAIDs; po= oral. Note: Appropriate resuscitation equipment shall be available at the bedside during administration, and a physician shall be readily accessible at all times. For more information, see CTCAE v5.0, website: http://ctep.cancer.gov.		

6.8 Guidelines for Management of Overlapping Toxicities

The prescribing information for sacituzumab tirumotecan and anlotinib each describes common treatment-related adverse events (AEs) as well as less frequent serious outcomes. The combination of sacituzumab tirumotecan and anlotinib carries potential overlapping toxicities, and such AEs may be difficult to attribute to a specific study treatment agent. Toxicity management measures for events with uncertain causality (i.e., dose reduction, treatment interruption or permanent discontinuation) shall apply to both sacituzumab tirumotecan and anlotinib. Investigators shall adhere to the most conservative dose adjustment guidelines outlined in Sections 6.1.3.1 and 6.1.3.2. If the toxicity fails to resolve, the investigator shall consider discontinuing the subject from study treatment.

7. Drug Management

7.1 Drug Packaging and Labeling

The labels of sacituzumab tirumotecan and anlotinib shall be subject to the actual supplied versions. All drug handling procedures (including preparation, storage, administration and

disposal) shall comply with relevant local guidelines and/or prescribing information. Refer to the prescribing information for full details.

7.2 Drug Preparation

sacituzumab tirumotecan

Drug preparation

After removing this product from the refrigerator, preparation of the drug solution may be performed. If immediate use is not possible, it is recommended to store it at room temperature for no more than 4 hours or under refrigeration for no more than 24 hours.

Reconstitution

• This product is a lyophilized preparation. Reconstitute each vial (200 mg) with 10 mL sterile water for injection.

• For reconstitution, slowly add sterile water for injection along the vial wall using a disposable syringe, then swirl gently to dissolve the powder. Allow to stand until foam dissipates; vigorous shaking is strictly prohibited. After visual confirmation of complete dissolution, swirl gently again to fully homogenize the solution.

• The reconstituted solution should be a clear, colorless to pale yellow liquid. Discard the solution if visible particles are observed. It is recommended to dilute the reconstituted solution as soon as possible. If immediate dilution is not feasible, the solution may be stored at room temperature or under refrigeration. The maximum storage time is 2 hours at room temperature, or 12 hours under refrigeration (2–8°C).

Dilution

• Calculate the volume of reconstituted solution required for preparation of the infusion solution based on body weight and administered dose using the reconstituted solution obtained from the above steps.

Volume of required reconstituted solution (mL) = [Body weight (kg) × Dose (mg/kg)] ÷ 20 mg/mL

• Refrigerated reconstituted samples do not require warming and may be directly diluted and compounded.

• Withdraw the required volume of reconstituted solution from the vial and add it into an infusion bag containing 250 mL 0.9% Sodium Chloride Injection.

• Gently invert the infusion bag several times to ensure thorough mixing of the solution.

• PVC or PP infusion bags shall be used for infusion, along with infusion sets made of PVC, PE or TPE materials. A sterile, pyrogen-free, low-protein-binding PES filter with a pore size of ≤ 5 μm shall be integrated into or attached to the distal end of the infusion set.

• Do not co-administer other medicines through the same infusion line.

• This product is for single use only. Any unused drug remaining after a single administration must be discarded. All residual or expired medicines and waste shall be disposed of in accordance with relevant local regulations.

The diluted solution is recommended to be used as soon as possible, with a maximum storage duration of 6 hours at room temperature (covering the time for dilution, storage and infusion). If immediate use after dilution is not feasible, the solution may be stored under refrigeration (2~8°C) for up to 24 hours. Following refrigeration, the solution shall be allowed to warm to room temperature, after which it must be used within a maximum of 6 hours at room temperature (including the time for dilution, storage, warming to ambient temperature and infusion).

Incompatibilities

This product must not be mixed with other medicinal products in the absence of compatibility studies. It shall not be co-infused with other medicinal products via the same intravenous access line.

For detailed operating procedures, please refer to the Instructions for Use.

7.3 Concomitant Medications and Concomitant Therapy

Concomitant medications and/or concomitant treatments shall be documented from the time of informed consent signature through 30 days after the last study drug administration. After 30 days following the last administration, only concomitant medications/treatments associated with adverse events (AEs) related to the study drug need to be recorded. All concomitant medications (including start/stop dates and indications for use) must be documented in the source documents of subjects and the corresponding sections of the Case Report Forms (CRFs).

7.3.1 Permitted Concomitant Therapy

• Subjects may receive optimal supportive care excluding anti-tumor therapy. Medications specified in the protocol as judged permissible by the Investigator may be administered (e.g., concomitant medications for disease-related symptoms and various adverse events).

• Drugs and therapies for treating or alleviating symptoms of complications are permitted, including antihypertensives, antibacterials, antihistamines, etc.

• Radiation therapy to non-target lesions is allowed (e.g., treatment of painful bone lesions primarily for pain relief), provided that target lesions are not included in the radiation field and the assessment of anti-tumor efficacy will not be interfered with. Palliative surgery or any other supportive care required for essential symptom control is also permitted.

• COVID-19 vaccination is allowed before or during the study. However, live attenuated vaccines and replicating vector vaccines are prohibited within 30 days prior to the initiation of study treatment and throughout the study treatment period.

7.3.2 Prohibited Concomitant Therapy

• From the time of signing the informed consent form through the End of Treatment (EOT) visit, subjects are prohibited from receiving anti-tumor therapies not specified in the protocol, including radiation therapy and/or local treatment targeting target lesions, surgical treatment, chemotherapy, biological agents, proprietary Chinese medicine preparations, or other investigational medicinal products.

• If a subject requires other systemic anti-tumor therapy during the study, the study drug shall be discontinued immediately, and the alternative systemic anti-tumor therapy may be administered only after completion of the End of Treatment (EOT) visit.

• Live vaccines.

• Strong CYP3A4 inhibitors or strong CYP3A4 inducers should be avoided whenever possible, unless their use is deemed necessary by the Investigator. Under such circumstances, subjects shall be closely monitored for reduced efficacy and increased toxicity resulting from drug-drug interactions.

• Systemic corticosteroids are prohibited except for the following indications:

• Management of suspected immune-related adverse events or infusion reactions;

• Prophylaxis of vomiting as needed;

• Premedication for intravenous contrast medium allergy or study treatment;

• Management of acute exacerbations of asthma or COPD (only short-term oral or intravenous prednisone at a daily dose >10 mg or equivalent dosage);

• Chronic systemic replacement therapy with prednisone at a daily dose no more than 10 mg or equivalent dosage;

• In addition, the following corticosteroid administrations are permitted:

Topical or ophthalmic administration;

Intra-articular administration;

Inhaled therapy for asthma or chronic obstructive pulmonary disease (COPD).

Treatments prohibited as specified in other inclusion and exclusion criteria.

7.4 Drug Accountability

Receipt and inventory of drugs

Designated personnel at the study site shall timely complete records concerning the receipt, dispensing, administration, inventory, destruction, recovery and damage of study drugs in accordance with applicable regulations and guidelines. Clinical research staff shall ensure that the study drug is used solely for the purpose of this clinical trial.

Lukosartuzumab used in this trial is supplied by Sichuan Kelun-Biotech Biopharmaceutical Co., Ltd. for use at the study sites. The study site shall promptly sign relevant drug receipt documents upon receiving the study drugs.

Study drugs shall be managed by dedicated personnel, who are required to maintain drug custody records. Expired drugs must not be administered. Fire prevention, anti-theft, heat protection, mildew prevention, insect control and rodent control measures shall be implemented. Study drugs shall be stored strictly in compliance with specified storage conditions.

Prior to the initiation of study drug administration, study nurses or pharmacists shall prepare the study drug and complete drug preparation records.

Investigators shall administer the study drug to subjects in accordance with the trial protocol and truthfully document actual drug administration after dosing. All study drugs are exclusively intended for this clinical trial and shall not be utilized for any other purposes.

In this study, containers of used study drugs may be destroyed on-site in accordance with applicable guidelines and standard operating procedures established by the study site and local institutions. All unused study drugs shall be recovered for centralized destruction upon trial completion/termination or upon expiry of the shelf life.

8. Study Methods and Procedures

8.1 Study Procedures

(1) Informed consent process

Investigators shall fully explain to each subject the nature, purpose, relevant procedures, expected duration, potential risks and benefits, as well as any possible discomforts of this study during the informed consent process. Each subject must be informed that participation in this study is voluntary, and he/she may request to discontinue treatment at any time without prejudice to his/her legitimate rights and interests.

After explaining the basic information about the study and confirming that each prospective subject fully understands the study objectives, each prospective subject, his/her legal guardian or impartial witness shall be required to sign and date the informed consent form. Prior to signing and dating the form, the subject, guardian or impartial witness shall carefully read and deliberate on the document, and sign the informed consent form only after understanding the study procedures and agreeing to participate; the investigator shall also sign the form concurrently. The informed consent form is made out in duplicate: one copy shall be filed by the national drug clinical trial institution for archiving, and the other shall be retained by the subject. No subject may be enrolled into the study without obtaining and signing the informed consent form. During the study, if there are any updates to the information contained in the informed consent form, the investigator shall promptly inform the subject, his/her guardian or impartial witness and complete re-signature of the revised informed consent form. Subjects undergoing re-screening are required to sign a new informed consent form.

(2) Visits during the study

Subjects may undergo screening examinations upon signing the informed consent form. The Investigator shall assess subject eligibility based on the latest screening evaluation results.

For details of all visit procedures and assessment timepoints during the study, refer to Section 1.2 Visit Flow Chart. The examinations to be performed throughout the study are listed in the table below.

Table 6-6 during the study of examination objective

Item	Requirements and Observation Indicators
Demographic Data	Date of birth, gender, ethnicity, age, etc
Medical history	Including prior tumor diagnosis information, history of anti-tumor therapies (together with corresponding treatment responses), current medical conditions (excluding neoplastic diseases), past medical history, surgical history, allergy history, smoking history, history of

	excessive alcohol consumption, history of drug abuse, etc
genetic testing	Next-generation sequencing covering 463 tumor pathway-related genes
blood sample collection	Pre-treatment blood samples
vital signs	Blood pressure, pulse, respiratory rate and body temperature
physical examination	Complete or targeted physical examination. The complete examination covers skin, mucous membranes, superficial lymph nodes all over the body, head and its organs, neck, chest, abdomen, spine and extremities, nervous system, and other items.
Height and weight	/
ECOG score	Record scores

Item	**Requirements and observation measures**
Routine blood test	Red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT), white blood cell count (WBC), neutrophil count (NEUT), lymphocyte count (LYM)
Serum biochemistry	Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), blood urea nitrogen (BUN)/urea, creatinine (Cr), uric acid (UA), lactate dehydrogenase (LDH), total protein (TP), albumin (ALB), glucose (GLU) – fasting or random, calcium (Ca), phosphorus (P), potassium (K), sodium (Na), chloride (Cl), magnesium (Mg)
Urinalysis	Urine protein (PRO), urine white blood cells (WBC), urine glucose (GLU), urine ketone bodies (KET), urine bilirubin (BIL), urobilinogen (URO), urine pH (PH), urine occult blood (BLD), and urine specific gravity (SG)

Coagulation function	Prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR)
Pregnancy test	Beta-human chorionic gonadotropin (β-HCG) or urine pregnancy test
Virological examination	Hepatitis B serological markers (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [HBsAb], hepatitis B e antigen [HBeAg], hepatitis B e antibody [HBeAb], hepatitis B core antibody [HBcAb]), hepatitis C virus antibody (Anti-HCV), and human immunodeficiency virus antibody (Anti-HIV).Subjects with positive HBsAg results shall undergo additional HBV-DNA testing, and those with positive Anti-HCV results shall receive supplementary HCV-RNA testing.Subjects with a medical history of syphilis are also required to undergo the rapid plasma reagin test (RPR) or toluidine red unheated serum test (TRUST) during the screening period
Electrocardiogram	12-lead electrocardiogram
Imaging examination	Computed tomography (CT) or magnetic resonance imaging (MRI), which must cover the chest, abdomen and pelvis. Contrast-enhanced CT or contrast-enhanced MRI is preferred if there are no contraindications
Brain imaging examination	Contrast-enhanced MRI or contrast-enhanced CT scan; contrast-enhanced MRI scan is recommended as the first choice if no contraindications exist
Bone scan	Radionuclide bone scan (ECT); PET-CT examination results are acceptable

Note: According to the research flowchart, except for the mandatory items during the baseline period and the efficacy assessment, the rest are determined by the researchers

Unplanned visitation

During the research period, considering the safety of the subjects, if any adverse events occur or abnormal results are found in laboratory tests,the researchers can increase the frequency of follow-up visits for the subjects as needed, which is referred to as unplanned visits. The researchers must accurately record each unplanned visit of the subjects in the original files and eCRF and other relevant materials under the unplanned follow-up section.

8.2 Biological Sample Collection and Testing

8.2.1 Blood Biomarkers

The metabolome, proteome and ctDNA genome of the plasma samples in this project were all handled by Nanjing Shihet Genomics Biotechnology Co., Ltd. Among them, the metabolome and proteome were detected through the OE480 high-resolution mass spectrometry platform (Orbitrap), while the ctDNA genome was analyzed by using the HuaDai high-throughput sequencer and the accompanying reagents for liquid biopsy NGS sequencing.

(I) Patient Enrollment and Sample Collection

According to the project inclusion/exclusion criteria, the patients are screened for inclusion. Each subject is selected for inclusion, and each subject will be assigned an independent ID.The first administration of the drug serves as the blood collection point. Blood samples of the enrolled population are collected.

Requirements for collecting fresh blood samples:

1) For each case, the venous blood collection volume is 20 ml, and it is placed in two EDTA anticoagulation blood collection tubes separately;

2) Mix the blood gently, check the condition of the blood: uniform without obvious hemolysis;

3) Mark the case name and the collection date on the anticoagulation blood collection tube;

4) The centrifuge is pre-cooled in advance. Within 2 hours after blood collection, centrifuge at 4℃ (or room temperature) at 1800g for 10 minutes;

After centrifugation, carefully collect the upper layer plasma into two new labeled 5ml low-adhesion centrifuge tubes;

The blood cell sediment is retained in the EDTA blood collection tube and transported at room temperature together with the plasma supernatant low-adhesion tube to the headquarters of Nanjing Shihao Gene. When the temperature is high, cold chain transportation is required.

(II) Sample cfDNA extraction

Collect plasma samples, centrifuge at 16000g for 10 minutes at 4℃; add 100 μL of proteinase K and 800 μL of lysis reagent to each 1 mL of plasma supernatant, and incubate at 60℃ for 30 minutes; after the water bath is completed, insert the sample into ice to cool. Each 1 mL of plasma supernatant was added to 1.8 mL of buffer; a diaphragm vacuum pump was installed, and the sample was eluted through the column; sample was washed with cleaning solution 1; sample was

washed with cleaning solution 2; sample was washed with absolute ethanol through the column; then the residual ethanol was removed: the adsorption column was taken out and placed in a collection tube, and centrifuged. The filter element of the adsorption column was transferred to a 1.5 mL DNA collection tube. Open the cover of the adsorption column tube, add enzyme-free water in the center of the filter membrane, and centrifuge to elute DNA. The filter element of the adsorption column was transferred to a 1.5 mL low-adsorption DNA collection tube. Open the cover of the adsorption column tube, add enzyme-free water in the center of the filter membrane, and centrifuge to elute DNA. After measuring the DNA concentration, it was stored for later use.

(III) Construction of DNA library

Based on the DNA extraction results, 0 - 50 ng of free cfDNA was taken for library construction. The specific operation steps are as follows:

1) Large fragment separation of ctDNA: Based on the principle that magnetic beads preferentially bind to large fragments, the large fragments are first separated. Add a specific proportion of purified magnetic beads to the 96-well plate containing the extracted DNA and mix well. Let it stand at room temperature and then place it on a magnetic stirrer for magnetic separation (at this time, the large fragment DNA has bound to the magnetic beads). Transfer the supernatant from this step to a new 96-well PCR plate (for the next step of small fragment recovery); then, wash the magnetic beads with 80% ethanol to remove impurities. After the magnetic beads are slightly dry, elute the large fragment DNA from the magnetic beads using enzyme-free water and recover it. Store the obtained large fragment DNA at -20 ℃.

2) cfDNA fragment recovery: Add a specific proportion of purified magnetic beads to the supernatant retained from the previous step and mix well. Let it stand at room temperature, then place it on a magnetic stirrer for magnetic separation and discard the supernatant. Then, use 80% ethanol to clean the magnetic beads to remove impurities. After the magnetic beads dry slightly, wash the DNA off the beads with enzyme-free water to achieve the recovery of small fragment DNA. Measure the concentration of the small fragment DNA for subsequent experiments.

3) Terminal repair with A: Add the terminal repair mixture prepared in proportion to the small fragment cfDNA, and perform the terminal repair reaction on the PCR instrument.

4) Adapter ligation: Add the Adapter mixture prepared in proportion to the cfDNA after the previous reaction step to the cfDNA, and perform the ligation reaction on the PCR instrument.

5) Add a specific proportion of purified magnetic beads to the reaction solution after ligation for DNA purification to remove unconnected DNA and Adapters and other impurities, and measure the concentration of the purified DNA;

6) PCR amplification: Add PCR primers and high-fidelity DNA amplification enzymes to the purified cfDNA for the PCR amplification reaction;

7) PCR product purification: Add a specific proportion of purified magnetic beads to the products after the above PCR reaction for purification to remove salt ions, primer dimers and other by-product impurities in the reaction solution, and then wash the DNA library off the magnetic beads with enzyme-free water to obtain the constructed library.

8) Measure the concentration of the purified library and calculate the total library quantity and PCR amplification efficiency;

9) Library product QC: Take 1 μL of the library for corresponding dilution and perform PCR amplification, and use gel electrophoresis to conduct QC testing on the PCR products of this step.

(IV) Probe enrichment

1) The DNA capture probe hybridizes with the library. In the genomic library, add blocking primers, hybridization solution, and capture probe for overnight hybridization.

2) The washing and recovery of the captured library products. Use Wash Buffer to wash the hybridized products after capture to reduce the background of non-specific hybridization.

3) The captured library binds to streptavidin magnetic beads. Mix the activated streptavidin magnetic beads with the hybridized products and incubate overnight.

4) Magnetic bead capture of the library cleaning. Remove the non-specifically bound library.

(V) High-throughput sequencing

The captured libraries were sequenced on the Hada high-throughput sequencing platform according to the kit instructions. DNA formed clusters on the kit's Flew cell. The sequencing platform completed the DNA high-throughput sequencing through a cycle of single-base synthesis, pause, fluorescence detection, and synthesis recovery. The sequencing depth of ctDNA was no less than 9000X, and the sequencing depth of white blood cell DNA was no less than 100X.

The sequenced data after extraction was in the bcl format. The bcf2fastq (v2.17.1.14) software was used to convert it to FASTQ format. Data quality control was performed using Trimmomatic. After quality control, the data was processed using BWA (0.7.12) with the MEM algorithm and default parameters to align to the human reference genome (hg19, GRCh37 Genome Reference Consortium Human Reference 37) and generate a SAM file. Picard (1.119) was used to convert the SAM file to BAM format and sort it according to chromosome coordinates. The alignment results were optimized locally using Genome Analysis Toolkit (GATK, version 3.4-0).
SNVs/Indels/CNVs analysis: analysis of point mutations (SNVs) and insertions/deletions (Indels), using VarScan2 (2.3.9), with a minimum detection frequency of 0.01 and a p-value of 0.05, generating VCF results. ANNOVAR was used to annotate the VCF results, and IGV was used for further manual inspection of the results. Copy number variation (CNVs) analysis used ADTEx (1.0.4). Finally, a list of patient gene mutations was generated.

(VI) Metabolomics and proteomics on-machine analysis

After the plasma samples were extracted, the Vanquish Flex-Orbitrap Exploris™ 480 ultra-high liquid chromatography mass spectrometer was used for the detection of the metabolic and proteomic profiles.

8.2.2 Storage and destruction of biological samples

The research team, in collaboration with other relevant departments, will handle or destroy the samples, and conduct combined anonymization processing. It is possible to conduct additional analyses on the anonymized and combined samples to further evaluate and validate the analysis. Method.

9. Evaluation Measures

9.1 Efficacy evaluation and follow-up

Tumor assessment is conducted using RECIST v1.1, based on the predetermined fixed time points for tumor assessment as planned.

The baseline assessment should be completed within 28 days prior to enrollment and administration.If there are no contraindications, the preferred method for tumor assessment in this study is enhanced CT or enhanced MRI (except for bone). During the entire study period, tumor assessment should include chest, head, neck/ supraclavicular lymph nodes, upper abdomen, and whole-body bone scans.If there is any evidence of metastasis in any other part during the screening process, imaging examinations of these parts should be conducted based on the investigator's judgment or the clinical symptoms and signs of the individual subject, and follow-up should be carried out throughout the study. During the screening process, all known lesion sites must be recorded. During each subsequent tumor assessment, they should be re-evaluated, and the study investigator should determine whether to test and record new lesions based on the patient's clinical manifestations. For lesions detected at baseline, the same examination method and the same investigator should be used for assessment in all subsequent tumor assessment visits. From the first administration of the drug, an imaging assessment should be conducted every 6 weeks (±7 days) within 48 weeks, and once every 12 weeks (±7 days) after one year until imaging disease progression, initiation of new anti-tumor treatment, subject withdrawal of informed consent, loss to follow-up, death, or termination of the study by the sponsor, whichever occurs first. Regardless of whether the administration is delayed or adjusted, the imaging examinations will be conducted according to the original plan. For subjects who terminate treatment for reasons other than imaging disease progression, death, or loss to follow-up, if the time interval between the last imaging examination is greater than 4 weeks, a re-examination should be conducted at the EOT visit. Subsequent imaging examinations should be carried out as much as possible according to the original plan until imaging disease progression, initiation of new anti-tumor treatment, subject withdrawal of informed consent, loss to follow-up, death, or termination of the study, whichever occurs first. Efforts should be made to obtain information on disease progression before initiating new anti-tumor treatment.

During the screening period, enhanced MRI or enhanced CT scans of the brain should be performed. If there are no contraindications, enhanced MRI scans are recommended as the preferred method. The examination results of this center before signing the informed consent (within 28 days before enrollment and administration) can be accepted; subsequent examinations should be conducted based on the investigator's judgment according to clinical indications.

During the screening period, a radioactive nuclide bone scan (ECT) was used for examination. The examination results before signing the informed consent form (within three months before enrollment and administration of the drug) were acceptable, and positron emission computed tomography (PET-CT) was also allowed. Then it will be carried out based on the clinical indications by the researchers. For patients who have been confirmed to have bone metastasis, if the researchers' efficacy evaluation is CR or if there is suspicion of bone lesion progression, bone imaging examinations must be conducted.

9.2 Safety Evaluation

Safety assessments will be performed during the screening period, throughout the study, at the End-of-Treatment (EOT) visit, and during the safety follow-up.

Safety observation indicators include: adverse events (assessed using NCI CTCAE v5.0), vital signs, physical examination, laboratory tests, electrocardiogram (ECG), and echocardiogram, among others.

9.3 Evaluation of Other Study Objectives

To evaluate the correlation between the characteristics of plasma metabolome, proteome and ctDNA genome and the efficacy of luconasertumab combined with anlotinib in the second-line treatment of patients with driver gene-negative locally advanced or metastatic nsq -NSCLC.

Tissue/Blood Biomarkers: If the subjects can provide, provide tumor tissue samples (tissue blocks or unstained sections) that meet the requirements at the treatment baseline for analysis such as TROP 2 immunohistochemical expression. The following two types of tissues are acceptable: 1. Archived tumor tissues that meet the quality control standards of the pathology department; 2.The number of unstained slides is at least 5-15, and the slide thickness is 4-5 micrometers. If the subject is willing to provide, a 20 ml whole blood sample should be provided before the first administration.

10. Safety Monitoring, Reporting, and Medical Management

10.1 Adverse Events

According to GCP, an adverse event (AE) refers to all adverse medical events that occur in a subject after receiving the investigational drug.These can manifest as symptoms, signs, diseases or abnormal laboratory test results, but they do not necessarily have a causal relationship with the investigational drug.

The criteria for determining whether a laboratory test result should be reported as an AE are as follows:

The test result is accompanied by symptoms, and/or;

The test result requires further diagnostic tests or drug/surgical intervention, and/or;

The test result leads to a reduction in the investigational drug dose, suspension of administration, or permanent discontinuation of the drug, and/or;

The investigator considers the test result to constitute an AE.

If it is merely a review of an abnormal test result and does not meet any of the above conditions, it does not constitute an AE. Any abnormal laboratory test result that is determined to be an error does not need to be reported as an AE.

10.2 Adverse Drug Reactions

It refers to harmful reactions that occur with qualified drugs under normal usage and dosage, and which are not related to the intended therapeutic effect.

10.3 Serious Adverse Events

Serious Adverse Event (SAE) refers to any adverse event that meets any of the following criteria:

1) Results in death;

2) Threatens life*;

3) Requires hospitalization or prolongs hospital stay;

4) Leads to permanent or severe disability/function loss;

5) Causes congenital abnormalities/birth defects;

6) Other significant medical events. For adverse events that may not immediately endanger life, death, or hospitalization, but according to appropriate medical diagnosis, are considered to potentially endanger the subject or require medical measures to prevent the occurrence of any of the above situations, they are also generally regarded as serious adverse events.

*Note: “Threatening life” means that the subject immediately has the risk of death upon the occurrence of the adverse event, not that it is assumed that it will develop severely in the future and potentially lead to death.

10.4 Hospitalization

Adverse events that lead to hospitalization or prolong hospital stay in clinical studies are regarded as serious adverse events. Any situation where a patient is admitted to a hospital for the first time by a medical institution (even if for less than 24 hours) meets this criterion.

Hospitalization does not include the following situations:

1. Regular emergency room admission;
2. On-site surgeries (such as outpatient/ on-the-day/ non-bedridden surgeries) (observation in the outpatient department is less than 24 hours);
3. Nursing home;
4. Rehabilitation institutions;
5. Hospice care institutions (such as nursing homes);
6. Hospitalization due to pre-existing illness without any new adverse events or worsening of the original disease (such as: to check for ongoing laboratory abnormalities since the start of the study);
7. Hospitalization due to management or social reasons (such as: annual routine physical examinations, or for the convenience of certain examinations, etc.);
8. Hospitalization as per the trial protocol during clinical research (such as: performing operations as required by the trial protocol);
9. Elective hospitalization not related to the deterioration of adverse events (such as: elective cosmetic surgery);
10. Planned treatments or surgical procedures should be recorded in the entire trial protocol and/or the baseline data of the subjects.

Diagnostic or therapeutic invasive procedures (such as surgeries), and non-invasive operations should not be reported as adverse events. However, if the disease condition that led to this operation meets the definition of an adverse event, it should be reported. For example, acute appendicitis that occurs during the reporting period of adverse events should be reported as an adverse event, and the appendectomy performed as a result should be recorded as the treatment for this adverse event.

10.5 Disease Progression and Death

Disease progression is defined as the deterioration or worsening of the subject's condition as caused by the study's indication, including the imaging progression and clinical symptoms and

signs of the original disease. New metastatic lesions of the primary tumor, or the progression of existing metastatic lesions, are all considered as disease progression.

In the study population of this trial, “disease progression” is an expected occurrence and should not be reported as an AE term unless the disease progression is more severe than expected and/or the investigator believes that the tumor progression is related to the administration of the investigational drug or the study procedures. For events diagnosed as disease progression, even if they meet the criteria of being life-threatening, requiring hospitalization, or prolonging hospital stay, causing permanent or severe disability/function loss (seriously impairing the ability to perform normal life functions), or being an important medical event, they do not need to be reported as SAEs. However, if it is uncertain whether the AE is caused by disease progression, it should be reported as an SAE.

In clinical trials, if a subject dies within 30 days after the last administration, it must be reported as an SAE, regardless of whether the investigator assesses that it may be related to disease progression, or whether the subject has received other anti-tumor treatments. The term “death” should not be used as an SAE term; instead, it should be regarded as the outcome of the event. The investigator must record the event that led to the death result as an SAE. If the cause of death cannot be determined at the time of reporting, the SAE term should be recorded as “unexplained death”, and the investigator should obtain more information to clarify the cause of death.

10.6 Collection, Recording, and Description of Adverse Events/Serious Adverse Events

10.6.1 Window period for collecting adverse events

During the trial, close monitoring should be conducted to determine if any adverse events occur in the subjects. Only adverse events related to the research process should be recorded from the signing of the informed consent form until the first administration of the drug. All adverse events should be observed and recorded from the first administration until 30 days after the last administration.

10.6.2 Record and description of adverse events / serious adverse events

All adverse events observed during the study or mentioned by the subjects were accurately recorded by the researchers in the original medical records and entered into the “Adverse Events” page of the CRF.

In order to fully assess the safety of the investigational drug, this study stipulates the following: adverse events will be observed and recorded from the moment the informed

consent form is signed. From the time of signing the informed consent form until the first administration of the drug, only adverse medical conditions related to the study procedures (such as invasive operations for blood collection, bone marrow puncture/biopsy, etc.) will be recorded as AE. New abnormal observations by the investigators that are considered clinically significant after the first administration will be recorded as AE, including physical examination results, clinical-related abnormal vital signs, clinical-related laboratory test abnormalities, clinical-related electrocardiogram results, etc. If the original condition worsens in terms of nature, severity, or frequency after the first administration, it should also be recorded as an AE. Diseases, conditions, or laboratory test abnormalities that existed prior to the screening visit or were detected during the screening visit are considered to be pre-existing and should be recorded as a history or baseline status in the source file and eCRF based on the investigator's judgment.

The principles for recording and describing adverse events should be as follows: (1) Completeness: In the original medical record description, it should include, but not be limited to, the basic information of the trial and the subjects, the usage of the investigational drug, the occurrence of adverse events, the treatment measures taken for the adverse events, the measures taken for the investigational drug, the outcome of the adverse events, the judgment and basis of causality, concurrent medications, etc.; (2) Readability: For medical terms and other abbreviations, efforts should be made to avoid their use and reduce ambiguity.

The contents to be entered into the CRF include but are not limited to:

1 Name of adverse event;

2 Start date;

3 Severity;

4 Whether to take therapeutic measures;

5 The measures taken for the test drugs;

6 The causal relationship with the investigational drug;

7 Whether it is a serious adverse event

8 Outcome of adverse events

9 End date

10.6.3 Identification of adverse events / serious adverse events

All adverse events will be coded using the International Medical Terminology Dictionary (MedDRA). If possible, adverse events should be recorded according to the exact diagnosis. If it is not possible to record an exact diagnosis, separate signs and symptoms should be recorded. Each diagnosis/symptom should be recorded separately. When the later diagnosis is clear, the records should be updated with the diagnosis instead of the previous symptoms/signs.

When determining the name of an adverse event, ensure that each adverse event name consists of a single event. One diagnosis, one sign/symptom is one adverse event.

Terms such as hospitalization, surgery, death, etc. themselves are not adverse events. The causes that lead to these situations need to be recorded as adverse events. When the causes of the above situations are not yet clear, known information such as unexplained hospitalization, unexplained death, etc. can be used as the name of the adverse event and updated and refined in subsequent follow-ups.

10.6.4 Start and end times of adverse events / serious adverse events

Use the “date of symptom onset” as the start date of the adverse event; if the adverse event is a clinically significant abnormal laboratory test or examination result, the start date is the sampling date. Use the date when the event reaches the criteria for a serious adverse event as the start date of the serious adverse event.

The end time of the adverse event should be when the adverse event is cured, cured with sequelae, improved to the baseline or in a stable state. The time should be as precise as possible to the year, month and day, and if the information is incomplete, it should also be specific to the year and month. If the subject dies, and the end date is not collected (regardless of whether it is caused by an SAE), the end date of the adverse event should be left blank and the status should be “ongoing”.

10.6.5 Severity of Adverse Events/Serious Adverse Events

The researchers will refer to the NCI CTCAE v5.0 to assess adverse events and grade the severity of each adverse event. If the severity of a certain adverse event is not specified in the guidelines, the researchers can assess it based on the general definitions of 1 to 5 levels and combined with medical judgment. General grading of adverse events:

Level 1: Mild; asymptomatic or mild; only observable by clinical or diagnostic means; no treatment required.

Level 2: Moderate; requires minor, local or non-invasive treatment; limited in instrumental daily activities of a comparable age*.

Level 3: Severe or of significant medical significance but not immediately life-threatening; leading to hospitalization or prolonged hospital stay; disabling; limited in self-care daily activities**.

Level 4: Life-threatening; requiring emergency treatment.

Level 5: Deaths related to AE.

* Instrumental daily living activities refer to cooking, purchasing clothes, using the phone, managing finances, etc.

** Autonomously performed daily living activities refer to bathing, getting dressed and undressed, eating, washing, taking medicine, etc., without being bedridden.

10.6.6 Causality Assessment of Adverse Events/Serious Adverse Events

According to the five criteria for adverse event analysis, the relationship between the adverse event and the investigational drug is judged on a five-level scale: “definitely related, likely related, possibly related, possibly unrelated, and unrelated”. Among these, “definitely related, likely related, and possibly related” are considered to be related to the investigational drug. The judgment of the correlation should be completed by the authorized clinical researcher. In addition to making a judgment on whether it is related to the investigational drug, the researcher should also try to provide the basis for the judgment.

Causal Relationship Terminology	**Evaluation criteria**
Definitely related	· There is a reasonable temporal relationship · Consistent with the known mechanism of action, properties or known adverse reactions of the drug · Positive dechallenge · Positive rechallenge · No other reasonable alternative explanations
Probably related	· A reasonable temporal association exists · Consistent with the drug’s known mechanism of action, pharmacological properties or established adverse reactions · Positive dechallenge · Absence of evidence of positive rechallenge

	· No other plausible alternative explanations
Possibly related	· There is a reasonable temporal relationship · No evidence of positive rechallenge · Presenting with any of the following conditions: Consistent with the drug's known mechanism of action, properties or known adverse reactions, with positive dechallenge, but there are other reasonable alternative explanations; Consistent with the drug's known mechanism of action, properties or known adverse reactions, with no evidence of positive dechallenge and no other reasonable alternative explanations; Inconsistent with the drug's known mechanism of action, properties or known adverse reactions, with positive dechallenge and no other reasonable alternative explanations; Inconsistent with the drug's known mechanism of action, properties or known adverse reactions, with no evidence of positive dechallenge and no other reasonable alternative explanations.
Probably unrelated	· The temporal relationship cannot be ruled out · No evidence of positive dechallenge · No evidence of positive rechallenge · Presenting any of the following scenarios: Although consistent with the known mechanism of action, properties or known adverse reactions of the drug, the event can be explained by other more reasonable causes; Inconsistent with the known mechanism of action, properties or known adverse reactions of the drug, and the event can be explained by other more reasonable causes.
Unrelated	· No reasonable temporal relationship · Inconsistent with the drug's known mechanism of action, properties or known adverse reactions

Causal Relationship Terminology	Evaluation criteria
	· No evidence of positive dechallenge · No evidence of positive rechallenge · The event can be explained by other reasonable causes

10.6.7 Follow-up of adverse events / serious adverse events

Follow up on all adverse events that have occurred until the event is judged by the investigator to have healed, with or without sequelae, the condition stabilizes, the laboratory test values return to baseline levels, the subject is lost to follow-up, the subject dies, or the event can be otherwise explained. If follow-up cannot be conducted for some reason, the reason must be recorded in the source file.

10.6.8 Serious adverse event reports or other non-serious adverse drug reactions

When an SAE occurs, researchers should immediately take appropriate and proactive rescue and protection measures for the subjects, and can decide whether to terminate the study based on the condition. For any SAE that occurs within the adverse event collection time window for the subjects, whether it is the first report or a follow-up report, the researcher or the designated personnel must complete the “Severe Adverse Event Report Form” within 24 hours after learning about it. At the same time, the SAEs that occurred after using Lu Kang Shatuzumab should be reported to the Kelenbo Tai Drug Safety Department (email: klbio_pv@kelun.com), and the SAEs that occurred after using Anlotinib should be reported to the Zhengda Tianqing Drug Safety Department (email: PV@cttq.com). All relevant materials of the severe adverse event reports (such as the original report form, emails, case report forms, etc.) must be properly preserved at the research center. At the same time, the researcher should promptly fill out the “Individual Adverse Reaction Report Form” for other non-serious adverse drug reactions that occurred after using Lu Kang Shatuzumab and send it to the Kelenbo Tai Drug Safety Department (email: klbio_pv@kelun.com).

For reports involving death incidents, researchers must report to the sponsor and the ethics committee (if necessary) within 24 hours of learning about the incident, and should also provide other necessary materials to the sponsor and the ethics committee (if necessary), such as autopsy reports and final medical reports.

10.7 Pregnancy Reporting

Within 6 months after the first administration of the investigational drug to the female subject or the male subject's partner and up to the last treatment (based on the last used drug), if pregnancy occurs, the female subject or the male subject's partner must fill out the “Pregnancy Report Form” within 24 hours of learning about it, and report it to the Ethics Committee of the research center. At the same time, the pregnancy events that occurred after

using Lu Kang Shaozhuantu monoclonal antibody should be reported to the Pharmacovigilance Department of Kelunbo Tai (email: klbio_pv@kelun.com), and the pregnancy events that occurred after using Anlotinib should be reported to the Pharmacovigilance Department of Chentai Tianqing (email: PV@cttq.com).

After a female subject becomes pregnant, the treatment must be terminated and the procedures and follow-up required by the trial protocol at the time of termination must be completed, unless these procedures are pregnancy contraindications. For male subjects whose partners become pregnant, the male subject is not required to terminate the treatment, but the partner's situation must be recorded, reported, and followed up in the same manner as for a female subject who becomes pregnant.

Researchers should discuss with the subjects the risks of continuing the pregnancy and the possible effects on the fetus. The pregnancy events should be followed up until the pregnancy outcome (including any premature termination of pregnancy or delivery), and the delivery should be followed up until 3 months after the birth of the fetus. Within 24 hours after learning the pregnancy outcome, it is necessary to report to the ethics committee of the research center (if the ethics committee requires), and also to report the follow-up information of pregnancy events that occurred after using Luconasertumab to the Kelenbo Tai Drug Safety Department (email: klbio_pv@kelun.com), and to report the follow-up information of pregnancy events that occurred after using Anlotinib to the Chentai Tianqing Drug Safety Department (email: PV@cttq.com).

Pregnancy itself is not regarded as an adverse event or a serious adverse event, but any of the following complex situations during pregnancy will be recorded, reported and followed up as a “serious adverse event” according to the protocol.

Complex situations include:

1. Spontaneous abortion (including threatened abortion, inevitable abortion, complete abortion, incomplete abortion, recurrent spontaneous abortion, etc.);
2. Stillbirth;
3. Congenital anomalies or malformations of the fetus/neonate;
4. Ectopic pregnancy;
5. Termination of pregnancy for medical reasons;
6. Fetal death;
7. Neonatal death (all neonatal deaths occurring within one month after birth shall be reported as SAEs regardless of the cause of death. Any infant death occurring more than one

month after birth shall also be reported if the investigator considers that such death may be related to the investigational product), etc.

11. Criteria for Trial Termination/Suspension

11.1 Sponsor's Right to Terminate/Suspend This Trial

Before terminating/suspending a clinical trial, the sponsor must notify the investigator, the ethics committee and the national food and drug administration, and state the reasons. After prematurely terminating/suspending the study, the resumption of the study must be approved by the ethics committee for review.

11.2 Termination/Suspension Requested by the Ethics Committee

11.3 Provisions for Completion of the Clinical Trial

The trial will be concluded when all the subjects meet the following conditions:

1) All the subjects have completed at least 6 months of survival follow-up;

2) Or all the subjects have died, been lost to follow-up, or withdrawn their informed consent.

12. Data Management

12.1 Data Management

1) Researchers must ensure that the data is true, complete and accurate. The original medical treatment data shall not be sent out.

2) Any corrections to the trial records can only be made by drawing lines, noting the revised data beside, explaining the reason, signing by the researcher and indicating the date. It is not allowed to erase or cover the original records.

3) All laboratory examination items are complete.

12.2 Data Recording and Document Retention

The documents in clinical trials (such as the protocol and protocol revisions, completed CRFs, signed ICFs, etc.) need to be stored and managed in accordance with GCP requirements. The research center should keep these documents for 5 years after the completion of the study.

The research documents should be properly stored to facilitate future access or data traceability. When storing the documents, safety and environmental risks should be taken into consideration.

12.3 Access to Source Data/Documents

The researchers agreed that the relevant regulatory authorities should have direct access to all the research-related documents, including the medical records of the participants.

12.4 Protocol Amendments

Any possible revisions to the protocol that may be necessary during the research process should be communicated to the sponsor and the investigator, and their consent should be obtained.

All revisions to the protocol should be kept as supplementary documents of the protocol. Any modification to the protocol must be submitted to the ethics committee, and approval or filing should be carried out in accordance with the regulations of the ethics committee.

13. Statistical Analysis

13.1 Sample Size Determination

This study adopted a single-arm design. The objective response rate (ORR) of the docetaxel monotherapy regimen was 13.3%. 13% was selected as the historical control (p0 = 13%), and it was predicted that the ORR of the lapatinib combined with anlotinib could reach 33% (p1 = 33%). Using Simon's two-stage optimal design, with a one-sided type I error rate of 0.05 and a test power of 80%, under this assumption, 9 subjects were enrolled in the first stage. If ≤ 1 subject achieved CR/PR, the study would be terminated; if > 1 subject achieved CR/PR, it would proceed to the second stage, with a total sample size of 34 subjects. Considering about 10% dropout in the actual study, a total of 38 subjects were ultimately required to be enrolled.

13.2 Definition and Selection of Analysis Sets

The Full Analysis Set (FAS) refers to the collection of all subjects who were enrolled in the study and received at least one dose of the investigational drug and had at least one efficacy assessment conducted.

Safety Set (SS): A collection of all subjects who received at least one dose of the investigational drug after enrollment and had at least one evaluation of safety indicators.

Per Protocol Set (PPS): A collection of all cases in the full analysis set that completed the treatment as per the protocol and had no serious violations of the trial protocol.

13.3 Statistical Methods

All statistical analyses will be conducted using PASS (version 15 or above) statistical analysis software. Unless otherwise specified, measurement data will be statistically described using the mean ± standard deviation or median (minimum value, maximum value). Count data will be described using frequency (percentage). Survival time will be estimated using the Kaplan-Meier method for the median PFS/OS and its 95% confidence interval (95% CI), and survival curves will be plotted. ORR/DCR will be summarized based on the number and percentage of response cases, and its 95% CI will be calculated using the Clopper-Pearson method.

14. Trial Management

14.1 Compliance With GCP Requirements

The situation of the management body and the implementation of GCP:

1) The drug registration applicant and the researcher should both implement the quality control and quality assurance system for clinical trials using standard operating procedures;

2) The original data must comply with the requirements of GCP in China;

3) The laboratory test results must be accurate and reliable;

4) All observed results and findings should be verified to ensure the reliability of the data;

5) Establish a complete trial organization structure and clearly define the responsibilities of each level of personnel;

6) The main researcher is responsible for the overall quality control and implements the responsibilities of each level of personnel;

7) The main researcher is responsible for designing the trial plan and informed consent form, and implements it after obtaining the consent of the drug registration applicant. The trial summary report is written by the main researcher after the trial is completed;

8) The designated researcher is responsible for formulating the trial implementation rules and SOPs, and using them in the trial;

9) Before the trial, the trial group organizes all participants to study the plan, and all trial participants must undergo GCP training;

10) The physicians and nurses participating in the trial must strictly abide by the provisions of the plan, follow the procedures, and shall not arbitrarily change;

11) The designated statistician is responsible for the comprehensive statistical processing of the data.

14.2 Protection of Subject Privacy

All the data of the subjects during the trial will be entered into the computer for confidential storage and analysis. If necessary, relevant institutions may review the records to verify the authenticity, accuracy and completeness of the data. The obtained trial data may also be published in academic journals, but the names of the subjects will not be disclosed. The privacy of the subjects will be kept confidential.

Additional preventive measures are taken to ensure the confidentiality of the documents and to prevent the identification of the subjects through genetic data. However, in special circumstances, certain personnel may see the genetic data and personal identification codes of a particular subject. For example, in cases of medical emergencies, the sponsor, their representative doctor or the researcher will know the subject's identification code and have access to the subject's genetic data. Additionally, relevant regulatory agencies require access to the relevant documents.

14.3 Problems Occurring During the Trial and Management Measures

1) Revision of the plan: After this plan is approved by the ethics committee, if it needs to be revised, a "Plan Revision Explanation" must be formulated and signed by the principal investigator. The plan can only be revised with the agreement of the investigator and the drug registration applicant;

2) After the plan is revised, it must be submitted to the ethics committee for review and approval before it can be implemented;

3) No participant in the trial is allowed to violate the plan. If a violation of the plan occurs, an explanation must be written and notified to the drug registration applicant. The latter has the right to decide whether the trial should continue;

4) If a subject slightly violates the plan, the researcher should promptly notify the drug registration applicant, jointly discuss, and handle the violation situation and whether the subject has dropped out for this case; Minor deviations that do not affect the safety of the subject or the completeness of the trial plan are called minor violations of the plan;

5) Any adverse event (AE) or severe adverse event (SAE) that occurs during the trial will be reported and handled in accordance with the requirements of this plan and SOP.

14.4 Quality Control and Quality Assurance

14.4.1 Quality Assurance

The sponsors, as well as the collaborating units (including CROs, SMOs, statistical units, clinical centers, etc.) entrusted by the sponsors to undertake all or part of the responsibilities and tasks related to this study, should establish their own quality assurance systems, fulfill their respective duties, and strictly follow the clinical trial protocol, adopt corresponding standard operating procedures, in order to ensure the implementation of the clinical trial quality control and quality assurance systems.

14.4.2 Quality Assurance During Clinical Trial Conduct

Before the clinical trial is initiated, the researchers should receive training on the trial protocol to ensure that they have a thorough understanding and knowledge of the specific contents of the clinical trial plan and its indicators. The quality control personnel should verify the basic conditions of the clinical trial to ensure that the clinical trial conditions meet the requirements of the protocol. During the trial process, the researchers should carefully carry out clinical operations and other tasks in accordance with the institutional SOP and the trial protocol requirements, and record them truthfully, timely, completely and in a standardized manner. The quality control personnel should conduct quality checks on the trial process and the corresponding original records. After the trial is completed, the research unit should organize the corresponding project files, which will be checked by the quality control personnel, and then archived and preserved. The quality assurance department of the clinical research unit conducts feasibility audits on the trials being conducted. When non-compliance is found, it should be promptly notified to the researchers and the unit's responsible person for correction, and the correction situation should be tracked.

14.5 Expected Clinical Trial Progress and Completion Date

From September 2025 to March 2026: 9 cases completed

From March 2026 to September 2027: 29 cases completed

14.6 Sponsor Responsibilities

The sponsor is responsible for initiating, applying for, organizing this clinical trial, and providing the trial funds. The sponsor, in accordance with the regulations stipulated in Chinese

GCP, Drug Registration Management Measures and other relevant documents, submits the application for the clinical trial to the National Food and Drug Administration. They may also entrust a contract research organization (CRO) to carry out certain tasks and responsibilities in the clinical trial.

The sponsor selects the institutions and researchers for the clinical trial, and recognizes their qualifications and conditions to ensure the completion of the trial.

The sponsor provides the investigational drug to the researcher and should establish a management system and record system for the investigational drug.

14.6.1 Investigator Responsibilities

This clinical research will be conducted in accordance with the ethical, legal and scientific principles stipulated in the Helsinki Declaration and Chinese GCP regulations, as well as the design and provisions of the research plan.

The researchers are responsible for making medical decisions related to the clinical trial and ensuring that subjects receive timely treatment when adverse events (AEs) occur during the trial. The researchers should be aware of the procedures and requirements for reporting SAEs, and record and report these events as required.

The researchers should accurately, completely, timely and legally enter the data into the eCRF, accept the inspections or audits by the monitors or inspectors dispatched by the sponsor or CRO company, as well as the inspections and visits by the drug supervision and administration department, to ensure the quality of the clinical trial.

14.6.2 Sponsor’s Method for Publication of Study Data

The sponsor has exclusive rights to the data of this study. Before the final report of the multi-center research is completed, no individual should publish the manuscript without the written consent of the sponsor. Regarding the manuscript and publication, the sponsor has the final decision-making power.

Regarding the collection, collection, trading, export, and exit of human genetic resources, compliance with the “Regulations on the Management of Human Genetic Resources of the People's Republic of China” Order No. 717 (May 2019) is required.

15. Trial-Related Ethics

15.1 Ethics Committee

Before the start of the research, the researchers need to submit the research plan, informed consent form and other relevant materials to the ethics committee for approval. Any revisions made to the research plan must also be approved by the ethics committee.

15.2 Informed Consent

Qualified researchers must explain in detail to each subject the nature, purpose, relevant procedures, expected time, potential risks and benefits, as well as any possible discomforts of this trial in the informed consent document. Each subject must be aware that participating in this trial is voluntary, and they can withdraw from the trial and revoke the informed consent at any time without affecting their subsequent treatment or relationship with the treating doctor.

The informed consent form should be presented in a standard writing format and use as little professional language as possible. Each informed consent form must include all the above relevant contents and include a voluntary statement. The informed consent form must be submitted to the ethics committee for approval.

After explaining the basic content of the trial and the researcher is convinced that each subject who will participate in the trial understands the purpose of the trial, each subject should sign their name and date on the informed consent form. Before signing and noting the date, the subject should read and consider their statement and should receive a copy of the informed consent form to keep after signing. Without obtaining informed consent or signing the informed consent form, the subject cannot enter the trial.

15.3 Others

When the subject is unable to independently participate in the informed consent process, throughout the entire informed consent procedure, there must be a reliable and impartial witness/legal representative present. The selection of the impartial witness/legal representative must not infringe upon the subject's right to confidentiality. After the subject gives verbal consent, the impartial witness/legal representative should sign and date the informed consent form to confirm that the information is accurate.

16.References

1. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May-Jun;74(3):229-263.
2. Cancer incidence and mortality in China, 2022. J Natl Cancer Cent. 2024 Feb 2;4(1):47-53.
3. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol. 2013 Mar 10;31(8):992-1001.
4. [中国临床肿瘤学会 (CSCO). (2024)] 非小细胞肺癌诊疗指南.
5. 65O Phase III LEAP-008 study of lenvatinib plus pembrolizumab versus docetaxel for metastatic non-small cell lung cancer (NSCLC) that progressed on a PD-(L)1 inhibitor and platinum-containing chemotherapy. Immunooncol Technol 20:100537, 2023
6. CONTACT-01: A Randomized Phase III Trial of Atezolizumab + Cabozantinib Versus Docetaxel for Metastatic Non-Small Cell Lung Cancer After a Checkpoint Inhibitor and Chemotherapy. J Clin Oncol. 2024 Jul 10;42(20):2393-2403.
7. Injectable Reactive Oxygen Species-Responsive SN38 Prodrug Scaffold with Checkpoint Inhibitors for Combined Chemoimmunotherapy. ACS Appl Mater Interfaces. 2020 Nov 11;12(45):50248-50259.
8. Recent advances in trophoblast cell-surface antigen 2 targeted therapy for solid tumors. Drug Dev Res. 2021 Dec;82(8):1096-1110.
9. The emergence of trophoblast cell-surface antigen 2 (TROP-2) as a novel cancer target. Oncotarget. 2018 Jun 22;9(48):28989-29006.
10. Impact of Endocytosis Mechanisms for the Receptors Targeted by the Currently Approved Antibody-Drug Conjugates (ADCs)-A Necessity for Future ADC Research and Development. Pharmaceuticals (Basel). 2021 Jul 15;14(7):674.
11. TROP2 Is Associated with Primary Resistance to Immune Checkpoint Inhibition in Patients with Advanced Non-Small Cell Lung Cancer. Clin Cancer Res. 2024 Feb 16;30(4):779-785.
12. Preclinical profiles of SKB264, a novel anti-TROP2 antibody conjugated to topoisomerase inhibitor, demonstrated promising antitumor efficacy compared to IMMU-132. Front Oncol. 2022 Dec 23:12:951589.
13. Updated efficacy and safety of anti-TROP2 ADC sacituzumab tirumotecan (sac-TMT) for previously treated advanced NSCLC in Phase 2 study. Cancer Res (2024) 84 (7_Supplement): CT247.
14. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. Lancet. 2014 Aug 23;384(9944):665-73.
15. Efficacy and safety of second-line therapy of docetaxel plus ramucirumab after first-line platinum-based chemotherapy plus immune checkpoint inhibitors in non-small cell lung cancer (SCORPION): a multicenter, open-label, single-arm, phase 2 trial. EClinicalMedicine. 2023 Nov 10:66:102303.
16. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol. 2014 Feb;15(2):143-55.

17. EP08.02-158 Final Analyses of ALTER-L018: A Randomized Phase II Trial of Anlotinib Plus Docetaxel vs Docetaxel as 2nd-line Therapy for EGFR-negative NSCLC. J Thorac Oncol, 2022，17(9):S480

18. 1036P Anlotinib plus docetaxel vs. docetaxel as 2nd-line treatment of advanced non-small cell lung cancer (NSCLC): Updated results from ALTER-L016. Ann Oncol, 2022,33(suppl_7): S448-S554.

19. Anlotinib plus docetaxel in advanced NSCLC progressing on immunotherapy: A pooled analysis of two randomized trials. J Clin Oncol 42, 2024 (suppl 16; abstr 8062).

20. Antibody-drug conjugates: in search of partners of choice. Trends Cancer. 2023 Apr;9(4):339-354.

21. Targeting Trop-2 in cancer: Recent research progress and clinical application. Biochim Biophys Acta Rev Cancer. 2023 Jul;1878(4):188902.

22. Safety, pharmacokinetics, and antitumor properties of anlotinib, an oral multi-target tyrosine kinase inhibitor, in patients with advanced refractory solid tumors. J Hematol Oncol. 2016 Oct4;9(1):105.

23. Effect of Anlotinib as a Third-Line or Further Treatment on Overall Survival of Patients With Advanced Non-Small Cell Lung Cancer: The ALTER 0303 Phase 3 Randomized Clinical Trial. JAMA Oncol. 2018 Nov 1;4(11):1569-1575.

临床试验方案确认签字页

评估芦康沙妥珠单抗联合安罗替尼二线治疗驱动基因阴性局部晚期或转移性非鳞状非小细胞肺癌患者的有效性和安全性的多中心临床研究

主要研究者关于方案的同意书：

我已经认真阅读过本方案，我同意方案中包括的所有用来进行研究的必要的信息，并且我同意按方案所描述的内容执行。我明白缺少伦理委员会批准的情况下，试验不得启动，并且要完全遵守本单位的相关规定。

主要研究者签字：[illegible]　　日期：2025.10.30