A phase II clinical trial to evaluate the safety and efficacy of AS1501 for the treatment of chronic and acute liver failure (ACLF)

Clinical trial protocol

Programme number:	AS1501-CTP-II-01
version number:	V 2.0
Version date:	202 5/ 3/ 3
Lead Investigator:	
Lead unit:	Shenzhen Third Peoples Hospital
Sponsor:	Shenzhen Zhongke Amshenn Pharmaceutical Co., LTD
contract research organization:	Kunling Enterprise Management Shanghai Co., LTD

The signature page of the principal investigator

We have read and confirmed this protocol (Protocol number: AS1501-CTP-ii-01, version number: v 2.0, version date: 202 5/3/3). We agree to perform the relevant duties in accordance with the Helsinki Declaration, GCP, relevant laws and regulations and this study protocol, and are responsible for carrying out the clinical trial.

clinical research unit:

Principal Investigator signature:

_____ _____

sign ones name date

Signatory page of the sponsor

We have read and confirmed this protocol (Protocol Number: AS1501-CTP-ii-01, Version Number: v 2.0, Version Date: 202 5/3/3). We agree to fulfill our responsibilities in accordance with the Helsinki Declaration, Good Clinical Practice (GCP), relevant laws and regulations, as well as this study protocol. We will be responsible for initiating, applying for, organizing, and funding this clinical trial, and conducting audits of the clinical trial.

Applicant: Shenzhen Zhongke Amshenn Pharmaceutical Co., LTD

Signature of the sponsor representative:

_____ _____

sign ones name date

Abbreviations

Abbreviations and terminology	English full name	Chinese interpretation
AARC	AsianAcute-on-Chronic Liver Failure Research Working Group	APAC Research Alliance for Hepatology
ACLF	Acute-on-chronic liver failure	Chronic acute liver failure
ADA	A nti drug antibody	Drug-resistant antibodies
AE	A dverse event	adverse event
AESI	Adverse Event of Special Interest	Special attention to adverse events
AIH	A utoimmune hepatitis	autoimmune hepatitis
ALB	A lbumin	albumin
ALF	A cute liver failure	acute hepatic failure
ALT	alanine aminotransferase	AAT
ALP	alkaline phosphatase	alkaline phosphatase
ANC	absolute neutrophil count	Neutrophil count
APTT	activeated partial thromboplastin time	Activates part of the clotting activase time
AST	aspartate aminotransferase	Aspartate aminotransferase of the Tianmen
AUC	Area under curve	area under the curve
AUC_{last}	Area Under the Curve from time zero to the last measurable concentration	The area under the curve from the time of administration to the last measurable concentration point
AUC_{ss}	Area Under the Plasma Concentration-Time Curve at Steady State	Area under the steady-state plasma concentration-time curve
BMI	Body Mass Index	Body mass index
BUN	blood urea nitrogen	usea nitrogen
Ca	Calcium	calcium
C max	Maximum concentration	Maximum plasma drug

		concentration
Css-max	M aximum concentration at Steady State	Steady-state plasma peak concentration
Css-min	M inimum concentration at Steady State	Steady-state plasma trough concentration
Cl	Chlorine	chlorine
CL	Plasma clearance	plasma clearance
Cr	Creatinine	creatinine
CRF	case report form	Case report form
CRP	C-reactive protein	C reactive protein
CTCAE	Common Terminology Criteria for Adverse Events	Standard of general terminology for adverse events
DAMP	D amage— associated molecular patterns	Damage-related pattern molecules
DBil	Direct bilirubin	bilirubin direct
DISC	D eath inducing signaling complex	Death induction signal complex
DMC	Data Monitoring Committee	Data Monitoring Committee
DNA	Deoxyribonucleic acid	deoxyribonucleic acid
EDC	Electronic Data Capture System	Electronic data acquisition system
ELISA	Enzyme-linked immunosorbent assay	Enzyme-linked immunosorbent assay
FAS	full analyse set	Full analysis set
Fas L	Fas ligand	Fas part
GCP	Good Clinical Practice	Clinical trial management standards
GGT	Glutamyltransferase	γ-glutamyltranspetidase
h	hour	hour
Hb	Hemoglobin	hemoglobin
HBcAb	H epatitis B central ant ibody	Hepatitis B core antibody
HBsA b	H epatitis B surface ant ibody	Hepatitis B surface antibody
HBsAg	H epatitis B surface antigen	HBsAg

HBV	Hepatitis B virus	hepatitis B virus
HCV	Hepatitis C virus	HCV
HDS	H erbal and dietary supplement	Herbs and dietary supplements
HIV	human immunodeficiency virus	human immunodeficiency virus
IAIHG	I nternational autoimmune hepatitis group	International Autoimmune Hepatitis Group
INR	International Normalized Ratio	international normalized ratio
ITT	intend to treat	Intentional treatment
K	potassium	potassium
LVEF	Left Ventricular Ejection Fractions	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities	International Medical Terminology Dictionary
MELD	Model For End Stage Liver Disease	End-stage liver disease model
Mg	Magnesium	magnesium
Min	minute	minute
MRI	M agnetic resonance imaging	magnetic resonance
MSD	Supersensitive Electrochemiluminescence	Electrochemiluminescence
Na	Sodium	sodium
NAFLD	N onalcoholic fatty liver disease	Nonalcoholic fatty liver disease
NOAEL	no observable adverse effect level	No toxic effects were observed at the dose
P	Phosphorus	phosphorus
PAMP	P athogen— associated molecular patterns	Pathogen-associated pattern molecules
PD	P harmacodynamics	pharmacodynamics
PLT	Platelet	blood cells
PK	pharmacokinetics	pharmacokinetics
PT	prothrombin time	prothrombin time
PTA	P rothrombin activity	Prothrombin activity
RBC	red blood cell	RBC
RNA	Ribonucleic acid	ribonucleic acid
RP2D	recommended phase 2 dose	Recommended dose for phase 2 clinical trial

RUCAM	the Roussel Uclaf Causality Assessment Method	RUCAM causality evaluation method
SAE	serious adverse event	Serious adverse events
SOP	Standard Operation Procedure	standard practice
SS	Safety Analysis set	Set of security analysis
TBil	Total bilirubin	total bilirubin
TCM	Traditional Chinese Medicine	Traditional Chinese medicine
TEAE	Treatment-Emergent Adverse Events	Adverse events that occur after medication
Tmax	Time to Maximum Concentration	Peak time
TNF-α	Tumor Necrosis Factor-α	TNF-α
TRAIL	TNF-related apoptosis inducing ligand	TNF-related apoptotic induction ligand
TRAIL-DR5	TNF-related apoptosis inducing ligand - death receptor 5	TNF-related apoptotic induction ligand-death receptor 5
ULN	upper limit of normal	Upper limit of normal value
UREA	——	urea
WBC	white blood cell	leucocyte count

catalogue

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1. scenario summary

1.1. Programme summary

Program number	AS1501-CTP-II-01
Study name	A phase II clinical trial to evaluate the safety and efficacy of AS1501 for the treatment of chronic acute liver failure (ACLF)
Version number/date	V 2.0/202 5- 3- 3
Registration classification	Class 1 biological products for therapeutic use
Study phases	II designated time
The sponsor	Shenzhen Zhongke Amshenn Pharmaceutical Co., LTD
Lead researcher	Professor Chen Jun
Clinical trial unit	Shenzhen Third Peoples Hospital
purpose of research	**IIa designated time** **fundamental purpose:** • To evaluate the safety and tolerability of AS1501 for injection in subjects with early chronic acute liver failure (ACLF) and to explore a rational dosing regimen. • Determine the recommended dose and frequency for stage IIb. **Secondary objective:** • To preliminarily evaluate the efficacy, immunogenicity and pharmacokinetic (PK) characteristics of AS1501 for injection in the treatment of early ACLF subjects. • To preliminarily explore the changes in serum TRAIL expression levels before and after administration of AS1501 for injection in early ACLF subjects. **IIb designated time** **fundamental purpose:** • To evaluate the efficacy of AS1501 for injection in ACLF subjects. **Secondary objective:** • To evaluate the safety and PK characteristics of AS1501 for injection in ACLF subjects. • To explore the changes in serum TRAIL (TNF-related apoptotic induction ligand) expression levels before and after administration of AS1501 for

	injection in ACLF subjects.
End of study	**Ⅱa designated time** **Main endpoint:** 1) Incidence of all types of adverse events (AE) and serious adverse events (SAE), and incidence of AE and SAE related to the study product. **Secondary endpoint:** 1) AS1501 Changes in the MELD (end-stage liver disease model) score and the ACLF Research Alliance (AARC) of the Asia Pacific Association for Research in Liver Diseases at the end of treatment compared to baseline. 2) AS1501 Survival rate of ACLF patients at the end of treatment. 3) AS1501 The cure rate of ACLF patients at the end of treatment; Cure criteria: ① disappearance of clinical symptoms such as fatigue, poor appetite, abdominal distension, reduced urine output, tendency to bleed, and hepatic encephalopathy; ② resolution of jaundice, liver size returning to normal; ③ basic normalization of liver function indicators; ④ normalization of PTA. 4) AS1501 The improvement rate of ACLF patients at the end of treatment; Improvement criteria: ① Significant improvement in clinical symptoms such as fatigue, poor appetite, abdominal distension, and bleeding tendency, with the disappearance of hepatic encephalopathy; ② Significant improvement in physical signs such as jaundice and ascites; ③ Significant improvement in liver function indicators (TbiL <5 ULN, PTA> 40%, or INR <1.5). 5) AS1501 The proportion of ACLF patients with serum biochemical response rate (ALT, AST, Tbil, PTA are normal) at the end of treatment. 6) Incidence of switching to other treatments (artificial liver, liver transplantation, cell therapy, etc.). 7) AS1501 Pharmacokinetic parameters after single and multiple dosing, including: apparent volume of distribution (Vd/f), plasma clearance rate (CL/F), plasma elimination half-life (t1/2), plasma peak concentration (Cmax), time to peak (Tmax), area under the plasma concentration-time curve (AUC), steady-state plasma peak concentration (Css-max), time to steady-state peak (Tss-max), steady-state plasma trough concentration (Css-min), area under the steady-state plasma concentration-time curve (AUCss), and accumulation ratio (Rac).

8) Immunogenicity related indicators, such as: ADA antibodies.
9) The changes in serum TRAIL expression levels before and after administration of AS1501 for injection to ALCF subjects were preliminarily explored.

IIb designated time

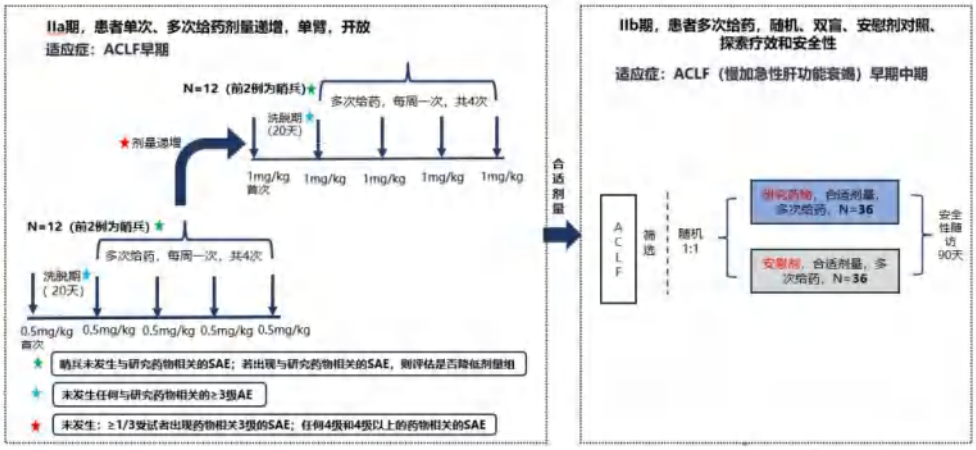
Main endpoint:

Survival of ACLF patients at day 28 after the first dose.

Secondary endpoint:

1) Survival of ACLF patients at 8 and 12 weeks after the first dose.
2) The cure rate of ALCF patients at weeks 4, 8, and 12 after the first dose. Cure criteria: ① disappearance of clinical symptoms such as fatigue, poor appetite, abdominal distension, reduced urine output, bleeding tendency, and hepatic encephalopathy; ② resolution of jaundice, with the liver returning to normal size; ③ basic normalization of liver function indicators; ④ normalization of PTA.
3) The improvement rate of ACLF patients at 4, 8, and 12 weeks after the first dose. Improvement criteria: ① Significant improvement in clinical symptoms such as fatigue, poor appetite, abdominal distension, and bleeding tendency, with hepatic encephalopathy disappearing; ② Significant improvement in physical signs such as jaundice and ascites; ③ Significant improvement in liver function indicators (TbiL <5 ULN, PTA> 40%, or INR <1.5).
4) The percentage of ALCF patients with serum biochemical response (ALT, AST, Tbil, PTA were normal) at 4, 8, and 12 weeks after the first dose.
5) Changes in the MELD (end-stage liver disease model) score and the Asia Pacific Association for Research in Liver Diseases ACLF Research Consortium (AARC) score of ALCF patients at 4, 8, and 12 weeks after the first dose compared to baseline.
6) Incidence of switching to other treatments (artificial liver, liver transplantation, cell therapy, etc.).
7) Liver transplant rate at day 90;
8) AS1501 Pharmacokinetic parameters after multiple dosing, including: apparent volume of distribution (Vd/f), plasma clearance (CL/F), plasma elimination half-life (t1/2), plasma peak concentration (Cmax), time to peak (Tmax), plasma trough concentration (Cmin), plasma concentration-time curve area under the curve (AUC), steady-state plasma peak concentration (Css-max), steady-state time to peak (Tss-max), steady-state plasma trough concentration (Css-min), steady-state

	plasma concentration-time curve area under the curve (AUCss), and accumulation ratio (Rac). 9) The changes in serum TRAIL expression levels before and after administration of AS1501 for injection in ACLF subjects were preliminarily explored.
Subject population and number of cases	Phase IIa: It is planned to include early ALCF population, and two dose groups of 0.5mg/kg and 1mg/kg are planned to be carried out. Each group is planned to include 12 subjects, a total of 24 subjects. Phase IIb: A total of 72 subjects were planned to be included in the ACLF (early and middle) trial, with a 1:1 randomization to receive appropriate doses of study drug or placebo. Each group was planned to include 36 subjects.
research design	This trial is a phase II clinical study, which is divided into two stages: Phase IIa: A sentinel and single-arm design is adopted, with an expected enrollment of 12 early-stage ACLF participants receiving a dose group of 0.5 mg/kg. The first two participants will be sentinels, sequentially enrolled for a single intravenous administration. If these two sentinels do not experience any SAEs related to the study drug within 2 weeks after the initial administration, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to further explore the study. After receiving a single intravenous administration, participants must fast for 20 days. During this 20-day fasting period, if no SAEs related to the study drug occur at grade 3 or higher, and after joint evaluation by the investigator and sponsor, the participant will enter the multiple-dose phase (once a week [D21 is the first dose of multiple-dose administration], for 4 consecutive weeks). Otherwise, the dose will be reduced to further explore the study, with the specific dose to be determined through discussion between the sponsor and the investigator. If any participant drops out during the fasting period after a single administration, additional participants can be added to ensure that no fewer than 12 participants enter the multiple-dose phase. After all 12 early ACLF subjects in the 0.5mg/kg dose group have completed continuous dosing, the DMC (Data Monitoring Committee) will evaluate the safety of this dose group. If any of the following conditions occur in the 0.5mg/kg dose group, the DMC will discuss whether to continue dose escalation: 1) More than 1/3 of the subjects had a drug-related grade 3 SAE; 2) Any drug-related SAE of grade 4 or above. If the DMC evaluation confirms compliance with the dose escalation criteria, the remaining 12 early ACLF participants will receive the 1 mg/kg dose group. The inclusion rules for the 0.5 mg/kg dose group apply here as well; the first two participants serve as sentinels, followed by the rest in sequence, receiving a single intravenous dose. If these two sentinel participants do not experience any SAEs related to the study drug within 2 weeks after the initial administration, the remaining 10 participants can proceed; otherwise, the dose will be reduced to

	continue exploring the study. After receiving a single intravenous dose, participants must fast for 20 days. During this 20-day fasting period, if no grade 3 or higher AE related to the study drug occurs, and after joint assessment by the investigator and sponsor, they will enter the multiple-dose phase (once a week [D21 is the first dose of the multiple-dose phase], for 4 consecutive weeks). If any participant drops out during the fasting period after a single dose, additional participants will be added to ensure that at least 12 participants enter the multiple-dose phase. After completing the 0.5mg/kg and 1mg/kg dose exploration studies, investigators and sponsors can determine the recommended appropriate dose for phase IIb studies based on cumulative safety, efficacy, and possible PK/PD results, or may explore other dose groups or other dosing frequencies. Phase IIb: A randomized (1:1), double-blind, placebo-controlled parallel design is adopted, with an expected enrollment of 72 ACLF participants receiving appropriate doses and frequencies of AS1501 or placebo to further evaluate the efficacy and safety of injectable AS1501. The specific design will be determined by the investigators and sponsors in consultation based on the results from Phase IIa. The research process of each stage of this study was divided into screening period, treatment period and safety follow-up period. The specific design is shown in the figure below. 
Selection criteria and exclusion criteria	➢ **Stage IIa-main inclusion criteria** (1) Age of 18 to 75 years (including the critical value) when signing the informed consent; (2) According to the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Liver Failure and Artificial Liver Group of the Infection Disease Branch of the Chinese Medical Association and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure was diagnosed, and specific indicators included:

a) Chronic liver disease (chronic hepatitis B, autoimmune hepatitis, drug-induced hepatitis, etc.) with acute impact factor of drugs;
b) Serum TBil ⩾ 10 times ULN or mean daily increase of ⩾ 17.1 μ mol/L;
c) Meets any of the following three criteria: A bleeding tendency with PTA ⩽ 40% (or INR ⩾ 1.5); B hepatic encephalopathy; C hepatorenal syndrome or ascites.

(3) The screening was in the early stage of liver failure and did not meet the conditions for liver transplantation;

- Early signs of liver failure:
  - Extremely weak, with obvious loss of appetite, vomiting and abdominal distension and other serious digestive symptoms;
  - ALT and/or AST continued to rise significantly, and jaundice progressively deepened (TBil> 171μmol/L or daily increase> 17.1μmol/L);
  - Tenderness of bleeding, 30% <PTA⩽40% (or 1.5⩽INR<1.9);
  - No complications and other extrhepatic organ failure.

(4) The serum TRAIL increased during screening, and was more than 3 times that of normal people;

(5) Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form;

(6) Able to follow the protocol and complete the trial;

(7) Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration.

➢ **Stage IIa-primary exclusion criteria**

(1) Patients with a history of allergic constitution or severe allergy to protein drugs (CTCAE v5.0 points>3 levels);

(2) Patients who have undergone liver transplantation or are scheduled for liver transplantation within one month.

(3) ACLF patients in the middle and late stages;

(4) Severe grade III ascites or refractory ascites with stage III-IV hepatic encephalopathy.

(5) Patients who had received artificial liver treatment within 1 week prior to screening.

(6) Patients with malignant tumors or a history of malignant tumors; those who were diagnosed with lung cancer, liver cancer, pancreatic cancer or gastrointestinal tumors by imaging (ultrasound, CT or MRI) and tumor markers (AFP, CEA, CA125 or CA199, etc.) during the screening period or within 1 month before the screening period.

(7) Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results

	indicating a risk of severe varices with bleeding during or within 1 month prior to screening. (8) Subjects with acute kidney injury (AKI) defined by KDIGO criteria:(1) Scr increased by 26.5μmol/L(0.3mg/dL or more within 48h,1mg/dL=88.4μmol/L); (2) Scr increased by 1.5 times or more than baseline value within 7d; (3) Urine output decreased (<0.5ml/kg/h) and lasted for more than 6h. (9) The following laboratory test values or abnormal test values are present: a.Blood routine: platelet (PLT) <75 x 10^9L/L, hemoglobin (HGB) <80g/L; b. PT-INR> 1.9 or PTA <30%; c.Left ventricular ejection fraction (LVEF) <50%; serum creatinine> 1.5 x ULN. (10) Associated with severe respiratory dysfunction, dyspnea or failure. (11) Severe infections that cannot be controlled by concomitant drugs, including major organs such as the abdominal cavity, lungs, urinary tract, and skin. (12) HIV positive, or active tuberculosis or syphilis infected. (13) Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history. (14) Subjects with uncontrolled severe hypertension or diabetes. (15) Women who are pregnant or breastfeeding, or who have a positive pregnancy test. (16) Participants who participated in clinical trials of other drugs or medical devices within the first 30 days of randomization or within five drug half-lives. (17) Subjects who have had trauma or major surgery (e.g., requiring general anesthesia) within 28 days prior to the first dose of the study drug. Note: Subjects who plan to undergo surgical procedures under local anesthesia may participate in the study. (18) Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia, or mental status change; or any problem that may impair the subjects ability to receive or tolerate planned treatment at the research center, understand informed consent, or the investigators belief that the subject is contraindicated from participating in the study or confused about the assessment or study results as specified in the protocol. (19) Other conditions that the researchers did not think were suitable for this study. ➢ **Stage IIb-main inclusion criteria** Those who do not meet one of the following conditions shall not be selected as subjects. (1) Age of 18 to 75 years (including the critical value) when signing the informed

	consent; (2) According to the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Liver Failure and Artificial Liver Group of the Infection Disease Branch of the Chinese Medical Association and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure was diagnosed with specific indicators including: d) History of chronic liver disease; e) Serum TBil ≥ 10 times ULN or mean daily increase of ≥ 17.1μmol/L; f) Meets any of the following three criteria: A bleeding tendency with PTA ≤ 40% (or INR ≥ 1.5); B hepatic encephalopathy; C hepatorenal syndrome or ascites. (3) The screening was in the early or middle stage of liver failure and did not meet the conditions for liver transplantation; ◆ Early signs of liver failure: • Extremely weak, with obvious anorexia, vomiting and abdominal distension and other serious digestive symptoms; • ALT and/or AST continued to rise significantly, and jaundice progressively deepened (TBil> 171μmol/L or daily increase> 17.1μmol/L); • Tenderness of bleeding, 30% <PTA≤40% (or 1.5≤INR<1.9); • No complications and other extrhepatic organ failure. ◆ Mid-stage manifestations of liver failure: • On the basis of early manifestations of liver failure, the disease progresses further; • ALT and/or AST decreased rapidly, TBil continued to rise; • Significant bleeding (petechiae or ecchymosis), 20% <PTA ≤ 30% (or 1.9 ≤ INR <2.6); • There was 1 complication and/or 1 failure of extrhepatic organ function. (4) The serum TRAIL increased during screening, and the TRAIL content was more than 3 times that of normal people; (5) Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form; (6) Able to follow the protocol and complete the trial. (7) Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration. ➢ **Stage IIb-primary exclusion criteria** Subjects are not eligible for inclusion in this study if they meet one of the

	following criteria. (1) Patients with a history of allergic constitution or severe allergy to protein drugs (CTCAE v5.0 points>3 levels); (2) Patients who have undergone liver transplantation or are scheduled for liver transplantation within 1 month. (3) Severe grade III ascites or refractory ascites. (4) Patients with associated stage III-IV hepatic encephalopathy. (5) Patients who had received artificial liver treatment within 1 week prior to screening. (6) Patients with malignant tumors or a history of malignant tumors; those who were diagnosed with lung cancer, liver cancer, pancreatic cancer, gastrointestinal tumors, etc., by imaging (ultrasound, CT or MRI) and tumor markers (AFP, CEA, CA125 or CA199, etc.) during the screening period or within 1 month prior to the screening period. (7) Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results indicating a risk of severe varices with bleeding during the screening period or within 1 month prior to screening. (8) Subjects with acute kidney injury (AKI) as defined by KDIGO criteria:(1) Scr increased by 26.5μmol/L(0.3mg/dL or more within 48 h, 1mg/dL =88.4μmol/L); (2) Scr increased by 1.5 times or more than baseline value within 7 d; (3) Urine output decreased (<0.5ml/kg/h) and lasted for more than 6 h. (9) Severe coagulation failure with PT-INR> 2.5 or PTA <20%. (10) Associated with severe respiratory dysfunction, dyspnea or failure. (11) Severe infections that cannot be controlled by concomitant drugs, including major organs such as the abdominal cavity, lungs, urinary tract and skin. (12) HIV positive, or active tuberculosis or syphilis infected. (13) Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history. (14) Subjects with uncontrolled severe hypertension or diabetes. (15) Women who are pregnant or breastfeeding, or who have a positive pregnancy test. (16) Participants who participated in clinical trials of other drugs or medical devices within the first 30 days of randomization or five drug half-life periods. (17) Subjects who have had trauma or major surgery (e.g., requiring general anesthesia) within 28 days prior to the first dose of the study drug. Note: Subjects who plan to undergo surgical procedures under local anesthesia may participate in the study.

	(18) Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia, or mental status change; or any problem that may impair the subjects ability to receive or tolerate planned treatment, understand informed consent at the research center, or that the investigator considers contraindicated for participation in the study or confuses the assessment or study results as specified in the protocol. (19) Other conditions that the researchers did not think were suitable for this study.
research on drug	**Test Drug: Injectable AS1501, Formulation: lyophilized powder for injection; Specifications: 25mg per vial; Appearance: white loose body; Batch number: ****; Expiry date: ** year ** month ** day; Storage conditions: sealed, protected from light, refrigerated at 2℃~8℃; Provided by Shenzhen Zhongke Amshenn Pharmaceutical Co., Ltd.** **Basic treatment drugs: ACLF patients can receive targeted treatment or management based on the cause and triggers of the disease. This includes, but is not limited to, necessary nutritional support, plasma transfusions (excluding those within 48 hours before AARC or MELD score assessment), albumin supplementation, venous catheterization, dynamic monitoring, fluid resuscitation, microecological regulation, analgesics, sedatives, antibiotics, oxygen administration, and mechanical ventilation. During the trial, artificial liver support, glucocorticoids (except for pre-treatment and treatments for infusion reactions/cytokine release syndrome), and hepatocyte growth factors are not allowed.**
End of treatment criteria	Subjects may request to terminate treatment at any time, and the reasons for such termination shall be recorded in the case report form (CRF), including but not limited to: · The subject withdrew the informed consent; · Unacceptable toxicity occurs; · Poor compliance; · The subject requests termination of treatment (e.g., consider receiving an artificial liver); · Disappearance; · die; · encyesis; · other;
Withdraw study criteria	Subjects may request to discontinue or withdraw from the study at any time, and the reasons shall be recorded in the case report form (CRF), including but not limited to the following aspects: The subject withdrew from the study · The subject withdraws the informed consent; · encyesis; · Missing persons;

	· die; · other; The subject was discontinued or withdrew from the study by the investigator · Major protocol deviations or protocol violations occurred during the study that had a significant impact on drug tolerance, safety, or PK evaluation; · Researchers consider it necessary to stop the trial from a medical ethics perspective, such as if there is an appropriate liver donor and an emergency liver transplant is needed; · Those who experienced life-threatening serious adverse events (SAE) related to the study drug and were not suitable to continue the trial; · After the investigator discontinued the study drug treatment, it was determined that withdrawal from the study was most beneficial to the subject; · Poor subject compliance (e.g., failure to take prescribed medications and receive examinations, use of other drugs that affect the evaluation of efficacy and safety); and serious protocol violations/biases; · Clinical adverse events (AE), laboratory abnormalities or other medical conditions that continue to participate in the study may endanger the safety or health of the subject and will not be in the best interest of the subject; · Subjects who meet the exclusion criteria (new or previously undetected) cannot continue to participate in the study.
End of research definition	**Phase IIa: The last subject completed the follow-up as specified in the protocol. Phase IIb: The last subject completed the 4-week efficacy and safety assessment or withdrew from the study earlier (whichever occurred first), and long-term follow-up data were continuously collected.**
dosage regimen	Administration method: Intravenous infusion is used. When administering, dissolve the injectable AS150 1 in sterile water for injection and then add it to 0.9% sodium chloride injection (the total volume of the test drug, sterile water for injection, and 0.9% sodium chloride injection is 200 ml). The infusion time should be no less than 2 hours. In Phase IIb, the placebo administration method is the same as for AS1501. AS1501 Pre-treatment before administration: In the Phase I healthy population clinical study of AS1501,2 mg/kg dose group experienced 2 cases of grade 3 hypersensitivity reactions, and the 3 mg/kg dose group had 2 cases of cytokine release syndrome (CRS). Therefore, in this study, if any dose group in Phase IIa observes a study drug-related hypersensitivity reaction or infusion reaction of grade 2 or higher, or any level of CRS, the subsequent dosing to that subject and other subjects entering the study can be preceded by pre-treatment with at least glucocorticoids (dexamethasone 5-10mg or

	equivalent), along with antihistamines (oral or intravenous diphenhydramine 25 mg³50 mg) and analgesics (oral acetaminophen 650 mg³1000 mg) as per standard procedures at each center. Dosage frequency: Initially planned for once every 7 days, with 5 doses planned for Phase IIa (initial single dose, 4 consecutive doses). For Phase IIb, it is initially planned for once a week, totaling 4 doses. The specific dosing regimen (including dosage frequency) will be determined based on data from Phase IIa.
cell factor	Time point of phase IIa detection: D1/D21/D28/D35/D42 (within 0.5h ± 5min before each infusion), 0h (± 30min) and 24h (± 30min) after each infusion, samples were collected by venous blood collection of 4ml each time. Time point for phase II b detection: before each infusion (within 0.5h ± 5min), 0h (± 30min) and 24h (± 30min) after each infusion, samples were collected from 4ml of venous blood;
PK draw blood	Stage IIa: Blood samples were collected at D1 (before the start of the first infusion), 1h ± 5min after the start of the infusion on D1, 0h ± 5 min, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 30min (D2),72h ± 30min (D4),144h ± 30min (D7),312h ± 30min (D14), before D21/D28/D35, before D42, and 1h ± 5min after the start of the infusion, 0h ± 5 min, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 30min (D43),72h ± 30min (D45),144h ± 30min (D48), and 312h ± 30min (D55). A total of 23 PK blood sampling time points were tentatively determined, with 4ml of venous blood collected and placed in serum tubes for PK analysis. Phase IIb: Subjects were collected blood samples for PK of glutamic acid concentration before infusion on D8, D15 and D22, and peak concentration PK blood samples were collected immediately after infusion on D1 and D22. A total of 5 PK blood sampling time points were tentatively determined. During the trial, the design of subsequent PK blood sampling points may be adjusted according to the results of previous PK tests.
immunogenicity	Stage IIa: Before D1 (within 0.5h±5min), before D7, D14, D21/D28/D35/D42, and at the end of treatment visit, immunogenic blood samples were collected respectively. Eight sampling time points were tentatively determined, and 4ml venous blood was collected for each sample. Phase IIb: Before D1/D8/D15/D22 administration, immunogenic blood samples were collected at 4 time points tentatively. During the trial, the design of subsequent immunogenicity blood sampling points may be adjusted according to the results of previous immunogenicity tests.
TRAIL sample collection	Stage IIa: Screening period, before infusion on D1 (within 0.5h ± 5min), 0h ± 5min after infusion on D1, 144h ± 30min (on D7),312h ± 30min (on D14), before administration on D21/D28/D35, before and 0h ± 5min after infusion on D42 (the 4th consecutive administration), 144h ± 30min (on D48), and 312h ± 30min (on D55), a total of 12 PD blood sampling time points, with 4ml of venous blood collected from each. Phase IIb: TRAIL blood samples were collected at 6 time points, including

	screening, before D1/D8/D15/D22 administration and end of treatment visit. During the trial, the design of subsequent PD blood sampling points may be adjusted according to the previous results.
Biological sample analysis methods	The concentration of AS1501 in serum was measured by validated methods such as enzyme-linked immunosorbent assay (ELISA) or electrochemiluminescence (MSD). ADA in serum will be detected by validated methods such as ELISA or MSD. ELISA, a validated method, was used to detect the concentration of TRAIL in serum.
safety evaluation	Safety observation: During the trial, the physical examination, vital signs, electrocardiogram, blood routine, urine routine, blood biochemical, coagulation function and other indicators of the subjects and adverse events were closely observed.
statistical method	Statistical analysis will be conducted using SAS 9.4 or above to describe safety and efficacy data. Adverse events will be coded using the MedDRA (International Classification of Adverse Events). The analysis of adverse events will be based on post-administration adverse events (TEAEs). The number of subjects and incidence rates of adverse events will be calculated. Descriptive statistics will summarize vital signs and laboratory tests. A shift table will describe changes in normal or abnormal laboratory test results before and after administration.

1.2. Phase IIa trial flow chart (SOA)

		stage of therapy									follow-up period	
Interview description	Screening period	Single-dose period + elution period after single-dose (D1-20)					Multiple dosing period (D21-42)				End treatment / exit study	Safety follow-up
Date of visit (days)	D-14~-1	D1	D2	D4	D7	D14	D21	D28	D35	D42	Within 14 days of the last dose	28 days after the last dose
Time window (± days)	-	-	±1	±1	±1	±2	±2	±2	±2	±2	±3	±7
Sign the informed consent form[1]	×											
Demographic data[2]	×											
Past medical history and treatment history[3]	×											
Inclusion and exclusion criteria[4]	×											
vital sign[5]	×	×			×	×	×	×	×	×	×	
check-up[6]	×	×					×	×	×	×	×	
Height, weight, BMI[7]	×	×					×	×	×	×	×	
Pregnancy test (women of childbearing age)[8]	×	×									×	
Virus screening[9]	×											
Abdominal CT (digestive system)[10]	×											
echocardiogram	×											
12 lead electrocardiogram[11]	×	×			×	×	×	×	×	×	×	
routine blood test[12]	×	×			×	×	×	×	×	×	×	
Blood biochemistry[13]	×	×			×	×	×	×	×	×	×	

		stage of therapy									follow-up period	
Interview description	Screening period	Single-dose period + elution period after single-dose (D1-20)					Multiple dosing period (D21-42)				End treatment / exit study	Safety follow-up
Date of visit (days)	D-14~ 1	D1	D2	D4	D7	D14	D21	D28	D35	D42	Within 14 days of the last dose	28 days after the last dose
Time window (± days)	-	-	±1	±1	±1	±2	±2	±2	±2	±2	±3	± 7
coagulation function[14]	×	×			×	×	×	×	×	×	×	
routine urine test[15]	×	×			×	×	×	×	×	×	×	
arterial blood gas analysis[16]	×	×					×	×	×	×	×	
Indicators of inflammation (CRP/Procalcitonin)[17]	×	×									×	
Child-Pugh score[18]	×	×					×	×	×	×	×	
APAC Liver Disease Research Society ACLF Research Consortium (AARC) score [19]	×	×					×	×	×	×	×	
End-stage liver disease model (MELD) score[20]	×	×					×	×	×	×	×	
SIRS diagnose[21]	×	×					×	×	×	×	×	
AS1501 Medication[22]		×					×	×	×	×		
cell factor[23]		×	×				×	×	×	×		
immunogenicity[24]		×			×	×	×	×	×	×	×	
Pharmacokinetics (PK)[25]	×	×	×	×	×	×	×	×	×	×		
PD marker (TRAIL)[26]	×	×			×	×	×	×	×	×		
Record adverse events[27]	×											
Combination medication[28]	×											

1 Subjects are required to sign an informed consent form prior to any study-related procedures.

2 Demographic data include age, gender and ethnicity.

3 Past Medical History and Treatment History: This includes the history of previous liver diseases and other medical conditions, as well as their treatment history; the history of previous liver diseases should include specific diagnoses, dates of diagnosis, and any treatments previously administered. Inquire about and record the subjects history of other diseases and treatments. Personal History: This includes smoking history, alcohol consumption history, surgical and trauma history, allergy history, infectious disease history, and drug abuse history

4 The eligibility of the study drug was reconfirmed before the first dose.

5 The vital signs examination items include temperature, respiration, pulse/heart rate, and blood pressure; measurements should be taken after a 10-minute rest period. Examination time points: screening period (within 3 days before the first dose), D1 (within 0.5h ± 5 min before the dose, 1h ± 10min, 2h ± 10min, and 4h ± 20min after infusion starts), D7, D14, D21/D28/D35/D42 (within 0.5h ± 10min before the dose, 1h ± 10min, 2h ± 10min, and 4h ± 20min after infusion starts), and end-of-treatment visit.

6 A comprehensive physical examination includes general condition, head, neck, lymph nodes, chest, abdomen, musculoskeletal system, skin, nervous system, and other areas. A full physical examination will be conducted during the screening visit. In subsequent visits, a simplified examination will be performed, including but not limited to general condition and any abnormal signs that the investigator deems necessary to monitor.

7 Height and BMI were collected only during the screening period. Weight: The examination time points were during the screening period, before D1 administration, before D21/D28/D35/D42 administration, and at the end of treatment visit.

8 Only women of reproductive age need to undergo blood pregnancy tests; time points for testing: screening, before D1 administration (results can be accepted within 3 days prior to first administration), and end-of-treatment visit. Researchers may perform additional serum pregnancy tests as indicated by clinical indications.

9 Virus screening includes: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), hepatitis C antibody (Anti-HCV), hepatitis B virus DNA (HBV-DNA), hepatitis C virus RNA (HCV-RNA), *HBV-DNA should be tested when HBsAg is positive or at the discretion of the investigator, HCV-RNA should be tested when the investigator deems it necessary; human immunodeficiency virus antibody (HIV), Treponema pallidum antibody (RPR or TRUST); the testing time point is: screening period. For patients with chronic hepatitis B infection, the investigator can assess and regularly recheck viral-related indicators.

10 Abdominal CT (digestive system): Abdominal CT should be completed during the screening period. Results of good quality within 1 month from each center are acceptable.

11 12-Lead ECG: This includes indicators such as heart rate, RR interval, PR interval, QT interval, QRS interval, and QTc interval. The examination time points are: screening period (within 14 days before the first dose), 0.5h ± 10min before D1 administration, 0 h ± 10min, 1h ± 10min, 2h ± 10min, and 8h ± 20min after D1 infusion completion, D7, D14, D21/D28/D35/D42 before administration (within 0.5h ± 10min), and 0 h ± 10min, 1h ± 10min, 2h ± 10min, and 8h ± 20min after D21/D28/D35/D42 infusion completion, followed by a final treatment visit. If participants exhibit abnormalities such as prolonged QTcF interval, researchers may increase the frequency of examinations based on their condition; after administration, additional ECG checks can be conducted by the researcher. Participants should rest for at least 10 minutes before each examination. If blood is drawn on the day of the ECG, it must be completed within 30 minutes prior to blood collection.

12 The routine blood test items include: white blood cell count (WBC), neutrophil count (ANC), percentage of neutrophils, percentage of eosinophils, percentage of basophils, lymphocyte count (LYM), percentage of lymphocytes, monocyte count (MONO), percentage of monocytes, red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT); testing time points: screening period, before D1 administration (results acceptable within 3 days prior to the first dose), D7, D14, D21/D28/D35/D42 before administration, end-of-treatment visit. During the treatment period, additional tests may be conducted by the investigator based on the subjects condition.

13 Blood biochemical test items: including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin (ALB), prealbumin (Pre-ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), creatinine (CREA), urea (UREA), uric acid (UA), potassium (K), sodium (Na), chloride (Cl), calcium

(Ca), magnesium (Mg), phosphorus (P), glucose, lactate dehydrogenase (LDH), and creatine kinase; test time points: screening period, before D1 administration (results acceptable within 3 days prior to the first dose), D7, D14, before D21/D28/D35/D42 administration, and end-of-treatment visit. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

14 Coagulation function includes: Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), International Normalized Ratio (INR), Fibrinogen (FIB); testing points are during the screening period, before D1 dosing (results acceptable within 3 days prior to first dose), D7, D14, D21/D28/D35/D42 before dosing, and at the end-of-treatment visit. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

15 Routine urinalysis includes: pH, glucose, protein, occult blood, ketones, bilirubin, urobilinogen, nitrite, white blood cells, and specific gravity; testing time points: screening period, before D1 administration (results can be accepted within 3 days prior to the first dose), D7, D14, D21/D28/D35/D42 before administration, and at the end-of-treatment visit. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

16 The arterial blood gas analysis includes: pH (pH), partial pressure of oxygen (PO2), arterial oxygen saturation (SaO2), partial pressure of carbon dioxide (PCO2), lactate, etc.; examination time points: screening period, before D1 administration (results can be accepted within 3 days prior to the first dose), before D21/D28/D35/D42 administration, and at the end-of-treatment visit. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

17 Indicators of inflammation: including C-reactive protein (CRP), procalcitonin, etc.; time points for examination: screening period, before D1 administration (results can be accepted within 3 days before the first administration), end of treatment visit. During the treatment period, additional examinations may be conducted by the investigator according to the condition of the subject.

18 Child-Pugh Score: During the screening period, liver function classification should be performed according to the modified Child-Pugh classification method. Reference indicators include hepatic encephalopathy, ascites, total bilirubin, albumin, and prolonged prothrombin time. Based on these indicators, patients are classified as follows: Grade A (5-6 points), Grade B (7-9 points), and Grade C (≥10 points).

19 APAC Liver Disease Research Society (AARC) Study Alliance score: including total bilirubin, INR, serum creatinine, etiology (0 for cholestatic or alcoholic liver disease, 1 for others). Time points: screening period, pre-dose on D1 (results acceptable within 3 days before the first dose), pre-dose on D21/D28/D35/D42, end-of-treatment visit.

20 End-stage liver disease model (MELD) score: including bilirubin, hepatic encephalopathy staging, INR, lactate, serum creatinine. Time points: screening period, before D1 administration (results can be accepted within 3 days before first administration), before D21/D28/D35/D42 administration, end-of-treatment visit.

21 SRIS: including temperature, heart rate, respiration, and white blood cell count. Time points: screening period, pre-dose on D1 (results are acceptable within 3 days prior to the first dose), pre-dose on D21/D28/D35/D42, and end-of-treatment visit.

22 AS1501 Medication: A sentinel and single-arm design is adopted, with an expected enrollment of 12 early-stage ACLF participants, receiving a dose group of 0.5 mg/kg. The first two participants will be sentinels, sequentially enrolled, and receive a single intravenous dose. If these two sentinels do not experience any SAEs related to the study drug within 2 weeks after the initial dose, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to continue exploring the study. After receiving a single intravenous dose, participants must fasten for 20 days. During this 20-day fasting period, if no SAEs related to the study drug occur at grade ≥ 3, and after joint evaluation by the investigator and sponsor, the participant will enter the multiple-dose phase (once a week [D21 is the first dose] for 4 consecutive weeks); otherwise, the dose will be reduced to continue exploring the study, with the specific dose determined through discussion between the sponsor and the investigator. If any participant drops out during the fasting period after a single dose, additional participants can be added to ensure that no fewer than 12 participants enter the multiple-dose phase. Once all 12 early-stage ACLF participants in the 0.5 mg/kg dose group complete their continuous dosing,

the DMC (Data Monitoring Committee) will assess the safety of this dose group. If any of the following situations occur in the 0.5 mg/kg dose group, the DMC will discuss whether to continue dose escalation: 1) ≥ one-third of participants experience drug-related grade 3 SAEs; 2) any grade 4SAE related to drugs at grade 4 or above. If the DMC evaluates that they meet the criteria for dose escalation, the 12 early ACLF participants who continue to be enrolled will receive the 1 mg/kg dose group. The enrollment rules for the 0.5 mg/kg dose group apply; the first two participants serve as sentinels, followed by the remaining participants who receive a single intravenous dose. If these two sentinel participants do not experience any SAE related to the study drug within 2 weeks after the initial dose, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to further explore the study. After receiving a single intravenous dose, participants must be followed for 20 days. During this 20-day follow-up period, if no grade 3 or higher AE related to the study drug occurs, and after joint evaluation by the investigator and sponsor confirms safety and tolerability, participants will enter the multiple-dose phase (once a week [D21 is the first dose] for 4 consecutive weeks). If any participant drops out during the follow-up period after a single dose, additional participants can be added to ensure that at least 12 participants enter the multiple-dose phase. After completing the 0.5 mg/kg and 1 mg/kg dose exploration studies, the investigator and sponsor can determine the recommended appropriate dose for phase IIb studies based on cumulative safety, efficacy, and potential PK/PD results. They may also explore other dose groups or different dosing frequencies.

23 Cytokines: At least include TNF-α and IL-6. Researchers can add other cytokine tests based on the centers feasible testing spectrum and the condition of the participants. Testing time points: before each infusion on D1/D21/D28/D35/D42 (within 0.5h ± 5min), and 0h (± 30min) and 24h (± 30min) after each infusion on D1/D21/D28/D35/D42 for sample collection, collecting 4ml of venous blood from each participant. During the treatment period, additional tests can be conducted by the researcher based on the participants condition.

24 Immunogenicity: Blood samples for immunogenicity were collected at 8 sampling time points before D1 (within 0.5h ± 5min), D7, D14, D21/D28/D35/D42, and at the end of treatment visit, with 4ml venous blood collected from each time point.

25 Pharmacokinetics (PK): Blood samples were collected at the following time points before D1 (first dose) infusion start (within 0.5 h 5 min), 1h ± 5 min after D1 infusion start, 0h ± 5 min after infusion completion, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 3 0min (D2),72h ± 3 0min (D4),144h ± 3 0min (D7),312h ± 3 0min (D14), before D21/D28/D35 dosing, before D42 dosing, and 1 h ± 5 min after infusion start, 0 h ± 5 min after infusion completion, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 3 0min (D43),72h ± 3 0min (D45),144h ± 3 0min (D48),312h ± 3 0min (D55). A total of 23 PK blood sampling time points were planned, with 4ml of venous blood collected and placed in serum tubes for PK analysis.

26 TRAIL: The main test is to detect serum TRAIL levels. Blood samples are required at 12 PD blood draw time points: before screening, 0.5 h 5 min prior to D1 infusion (before D1 infusion), 0h ± 5min after D1 infusion, 144h ± 30min (D7),312h ± 30min (D14), before D21/D28/D35 dosing, before and 0h ± 5min after D42 (the 4th consecutive dose), and 144h ± 30min (D48),312h ± 30min (D55) after D42 infusion. Each time point involves collecting 4ml of venous blood.

27 Follow up and record all adverse events from the date of signing the informed consent form to the safety follow-up period or until the subject starts new liver protection treatment (or artificial liver, liver transplantation, etc.), whichever occurs first.

28 Record all concomitant drugs and treatments from 14 days prior to the first dose to the safety follow-up period.

1.3. Phase IIb trial flow chart (SOA)

Interview description	Screening period	stage of therapy				End of treatment / early withdrawal	Safety follow-up	long term follow-up	
Interview time	D-14~ 1	W1	W2	W3	W4	14 days after the last dose	28 days after the last dose	W8	W12

		D1	D8	D15	D22	D 36	D50	D60	D90
Time window (days)	-	-	±2	±2	±3	±3	±7	±7	±7
Sign the informed consent form[1]	×								
Demographic data[2]	×								
Medical and personal history[3]	×								
Inclusion and exclusion criteria[4]	×								
Height, weight, BMI[5]	×	×	×	×	×	×		×	×
vital sign[6]	×	×	×	×	×	×		×	×
check-up[7]	×	×	×	×	×	×			
randomization[8]		×							
Pregnancy test (for women of childbearing age only)[9]	×	×				×			
Virus screening[10]	×								
Abdominal CT (digestive system)[11]	×								
echocardiogram	×								
12 lead electrocardiogram[12]	×	×	×	×	×	×		×	×
routine blood test[13]	×	×	×	×	×	×		×	×
Blood biochemistry[14]	×	×	×	×	×	×		×	×
coagulation function[15]	×	×	×	×	×	×		×	×
routine urine test[16]	×	×	×	×	×	×		×	×
arterial blood gas analysis[17]	×	×	×	×	×	×		×	×
Indicators of inflammation (CRP, procalcitonin)[18]	×	×							
cell factor[19]		×	×	×	×				
immunogenicity[20]		×	×	×	×				

Interview description	Screening period	stage of therapy				End of treatment / early withdrawal	Safety follow-up	long term follow-up	
Interview time	D-14~ 1	W1	W2	W3	W4	14 days after the last dose	28 days after the last dose	W8	W12
		D1	D8	D15	D22	D 36	D50	D60	D90
Time window (days)	-	-	±2	±2	±3	±3	±7	±7	±7
Pharmacokinetics (PK)[21]		×	×	×	×				
PD marker (TRAIL)[22]	×	×	×	×	×	×			
Study drugs and medications [23]		×	×	×	×				
Child-Pugh score[24]	×	×	×	×	×	×		×	×
APAC Liver Disease Research Society ACLF Research Consortium (AARC) score [25]	×	×	×	×	×	×		×	×
End-stage liver disease model (MELD) score[26]	×	×	×	×	×	×		×	×
SIRS diagnose[27]	×	×	×	×	×	×		×	×
Record adverse events[28]	×								
Combination medication[29]	×								

1 Subjects are required to sign an informed consent form prior to any study-related procedures.

2 Demographic data include age, gender and ethnicity.

3 Past Medical History and Personal History: This includes the history of past liver diseases, other illnesses, and their treatment; the history of past liver diseases should include specific diagnoses, dates of diagnosis, and any treatments previously administered. Inquire about and record the subjects history of other diseases and treatments. Personal history includes smoking history, alcohol consumption history, surgical or trauma history, allergy history, infectious disease history, and drug abuse history.

4 The eligibility of the study drug was reconfirmed before the first dose.

5 Height and BMI were measured only during the screening period. Weight: during the screening period, before dosing on D1/D8/D15/D22, at the end of treatment visit, D60, and D90.

6 The vital signs examination items include: body temperature, respiration, pulse/heart rate, and blood pressure; measurements should be taken after a quiet rest of 10 minutes; the time points for examination are: screening period, 0.5h ± 10min before each dose on D1/D8/D15/D22,1h ± 10min, 2h ± 10min, 4h ± 20min after infusion starts, end-of-treatment visit, D60, and D90.

7 A comprehensive physical examination includes general condition, head, neck, lymph nodes, chest, abdomen, musculoskeletal system, skin, nervous system, and other areas. A full physical examination will be conducted during the screening visit. In subsequent visits, a simplified examination will be performed, including but not limited to general condition and any abnormal signs that the investigator deems necessary to monitor.

8 Randomized grouping: The 72 ACLF subjects were randomly divided into groups 1:1 to receive either AS1501 or placebo.

9 Only women of reproductive age need to undergo blood pregnancy tests; time points for testing: screening, before D1 administration (results can be accepted within 3 days prior to the first dose), and end-of-treatment visit. Researchers may perform additional serum pregnancy tests as indicated by clinical indications.

10 Virus screening includes: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), hepatitis C antibody (Anti-HCV), hepatitis B virus DNA (HBV-DNA), hepatitis C virus RNA (HCV-RNA), *HBV-DNA should be tested when HBsAg is positive or at the discretion of the investigator; HCV-RNA should be tested when the investigator deems it necessary; human immunodeficiency virus antibody (HIV), Treponema pallidum antibody (RPR or TRUST); the testing time point is during the screening period. For patients with chronic hepatitis B infection, regular retesting of viral markers can be assessed by the investigator.

11 Abdominal CT: Abdominal CT should be completed during the screening period. Results of good quality within one month from each center are acceptable.

12 12-Lead ECG: This includes indicators such as heart rate, RR interval, PR interval, QT interval, QRS interval, and QTc interval. The proposed examination time points are: screening period, within 0.5h ± 10min before each dose on days D1/D8/D15/D22,0 h ± 10min, 1h ± 10min, 2h ± 10min, and 8 h ± 20min after each infusion on days D1/D8/D15/D22, end-of-treatment visit, day D60, and day D90. If the participant exhibits an extended QTcF interval, the investigator may increase the frequency of examinations based on the participants condition. Participants should rest for at least 10 minutes before each examination. If blood is drawn on the day of the ECG, the ECG should be completed within 30 minutes before blood collection. Additional ECGs will be performed as clinically indicated.

13 The routine blood test items include: white blood cell count (WBC), neutrophil count (ANC), percentage of neutrophils, percentage of eosinophils, percentage of basophils, lymphocyte count (LYM), percentage of lymphocytes, monocyte count (MONO), percentage of monocytes, red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT);; tentative test time points: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, D90. During the treatment period, additional tests may be conducted by the investigator based on the subjects condition.

14 The biochemical blood test items include: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin (ALB), pre-albumin (Pre-ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), creatinine (CREA), urea (UREA), uric acid (UA), potassium (K), sodium (Na), chloride (Cl), calcium (Ca), magnesium (Mg), phosphorus (P), glucose, lactate dehydrogenase (LDH), and creatine kinase; provisional test time points: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, D90. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

15 Coagulation function includes: Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), International Normalized Ratio (INR), Fibrinogen (FIB); testing time points are: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, D90. During the treatment period, additional tests may be conducted by the investigator based on the subjects condition.

16 Routine urinalysis includes: pH, glucose, protein, occult blood, ketones, bilirubin, urobilinogen, nitrite, white blood cells, and specific gravity; examination time points: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, and D90. During the treatment period, additional tests may be conducted by the investigator based on the participants condition.

17 The arterial blood gas analysis includes: pH (pH), partial pressure of oxygen (PO2), arterial oxygen saturation (SaO2), partial pressure of carbon dioxide (PCO2), lactate, etc.; examination time points: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, D90. During the treatment period, additional tests can be conducted by the investigator based on the subjects condition.

18 Indicators of inflammation: including C-reactive protein (CRP), procalcitonin, etc.; tentative test time points: screening period, before D1 administration (if the screening period is less than 3 days away, it can be omitted). During treatment, additional tests may be conducted by the investigator according to the condition of the subject.

19 Cytokines: At least include TNF-α and IL-6. Researchers can add other cytokine tests based on the centers feasible testing spectrum and the condition of the participants. Testing time points: before each infusion at D1/D8/D15/D22 (within 0.5h ± 5min), 0h (± 30 min) after each infusion, and 24h (± 30 min) after each infusion for sample collection, collecting 4ml of venous blood each time; during the treatment period, additional tests can be conducted by the researcher based on the participants condition.

20 Immunogenicity test: 4ml of venous blood was collected before D1/D8/D15/D22 administration. During the test, the design of subsequent immunogenicity blood sampling points may be adjusted according to the results of previous immunogenicity test.

21 Pharmacokinetics sample collection: Subjects were collected the trough concentration PK blood samples before infusion on D8, D15 and D22, and the peak concentration PK blood samples were collected immediately after infusion on D1 and D22, a total of 5 PK blood sampling time points were tentatively determined.

22 TRAIL blood sample: The serum TRAIL content was mainly tested. TRAIL blood samples were collected at six time points in total, including the screening period, before D1/D8/D15/D22 administration, and at the end of treatment visit.

23 Study drug administration: The 72 enrolled ACLF subjects were given the appropriate dose and frequency of study drugs in a random 1:1 (AS1501: placebo) ratio. The specific dosage and frequency will be determined by the results of phase IIa.

24 Child-Pugh Score: Liver function classification should be performed according to the modified Child-Pugh classification. Reference indicators include hepatic encephalopathy, ascites, total bilirubin, albumin, and prolonged prothrombin time. Based on these indicators, the scores are divided into: Grade A (5-6 points), Grade B (7-9 points), and Grade C (≥10 points). Time points: screening period, before dosing on D1/D8/D15/D22, end-of-treatment visit, D60, and D90.

25 APAC Liver Disease Research Society ACLF Research Alliance (AARC) score: including total bilirubin, INR, serum creatinine, etiology (biliary or alcoholic liver disease is 0, other is 1). Time points: screening period, before D1/D8/D15/D22 administration, end of treatment visit, D60, D90.

26 End-stage liver disease model (MELD) score: including bilirubin, hepatic encephalopathy staging, INR, lactate, serum creatinine. Time points: screening, before administration on D1/D8/D15/D22, end of treatment visit, D60, D90.

27 SRIS: including body temperature, heart rate, respiration, and white blood cell count. Time points: screening period, before dosing on D1/D8/D15/D22, end of treatment visit, D60, and D90.

28 Follow up and record all adverse events from the date of signing the informed consent form to the safety follow-up period or until the subject starts new liver protection treatment (or artificial liver, liver transplantation, etc.), whichever occurs first.

29 Record all concomitant medications and treatments from 14 days prior to the first dose until the end of treatment.

2. foreword

2.1. Study the theoretical basis

2.1.1. Chronic acute liver failure

Chronic-on-acute liver failure (ACLF) is a syndrome that develops on the basis of chronic liver disease, characterized by acute exacerbation of jaundice and coagulation disorders. It can be accompanied by complications such as hepatic encephalopathy, ascites, electrolyte disturbances, infection, hepatorenal syndrome, hepatopulmonary syndrome, and failure of extrhepatic organs. According to the 2018 edition of Chinas "Guidelines for the Diagnosis and Treatment of Liver Failure."[1]The diagnostic criteria are: rapid deepening of jaundice, serum TBil> 10 ULN or daily increase ≥ 17.1 μ mol/L; signs of bleeding, PTA ≤ 40% (or INR ≥ 1.5). Depending on the underlying chronic liver disease, ACLF is divided into three types: Type A: acute-on-chronic liver failure occurring on the basis of chronic non-cirrhosis liver disease; Type B: acute-on-chronic liver failure occurring on the basis of compensated cirrhosis, often within 4 weeks; Type C: acute-on-chronic liver failure occurring on the basis of decompensated cirrhosis.

ACLF is a major worldwide medical problem[2]In a large retrospective study, the prevalence of ACLF in patients with decompensated cirrhosis was 35% (95% CI: 33% ~38%) and was highest in South Asia at 65% (95% CI: 47% ~84%), and 15% (95% CI: 13% ~18%) in East Asia[3]Its true prevalence may be underestimated. In our country, the incidence of ACLF in patients with decompensated cirrhosis due to hepatitis B infection is approximately 34%. With active prevention and good control of chronic viral hepatitis B, the incidence of ACLF in China has been declining year by year. A prospective cross-sectional study in China (involving all hospitalized patients from 2005 to 2014) showed that the overall incidence of ACLF was 2.53 per 100,000 per year (95% CI 2.16-2.91), a significant decrease compared to the overall incidence of 3.35 per 100,000 per year in 2005. The incidence in males was notably higher than in females (3.83 vs. 1.25 per 100,000 per year). The peak incidence for males occurred around ages 35 to 39 and 65 to 69 years (with a smaller peak at age 65 to 69), while for females, the two peaks were at ages 50 to 54 and 65 to 69 years [4]。

In our country, the main causes of ACLF are: viral hepatitis B, alcohol, and hepatotoxic substances such as drugs (including traditional Chinese medicine, anti-tuberculosis drugs, antibiotics, antitumor drugs, and non-steroidal anti-inflammatory drugs). In a prospective cross-sectional study in China that included all hospitalized patients (2005-2014), the various underlying chronic liver diseases of ACLF patients were: viral hepatitis B, autoimmune liver disease, alcoholic liver disease, and drug-induced liver injury, with their respective proportions being: 91%,3%,2.74%, and 1.19%[4]。

The clinical course of ACLF is rapid, the clinical manifestations are diverse, and the short-term mortality rate is high[2]28-day mortality was approximately 30% to 45% (95% CI 41% to 48%) and 90-day mortality was approximately 58% (95% CI 55% to 61%)[3], And the more organ failures that are combined, the more severe the disease and the higher the mortality. Over the past two decades, no

significant reduction in mortality has been observed among Asian patients with ACLF, and mortality remains close to 50%[5]。

The pathogenesis of ACLF is comprehensive and complex, which has not been fully elucidated. The pathogenesis of ACLF is usually described by the PIRO concept, including susceptibility, injury strike, body response and organ failure[6]The ACL refers to the condition where, on the basis of long-term chronic liver damage caused by fundamental liver diseases, patients first experience acute decompensation of liver function after suffering from various acute intrahepatic or extrahepatic injuries. This manifests as hyperbilirubinemia, coagulation disorders, hepatic encephalopathy, and ascites. The bodys response to such injury can be divided into two parts: one part is due to the acute liver injury leading to dysregulation of cellular immunity and the reticuloendothelial system within the liver, reducing the livers ability to synthesize various anti-infective proteins, thereby increasing the risk of bacterial infections, such as spontaneous bacterial peritonitis caused by gut microbiota translocation, and other systemic infections like urinary tract infections, pneumonia, and skin and soft tissue infections, which result in a large presence of PAMPs (pathogen-associated molecular patterns) in the patients circulation; the other part is the release of large amounts of DAMPs (damage-associated molecular patterns) from necrotic intrahepatic cells into the systemic circulation, where PAMPs bind to specific pattern recognition receptors (PRRs) on innate immune cells, activating the immune system and releasing large amounts of cytokines such as TNF, IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IFN- γ , leading to a "cytokine storm" that triggers systemic inflammatory responses. At the same time, the DAMPs released from damaged intrahepatic cells exacerbate this bodily response, causing excessive inflammationExtrhepatic organ damage; after the early excessive systemic inflammatory response, ACLF enters the state of immune exhaustion or immune paralysis in the late stage, and bacterial infection is further aggravated, which eventually leads to ACLF evolving into multi-organ failure and death [7][8][9][10][11][12][13][14][15]。

Currently, there is no specific targeted treatment for ACLF patients. Treatment methods mainly include comprehensive medical management, artificial liver support systems, liver transplantation, and stem cell transplantation. Comprehensive medical management is a crucial foundation for treating liver failure; however, it lacks effective drugs and methods. Artificial liver support systems, also known as artificial livers, are primarily divided into non-biological, biological, and hybrid types. Their main function is to remove various harmful substances from the body, replenish essential substances, temporarily replace some functions of the failed liver, and improve the internal environment, thereby creating conditions for hepatocyte regeneration and liver function recovery or waiting for opportunities for liver transplantation[16]However, the non-biological artificial liver mainly removes

toxic substances in the patients blood, and cannot completely complete the metabolic, secretory and synthetic functions of the liver, and cannot fundamentally reverse the pathological damage of the liver. It can not significantly improve the survival rate of patients with late-stage liver failure[17]Although biological and hybrid artificial livers have great potential for application due to their liver-specific detoxification, biosynthesis and transformation functions, cell source, cell culture and bioreactor are the main limitations of their clinical application[18]Liver transplantation is the most effective treatment for moderate to late-stage liver failure after comprehensive medical treatment and artificial liver support. However, liver transplantation has insurmountable drawbacks: one is the shortage of donors, which is particularly prominent in China; the other is that recipients need to use immunosuppressants long-term, which are expensive and come with many side effects[19]With the advancement of modern medicine and the deepening of stem cell research, although stem cell therapy for liver failure has achieved some therapeutic effects, many questions remain unresolved: (1) timing of transplantation; (2) number of transplants; (3) route of transplantation; (4) the impact of immune status, endotoxins, and high bilirubin levels on hepatocyte homing and colonization in patients with liver failure[20]In addition, the function and safety of stem cells from different sources still need to be further evaluated scientifically and objectively[21]。

2.1.2. TRAIL- DR5

The core event in the progression from liver injury to liver failure is a large number of hepatocyte deaths. When hepatocyte death exceeds the livers regenerative capacity, liver failure occurs. The classic pattern of hepatocyte death is divided into two categories: necrosis and apoptosis[22]。

Apoptotic defects are typically the primary causative factors, primarily involving both exogenous and endogenous pathways. In these two apoptotic pathways, death receptors Fas, tumor necrosis factor (TNF)-R1, and TRAIL (TNF-related apoptosis-inducing ligand) -death receptor 4 (DR4)/TRAIL-death receptor 5 (DR5) are all expressed on hepatocytes. They activate multiple death domains by binding to their respective ligands, such as Fas ligand (FasL), TNF- α , or TRAIL, initiating the apoptosis process. The endogenous pathway originates from the mitochondria and can be influenced by members of the Bcl-2 family. These two pathways interweave and play physiological roles in the liver. Dysregulation of apoptotic pathways can lead to hepatocellular carcinoma, viral hepatitis, autoimmune hepatitis, ischemia-reperfusion injury, iron or copper deposition disorders, toxic liver injury, and acute liver failure[23][24]*The detailed mechanisms of death receptor-mediated endogenous and exogenous apoptosis in hepatocytes are shown in the figure below.*

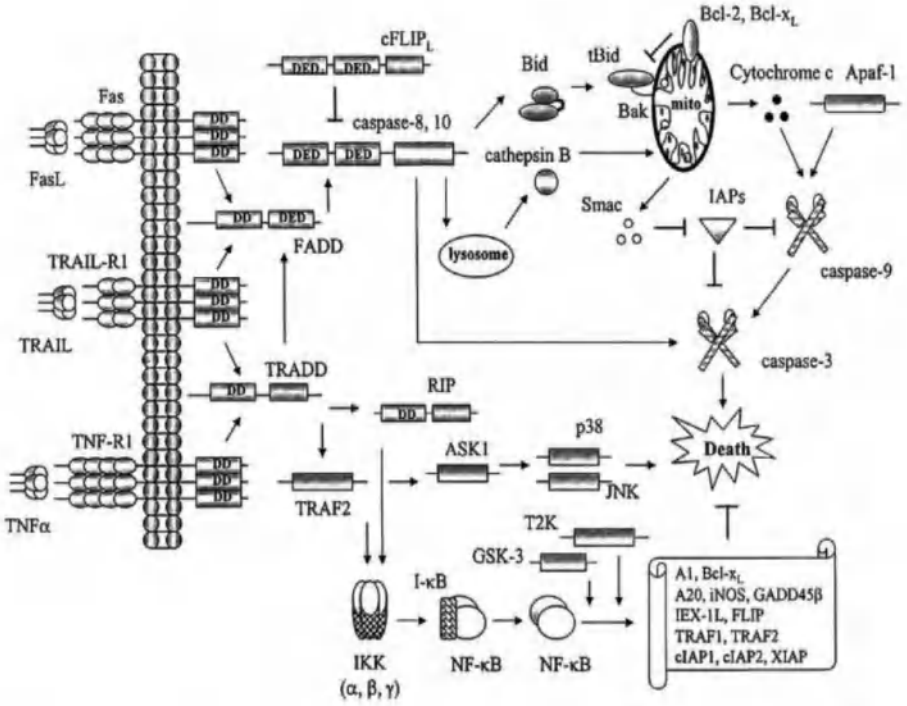


Figure 1 Mechanisms of endogenous and exogenous apoptosis in hepatocytes mediated by death receptors [24]

Among them, TRAIL is also known as Apo2L, first discovered by Wiley et al. in 1995. It is the third apoptosis molecule of the TNF family, following TNF and FasL. As a type II membrane protein, TRAIL consists of a non-conserved N-terminal (cytoplasmic) domain and a significantly conserved C-terminal (extracellular) domain. It can bind to DR4 and DR5, activating their cytoplasmic death domains to form homotetramers and recruit the fas-associated death domain (FADD) assembled in the Death-Inducing Signaling Complex (DISC). [25][26][27] DISC interacts with caspase-8 and caspase-10, activating the pro-apoptotic signaling cascade through receptor-dependent or mitochondrial-dependent pathways. In the mitochondrial-dependent pathway, active caspase-8/10 converts Bcl2 inhibitory bh3 domain protein (Bid) into tBid[28][29]The synergistic effect of BCL2-related X (BAX) and Bcl-2 homologous antagonists/kinases (BAK) leads to tBid mitochondrial translocation and cytoplasmic release of cytochrome c, which then assembles pro-cathepsin-9 and apoptotic protease activator-1 (APAF-1) to form an apoptotic complex, ultimately activating caspase-3/6/7. In the receptor-dependent pathway, activated caspase-8 directly activates the executioner cathepsin to induce apoptosis[30][31]The activated executioner cysteine aspartate leads to apoptosis-related events, including DNA fragmentation and the lysis of cytoplasmic, cytoskeletal and nuclear proteins[32]See the figure below for details.

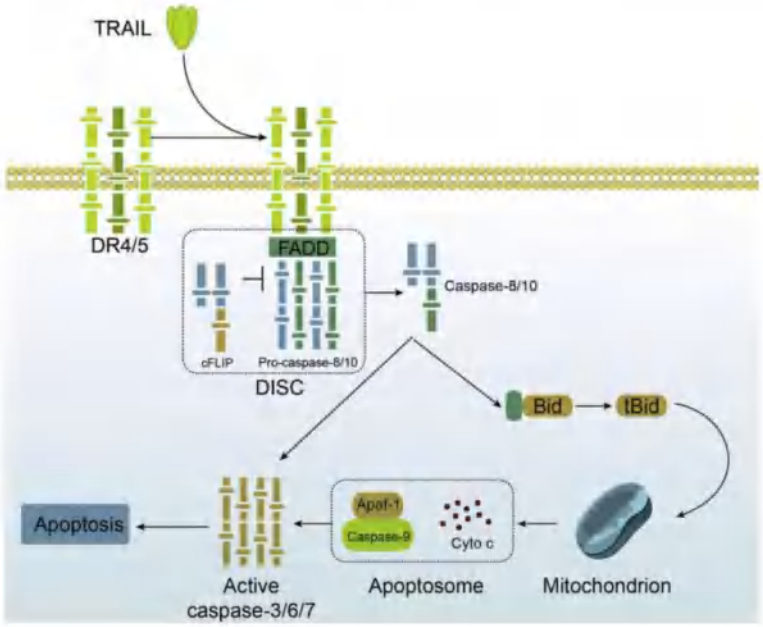


Figure 2 The classic signaling pathways of endogenous and exogenous apoptosis in hepatocytes mediated by TRAIL

Studies on liver injury have reported that TRAIL plays an important role in viral hepatitis, fatty liver, autoimmune liver disease and other diseases[32]TRAILs mechanisms of action in liver disease include: 1) By downregulating the transcription factors HNF4 α , PPAR α , and RXR α enriched in the liver, it inhibits HBV replication and expression. Similar to HBV, TRAIL levels are higher in HCV-infected patients compared to healthy controls, and this upregulation is associated with disease progression; 2) In non-alcoholic fatty liver disease (NAFLD) patients, soluble TRAIL levels are higher than in healthy controls, and these levels correlate positively with triglyceride levels[33]TRIAL deficiency can induce systemic insulin resistance, increase liver cholesterol and glucose synthesis, make the liver more prone to triglyceride accumulation and liver damage, resulting in more severe NAFLD[33][34]TRAIL can significantly reduce diet-induced metabolic abnormalities and can counteract impaired glucose tolerance and NAFLD[35]; 3) Inhibit autoimmune inflammation, maintain immune homeostasis and inhibit immune response (closely related to CD95L or CD30L[36]In a study on primary biliary cholangitis (PBC), TRAIL fails to induce apoptosis of inflammatory cells but inhibits autoimmune inflammation by suppressing DNA synthesis and blocking the cell cycle process of lymphocytes; 4) TRAIL plays a crucial role in improving liver fibrosis and cirrhosis by selectively inducing apoptosis of activated hepatic stellate cells[37][38]。

In vitro and in vivo studies have shown that normal hepatocytes are tolerant to TRAIL-mediated apoptosis, while chronic viral infection, steatosis and toxic substances can upregulate the expression of TRAIL in hepatocytes[22]In addition, studies on immune-related mechanisms in liver failure have shown that the activation of immune cells such as Kupffer cells, dendritic cells, natural killer cells,

cytotoxic T lymphocytes, and regulatory T cells, along with the production of cytokines, plays a crucial role in the development of liver failure. During liver injury, natural killer cells expressing TRAIL also exhibit strong hepatocyte toxicity[39]。

2.2. background

2.2.1. research on drug

2.2.1.1. mechanism of action

Research drug AS1501 is a Class 1 therapeutic biologic that competitively binds to TRAIL-R2 (DR5) on the cell surface. The SDR5 region of AS1501 can bind to its ligand TRAI L, but due to the lack of a transmembrane domain and intracellular death domains, it cannot transmit apoptotic signals into the cell. Therefore, SDR5 can competitively bind to death receptors on the cell membrane surface via TRAIL, thereby blocking TRAIL-mediated apoptosis. See the figure below.

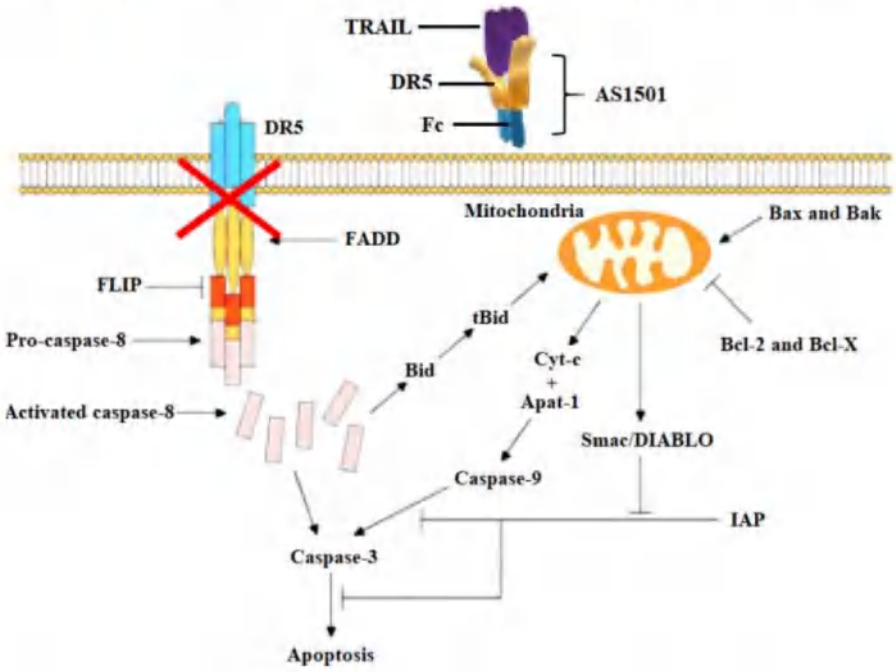


Figure 3 AS1501 and TRAIL combined to block apoptosis signaling

Viral hepatitis and autoimmune hepatitis-induced liver damage is primarily cell-mediated, involving the release of cytokines such as TRAIL, FasL (CD95L), TNF-, and interferon- γ (Interferon- γ , IFN- γ) by immune cells, which induce apoptosis through multiple signaling pathways. Extensive experimental results have shown that although various signaling pathways can induce apoptosis, their downstream pathways are shared, all of which involve activating Caspases and degrading DNA. Additionally, when inducing hepatocyte death, TRAIL and other signaling pathways are interdependent and synergistic; only when both TRAIL and other signaling pathways are present can immune cells effectively kill hepatocytes. In other words, when TRAIL is blocked, other

cytokines cannot activate sufficient downstream (shared) Caspases in the body, leading to DNA degradation and hepatocyte death. For example, in a human hepatitis B virus transgenic mouse model, blocking TRAIL alone without blocking other cytokines can effectively prevent hepatocyte DNA degradation and death. Similarly, in Con-A-induced acute immune hepatitis, blocking TRAIL or knocking out the TRAIL gene can effectively reduce hepatocyte DNA degradation and death.

In conclusion, hepatocyte death caused by viral and immune hepatitis is mediated by TRAIL and several interdependent cytokines, and blocking TRAIL alone can effectively prevent hepatocyte death caused by viral or immune hepatitis.

2.2.1.2. physicochemical property

The detailed properties of AS1501 are shown in the table below.

The common name of the Chinese language	Injection of AS1501
The generic name of the English word	AS1501 for Injection
active ingredient	Human recombinant AS1501 fusion protein
accessories	Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, mannitol and polysorbate 20
constitutional formula	The dimer fusion protein consists of a truncated human death receptor 5 (DeathReceptor5) extracellular region and a human IgG1Fc segment (hinge, CH2 and CH3 regions)
formula weight	About 79KD
physical characteristics	The pH value of the drug is 6.7~7.3; the osmotic pressure molar concentration is 270~330mOsmol/kg; the water content is not greater than 3.0%.
stability	The validity period is tentatively set at 3 years
the form of a drug	Lyophilized powder for injection
shape and properties	White loose body
specifications	25mg per dose
storage procedures	Sealed, lightproof, 2℃~8℃ refrigerated

2.2.2. Overview of preclinical studies

2.2.2.1. Pharmacodynamics studies

2.2.2.1.1. In vitro pharmacological studies

In vitro studies have demonstrated the mechanism by which AS1501 can inhibit TRAIL killing activity and that AS1501 can specifically bind to TRAIL.

1) AS1501 In vitro inhibition of TRAIL killing activity biological activity estimation test

The in vitro inhibition of TRAIL killing activity was assessed through a biological activity estimation test. To better evaluate the biological activity of AS1501 in different tissue cells, multiple cell lines were selected. After incubation with TRAIL protein, MTS (3-(4,5-dimethylthiazol-2-yl)-5-

(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) was used to detect the number of live cells, as described in A_{490}-A_{630}The absorbance ratio was used to determine the results of TRAIL killing experiments.

The experimental results showed that TRAIL induced apoptosis in human hepatocellular carcinoma cells (HepG2), human leukemic T lymphocytes (Jurkat) and mouse fibroblasts (L929), with high sensitivity EC_{50}Values are at the ng level.

2) Specific detection experiments of AS1501 and TRAIL binding

In the experiment, MTT method was used to detect whether AS1501 could block TNF-α induced apoptosis to determine whether AS1501 had cross-reactivity with TNF-α, and ELISA method was used to detect whether AS1501 could bind with Fas L to determine whether AS1501 had cross-binding activity with Fas L.

The results showed that at physiological concentrations, AS1501 did not cross-react with TNF-α and Fas L, reflecting the specificity of AS1501 binding to TRAIL.

3) AS1501 Detection of TRAIL-induced apoptosis inhibition experiments

In the detection experiment of AS1501 blocking TRAIL-induced apoptosis, HepG2 cells were used as test cells, and TRAIL was induced to induce apoptosis in HepG2 cells. The experimental groups were divided into high concentration AS1501 group (35ng/ml), medium concentration group (3.5ng/ml), low concentration group (0.35ng/ml), and blank control group (no AS1501). The changes in the caspase cascade reaction proteins were detected using Western blotting (Western blot method).

The results showed that AS1501 could block the binding of TRAIL to death receptors on cell membrane, thus downregulating the activation of caspase cascade protein in cells and reducing apoptosis.

4) Affinity experiments of TRAIL protein with AS1501 in different species

In the affinity test of TRAIL protein and AS1501 in different species, the affinity of AS1501 was tested with human TRAIL, macaque TRAIL and mouse TRAIL respectively. The data results are shown in the following table.

Table 1 Affinity and kinetic data of TRAIL and AS1501 in different species

part	analyte	k_a （1/Ms）	k_d （1/s）	K_D （M）	Chi² （RU²）
AS1501	human being TRAIL	2.91E+06	3.01E-04	1.03E-10	2.24E-01
	monkey TRAIL	2.89E+06	2.63E-04	9.12E-11	1.88E-01
	mouse TRAIL	1.73E+04	3.83E-04	2.22E-08	7.02E-01

From the affinity data results, it is evident that AS1501 has strong binding activity with TRAIL. The binding affinities of human TRAIL and monkey TRAIL with AS1501 are similar to those of mouse TRAIL with AS1501, and stronger than those of mouse TRAIL with AS1501. This indicates

that pharmacological and safety data from AS1501 in mouse and monkey models can provide reference for data in humans, and the data from the monkey model should be closer to human data.

- **Affinity experiments of TRAIL protein with TRAIL-R2**

In the affinity test of TRAIL protein and TRAIL R2, the binding affinity of human TRAIL with TRAIL R2 (Fc Chimera) was 4.23E-11 M.

- **Fc gamma Affinity measurement of receptor protein with AS1501**

In the affinity test between Fc gamma receptor protein and AS1501, Fc gamma RI CD64 and AS1501 bind very well.

5) TRAIL markers as a companion diagnostic study for AS1501

The results of the TRAIL marker as a companion diagnostic for AS1501 indicate that in patients with liver failure, TRAIL is positively correlated with the dynamic changes of alanine aminotransferase (Alanine Transaminase, ALT) and aspartate aminotransferase (Aspartate Transaminase, AST), and negatively correlated with prothrombin activity (Prothrombin time activity, PTA). This correlation is more pronounced within the dynamic range, indicating that TRAIL is highly consistent with the dynamic monitoring of liver injury and severity markers. TRAIL monitoring plays a crucial role in predicting both hepatic inflammation and the severity of critical conditions.

2.2.2.1.2. In vivo pharmacology studies

In order to prove the effectiveness of AS1501 for viral, drug-induced, autoimmune and other causes of liver injury and/or liver failure, we combined the literature and carried out a series of formal tests for comprehensive analysis.

1) AS1501 Pharmacological evaluation of acetaminophen in mouse liver failure model

In this experiment, the pharmacological effects of AS1501 were evaluated in a mouse liver failure model induced by acetaminophen. During the trial period, no animals in the non-modeling group showed signs of death or near-death conditions. The survival rate of mice in the APAP modeling and saline administration group decreased compared to the 16.7%,15.0mg/kg control. AS1501 significantly increased the survival rate of APAP-induced mice to 55.6%, and within the dose range of 5.0~15.0mg/kg, the efficacy of AS1501 was positively correlated with the drug dose. Positive drug NAC at 250 mg/kg demonstrated good therapeutic effects, while the anti-TNF-α monoclonal antibody 10.0mg/kg showed no significant therapeutic effect. The survival rates and survival curves of mice in the APAP-induced liver failure model treated with AS1501 are shown in Table 2 and Figure 4.

Under the experimental conditions, the test sample AS1501 can treat acute liver failure induced by APAP and improve the survival rate of mice.

Table 2 AS1501 Survival rate of mice in the acetaminophen-induced mouse liver failure model

group		Number of animals (per group)	Number of surviving animals (per group) at 28 days after administration	fraction surviving	P price (Compare with G3)
G1	Saline-saline	20	20	100%	< 0.0001
G2	Sodium chloride-AS1501 (15 mg/kg)	20	20	100%	< 0.0001
G3	AP-saline solution	30	5	16.7%	/
G4	APAP-NAC（250 mg/kg）	28	24	85.7%	< 0.0001
G5	APAP-Anti-TNF-α（10mg/kg）	21	7	33.3%	0.1676
G6	APAP-AS1501（5 mg/kg）	26	8	30.8%	0.4035
G7	APAP-AS1501（10 mg/kg）	26	11	40.7%	0.0466
G8	APAP-AS1501（15 mg/kg）	27	15	55.6%	0.0025

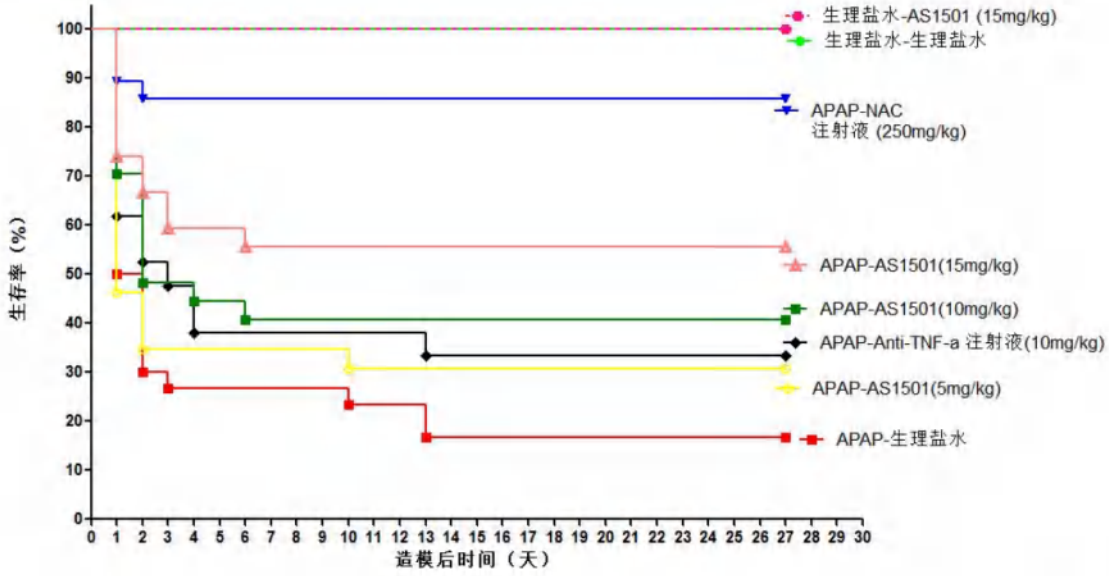


Figure 4 AS1501 Survival curve of mice induced by acetaminophen in liver failure model

2) <u>AS1501 Pharmacological evaluation of acetaminophen-induced drug-induced liver injury in mice</u>

In this experiment, the pharmacological effects of AS150 on AS1501 were evaluated in an acetaminophen-induced drug-induced liver injury model in mice. During the trial, no deaths or near-death cases related to the test product were observed in all animals.

General observation revealed: (1) During animal dissection, no lesions were found in the livers and spleens of 1 to 2 groups of animals, indicating that the dose of AS150127.0mg/kg is safe for mice.

(2) In the negative control group of the modeling group, the animals showed severe liver lesions, suggesting that the drug-induced liver injury model was successful. (3) The liver lesions in the dose group of the test samples were milder than those in the negative control group, indicating that when the dose of AS1501 is 9.0 mg/kg, it has therapeutic effects on APAP-induced hepatitis in mice.

Biochemical indicators revealed: (1) The ALT and AST levels in the G1 and G2 groups without modeling were within the normal range, indicating that the dose of AS150127.0 mg/kg is safe for mice. (2) The ALT and AST levels in the saline group with modeling were significantly higher compared to the unmodelled group, suggesting that the modeling was successful. The ALT and AST levels in the dose group of the test substance showed a significant decrease, indicating that at a dose of 9.0 mg/kg, AS1501 has therapeutic effects on APAP-induced drug-induced liver injury in mice.

The H&E staining results of the pathological sections show: (1) No modeling groups G1 and G2 showed any pathological areas, indicating that the dose of AS1501 within 27.0mg/kg is a safe dose. (2) Compared to the non-modeling groups G1 and G2, the modeling group showed significant liver damage areas, with the largest area in the negative control group G3. The damaged area in the test group was significantly smaller than that in G3, suggesting that AS15101 has therapeutic effects on acute liver injury induced by APAP at the dose of 3.0mg/kg, 9.0mg/kg, 27.0mg/kg. See Table 3 and Figure 5 for the ALT and AST values and levels of each group.

Table 3 AS1501 ALT and AST values in mice induced by acetaminophen drug-induced liver injury model

group		ALT (U/L)	P price (Compared with G3 group)	AST (U/L)	P price (Compared with Group G3)
G1	Saline-saline	56.76±8.608	< 0.0001	161.2±43.84	< 0.0001
G2	Saline AS1501 (27 mg/kg)	51.90±4.118	< 0.0001	111.9±7.509	< 0.0001
G3	AP-saline	10619±874.2	/	5151±535.9	/
G4	APAP –NAC (27 mg/kg)	8331±420.3	0.0159	4167±273.4	0.0906
G5	APAP-AS1501 (3 mg/kg)	8775±708.4	0.1061	4241±462.1	0.2033
G6	APAP-AS1501 (9 mg/kg)	7654±596.4	0.0092	3727±368.3	0.0384
G7	APAP-AS1501 (27 mg/kg)	10926±954.1	0.8130	6061±865.8	0.3722

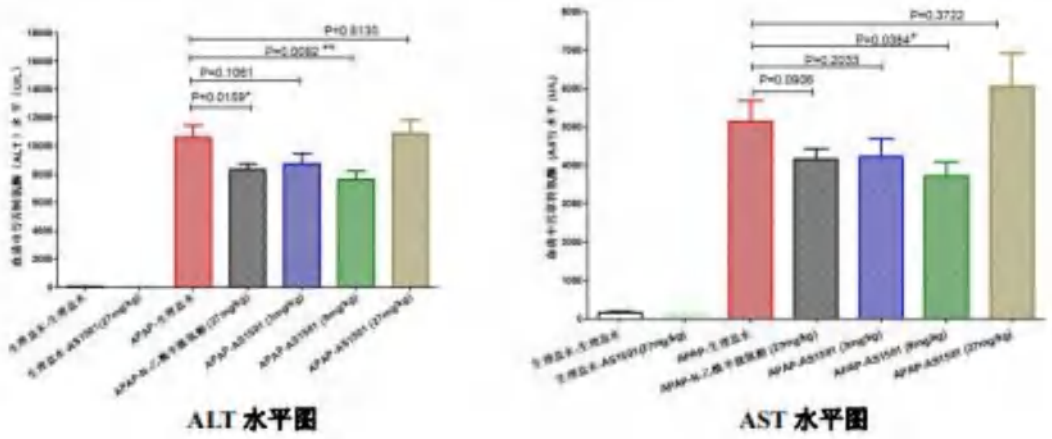


Figure 5 ALT and AST levels

3) <u>AS1501 Pharmacological evaluation of jack bean protein A (ConA) in mouse liver failure model</u>

In the pharmacological evaluation of AS1501 in the Con A-induced mouse liver failure model, no animals in the non-modeling groups (groups 1-2) showed signs of death or near-death. Under non-modeling conditions, mice administered the test dose of 30.0mg/kg did not die, indicating that the test dose of 30.0mg/kg is safe for mice. The survival rate of mice in the Con A-induced and saline group decreased to 2.9%. In the modeling condition, the test group (AS1501 dose of 10.0mg/kg, 20.0mg/kg, 30.0mg/kg) showed an increased survival rate compared to the negative control group, with statistically significant differences, showing better effects than the positive control group. Among these, 30.0mg/kg AS1501 can increase the survival rate to 32.3%, and the positive drug Ganlixin at 15 mg/kg also demonstrated therapeutic effects, though not as effective as 10.0mg/kg AS1501. The test substance from 10.0~30.0mg/kg showed significant therapeutic effects in the Con A-induced acute liver failure model in mice. See Table 4 and Figure 6 for details.

Therefore, under the experimental conditions, the test sample AS1501 can treat acute liver failure induced by Con A in mice and improve the survival rate of mice.

Table 4 shows the survival rate of mice in the mouse liver failure model induced by broad bean protein A

group		Number of animals (per group)	The drug was given after 33 days Number of survivors (per cent)	fraction surviving	P price (Compare with G3)
G1	Saline-saline	20	20	100%	< 0.0001
G2	Sodium saline-AS1501 (30.0 mg/kg)	20	20	100%	< 0.0001

G3	Con A-normal saline	34	1	2.9%	/
G4	Con A-AS1501（10.0 mg/kg）	33	10	30.3%	0.0026
G5	Con A-AS1501（20.0 mg/kg）	31	9	29.0%	0.0341
G6	Con A-AS1501（30.0 mg/kg）	31	10	32.3%	0.0069
G7	Con A-Ganlixin (15.0 mg/kg)	33	8	24.2%	0.0100

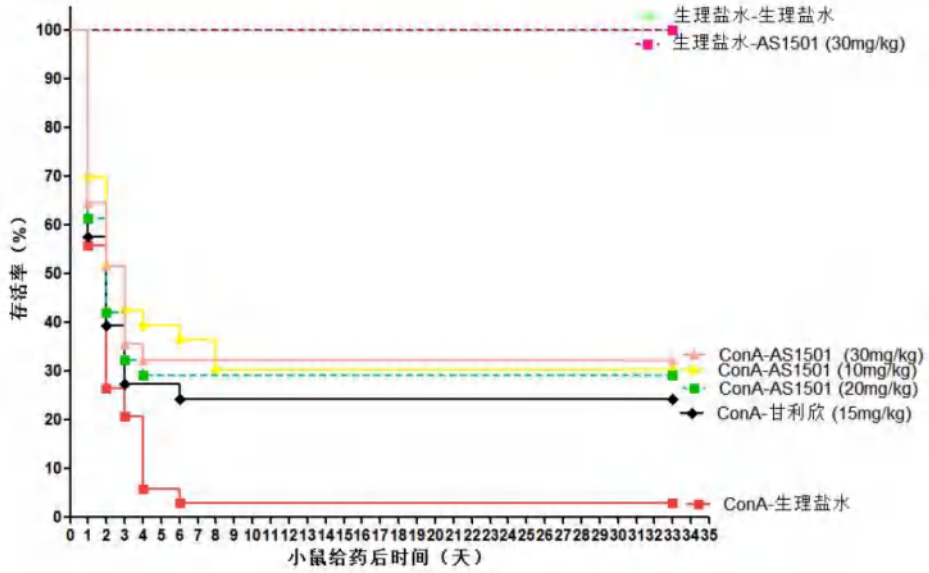


Figure 6 AS1501 Survival curve of mice induced by jack bean protein A in the model of liver failure

4) <u>AS1501 Pharmacological evaluation of jack bean protein A in mouse autoimmune hepatitis model</u>

In the pharmacological evaluation of AS1501 in the cowpea protein A-induced autoimmune hepatitis model in mice, gross observations revealed (1) during the trial period, although all animals showed weight loss, no abnormal changes were observed compared to the uninduced groups G1 and G2. (2) Upon dissection, gross examination found that there were no lesions in the livers and spleens of animals from groups G1 to G2, indicating that the dose of AS150127.0mg/kg was safe for mice. (3) The livers and spleens of animals in the induced group G3 showed severe lesions, suggesting that the Con A-induced hepatitis model was successful. (4) The livers and spleens of animals in the test group showed milder lesions compared to the negative control group G3, indicating that the dose of AS15013.0mg/kg, 9.0mg/kg, 27.0mg/kg had a therapeutic effect on liver damage in mice.

The results of the biochemical indicators alanine transaminase (ALT) and aspartate transaminase (AST) are as follows: (1) The ALT and AST levels in groups G1 and G2 without modeling were within normal ranges, indicating that the dose of AS150127.0mg/kg is safe for mice. (2) The elevated levels of ALT and AST in mice given physiological saline after modeling suggest that the modeling

was successful. The high and medium dose groups G7 and G6 showed relatively lower ALT and AST levels compared to the negative control group G3, with statistically significant differences, suggesting that AS1501 at a dose of 9.0mg/kg, 27.0mg/kg has therapeutic effects on Con A-induced autoimmune hepatitis in mice. Moreover, AS1501 at a dose of 27.0mg/kg resulted in the most significant reduction in ALT and AST levels, indicating that the effect of AS150127.0mg/kg at this dose is optimal under these experimental conditions.

The H&E staining results of the pathological sections show: (1) No modeling group G1 and G2 showed any pathological areas, indicating that AS150127.0 mg/kg is a safe dose for mice. (2) Compared with the non-modeling groups G1 and G2, the modeling groups G3 to G7 clearly showed liver injury areas, with the largest liver injury area in the negative control group G3. The injury areas in the test groups G5 to G7 were significantly smaller than those in G3, suggesting that the AS15101 doses of 3.0mg/kg, 9.0mg/kg, 27.0mg/kg had therapeutic effects on acute liver injury induced by Con A in mice. See Table 5 and Figure 7 for ALT and AST data.

According to the above experimental results, AS1501 has therapeutic effect in the autoimmune hepatitis model induced by Con A under the experimental conditions, and the efficacy of AS1501 is positively correlated with the dose range of 3.0~27.0mg/kg.

Table 5 AS1501 ALT and AST values in mice with autoimmune hepatitis induced by jack bean protein A

group		ALT（U/L）	P value (compared with the G3 group)	AST（U/L）	P value (compared with the G3 group)
G1	Saline-saline	60.24±3.856	< 0.0001	125.60±12.46	< 0.0001
G2	Saline AS1501（27 mg/kg）	66.16±5.512	< 0.0001	139.70±13.69	< 0.0001
G3	Con A-normal saline	3047.00±395.5	/	2299.00±335.4	/
G4	Con A-Glycyrrhizin（27 mg/kg）	1475.00±559.2	0.0277	1142.00±530.3	0.0733
G5	Con A-AS1501（3 mg/kg）	2227.00±467.2	0.1873	1551.00±350.0	0.1316
G6	Con A-AS1501（9 mg/kg）	1388.00±333.7	0.0026	925.4±208.2	0.0010
G7	Con A-AS1501（27 mg/kg）	1448.00±257.0	0.0015	976.9±166.0	0.0010

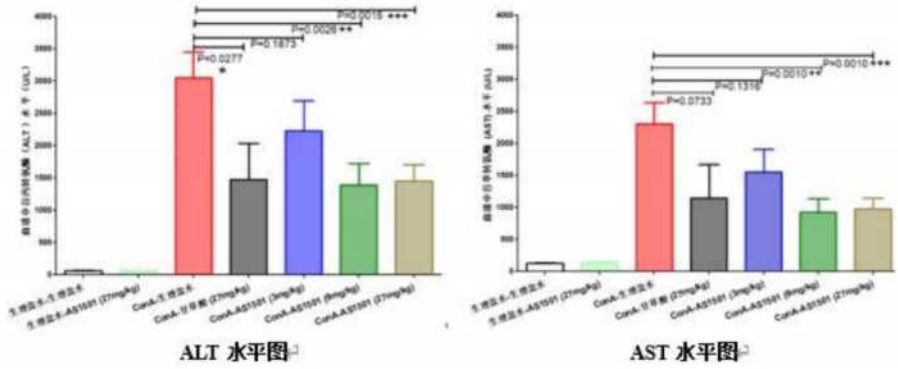


Figure 7 ALT and AST levels

5) <u>AS1501 Study on the molecular mechanism of immune regulation in drug-induced hepatitis and acute liver failure</u>

The above animal experiments show that AS1501 is effective for drug-induced hepatitis and acute liver failure. In order to clarify the mechanism of action of AS1501 more clearly, we further explore the molecular mechanism of immune regulation of AS1501 by combining another group of in vivo studies on animals.

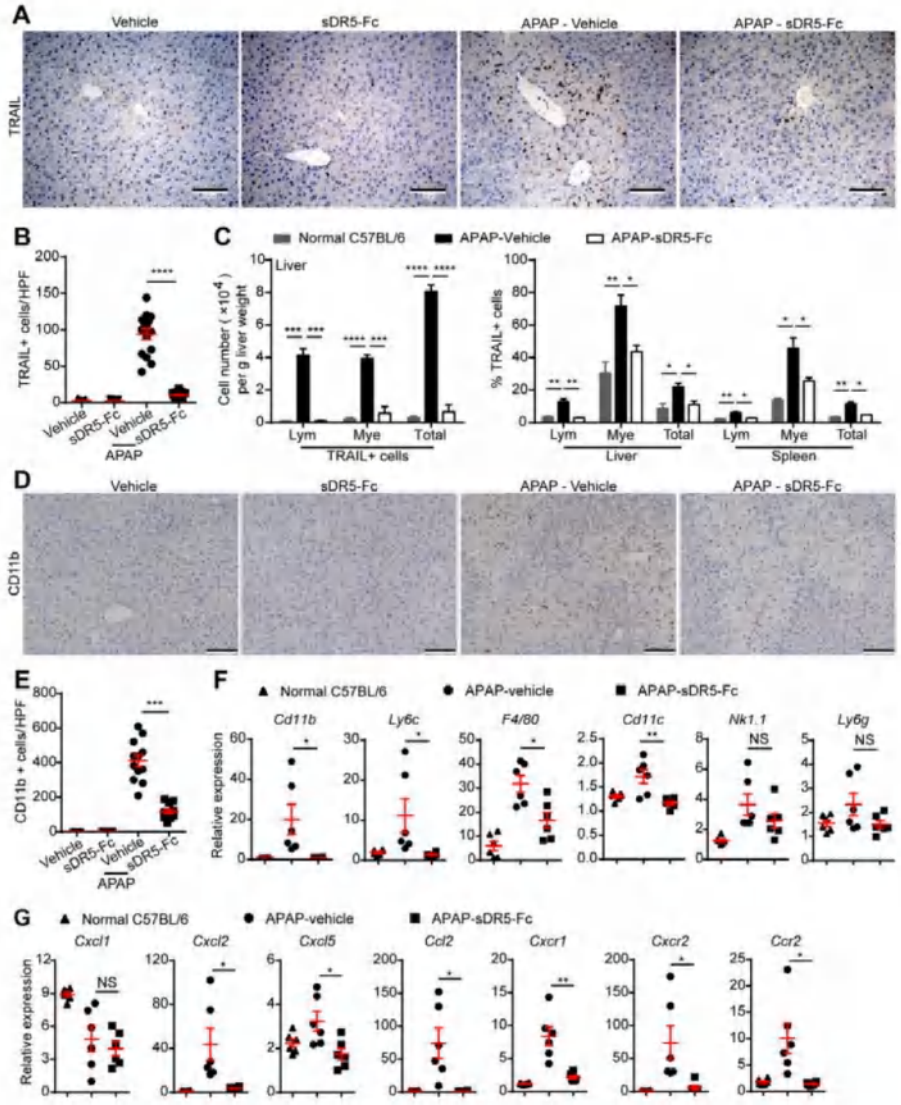


Figure 8 AS1501 Reduced leukocyte infiltration induced by APAP

Intraperitoneal injection of APAP (300 mg/kg) was administered to mice (n = 6), followed by administration of 9 mg/kg AS1501 (expressed as sDR5-Fc in the published article) or saline as a control one hour later. The liver was collected for histopathological analysis (A, D) and RNA expression analysis (F, G) 24 hours after APAP injection, or the liver and spleen samples were collected for flow cytometry analysis 8 hours after APAP injection (C).

(TRAIL) was measured by immunohistochemistry in liver samples (A and D)$^{+}$And CD11b^{+}White blood cells. Scale: 100μ m.

(B,E) TRAIL in each high-power field (HPF) of liver sections as shown in A and D^{+}And CD11b^{+}The number of white blood cells.

(C) TRAIL in liver and spleen^{+}The number or percentage of white blood cells. Lym, lymphocytes; Mye, bone marrow cells.

(F, G) quantified liver Cd11b, Ly6c, F4/80, Cd11c, Nk1.1, Ly6g, Cxcl1, Cxcl2, Cxcl5, Ccl2, Cxcr1, Cxcr2, and Ccr2 mRNA expression via qPCR. The data shown are relative to gene expression levels. All data presented are the mean ±SEM of representative experiments from three trials. The results were analyzed using a two-tailed non-paired t-test,$^{*}P < 0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P < 0.0001$. NS, no significance ($P > 0.05$).

APAP induces a severe inflammatory response in the liver. Through immunohistochemistry, we observed that large TRAIL was concentrated in the severely damaged liver tissue of mice treated with APAP^{+}Cells (Figure 8A). These TRAILs were significantly reduced by treatment with AS1501 (designator sDR5-Fc)$^{+}$Number of cells (Figure 8B). Flow cytometry also revealed that APAP significantly increased TRAIL in the liver and spleen^{+}The number and percentage of white blood cells, and the use of AS1501 significantly reduced this effect (Figure 8C). Most TRAIL^{+}Bone marrow cells are CD11b^{+}We also observed a large number of CD11b^{+}The cells were concentrated in the severely damaged areas of the liver (Figure 8D), and AS1501 significantly reduced these CD11b^{+}Cell infiltration (Figure 8E). CD3 was observed in the liver of mice treated with APAP^{+}, CD4^{+}And CD8^{+}T cells, which suggests that most of the white blood cells infiltrating the damaged areas of the liver are not T cells.

Consistent with these results, APAP significantly upregulated the liver mRNA levels of inflammatory cell markers, and AS1501 treatment markedly attenuated this effect (Cd11b was 20.05 ± 8.94 vs. 1.14 ± 0.03, Ly6c was 11.17 ± 4.76 vs. 1.59 ± 0.22, F4/80 was 31.78 ± 5.16 vs. 17.36 ± 2.84; Figure 8F). Notably, APAP increased the levels of dendritic cell marker Cd11c (1.7 times), granulocyte marker Ly6g (2.3 times), and Nk1.1 (3.0 times). AS1501 treatment had no significant effect on Ly6g and Nk1.1 levels but reduced Cd11c levels. We observed that APAP significantly upregulated the levels of Cxcl2, Ccl2, Cxcr1, Cxcr2, and Ccr2 (Figure 8G), indicating that circulating MDSCs recruit CXCL2 and CCL2 to the liver in a CXCR-dependent manner, which AS1501 significantly normalized (Figure 8G).

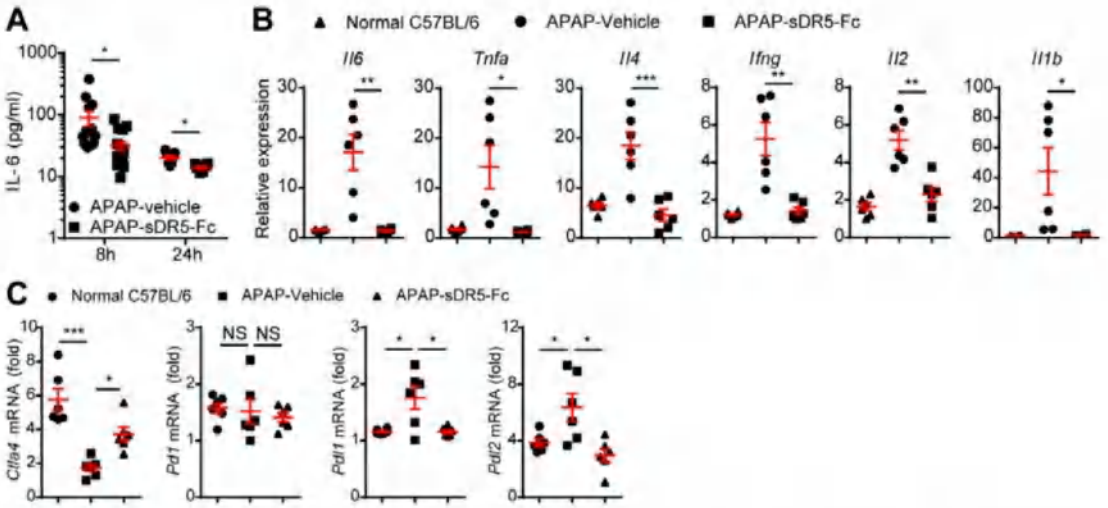


Figure 9 AS1501 Reduced APAP-induced inflammatory response

Mice were treated with APAP (300 mg/kg, intraperitoneal injection) and then given 9 mg/kg AS1501 or saline as a control group after 1 hour.

(A) Serum IL-6 was detected by cell counting bead array 8 and 24 hours after APAP injection. n=17 at 8 hours, n=6 at 24 hours.

(B, C) Quantification of liver Il6, Tnfa, Il4, Ifng, Il2, Il1b, Ctla4, Pd11, Pd12, and Pd1mRNA expression via qPCR. The data shown represent relative gene expression levels, n=6. All displayed data are averages ± SEM from representative experiments in three trials. Through a two-tailed non-paired t-test,*P<0.05，**P<0.01，***P <0.001. NS, no significant difference (P> 0.05).

Through AS1501 blocking TRAIL significantly reduced serum IL-6 levels (Figure 9A), but found no such effect on serum TNF-α, IFN-γ, IL-10, IL-17A, IL-4, or IL-2, which may be due to the low serum levels of these cytokines induced by APAP. However, we observed that compared to mice treated with AD1501 after APAP overdose and left untreated, the expression of cytokines such as Il6, Tnfa, Ifng, Il4, Il2, and Il1b in the liver of mice treated with AD1501 was significantly reduced (Figure 9B).

Programmed Death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) are important immune checkpoint receptors involved in inducing immune tolerance. We observed that APAP significantly reduced Ctla4 transcripts in the liver, which AS1501 could reverse (Figure 8C). However, APAP did not affect Pd1 mRNA levels. PD-1 has two ligands: PD-L1 and PD-L2. APAP increased the expression of Pdl1 and Pdl2 mRNA, which AS1501 could reverse. These results suggest that AS1501 attenuates the inflammatory response induced by APAP.

The results of this experiment were better combined with the in vitro test to verify the mechanism of action of AS1501. AS1501 mainly inhibits hepatocyte apoptosis by blocking TRAIL-DR5 pathway, and AS1501 can also reduce inflammatory response.

6) The role of the TRAIL-DR5 signaling pathway in viral hepatitis models

To determine the potential role of TRAIL in hepatitis induced by HBV, Professor Chen Youhais team tested the effects of HBV and its X protein (HBx) on TRAIL induced hepatocyte apoptosis in vivo and in vitro in 2007[40]The hepatitis and hepatocyte death in HBV-transgenic mice were blocked by the soluble DR5 receptor, which significantly inhibits TRAIL function. It was also found that HBV or HBx transfection of liver cancer cell lines significantly increased their sensitivity to TRAIL-induced apoptosis. The increase in TRAIL sensitivity was associated with a significant upregulation of Bax protein expression. Knocking down Bax expression using Bax-specific small interfering RNA can block HBV-induced hepatitis and hepatocyte apoptosis. Degradation of caspase 3 and 9, rather than Bid or caspase-8, is preferentially affected by Bax knockdown. These results confirm that HBV makes hepatocytes sensitive to TRAIL-induced apoptosis through Bax, and Bax-specific small interfering RNA can be used to inhibit HBV-induced hepatocyte death.

- **Establishment of HBV animal model**

Hepatitis B virus (HBV) is the smallest known DNA virus in humans, with a genome length of about 3.2kb and is partially double-stranded DNA. Additionally, it belongs to the family Deoxyribonucleic Acid of the liver, exhibiting strict hepatotropism and species specificity. It typically invades liver tissue, causing acute and chronic hepatitis, and naturally infects only humans and a few primates[4]Such characteristics limit the use of animal models for HBV research. Our company has also tried to use mice as an HBV animal model, but it was stalled due to unsuccessful gene transfer.

Table 6 HBV animal models and their advantages and disadvantages

model	merit	shortcoming	remarks
Non-human primate	Susceptible to natural HBV infection; extremely similar to human infection	Ethical constraints; high cost; large size	Similar to human genes
arbor	Small body size; direct infection with HBV	Low infection efficiency; only causes transient, mild infection; low viral load	Its close to primates in origin
Ducks, groundhogs, etc	Can be infected by HBV-related virus; primary hepatocytes are easily obtained	The size is relatively large; the immune background is unclear; HBV-related viruses are different from HBV, and the data obtained may not be applicable to HBV	Host of HBV-related virus
mouse	Small size, easy to control; cheap, easy to obtain; clear genetic background; short breeding cycle, many offspring; mouse gene modification technology and reagents are more	Mice have no HBV receptor and are not naturally infected; HBV genome transfer technology requires high requirements and complex operation; immunodeficient mice require strict feeding conditions	It is very different from human biology, and the results obtained are not completely applicable to human body

2.2.2.2. toxicologic study

2.2.2.2.1. Single-dose toxicity studies

- **AS1501 Toxicity test of SD rats given by single intravenous infusion**

In the toxicity test of SD rats given a single intravenous infusion of AS1501,40 rats (20 per sex) were randomly divided into 4 groups (5 per sex/group), namely the negative control group (sodium chloride injection, 0 mg/kg), low-dose test group (AS1501,375 mg/kg), medium-dose test group (AS1501,750 mg/kg), and high-dose test group (AS1501,1500 mg/kg). The drug was administered via tail vein using an injection pump with a volume of 15 mL/kg and a flow rate of approximately 5 mL/kg/min. Continuous observation was conducted for 4 hours after administration. During the trial period, regular clinical observations, weight checks, and food intake measurements were performed on the animals. After the observation period (Day 15), all animals were euthanized and subjected to gross autopsy examination.

The solutions of AS1501 with concentrations of 25,50, and 100 mg/mL prepared on day 1 were analyzed, with an accuracy range of 101.04% to 106.79%. The coefficient of variation (CV) for the upper, middle, and lower layers at each concentration ranged from 0.04% to 1.50%, indicating that the preparation of AS150 liquid was accurate and suitable for testing. No AS1501 was detected in the negative control group solution.

During the trial, no animals died or were near death. During the trial, no abnormal changes related to the test substance were observed in the clinical observation, weight and food intake of each animal. No abnormal changes related to the test substance were observed in the gross dissection of animals.

In conclusion, under the above experimental conditions, AS1501 was administered to SD rats by single intravenous infusion at 375,750 and 1500mg/kg. The dose without observed toxic reaction (NOAEL) was 1500mg/kg.

- **AS1501 Toxicity test of single intravenous infusion to crab-eating macaques**

In the toxicity test of AS1501 administered as a single intravenous infusion to cynomolgus monkeys, eight cynomolgus monkeys (four per sex) were randomly divided into four groups (one per sex/group): the negative control group (sodium chloride injection), the low-dose test substance group (300 mg/kg), the medium-dose test substance group (600 mg/kg), and the high-dose test substance group (1200 mg/kg). The drug was administered subcutaneously via the hind limb using an injection pump, with a dosing volume of 12 mL/kg and a flow rate of approximately 1 mL/kg/min. After dosing, all animals were observed continuously for 4 hours, during which clinical observations, body weight, temperature, ECG, blood cell count, coagulation function, biochemical blood tests, and urine analysis

were regularly conducted. At the end of the observation period (Day 16), all animals were euthanized and subjected to gross autopsy examination.

During the trial, no animals showed signs of death or near-death. During the trial, no abnormal changes related to the test substance were observed in the clinical observations, body weight, temperature, electrocardiograms, blood cell counts, coagulation function, biochemical blood tests, and urine analysis of animals in the 300,600, and 1200 mg/kg dose groups. Gross examination of the animals did not reveal any pathological changes related to the test substance.

In conclusion, under the above test conditions, AS1501 was administered to cynomolgus monkeys by single intravenous infusion of 300,600 and 1200 mg/kg, and no significant toxic reaction dose (NOAEL) was observed.

Table 7AS1501, single-dose toxicity

Species/strain	medication	Dose (mg/kg)	Sex and number per group	Maximum observed non-lethal dose (mg/kg)	Approximate lethal dose (mg/kg)	end of test
SD rat	The injection pump was used to inject the drug into the tail vein, with a dosage capacity of 15mL/kg and a dosage rate of 5mL/kg/min	0 375 750 1500	10 animals per group (5 males and 5 females)	1500	Not observed	1. During the trial, none of the animals died or were near death. 2. During the trial, no abnormal changes related to the test product were observed in the clinical observation, weight and food intake of each animal. 3. No abnormal changes related to the test items were observed in gross animal dissection.
machin	The injection pump was used to inject the drug into the tail vein, with a dosage capacity of 5mL/kg and a dosage rate of 5mL/kg/min	0 300 600 1200	Two animals per group (1 male, 1 female)	1200	Not observed	1. During the trial, none of the animals died or were near death. 2. During the trial, no abnormal changes related to the test product were observed in clinical observation, body weight, body temperature, electrocardiogram, blood cell count, coagulation function, blood

						biochemistry and urine analysis of the animals in each group. 3. No pathological changes related to the test articles were observed in gross examination of animal anatomy.

2.2.2.2.2. Repeated dose toxicity study

- **Dose exploration test: AS1501 Repeated intravenous infusion of SD rats for 4 weeks toxicity test**

See Table 10 for details.

- **Dose exploration: AS1501 Repeated intravenous infusion of crab-eating macaques for a 4-week toxicity trial**

See Table 10 for details.

- **AS1501 Toxicity tests were performed on SD rats for 13 weeks and recovery period of 4 weeks by repeated intravenous infusion**

In the toxicity test of SD rats receiving repeated intravenous administration of AS1501 for 13 weeks and a recovery period of 4 weeks, the aim was to observe potential toxic reactions and metabolic conditions in the body after SD rats received repeated intravenous infusions of AS1501, with one dose per week for 13 consecutive weeks, as well as the recovery of toxic reactions 4 weeks after the end of the dosing period. A total of 192 SD rats (96 males and females) were randomly divided into 8 groups. Groups 1, 2, 3, and 4 underwent toxicological studies (15 males and females per group), while Group 5 (6 males and females) and Groups 6, 7, and 8 (10 males and females per group) underwent pharmacokinetic studies. Animals in Groups 1 and 5 were given sodium chloride injection as a negative control, while those in Groups 2 and 6, Groups 3 and 7, and Group 48 received doses of AS150120mg/kg, 60 mg/kg, and 200 mg/kg, respectively. The animals were administered intravenously once weekly for 13 weeks, followed by a recovery period of 4 weeks. The infusion volume was 10 mL/kg, with an infusion rate of approximately 3.33 mL/kg/min. During the trial, clinical observations, body weight, food intake, temperature, ophthalmic examinations, blood cell counts, coagulation function, biochemical blood tests, urine analysis, T lymphocyte subsets, cytokines, serum complement, and serum anti-drug antibodies were all monitored. After the end of the administration period, the first 10 animals of each group 1-4 (except for the animals of group 11725377) were euthanized according to the plan on the day after the last dose (D93); Group 1-4The remaining 5 animals per sex/group were euthanized as planned at the end of the 4-week recovery

period (D120). All animals underwent gross dissection, organ weighing, and histopathological examination.

The test solutions with concentrations of 2, 6, and 20 mg/mL for D1, D5, and D92 were analyzed. The accuracy range of the prepared AS1501 solutions at each concentration was 96.00% to 105.54%, with the coefficient of variation (CV%) for the upper, middle, and lower layers ranging from 0.03% to 2.04%. This indicates that the preparation of AS1501 solutions was accurate and met the requirements. No AS1501 was detected in the negative control samples.

During this experiment, one male animal in the negative control group (animal number: 1725377) was found to have died unexpectedly on day D64. Before death, it showed mild kyphosis, pale ears and limbs. Gross examination revealed an enlarged liver and spleen. Histopathological examination showed myeloid leukemia derived from bone marrow, with metastasis observed in the liver, spleen, and lungs. Therefore, the cause of death was attributed to multi-organ failure. During the trial, no abnormal changes related to the test substance were observed in the clinical observations, body weight, food intake, temperature, ophthalmic examinations, blood cell counts, coagulation function, biochemical blood tests, urine analysis, T lymphocyte subsets, cytokines, and serum complement levels of animals in the 20,60, and 200 mg/kg dose groups.

SD rats were given 20,60 and 200mg/kg AS1501 once a week for 13 consecutive weeks. The plasma peak concentration of AS1501 (C_{max}) And plasma drug exposure were positively correlated with the dose administered. AS1501 There was no significant sex difference in the metabolic characteristics of the animal. After continuous administration, the accumulation factor (AUC_{last} , $_{13}AUC$ per time$_{last}$, For the first time) were 3.18,3.78 and 2.55, respectively. The accumulation factor (AUC) of female animals was 3.18,3.78 and 2.55, respectively $_{last}$, $_{13Again}/AUC_{last,\ for\ the\ first\ time}$) were 2.73,2.15 and 1.77, respectively. The specific toxicokinetic parameters are shown as follows:

Table 8 AS1501 Toxicokinetic parameters of repeated intravenous infusion to SD rats for 13 weeks

period	group mg/kg	sex	T_{max} h	C_{max} mg/mL	SE_C_{max} mg/mL	AUC_{last} h*mg/mL	SE_AUC_{last} h*mg /mL	AUC_{inf} h*mg/mL
D1	LDG	M	0.05	0.42	0.01	15.76	0.33	24.23
	20	F	0.05	0.40	0.02	14.82	0.41	24.35
	The medium-dose group	M	0.05	1.08	0.02	37.44	0.92	53.22
	60	F	0.05	1.07	0.04	44.82	0.82	61.22
	High-dose group	M	0.05	3.64	0.09	129.86	2.82	209.42
	200	F	0.05	3.65	0.25	142.77	1.08	235.77

D85	LDG	M	0.05	0.77	0.04	50.09	2.42	98.6
	20	F	0.05	0.63	0.03	40.39	1.04	82.4
	The medium-dose group	M	0.05	1.98	0.08	141.59	3.78	293.11
	60	F	0.05	1.74	0.08	96.15	5.07	182.08
	High-dose group	M	0.05	6.25	0.25	331.68	10.27	671.68
	200	F	0.05	5.43	0.24	252.61	11.56	492.18

During the experiment, no pathological changes related to the test products were observed in the organ weight, gross anatomy and histopathology of SD rats at the end of drug administration euthanasia and recovery period euthanasia.

In summary, AS1501 was administered to SD rats via repeated intravenous infusion at doses of 20,60, and 200 mg/kg, once weekly for 13 consecutive weeks. Under these experimental conditions, no significant toxic response was observed, with a no-observed-adverse-effect level (NOAEL) of 200 mg/kg. At this dose, the mean Cmax and AUC of AS1501 in male animals were observed on day 85_{last}The average C of AS1501 in female animals was 6.25mg/mL and 331.68h*mg/mL, respectively$_{max}$And AUC_{last}5.43mg/mL and 252.61h*mg/mL, respectively.

- **AS1501 Repeated intravenous administration of crab-eating macaques for 13 weeks and recovery period for 4 weeks**

In the toxicity test of repeated intravenous administration of AS1501 to cynomolgus monkeys for 13 weeks followed by a recovery period of 4 weeks, the possible toxic reactions and metabolic status in the body after repeated intravenous administration of AS1501 to cynomolgus monkeys, with one dose per week for 13 consecutive weeks, were evaluated. Additionally, the recovery of toxic reactions and potential delayed toxicity after 4 weeks post-administration were assessed. Forty cynomolgus monkeys (20 males/female) were randomly divided into four groups (5 males/female/group): the negative control group (sodium chloride injection), the low-dose test group (10 mg/kg), the medium-dose test group (30 mg/kg), and the high-dose test group (100 mg/kg). The animals were administered subcutaneously via the posterior limb vein, with one dose per week for 13 consecutive weeks and a recovery period of 4 weeks. During the trial, clinical observations were conducted on the animals, including body weight, food intake, temperature, ECG parameters, blood pressure, oxygen saturation, eye examination, blood cell count, coagulation function, biochemical blood tests, urine analysis, and lymphocyte subpopulations ($CD3^+$、 $CD3^+/CD4^+$、 $CD3^+/CD8^+$、 $CD4^+/CD8^+$The examination includes indicators such as ratios), cytokines (TNF-α, IFN-γ, IL-2, IL-4, IL-5, IL-6), serum complement (C3 and C4), and serum drug antibodies. The first three animals of each sex in each group

were euthanized at D93, and the remaining two animals of each sex in each group were euthanized at D120. All animals underwent gross necropsy, organ weighing, and histopathological examination.

The solutions of AS1501 prepared at concentrations of 2, 6, and 20 mg/mL on days 1, 50, and 92 were analyzed, with an accuracy range of 95.27% to 104.47%. The coefficient of variation (CV) for the upper, middle, and lower three layers at each concentration ranged from 0.02% to 1.79%, indicating that the preparation of AS1501 solutions was accurate and suitable for testing. No AS1501 was detected in the negative control group.

During the trial, no animals in groups 1 to 4 died or were near death.

During the trial, no abnormal changes related to the test product were observed in clinical observation, body weight, food intake, body temperature, electrocardiogram parameters, blood pressure, oxygen saturation, ophthalmological examination, blood cell count, coagulation function, blood biochemistry, urine analysis, lymphocyte subgroups, cytokines and serum complement in the animals of the 10,30 and 100 mg/kg dose groups.

Crab-eating macaques were given 10,30 and 100mg/kg AS1501 once a week for 13 weeks. The plasma peak concentration (C_{max}) And plasma drug exposure were positively correlated with the dose administered. AS1501 There was no significant sex difference in the metabolic characteristics of the animal body. Animals in the low, medium and high dose groups were given once a week for 12 weeks after continuous administration, and the accumulation factor ($AUC_{last,\ 12Again}/AUC_{last,\ for\ the\ first\ time}$) were 2.11,2.11 and 1.71, respectively, indicating no significant accumulation of AS1501 in animals. The specific toxicokinetic parameters are shown as follows:

Table 9 AS1501 Pharmacokinetic parameters of repeated intravenous infusion in crab-eating macaques for 13 weeks

period	group Dose (mg/kg)		C_{max} mg/mL	AUC_{last} h*mg/mL	AF
First dose	Low-dose group (n = 10) 10	Mean SD	0.21 0.04	11.38 1.12	
	Medium dose group (n = 10) 30	Mean SD	0.72 0.07	37.28 5.10	
	High-dose group (n = 10) 100	Mean SD	2.15 0.41	122.91 12.17	
13 times dose	Low-dose group (n = 10) 10	Mean SD	0.35 0.06	23.03 6.85	2.11 0.14
	Medium dose group (n = 10) 30	Mean SD	1.12 0.14	77.37 12.50	2.11 0.23
	High-dose group (n = 10) 100	Mean SD	3.12 0.39	210.94 38.63	1.71 0.24

During the trial, one animal in each of the negative control group and the test samples at doses of 30 and 100 mg/kg showed detectable anti-drug antibodies (confirmed positive) at D93, D120, and D-1, with titers all <1. One female animal in the negative control group (1725249) showed detectable anti-drug antibodies (confirmed positive) at D93 and D120, with titers of 1:4 and 1:2, respectively. One male animal in the test sample at a dose of 10 mg/kg (1725253) showed detectable anti-drug antibodies (confirmed positive) at D29, D50, and D64, with titers of <1, 1:2, and 1:32, respectively. No other animals showed detectable anti-drug antibodies during the trial.

After administration, euthanasia and recovery euthanasia, no pathological changes related to the test substance were observed in organ weight, gross anatomy observation and histopathology of group 1-4 animals.

In summary, AS1501 was administered to cynomolgus monkeys via repeated intravenous infusion at doses of 10,30, and 100 mg/kg, once a week for 13 consecutive weeks. No toxic reactions related to the test substance were observed in the animals. Under these experimental conditions, no significant toxic reaction dose (NOAEL) was observed with AS1501 administered via repeated intravenous infusion to cynomolgus monkeys at 100 mg/kg. At the 100 mg/kg dose, after the 13th administration (D85), the C_{max}And the AUC_{last}3.12mg/mL and 210.94 h*mg/mL, respectively.

Table 10 AS1501 Summary of 4-week toxicology studies with repeated dosing

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
rat,SD	venoclysis	Four weeks	The negative control group was 0mg/kg	5	yes	**1. Animal deaths and near-death situations** During the trial, no deaths or near-death conditions related to the test substance were observed in any of the animal groups. In the 20 mg/kg dose group, one male animal (animal number 16-8895) was found dead on D15 (the third dose). No abnormal clinical symptoms were observed before death, and gross necropsy revealed no abnormalities in major tissues or organs. Considering these circumstances, it is inferred that the animals death may have been due to asphyxiation caused by improper fixation during administration, which is unrelated to the test substance. **2. clinical observation** During the trial, no abnormality was observed in clinical observation of each group of animals. **3. weight** During the trial, there were no statistically significant changes in body
			The low dose group was 20mg/kg	5		
			The medium dose group was 60mg/kg	5		
			The high dose group was 200mg/kg	5		

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						weight and body weight gain in male and female animals at 20,60 and 200mg/kg doses compared with the concurrent excipient control group. **4. capacity for eating** During the trial, the food intake of the animals in the drug group was similar to that of the control group with auxiliary materials during the same period. **5. hematology** There were no statistically significant changes in blood cell counts in both male and female animals at the 20,60 and 200mg/kg doses compared to the control group. 6. **Blood biochemistry** Compared with the control group of adjuvants in the same period, there was no statistically significant change in blood biochemical indexes of male and female animals in the 20,60 and 200mg/kg dose groups. **7. Grandscopic anatomical examination** No pathological changes were observed in animals.
machin	venoclysis	Four weeks	The negative control group was 0 mg/kg	1	yes	**1. Animal deaths and near-death situations** During the trial, no animals in each group died or were dying. **2. clinical observation** duration of test, Group 1 (auxiliary material control group) One female animal was observed to have sparse fur all over the body from D-1 to D14; small amounts of soft feces were observed from D14 to D15. Group 2 (10 mg/kg): One male animal (15-8923) showed moderate yellow diarrhea on D 4, and a small amount of soft stool on D 6. One female animal (15-8924) had moderate wounds on the left forelimb from D 7 to D 30, a small amount of yellow diarrhea on D 9, a small amount of soft stool on D 19, moderate yellow diarrhea on D 20, and a small amount of brown diarrhea on D 21. No significant abnormal clinical symptoms were observed in group 3
			The low dose group was 10 mg/kg	1		
			The medium dose group was 30 mg/kg	1		
			The high dose group was 100 mg/kg	1		

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						(30mg/kg) animals. Group 4 (100mg/kg) One male animal (15-8927) had a small amount of yellow diarrhea from D-1 to D3, and a mild wound on the right forelimb from D15 to D21. In animals 15-8923, self-biting behavior was observed on days 8, 15, and 22 when administered (administered with the hind legs of restrained animals). In animal 15-8927, self-biting behavior was observed on day 15 when administered (administered with the hind legs of restrained animals), leading to forelimb wounds in both animals. Due to the limited number of animals, it is impossible to determine the correlation between this phenomenon and the test substance. The symptoms of soft stool, loose stool or sparse fur in the above groups of animals were not seen in an obvious dose-response relationship, or could be seen before the drug administration, and were considered to be unrelated to the administration. **3. weight** During the trial period, there was no significant abnormal change in the body weight of each group of animals. **4. temperature** During the trial, the body temperature of the animals in the test group fluctuated within the normal reference range, and no obvious abnormalities related to the test product were observed. **5. electrocardiogram** During the trial, there were no significant abnormalities in ECG waveform, heart rate, P-R interval, QRS time limit and QT interval of animals in the test group related to the test product. **6. hematology** During the trial, no significant difference related to the test product was found in the blood cell count of the animals in the test group. 7. **coagulation function**

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						During the trial period, no obvious abnormality related to the test product was found in the coagulation function index of the animals in the test group. **8. Blood biochemistry** During the trial, no obvious abnormalities related to the test products were found in the biochemical indexes of blood of animals in the test group. **9. URAN** During the trial, no obvious abnormalities related to the test products were found in the urine indexes of the test group animals. **10. Organ weight** During the trial, there was no obvious abnormality related to the test substance in the organ weight, visceral body ratio and visceral brain ratio of the animals in the test group. **11. A general observation** No abnormal changes were observed in the animals at the end of euthanasia.

Table 11 AS1501 Summary of repeated dosing 13-week recovery period and 4-week toxicology study

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
rat,SD	venoclysis	13 weeks	Toxicological studies		yes	**1. Animal deaths and near-death situations** During the experiment, one female animal in the negative control group (animal number: 17-25377) was found dead on day D64 of the trial. Prior to death, it showed mild kyphosis, pallor of the left ear and limbs. Gross examination revealed hepatomegaly and splenomegaly. Histopathological examination showed myeloid leukemia derived from bone marrow, with metastases observed in the liver, spleen, and lungs. Therefore, the cause of death was determined to be multi-organ failure. **2. clinical observation** During the trial, no abnormal changes related to the test product were observed in all animals. During the trial, the abnormal clinical symptoms observed in each group of
			The negative control group was 0 mg/kg	10+5		
			The low dose group was 20 mg/kg	10+5		
			The medium dose group was 60 mg/kg	10+5		
			The high dose group was 200 mg/kg	10+5		
			Toxicology studies			
			The negative control group was 0 mg/kg	4+2		
			Low dose	8+2		

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
			group 20 mg/kg			animals were scattered or sporadic. Due to the low incidence or no dose-response relationship, it was considered not related to the test substance. **3. weight** During the trial period, no abnormal changes related to the test substance were observed in the body weight and weight gain of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the weight gain of the animals, including: for the 20 mg/kg dose group, male animals showed an increase at W12 and a decrease at W14; female animals showed an increase at W12 and a decrease at W16; for the 60 mg/kg dose group, male animals showed an increase at W12 and a decrease at W16; female animals showed an increase at W1 and a decrease at W16; for the 200 mg/kg dose group, male animals showed an increase at W12 and a decrease at W11. These changes were considered unrelated to the test substance due to their small magnitude and fluctuation within the normal reference range for SD rats. 4. **capacity for eating** During the trial, there was no abnormal change in the animals food intake compared with the test product. **5. temperature** During the trial, no abnormal changes in body temperature were observed in any of the animals compared to the control group. Compared to the negative control group at the same time point, there were some slight but statistically significant differences (P≤0.05) in the animals body temperatures, including: an increase in D-1 body temperature in male animals and a decrease in female animals in the 200 mg/kg dose group. These changes occurred before the first administration and fluctuated within the normal reference range for SD rats, thus they
			Medium dose group 60 mg/kg	8+2		
			High dose group 200 mg/kg	8+2		

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						are considered unrelated to the test substance. **6. ophthalmic testing** During the trial, no abnormal changes were observed in the ophthalmological examination of animals. **7. blood count** During the trial, no abnormal changes related to the test substance were observed in the blood cell counts of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the blood cell counts of the animals, including: in the 20 mg/kg dose group for females, an increase in Mono (%) was observed at D93, and a decrease in red blood cell count (Red Blood Cell, RBC) was observed at D120; in the 60 mg/kg dose group for males, an increase in Eos (%) was observed at D93, and a decrease in RBC and an increase in MCH were observed at D120; in the 200 mg/kg dose group for females, a decrease in RBC and an increase in MCH were observed at D120. These changes, due to their small magnitude and fluctuation within the normal reference range for SD rats, are considered unrelated to the test substance. **8. coagulation function** During the trial, no abnormal changes in coagulation function were observed in all animals related to the test product. **9. Blood biochemistry** During the trial, no abnormal changes related to the test substance were observed in the biochemical blood of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the blood biochemical parameters of the animals, including: elevated Glu in female animals of the 20 mg/kg dose group on D120, and decreased urea (Urea) in female animals of the 60 mg/kg dose group on

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						D120. These changes, due to their small fluctuation and within the normal reference range for SD rats, were considered unrelated to the test substance. **10. URAN** During the trial, no abnormal changes related to the test product were observed in the animal urine analysis. **11. Lymphocyte subpopulations** During the trial, no abnormal changes related to the test product were observed in all animal lymphocyte subgroups. **12. cell factor** During the trial, no abnormal changes in cytokines were observed in the animals related to the test product. **13. SC** During the trial, no abnormal changes in cytokines were observed in animals related to the test product. **14. Drug-resistant antibodies** During the trial, no anti-AS1501 antibodies were detected in animals. **15. Toxicology analysis** After the first and 12th doses, toxic parameters of each group of animals (C_{max}reach AUC_{last}) No significant sex difference was observed. The dose ratio of animals in the low, medium and high dose groups (20,60,200mg/kg) was 1:3:10, and the C_{max}reach AUC_{last}All were positively correlated with the dose of administration. Animals in the low, medium and high dose groups of the test substance were given once a week for 12 consecutive times, and the plasma accumulation factor (AUC of male animals in each group was determined$_{last}$, 12 times /AUC_{last}For the first time), the accumulation factors were 3.18,3.78 and 2.55, respectively, and the accumulation factors for female animals were 2.73,2.15 and 1.77, respectively. **16. Organ weight** Compared to the negative control group of the same period, there were some statistically significant changes

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						in organ weight, visceral-to-body weight ratio, and visceral-to-brain weight ratio (P≤0.05) in the animals, including: in the 200 mg/kg dose group of male animals, a decrease in testicular weight and visceral-to-brain weight ratio was observed at D93. Due to the small fluctuation and no abnormal changes found in histopathological examination, this change is considered unrelated to the test substance. **17. Grandscopic anatomical observation** During the experiment, no pathological changes related to the test substance were observed in the gross examination of SD rats at the end of dosing euthanasia and recovery period euthanasia. In the high-dose group of animal 17-25465, renal lesions (renal cell carcinoma) were visible in the gross examination at the end of dosing euthanasia. In the high-dose group of animal 17-25480, renal lesions (renal cell carcinoma) were visible in the gross examination at the end of recovery period euthanasia. In the high-dose group of animal 17-25479, a breast mass (adenocarcinoma) was observed in the gross examination at the end of recovery period euthanasia. These pathological changes are considered spontaneous tumor-related lesions in SD rats and are not associated with the test substance. **18. Organize pathological examination** During this experiment, no pathological changes related to the test substance were observed in the histopathological examination of SD rats that underwent euthanasia at the end of drug administration and euthanasia at the end of recovery period. During this experiment, local injection reactions were visible in SD rats that underwent euthanasia at the end of drug administration and euthanasia at the end of recovery period, characterized by vascular degeneration and edema, thickening of the intima or entire vessel wall, vessel

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						wall necrosis or fibrosis, fibrosis of the arterial intima and/or perivascular areas, thrombosis, inflammatory cell infiltration and/or hemorrhage in the perivascular region. These pathological changes are believed to be associated with intravenous injection procedures.
machin	venoclysis	13 weeks	The negative control group was 0 mg/kg	3+2	yes	**1. Animal deaths and near-death situations** During the trial, no animals in each group died or were dying. **2. clinical observation** During the trial, no abnormal changes related to the test product were observed in all animals during clinical observation. In addition, some scattered or occasional symptoms were observed in animals during the trial, including: loose stools, sparse back hair, wounds and scabs. These symptoms were considered to be unrelated to the test product due to their mild degree and low incidence. **3. weight** During the trial period, no abnormal changes related to the test substance were observed in the body weight and weight gain of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant differences ($P \leq 0.05$) in the weight gain of the animals, including: a decrease in W9 body weight gain in female animals from the 30 mg/kg dose group, and a decrease in W9 body weight gain in female animals from the 100 mg/kg dose group. These changes, due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys, were considered unrelated to the test substance. **4. capacity for eating** During the trial, there was no abnormal change in the animals appetite. **5. temperature** During the trial, no abnormal changes in body temperature were observed in all animals related to the test

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						substance. **6. electrocardiogram** During the trial period, no abnormal changes related to the test substance were observed in the ECG parameters of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the ECG parameters of the animals, including: in the 2 mg/kg dose group of the test substance, heart rate decreased on days D1, D57, and D85, and Q-T interval increased on days D-1, D1, and D85; in the 10 mg/kg dose group of the test substance, heart rate decreased on day D1 and D57, and QRS duration shortened on day D1; in the 50 mg/kg dose group of the test substance, heart rate decreased on days D1, D57, and D85, and QRS duration shortened on day D1. These changes were considered unrelated to the test substance due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys. **7. blood pressure** During the trial, no abnormal changes in blood pressure were observed in all animals related to the test substance. **8. degree of blood oxygen saturation** During the trial, no abnormal changes in blood oxygen saturation were observed in all animals related to the test product. **9. ophthalmic testing** During the trial period, ophthalmological examination of the animals did not reveal any abnormal changes related to the test substance. In the 30 mg/kg dose group, one female animal (1725270) showed partial eyelid tissue defects at D-1, D90, and D119. Since this symptom occurred before the first administration, it was considered unrelated to the test substance. **10. blood count** During the trial period, no abnormal

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						changes related to the test substance were observed in the blood cell counts of all animals. Compared to the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the blood cell counts of the animals, including: in the male animals of the 10 mg/kg dose group, Baso (×10^9/L) and LUC (×10^9/L) increased on D2; in the female animals of the 100 mg/kg dose group, Baso (×10^9/L) decreased on D-1; on D4, hemoglobin (Haemoglobin, HGB) and hematocrit (Red blood cell specific volume, HCT) decreased; on D9, HGB, HCT, and mean corpuscular volume (Mean corpuscular volume, MCV) decreased. These changes were considered unrelated to the test substance due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys. **11. coagulation function** During the trial, no abnormal changes in coagulation function were observed in any of the animals compared to the control group. Compared to the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the coagulation function of the animals, including a decrease in APTT in male animals at the 10 mg/kg dose group on day 93. These changes, due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys, are considered unrelated to the test substance. **12. Blood biochemistry** During the trial period, no abnormal changes related to the test substance were observed in the biochemical blood of all animals. Compared with the negative control group at the same time, there were some slight but statistically significant changes (P≤0.05) in the animals biochemical blood, including: for the 10 mg/kg

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						dose group of males, D-1 showed elevated alkaline phosphatase (Alkaline Phosphatase, ALP), γ-glutamyl transferase (γ-Glutamyl-Transferase, GGT), and K+ levels; D2 showed elevated ALP and GGT levels; D44 showed elevated ALP levels. For females, D-1 showed decreased P levels. For the 30 mg/kg dose group of males, D44 showed increased Cl- levels; for females, D-1 and D2 showed decreased P levels. For the 100 mg/kg dose group of males, D-1 and D2 showed increased GGT levels; for females, D-1 and D2 showed decreased P levels. For D44 and D93, albumin (Albumin, ALB) and A/G ratios decreased. These changes, due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys, were considered unrelated to the test substance. **13. URAN** During the trial, no abnormal changes were observed in the animal urine analysis. **14. Lymphocyte subpopulations** During the trial, no abnormal changes related to the test product were observed in all animal lymphocyte subgroups. **15. cell factor** During the trial, no abnormal changes in cytokines were observed in the animals related to the test substance. In the 100 mg/kg dose group of the test substance, one male animal (1725272) showed elevated cytokines (TNF-α, IFN-γ, IL-2, IL-4, IL-5, and IL-6) at day D93. Since no similar increases were observed in the other animals of the high-dose group, this is considered an isolated event and not related to the test substance. **16. SC** During the trial period, no abnormal changes related to the test substance were observed in the animal serum complement. Compared with the negative control group at the same

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						time, some slight but statistically significant changes ($P \leq 0.05$) were noted in the animal serum complement, including: a decrease in serum complement C4 in male animals D-1 and D2 of the 10 mg/kg dose group; a decrease in serum complement C4 in male animals D-1 and D2 of the 30 mg/kg dose group; and a decrease in serum complement C4 in male animal D2 of the 100 mg/kg dose group. These changes, due to their small magnitude and fluctuation within the normal reference range for cynomolgus monkeys, are considered unrelated to the test substance. **17. Drug-resistant antibodies** During the trial, one animal in each of the negative control group and the 30 and 100 mg/kg dose groups tested positive for anti-drug antibodies (confirmed positive) at D93, D120, and D-1, respectively, with titers all <1; one female animal in the negative control group (1725249) tested positive for anti-drug antibodies at D93 and D120, with titers of 1:4 and 1:2, respectively. One male animal in the 10 mg/kg dose group (1725253) tested positive for anti-drug antibodies at D29, D50, and D64, with titers of <1, 1:2, and 1:32, respectively. No other animals tested positive for anti-drug antibodies during the trial period. Due to the low titer of the antibodies produced or their occurrence in the negative control group, they are considered to have little toxicological significance. **18. Toxicokinetics** The test substance was administered intravenously to the crab-eating macaque once a week. After the first dose, the test substance low-dose group male animal C_{max}Slightly higher than that of females ($P<0.05$, but the difference was not significant, the ratio was (male/female) 1.37), and there was no significant difference between different sexes in other groups. After

Species/strain	medication	Duration of administration	dosage	Number of animals/gender	GLP	end of test
						the 12th drug, there was no significant difference in pharmacokinetic parameters between different sexes in each group. The animal dosing ratio of low, medium and high dose groups (10,30,100mg/kg) was 1:3:10, and the average C of animals in low, medium and high dose groups after the first dose was 10_{max}The ratio and AUC_{last}The ratios were 1:3.35:10.01 and 1:3.28:10.80, respectively; the average plasma drug C of animals in the low, medium and high dose groups after the 13th $dose_{max}$The ratio and AUC_{last}The ratios were 1:3.2:9.74 and 1:3.36:9.16, respectively, indicating that the plasma peak concentration and plasma drug exposure were positively correlated with the dose of administration. Animals in the low, medium and high dose groups were given once a week for 13 weeks after continuous administration, and the accumulation factor ($AUC_{last,\ 12Again}/AUC_{last}$, For the first time) were 2.11,2.11 and 1.71 respectively. **19. Organ weight** During the trial, no abnormal changes related to the test substance were observed in the organ weight, visceral ratio and brain ratio of all animals. **20. A general observation** Euthanasia was performed on the day after administration (D93) and 4 weeks after discontinuation of administration (D120). No abnormal changes related to the test substance were observed in the gross anatomy of the animals. **21. Organize pathological examination** Euthanasia was performed on the day after administration (D93) and 4 weeks after discontinuation of drug (D120). No abnormal changes related to the test substance were observed in histopathological examination of animals.

2.2.2.2.3. Local tolerance studies

1) In vitro hemolysis test of human red blood cells with AS1501 for injection

In the in vitro hemolysis test of human red blood cells using injectable AS1501, a concentration of 10 mg/mL was used as the test sample concentration. Different volumes (0.5~0.1mL) of the test sample and different volumes (2.5~2.9mL) of 0.9% sodium chloride injection were added to a glass tube containing 2.0 mL of a 2% human red blood cell suspension. At the same time, 3.0 mL of 0.9% sodium chloride injection and 3.0 mL of sterilized water for injection were used as negative and positive controls, respectively. The total volume in each tube was 5.0 mL, and they were incubated at 37°°C for 3 hours.

On the day of the test, the concentration and uniformity of the test solution were analyzed. The theoretical concentration was 10mg/mL. The actual concentrations of the upper, middle, and lower layers of the test solution were 10.39808mg/mL, 10.39329mg/mL, 10.39508mg/mL, with accuracies of 103.98%,103.93%, and 103.95%, respectively. The coefficient of variation for the upper, middle, and lower layers was 0.02%. No test substance was detected in the negative control or positive control. The analysis results met the protocol requirements.

The negative control tube showed no hemolysis or agglutination, while the positive control tube exhibited complete hemolysis. All test tubes showed partial hemolysis without agglutination. Under these experimental conditions, an injection volume of 10mg/mL of AS1501 can cause partial hemolysis of human red blood cells in vitro, with no agglutination effect.

Although the in vitro hemolysis test of human red blood cells showed partial hemolysis of AS1501 for injection, repeated drug administration toxicity tests in SD rats and crab-eating macaques showed no abnormal changes in red blood cell count, and no abnormal changes related to the test substance were found in gross anatomy, so AS1501 was judged to be hemolytic.

2) Active whole-body allergy tests in guinea pigs with AS1501 for injection

In the active whole-body sensitization test using AS1501 for injection in guinea pigs, 34 guinea pigs (3 per group, male and female equally, 2 for supplementary experiments, males) were randomly divided into groups: negative control group, positive control group, low and high dose test substance groups, with 8 animals per group (male and female equally). The negative control group (Group 1) received sodium chloride injection; the positive control group (Group 2) received human albumin, with a sensitizing dose of 40 mg/kg and an eliciting dose of 80 mg/kg; the low (Group 3) and high dose test substance groups (Group 4) received AS1501 for injection. The sensitizing dose for the low test substance group was 1 mg/kg, and the eliciting dose was 2 mg/kg; the sensitizing dose for the high

test substance group was 3 mg/kg, and the eliciting dose was 6 mg/kg. Sensitization was administered via intraperitoneal injection, once every other day for a total of three times. Fourteen days after the last sensitization (D19), intravenous injection of the eliciting agent was performed on the first two animals per sex in each group. After the eliciting, allergic reactions were observed in the test substance group, while no abnormal reactions were seen in the 2 supplementary experiment animals. Therefore, the remaining animals in each group were elicited again on D19.

Before stimulation, no abnormal reactions were observed in the clinical observations of groups 1 to 4. After venous stimulation of the feet, none of the 8 animals in the negative control group showed signs of allergic reaction, indicating a negative result; the 8 animals in the positive control group exhibited various degrees of allergic reactions, including restlessness, piloerection, nose rubbing, coughing, rapid breathing, urination, respiratory distress, purpura, unsteady gait, convulsions, and Cheyne-Stokes respiration, with one animal dying, resulting in a weakly positive to strongly positive allergic reaction; for the low-dose group of the test substance, 8 animals exhibited varying degrees of allergic reactions, including piloerection, nose rubbing, coughing, rapid breathing, urination, respiratory distress, purpura, unsteady gait, convulsions, and Cheyne-Stokes respiration, with 5 animals dying, leading to a positive to strongly positive allergic reaction; for the high-dose group of the test substance, 8 animals exhibited various degrees of allergic reactions, including restlessness, piloerection, nose rubbing, sneezing, coughing, rapid breathing, urination, respiratory distress, purpura, unsteady gait, convulsions, and Cheyne-Stokes respiration, with 4 animals dying, resulting in a strongly positive to strongly positive allergic reaction. Under these experimental conditions, intraperitoneal injection of AS1501 at doses of 1 mg/kg and 3 mg/kg was used for sensitization three times, followed by intravenous injection at doses of 2 mg/kg and 6 mg/kg for stimulation. Fourteen days after the last sensitization, rapid-type hypersensitivity reactions were observed in guinea pigs.

The test sample AS1501 is a humanized protein, which is an allogeneic protein for guinea pigs. Since guinea pigs are animals that are prone to immediate allergic reactions and are sensitive to allergens, guinea pigs may have allergic reactions. The allergic reactions in animal experiments are not necessarily completely derived from humans.

Although the active whole-body sensitization test in guinea pigs showed allergic reactions after administration of AS1501 for injection, combined with repeated dosing toxicity tests in SD rats and cynomolgus monkeys, no abnormal changes related to the test substance were observed in gross anatomy or histopathological examination. Therefore, it is concluded that AS1501 for injection is non-sensitizing.

3) Local irritation

The formulation of AS1501 is lyophilized powder for injection, and it is administered intravenously in clinical settings. We conducted long-term toxicity tests involving repeated dosing over 13 weeks and a recovery period of 4 weeks in SD rats and cynomolgus monkeys to observe local irritation. During the rat trial, SD rats that were euthanized at the end of drug administration and the recovery period exhibited local reactions at the injection site, characterized by vascular degeneration and edema, thickening of the intima or entire vessel wall, necrosis or fibrosis of the vessel wall, fibrosis of the arterial intima and/or perivascular areas, thrombosis, inflammatory cell infiltration in the perivascular region, and/or hemorrhage. These pathological changes are believed to be related to the intravenous injection procedure. In the cynomolgus monkey trial, no local administration reactions to AS1501 were observed. The injection sites were also examined in detail through histopathology. Animals from groups 1 to 4 were euthanized on the day after dosing (D93) and 4 weeks after discontinuation of the drug (D120). Histopathological examination of the injection sites in these animals showed no abnormal changes associated with the test substance.

2.2.2.2.4. Other toxicity studies

- **immunogenicity**

In the long-term toxicity test involving repeated administration of SD rats for 13 weeks and recovery period for 4 weeks, no anti-AS1501 antibodies were detected in any animal. In the long-term toxicity test involving repeated administration of cynomolgus monkeys for 13 weeks and recovery period for 4 weeks, one animal in each of the negative control group, the 30 mg/kg and 100 mg/kg dose groups tested positive for anti-drug antibodies (confirmed positive) at D93, D120, and D-1, respectively, with titers all <1; one female animal in the negative control group (1725249) tested positive for anti-drug antibodies (confirmed positive) at D93 and D120, with titers of 1:4 and 1:2, respectively. One male animal in the 10 mg/kg dose group (1725253) tested positive for anti-drug antibodies (confirmed positive) at D29, D50, and D64, with titers of <1, 1:2, and 1:32, respectively. No anti-drug antibodies were detected in the remaining animals during the trial period. Due to the low titer of the antibodies produced or their occurrence in the negative control group, it is considered that they do not have significant toxicological significance. Moreover, AS1501 is a human protein and an exogenous protein for cynomolgus monkeys, so it cannot be extrapolated to human use.

- **immunotoxicity**

Immune toxicity in rats

Combining the long-term toxicity test of SD rats with repeated dosing for 13 weeks and a recovery period of 4 weeks, no abnormal changes related to the test substance were observed in the blood cell counts of all animals in groups 1 to 4. Compared with the negative control group at the

same time, there were no statistically significant changes in white blood cells, neutrophils, and lymphocytes in the blood cell counts of the animals. There were also no statistically significant changes in the weights of immune system organs such as thymus, spleen, and lymph compared with the negative control group at the same time. Histopathological examination of SD rats after euthanasia at the end of dosing and recovery period showed no pathological changes related to the test substance. The immune cells of SD rats, including subpopulations of lymphocytes, cytokines, and serum complement, also showed no abnormal changes related to the test substance.

During the trial, one female animal in the negative control group (animal number: 17-25377) was found dead on day D 64 of the experiment. Prior to death, it exhibited mild kyphosis, pallor of the left ear and limbs. Gross examination revealed hepatomegaly and splenomegaly. Histopathological examination showed myeloid leukemia derived from bone marrow, with metastases observed in the liver, spleen, and lungs. Therefore, the cause of death was attributed to multi-organ failure.

At the end of drug administration, euthanasia was performed on SD rats, and no pathological changes related to the test substance were observed in gross examination. In Group 4, animal No.17-25465 showed kidney lesions (renal cell carcinoma) at the end of drug administration during euthanasia. In Group 4, animal No.17-25480 showed kidney lesions (renal cell carcinoma) at the end of recovery period during euthanasia. In Group 4, animal No.17-25479 showed breast masses (adenocarcinoma) at the end of recovery period during euthanasia.

Considering that myeloid leukemia occurred in the negative control group during the experiment, rats with long toxic cycle (repeated administration for 13 weeks and recovery period for 4 weeks) were prone to tumor, and this phenomenon was not observed in the long-term toxicity of cynomolgus monkeys, it is inferred that the above pathological changes are spontaneous tumor lesions in SD rats and are not related to the test substance.

Immunotoxicity in crab-eating macaques

Combining the long-term toxicity test with repeated administration of the crab-eating macaque for 13 weeks and a recovery period of 4 weeks, no abnormal changes related to the test substance were observed in the blood cell counts of all animals. Compared to the negative control group during the same period, there were no statistically significant differences in white blood cells, neutrophils, and lymphocytes in the blood cell counts of the animals. There were also no statistically significant differences in the weights of immune system organs such as the thymus, spleen, and lymph nodes compared to the negative control group during the same period. Histopathological examination of SD rats at the end of euthanasia after drug administration and euthanasia at the end of the recovery period

showed no pathological changes related to the test substance. The immune cells of the crab-eating macaque, including subpopulations of lymphocytes, cytokines, and serum complement, showed no abnormal changes related to the test substance. On the day after drug administration (D 93) and 4 weeks after discontinuation (D 120), euthanasia was performed, and gross dissection of all animals showed no abnormal changes related to the test substance; histopathological examination of all animals also showed no abnormal changes related to the test substance, with no tumor occurrence observed.

- **Evaluation of ADCC effect in AS1501**

In the ADCC effect evaluation test of AS1501, PBMCs were used as effector cells and 293T cells transfected with TRAIL as target cells. The calcium yellow-green method was employed to detect the ADCC activity of AS1501. During the experiment, the same PBMC sample was used, with MabThera (a human-mouse chimeric antibody targeting CD20) and Daudi target cells serving as positive system controls, and anti-HA human antibody as the negative isotype control antibody (IgG1). The results showed that MabThera exhibited strong ADCC activity, showing a gradient relationship at different effector-to-target ratios. AS1501 either showed no or very weak ADCC activity. In the CD effect evaluation test of AS1501, different concentrations of AS1501 were added to 293T cells transfected with TRAIL, and the differences in killing efficiency between groups were compared under the action of 10% normal human serum complement. It is known that MabThera (a human-mouse chimeric antibody targeting CD20) has significant CDC activity, so Daudi target cells and MabThera were used as positive system controls. The results showed that MabThera significantly mediates CDC activity, achieving 100% specific killing efficiency against Daudi cells; whereas AS1501s specific killing efficiency was negative or very low.

In the AS1501 segment, theoretically, there should be ADCC and CDC effects. However, in several conducted experiments, either no or very weak ADCC and CDC effects were observed. This may be because TRAIL is a death-inducing protein, and its receptor is expressed on almost all cells. The high expression of TRAIL directly leads to cell suicide-like apoptosis, so the surface expression level of TRAIL is very low, insufficient to induce ADCC and CDC. This also suggests that ADCC and CDC may not be the mechanisms by which AS1501 functions.

2.2.2.3. Pharmacokinetic studies

2.2.2.3.1. absorb

Pharmacokinetics of a single intravenous infusion in SD rats

The pharmacokinetic characteristics of AS1501 single intravenous injection in SD rats were investigated.

Thirty-six SD rats were selected for the experiment, with an equal number of males and females. They were randomly divided into low-dose, medium-dose, and high-dose groups, with six males and females in each group. The rats were intravenously injected with AS15014.2 mg/kg, 20 mg/kg, and 42 mg/kg, respectively. Whole blood was collected at 5min before dosing, 2, 8, and 24h after dosing, and on days 2, 3, 5, 7, 11,14,21, and 28. Plasma was separated from the whole blood. The plasma drug concentration in each sample was measured using ELISA, with a detection limit of 2.5 ng/mL. The pharmacokinetic parameters of the drugs in each animal were calculated using the WinNonlin non-ventricular model (NCA).

During the trial, no abnormal clinical manifestations related to drug administration were observed.

The pharmacokinetic parameters of plasma in each group of animals are summarized as follows:

Table 12 AS1501 Pharmacokinetic parameters of SD rats after single intravenous infusion

group mg/kg		$t_{1/2}$ h	C_{max} μg/mL	AUC_{last} h·mg/mL	AUC_{inf} h·mg/mL	V mL/kg	Cl mL/h/kg	MRT h	$AUC_{(0-168h)}$ h·mg/mL
Low dose Quantity group 4.2	Mean	100.55	84.19	4.86	4.97	1520.06	0.85	145.23	4.71
	SD	53.29	8.02	0.41	0.46	59.89	0.08	21.19	0.36
Medium dose Quantity group 20	Mean	166.94	385.74	23.63	24.86	192.67	0.81	159.12	22.46
	SD	34.86	29.14	1.55	1.91	35.64	0.07	9.32	1.30
High doses Quantity group 42	Mean	141.66	767.18	46.88	48.80	174.79	0.87	158.85	44.56
	SD	35.39	57.01	3.93	4.63	39.48	0.08	6.01	3.32

The pharmacokinetic parameters of SD rats were not significantly different among genders when the same dose of test substance was administered intravenously ($p > 0.05$).

The peak concentration and drug exposure of SD rat plasma increased with the increase of drug dose. The ratio of low, medium and high dose groups was 1:4.76:10, and the average plasma C was 1_{max}The ratio was compared with the average $AUC_{(0-504h)}$ The ratios were 1:4.58:9.11,1:4.77:9.46

respectively, showing linear kinetic characteristics. The average plasma concentration-time curve of drugs in SD rats was shown in the figure below.

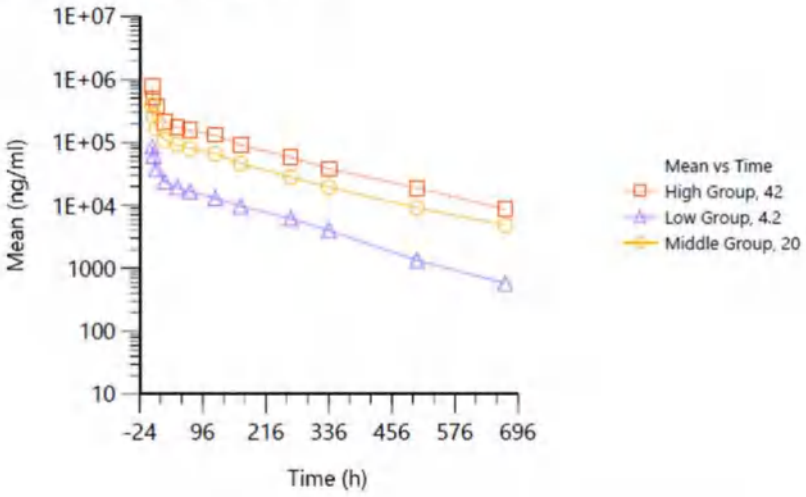


Figure 10 Average drug concentration —— time curve in plasma of SD rats

Pharmacokinetics of intravenous infusion in crab-eating macaques

In order to investigate the pharmacokinetic characteristics of AS1501 intravenous infusion, pre-experiment and formal experiment were carried out on crab-eating macaques.

In the formal pharmacokinetic study of intravenous administration to cynomolgus monkeys, 24 monkeys were selected, with an equal number of males and females. They were randomly divided into four groups: low dose, medium dose, high dose, and continuous dosing groups, receiving AS15013mg/kg, 10 mg/kg, 30 mg/kg, and 3 mg/kg (continuous dosing group, once weekly for four consecutive weeks). Blood samples were collected from Groups 1 to 3 at the following times: before administration, immediately after administration (± 1 min), 2 and 8 h after administration, 1, 2, 3, 5, 7, 10,14,17,21, and 28 d after administration. For Group 4, blood samples were collected before the first dose, immediately after administration (± 1 min), 2 and 8h after administration, 1, 2, 3, 5, and 7 d after administration; before the second and third doses, 2h after administration; and before the fourth (final) dose, immediately after administration (± 1 min), 2, 8 h after administration, 1, 2, 3, 5, 7, 10,14,17,21, and 28 d after administration. Plasma was separated from the blood samples.

No abnormal clinical manifestations were observed in all animals during the trial. The pharmacokinetic parameters of drugs in plasma of each group of animals are shown in the following table.

Table 13 AS1501 Pharmacokinetic parameters of crab-eating macaques after single and multiple intravenous infusions

group		$t_{1/2}$	C_{max}	AUC_{last}	AUC_{inf}	V	Cl	MRT	$AUC_{(0-168h)}$	AF
mg/kg		h	μg/mL	h·mg/mL	h·mg/mL	mL/kg	mL/h/kg	h	h·mg/mL	
Low dose Quantity group 3	Mean	103.45	68.62	3.05	3.05	150.97	1.02	51.70	2.89	
	SD	14.96	8.10	0.60	0.60	33.62	0.20	10.42	0.48	
Medium dose Quantity group 10	Mean	46.44	200.51	12.37	12.37	56.34	0.87	78.52	10.43	
	SD	7.28	31.27	3.40	3.41	11.05	0.29	14.94	2.33	
High doses Quantity group 30	Mean	47.25	638.72	45.35	45.39	45.30	0.67	114.65	32.87	
	SD	9.79	91.94	6.95	7.00	7.75	0.10	24.33	2.57	
Continuous series for the first time 3	Mean	34.11	60.88	2.49	2.58	57.80	1.20	42.86	60.88	
	SD	5.92	10.28	0.42	0.46	8.73	0.23	4.90	10.28	
Continuous series Last time 3	Mean	87.34	72.14	3.09	3.09	117.68	1.01	48.10	2.97	1.21
	SD	47.90	6.15	0.68	0.68	49.93	0.22	12.41	0.59	0.24

The plasma average C was 1:3.33:10 for the low, medium and high dose groups of the test samples$_{max}$The ratio was compared with the average $AUC_{(0\text{-}168\ h)}$ The ratios were 1:2.92:9.31 and 1:3.60:11.35, respectively. After administration, C_{max} - dosage, $AUC_{(0\text{-}168\ h)}$ --Dose regression analysis showed that the slope was 0.97 and 1.06 respectively, and the upper and lower limits of 90% confidence interval were 1.05,0.89 and 1.15,0.97, R^2The values were 0.98 and 0.97, respectively, indicating that the single intravenous injection of the test substance in crab-eating macaques showed linear pharmacokinetic characteristics within the dose range of 3~30 mg/kg. The average plasma drug concentration-time curve of the test substance is shown in the figure below.

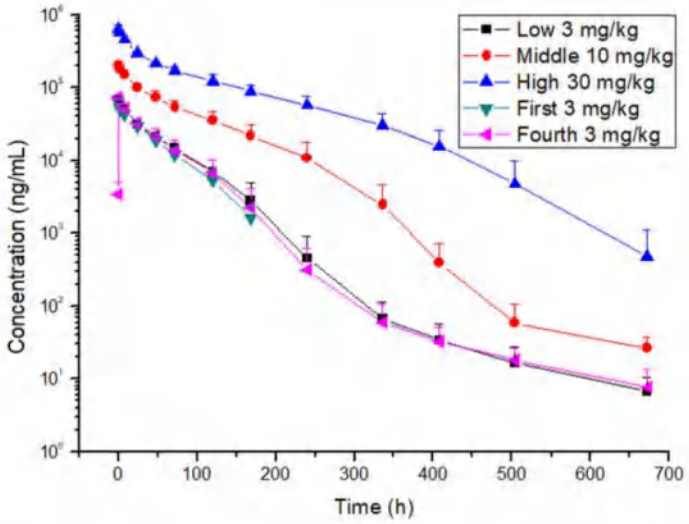


Figure 11 Average plasma drug concentration-time curve of the test sample

The test substance was administered intravenously once a week to cynomolgus monkeys (3 mg/kg) for four consecutive doses, with an average accumulation factor ($AUC_{(0\text{-}168h)\ quartic}/AUC_{(0\text{-}168h)\ for\ the\ first\ time}$) Is 1.21.

2.2.2.3.2. Distribution and excretion

In radionuclides^{125}The distribution and excretion of AS1501 intravenous injection in SD rats marked by M was determined by TCA precipitation of protein combined with SHPLC for the isotope (^{125}I) Study on the distribution of labeled AS1501 in postoperative tissues and excretion in fecal and urinary bile of SD rats.

Thirty-six SD rats were randomly divided into 6 groups, with 6 males and females in each group. The groups included: 4 tissue distribution groups (time point: 10 min, 2h,24h and 168h, dose: 4.2 mg/kg), and 2 excretion groups (bile, feces and urine, dose: 4.2mg/kg).

result display:

(1) ^{125}After intravenous administration of I-AS1501, the tissues ranked by total radiolabeled AUC from highest to lowest are (see Figure 11): plasma, thyroid, spleen, lung, kidney, gonads, heart, liver, stomach, pancreas, small intestine, muscle, fat, and brain; those ranked by precipitated radiolabeled AUC from highest to lowest are: plasma, thyroid, spleen, lung, kidney, gonads, heart, liver, pancreas, small intestine, stomach, fat, muscle, and brain. (AUC in urine is not provided).^{125}After I-AS1501 administration, it is mainly distributed in plasma, followed by spleen, lung, kidney, gonads, heart, liver, pancreas, small intestine, stomach, fat and muscle, with the least distribution in brain.

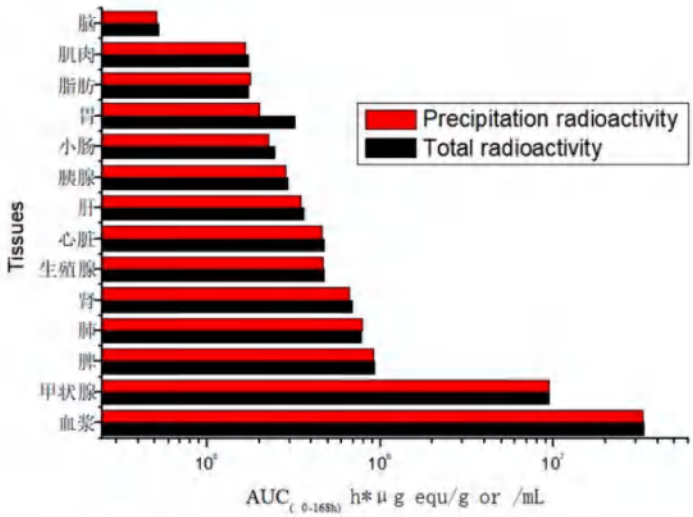


Figure 12 Distribution of drugs in tissues and plasma

(2) ^{125}After intravenous injection of I-AS1501 to rats, it was mainly excreted through urine, a small amount through feces, and little bile excretion. After 17 days, the urinary and fecal excretion of injected radioactivity was 93.65±4.94% and 3.28±0.74%, respectively. The urinary and fecal excretion were combined$^{pour\ into}$Radioactive 96.94 ± 5.37%. Administered intravenously to rats^{125}Within 8h after I-AS1501, the radioactivity of bile excretion accounted for 0.57±0.22% of the injected radioactivity.

(3) In plasma^{125}The concentration of I-AS1501 (precipitated radioactivity) reached the peak at 10min after administration and decreased with time. The precipitated radioactivity in other tissues was basically in a plateau period at 24h after administration and gradually decreased at 168h. The precipitated radioactivity in cardiac tissue increased slightly at 2h after administration compared with 10min after administration.

Radioactivity gradually increased with time after intratopic administration and reached the highest level at 168h due to iodine uptake by thyroid, so it did not represent the true distribution of 125I-AS1501 in this tissue.

2.2.2.3.3. Other pharmacokinetics

AS1501 Toxicokinetics of repeated intravenous infusion in SD rats

The experiment used 72 SD rats, with an equal number of males and females. They were divided into a negative control group and test substance low, medium, and high dose groups. The negative control substance, test substance at 20 mg/kg, 60 mg/kg, and 200 mg/kg were administered intravenously. Each rat received the drug once a week for 13 consecutive weeks. Blood samples were taken from the negative control group on D1 and D85,2 hours before and after dosing. For the low, medium, and high dose groups, blood samples were taken 3 minutes (± 1 min) after dosing on D1

and D85, and at 2, 8, 24,48,72,120, and 168h post-dosing. Plasma was separated from the blood samples.

The results show that the trends of plasma drug concentration changes were basically consistent among different sex groups of animals, with plasma drug concentrations in low, medium, and high dose groups positively correlated with the administered doses. The pharmacokinetic parameters of the drug were calculated using the WinNonLin 6.4 non-atrial model (NCA). The pharmacokinetic parameter results for each group of animals are as follows (see Table 14).

Low, medium and high dose groups of animals (20,60,200mg/kg), C_{max}reach AUC_{last}All were positively correlated with the dose of administration. Animals in the low, medium and high dose groups of the test substance were given once a week for 13 consecutive times, and the plasma accumulation factor (AUC of male animals in each group was determined$_{last,\ 13Again}$/$AUC_{last,\ for\ the\ first\ time}$) Were 3.18,3.78 and 2.55, and the accumulation factor of female animals were 2.73,2.15 and 1.77, respectively.

Table 14 AS1501 Toxicokinetic parameters of SD rats after repeated intravenous infusion for 13 weeks

Period	Group mg/kg	Sex	T_{max} h	C_{max} mg/mL	SE_C_{max} mg/mL	AUC_{last} h·mg/mL	SE_AUC_{last} h·mg/mL	AUC_{inf} h·mg/mL
D1	LDG	M	0.05	0.42	0.01	15.76	0.33	24.23
	20	F	0.05	0.4	0.02	14.82	0.41	24.35
	The medium-dose group	M	0.05	1.08	0.02	37.44	0.92	53.22
	60	F	0.05	1.07	0.04	44.82	0.82	61.22
	High-dose group	M	0.05	3.64	0.09	129.86	2.82	209.42
	200	F	0.05	3.65	0.25	142.77	1.08	235.77
D85	6	M	0.05	0.77	0.04	50.09	2.42	98.6
		F	0.05	0.63	0.03	40.39	1.04	82.4
	7	M	0.05	1.98	0.08	141.59	3.78	293.11
		F	0.05	1.74	0.08	96.15	5.07	182.08
	8	M	0.05	6.25	0.25	331.68	10.27	671.68
		F	0.05	5.43	0.24	252.61	11.56	492.18

AS1501 Toxicokinetics of repeated intravenous infusion in crab-eating macaques

The experiment used 40 cynomolgus monkeys, randomly divided into 4 groups, with 5 males and 5 females in each group. The negative control group received a sodium chloride solution; the test substance low, medium, and high dose groups were given intravenous infusions of the test substance at doses of 10 mg/kg, 30 mg/kg, and 100 mg/kg, respectively. Treatment was administered once weekly for a total of 13 weeks.

Table 15 AS1501 Pharmacokinetic parameters of repeated intravenous infusion in crab-eating macaques for 13 weeks

period	group Dose (mg/kg)		C_{max} mg/mL	AUC_{last} h·mg/mL	AF
First dose given	Low-dose group (n = 10)	Mean	0.21	11.38	
	10	SD	0.04	1.12	
	Medium dose group (n = 10)	Mean	0.72	37.28	
	30	SD	0.07	5.10	
	High-dose group (n = 10)	Mean	2.15	122.91	
	100	SD	0.41	12.17	
13 times dose	Low dose group (n = 10)	Mean	0.35	23.03	2.11
	10	SD	0.06	6.85	0.14
	Medium dose group (n = 10)	Mean	1.12	77.37	2.11
	30	SD	0.14	12.50	0.23
	High-dose group (n = 10)	Mean	3.12	210.94	1.71
	100	SD	0.39	38.63	0.24

Blood samples of approximately 1mL were drawn from the subcutaneous veins of the hind limbs on days D 1 and D 85, and plasma was separated. For the negative control group, blood samples were collected before dosing and 2h after the start of dosing. For the test group, blood samples were collected pre-dose, immediately after dosing, 0.5 h, 2 h, 8 h, 24 h, 48 h, 72 h, 120 h, and 16 8h after dosing. The AS1501 pharmacokinetic parameters were calculated using the WinnonLin 6.4 non-atrial model (NCA) (see Table 10).

The ratio of animal dosing dose of low, medium and high dose groups (10,30,100mg/kg) was 1:3:10. After the first dose, the average plasma drug C of animals in low, medium and high dose groups was tested$_{max}$The ratio and AUC_{last}The ratios were 1:3.35:10.01,1:3.28:10.80, respectively; the average C of animals in low, medium and high dose groups after 13 doses$_{max}$The ratio and AUC_{last}The ratios were 1:3.2:8.91 and 1:3.36:9.16, respectively, indicating that the plasma peak concentration and plasma drug exposure were positively correlated with the dose of administration.

Animals in the low, medium and high dose groups were given once a week for 4 weeks, and the accumulation factor (AUC was accumulated$_{last,\ Last}$/AUC$_{last,\ for\ the\ first\ time}$) Are 2.11,2.11 and 1.71 respectively.

2.2.2.4. Other in vitro tests

2.2.2.4.1. In vitro receptor occupancy test

The test steps are as follows:

(1) PBMC preparation: Take a frozen PBMC sample (source: whole blood from healthy volunteers), place it in a 37℃ water bath to rapidly thaw, then transfer the cell suspension to a 15ml centrifuge tube. Add 2ml of complete medium (RPMI-1640 + 10% FBS), gently pipette to mix well, and centrifuge at 1500rpm at room temperature for 5min. Then resuspend the cells in RPMI-1640 and count them, adjusting the cell density to 10x10^6^/ml;

(2) Take 12 clean 5ml flow tubes and number them, where tubes numbered 1 to 10 contain the test samples at concentrations of 1000 g/ml, 500 g/ml, 200 g/ml, 100 g/ml, 50 g/ml, 25 g/ml, 12.5g/ml, 6.25 g/ml, 3.125 g/ml, and 1.56 g/ml AS1501, respectively. Tube 11 is the negative control with physiological saline, and tube 12 is the flow antibody isotype control. Add the cell suspension, AS1501 test sample, physiological saline, and RPMI-1640 medium in sequence as shown in the table below, gently mix by pipetting, and incubate at room temperature for 30 min;μμμμμμμμμμ

Group number / Excipient or preparation	Test sample A		Test sample B				Test sample C				negative control group	Test sample B
	1	2	3	4	5	6	7	8	9	10	11	12
cell suspension (l)μ	50	50	50	50	50	50	50	50	50	50	50	50
A S1501 Test samples (l)μ	10	5	20	10	5	2.5	10	5	2.5	1.25	-	20
normal saline (l)μ	10	15	-	10	15	17.5	10	15	17.5	18.75	20	-
RPMI-1640	30	30	30	30	30	30	30	30	30	30	30	30

(3) After incubation, centrifuge at 1500 rpm at room temperature for 5 minutes to collect the cells. Wash the cells twice with FACS buffer (PBS + 1% BSA + 0.5 mM EDTA). Then add 100l FACS buffer of diluted Alexa Fluor 647 anti-human IgG Fc antibody (diluted 1:200) to tubes 1-11, and add an equal volume of diluted Alexa Fluor 647 Mouse IgG1 isotype control antibody to tube 12. Gently mix by pipetting, and incubate at room temperature for 30 minutes;μκ

(4) After the flow antibody staining is complete, wash the cells twice with FACS buffer. Centrifuge at 1500rpm for 5 min, discard the supernatant, and then add 100 l diluted PI dye (97l PBS

+ 3 l PI) to each tube. Incubate at room temperature for 1 5 min to remove dead cells. After incubation, resuspend the cells in 100 l FACS buffer, and immediately analyze the TRAIL levels occupied by AS1501 in the live cells of each group using a flow cytometer (hIgG Fcμμμμ^{+}The proportion of cells in living cells) and the total level of TRAIL on the cell surface (saturated concentration group hIgG Fc^{+}The proportion of cells in living cells), and calculate the receptor occupancy.

Receptor occupancy formula:

Receptor occupancy (%) = (hIgG Fc^{+}_{t}- hIgG Fc^{+}_{nc}）/（ hIgG Fc^{+}_{o}- hIgG Fc^{+}_{nc}） x 100%

hIgG Fc^{+}_{t}: Different concentrations AS1501 test group hIgG Fc^{+}The proportion of cells in living cells;

hIgG Fc^{+}_{nc}: Normal saline control group hIgG Fc^{+}The proportion of cells in living cells;

hIgG Fc^{+}_{o}: Saturated concentration group hIgG Fc^{+}The proportion of cells in a living cell.

Experimental results: the combination of different concentrations of AS1501 with PBMCs

In this experiment, compared to the physiological saline control group, as the concentration of AS1501 in the incubation increased, the proportion of PBMCs bound to AS1501 also increased. When the concentration was greater than or equal to 200g/ml, this proportion no longer increased (Figure 13). This indicates that at this point, the AS1501 bound to TRAIL on the cell surface has reached saturation, meaning that the TRAIL on the immune cell surface has been completely neutralized by AS1501.μ

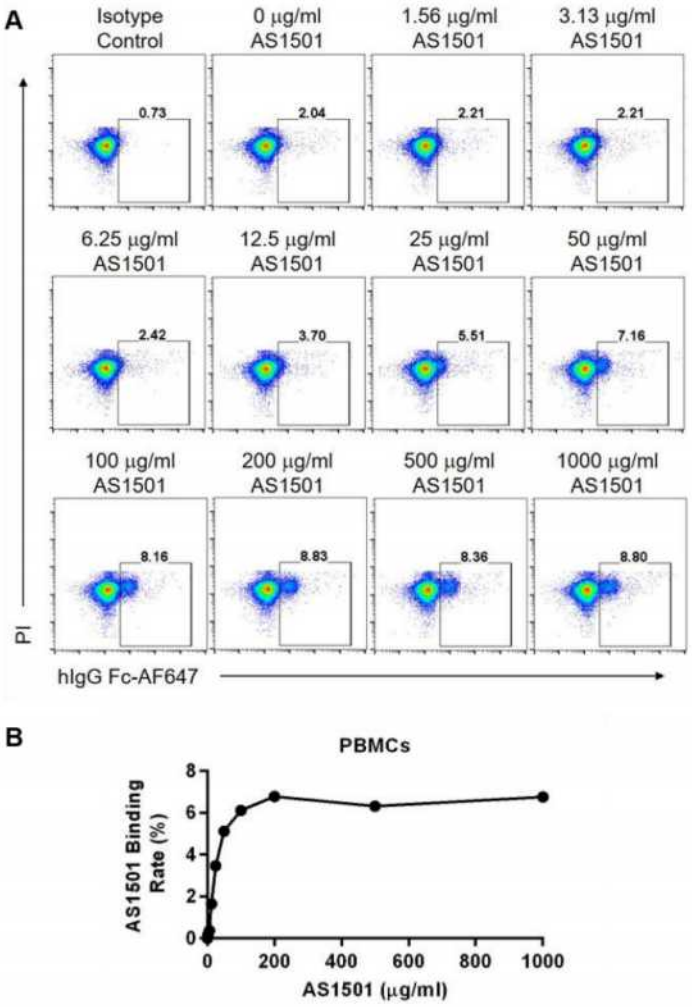


Figure 13 shows the binding of AS1501 and PBMCs at different concentrations

(A) Representative flow point diagram; (B) Statistical analysis diagram.

Results: The occupancy of TRAIL on PBMCs by different concentrations of AS1501 samples

The receptor level measured at saturation concentration of 200g/ml AS1501 was taken as the total TRAIL level on the surface of PBMCs. According to the formula, the receptor occupancy of AS1501 samples between 6.25g/ml-200g/ml was calculated as shown in Figure 14.μμμ

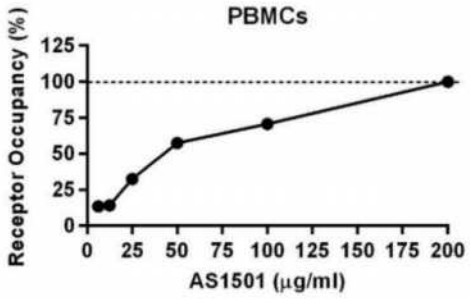


Figure 14 Occupancy of TRAIL on PBMCs surface by different concentrations of AS1501

2.2.2.4.2. In vitro stimulation of human PBMC release of cytokines (IL-2, IFN- γ)

The test steps are as follows:

(1) Preparation of PBMCs: Take two vials of cryopreserved PBMCs (source: whole blood from healthy volunteers), place them in a 37℃ water bath to rapidly thaw, then transfer the cell suspension to a 15ml centrifuge tube. Add 13mlRPMI-1640 medium, gently pipette to mix well, and centrifuge at 1500rpm at room temperature for 10min; discard the supernatant, wash the cells once with 14mlRPMI-1640 medium, resuspend the cells in RPMI-1640, count the cells, and adjust the cell density to 5×10^{6}/ml;

(2) After thoroughly mixing PBMCs, 96-well flat plate was laid down and 1×10 was added to each well^{6}Cells, i.e., a 200 μl cell suspension. After placing the PBMCs in a 37℃ incubator for 1h of rest, they were stimulated with 0 g/ml AS1501,15 μ g/ml AS1501,30 g/ml AS1501,60 g/ml AS1501,120 g/ml AS1501, and 240 g/ml AS1501, as well as 0.5 g/mlanti-human CD3e antibody for PBMC 0.5 h, 6 h, or 24 h.μ μ μ μ μ μ

group / Excipient or preparation	0μg/ml	15 μ g/ml	30 μ g/ml	60 μ g/ml	120 μ g/ml	240 μ g/ml	0.5 μg/ml anti-human CD3e
cell suspension (μ l)	200	200	200	200	200	200	200
AS1501 Test sample A (μl)	0	0.3	0.60	1.21	2.43	4.92	0
normal saline (μ l)	4.92	0	0	0	0	0	0
Anti-human CD3e （μl）	0	0	0	0	0	0	0.1

(3) After incubation, centrifuge at 400g and room temperature for 5 min. Collect the supernatant into a clean centrifuge tube and freeze it in-80℃ refrigerator.

(4) The content of IL-2 and IFN- γ in the culture supernatant was detected.

Results: Different concentrations of AS1501 treatment did not cause significant release of IL-2 and IFN- γ in PBMC

At different concentrations of AS1501, no IL-2 was detected in the supernatant after PBMC 0.5h,6h or 24h, while as a positive control, only after 24h of 0.5 μ g/ml anti-human CD3e treatment, high concentration of IL-2 (109pg/ml) could be detected in the supernatant.(Figure 15). Similarly,

different concentrations of AS1501 stimulated human PBMC 0 for 5 h or 6h in vitro, with extremely low IFN-γ levels (<20 pg/ml) in the supernatant. In the AS1501 24h treatment group, only at high concentrations (120 μg/ml and 240 μg/ml) was a relatively significant IFN-γ (over 20 pg/ml) detected in the supernatant. As a positive control, at 0.5 μg/ml anti-human CD3e for 0.5h and 6 h, the IFN-γ level in the supernatant was extremely low, while at 24 h, it reached 438 pg/ml. In summary, AS1501 treatment of PBMC does not cause significant release of IL-2 and IFN-γ.

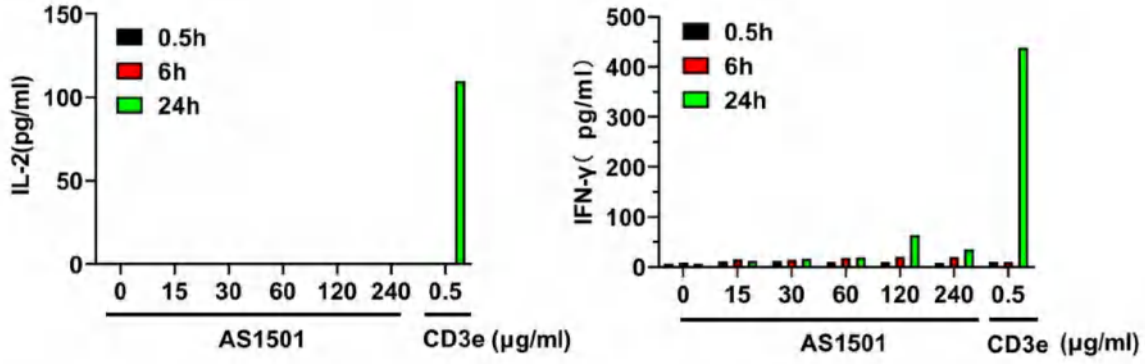


Figure 15 AS1501 Detection of IL-2 and IFN-γ release from human peripheral blood mononuclear cells induced by treatment

ELISA was used to detect the content of IL-2 and IFN-γ released into the supernatant after human PBMC was treated with AS1501 in vitro. 0.5 μg/ml anti-human CD3e was treated for the same time as a positive control group.

2.2.2.4.3. In vitro stimulation of human PBMC cytokine (IL-6) release detection experiment

The test steps are as follows:

(1) Preparation of PBMCs: Take a vial of cryopreserved PBMCs (source: whole blood from healthy volunteers) and place it in a 37℃ water bath to rapidly thaw. Transfer the cell suspension to a 15ml centrifuge tube, add 10ml RPMI-1640 medium, and gently resuspend by pipetting. Centrifuge at 1500 rpm at room temperature for 10 minutes; discard the supernatant, wash the cells once with 10ml RPMI-1640 medium, resuspend the cells in RPMI-1640, and count them. Adjust the cell density to 10 $\times 10^6$/ml;

(2) After thoroughly mixing PBMCs, 96-well U base plate was laid out, and 1×10 was added to each well^{6}Cells, i.e., a 100 μl cell suspension. PBMCs were placed in a 37° C incubator for 1h of rest, then stimulated with 0 g/ml AS1501,30 g/ml AS1501,60 g/ml AS1501,120 g/ml AS1501, and 240 g/ml AS1501, as well as 0.5 g/ml anti-human CD3e antibody for PBMC 0.5 h, 6 h, or 24 h, respectively.μμμμμμ

group / Excipient or preparation	0μg/ml	30μg/ml	60 μg/ml	120 μg/ml	240μg/ml	0.5 μg/ml anti-human CD3e
cell suspension (μl)	100	100	100	100	100	100
AS1501 Test sample A (μl)	0	0.30	0.60	1.21	2.46	0
normal saline (μl)	2.46	0	0	0	0	0
Anti-human CD3e (μl)	0	0	0	0	0	0.05

(3) After incubation, centrifuge at 400g at room temperature for 5 min, collect the supernatant into a clean centrifuge tube and freeze it in-80℃ refrigerator.

(4) The content of IL-6 in the culture supernatant was detected.

Results: AS1501 treatment of PBMC can cause significant IL-6 release

As shown in Figure 16, human PBMC does not release IL-6 when treated with different concentrations of AS1501 for 0.5 h. However, at 30 μg/ml AS1501 treatment for 6 h, human PBMC begins to release IL-6. At 60 μg/ml AS1501, the release of IL-6 increases rapidly, and at 120 μg/ml AS1501, PBMC releases the most IL-6. After 24h of AS1501 treatment, a large amount of IL-6 can also be detected in the supernatant. Notably, stimulation with 30 μg/ml AS1501 for 24h results in detectable IL-6 levels of 317 pg/ml in the supernatant. Higher concentrations of AS1501 also stimulate significant IL-6 release (>200 pg/ml) after 24 h. As a positive control, 0.5 μg/ml anti-human CD3e treatment for 6h results in about 100 pg/ml of IL-6 released by PBMC, and after 24 h, the IL-6 content in the supernatant is approximately 550 pg/ml. In summary, the experimental results indicate that AS1501 can bind to receptors on PBMC, causing the release of pro-inflammatory cytokine IL-6, and this effect is observed even at low concentrations (30 μg/ml).

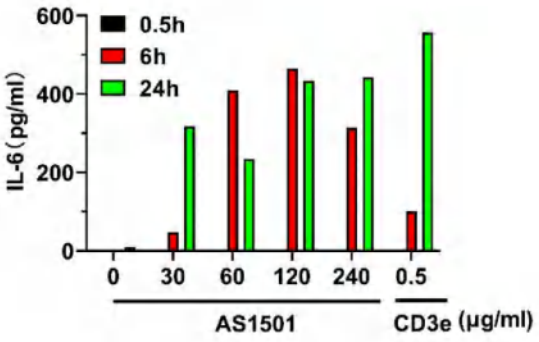


Figure 16 AS1501 Detection of IL-6 release from human peripheral blood mononuclear cells induced by processing

ELISA was used to detect the content of IL-6 released into the supernatant by human PBMC after AS1501 treatment in vitro, and 0.5μg/ml anti-human CD3e treated for the same time as positive control.

2.2.2.4.4. In vitro stimulation of human PBMC cytokine (TNF- α) release and apoptosis detection

The test steps are as follows:

(1) PBMC preparation: Take a frozen PBMC sample (source: whole blood from healthy volunteers) and place it in a 37℃ water bath to rapidly thaw. Transfer the cell suspension to a 15ml centrifuge tube, add 10ml RPMI-1640 medium, gently pipette to mix, and centrifuge at 1500rpm at room temperature for 10min. Discard the supernatant, wash the cells once with 10ml RPMI-1640 medium, resuspend the cells in RPMI-1640, and count the cells. Adjust the cell density to 10×10^{6}/ml;

(2) After thoroughly mixing PBMCs, 96-well U base plate was laid out, and 1x10 was added to each well^{6}Cells, i.e., a 100 μl cell suspension. PBMCs were placed in a 37℃ incubator for rest for 1 h, and then stimulated with 0 g/ml AS1501,30 g/ml AS1501,60 g/ml AS1501,120 g/ml AS1501, and 240 g/ml AS1501, as well as 0.5 g/ml anti-human CD3e antibody, for PBMC 0.5 h, 6 h, or 24 h.μμμμμμ

group / Excipient or preparation	0μg/ml	30μg/ml	60 μg/ml	120 μg/ml	240μg/ml	0.5 μg/ml anti-human CD3e
cell suspension (μl)	100	100	100	100	100	100
AS1501 Test sample A (μl)	0	0.30	0.60	1.21	2.46	0
normal saline (μl)	2.46	0	0	0	0	0
Anti-human CD3e （μl）	0	0	0	0	0	0.05

(3) Incubation is complete. Centrifuge at 400g and room temperature for 5 minutes, then transfer the supernatant to a clean centrifuge tube and store at-80℃C. Collect the cells and wash twice with pre-cooled 1x PBS, at 500x g and 4℃C for 5 minutes, discarding the supernatant. Resuspend the cells in 100 μl of pre-cooled 1x Annexin V Binding Buffer, adding 5 μ l Annexin V and 5 μ l PI per sample, gently mix, and incubate at room temperature in the dark for 15 minutes. After incubation, add 400 μl of pre-cooled 1x Annexin V Binding Buffer to each sample, gently mix, and place on ice. Perform flow cytometry detection within 1 hour.

(4) The content of TNF-α in the culture supernatant was detected.

Results: Flow detection results showed that AS1501 did not cause significant apoptosis of PBMC

In this experiment, different concentrations of AS1501 did not induce significant cell death in vitro at 2.5h,6h or 24h, while as a positive control, 0.5 μg/ml anti-human CD3e treatment could induce significant apoptosis (Figure 17 A).

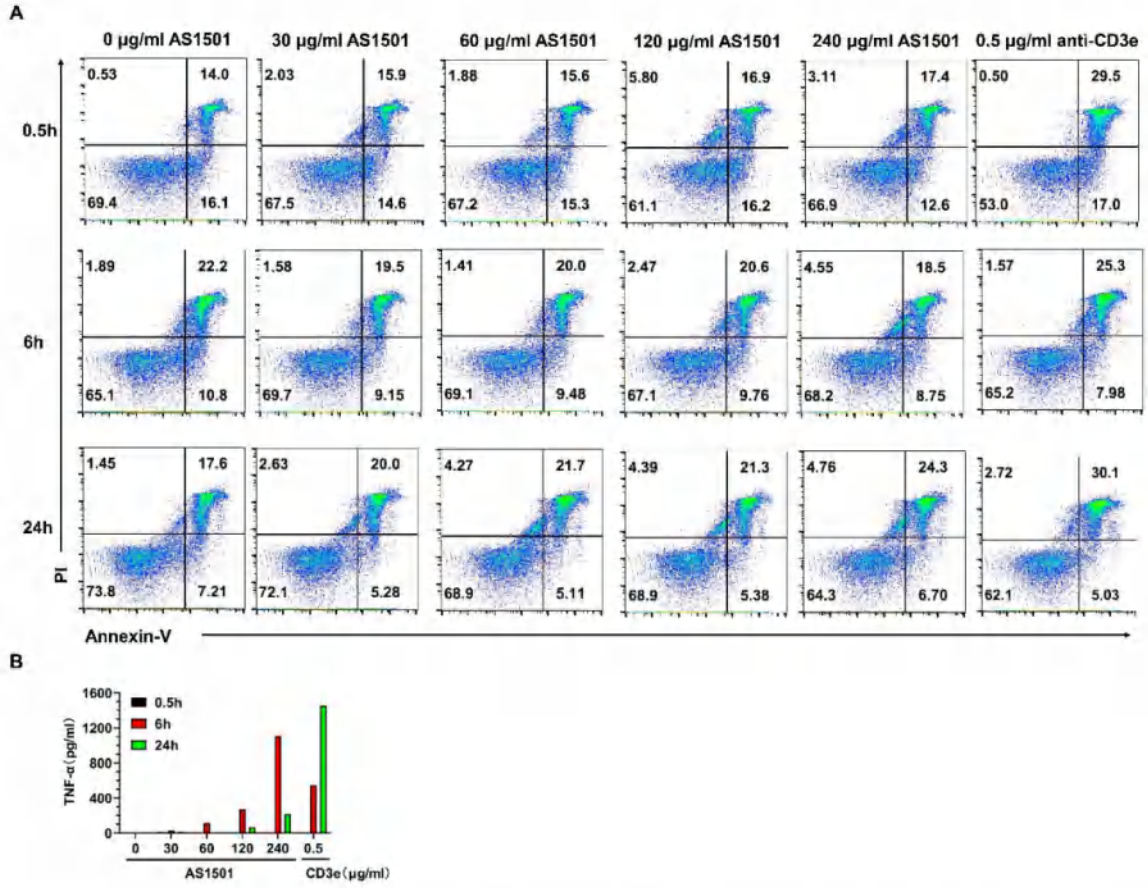


Figure 17 AS1501 Detection of apoptosis and TNF-α release in human peripheral blood mononuclear cells induced by processing

(A) Flow cytometry was used to detect apoptosis in human PBMC after AS1501 treatment, with anti-human CD3e as the positive control; (B) ELISA was used to measure the TNF-α content released into the supernatant from human PBMC after AS1501 treatment in vitro, with 0.5 μg/ml anti-human CD3e treated for the same time as the positive control.

2.2.2.4.5. In vitro stimulation of human PBMC cytokine (IL-10) release detection experiment

The test steps are as follows:

(1) Preparation of PBMCs: Take a vial of cryopreserved PBMCs and place it in a 37℃ water bath to rapidly thaw. Transfer the cell suspension to a 15 ml centrifuge tube, add 10ml RPMI-1640 medium,

gently pipette to mix, and centrifuge at 1500rpm at room temperature for 10 min. Discard the supernatant, wash the cells once with 10ml RPMI-1640 medium, resuspend the cells in RPMI-1640, count the cells, and adjust the cell density to 5×10^6/ml;

(2) After thoroughly mixing the PBMCs, 96-well flat plate was laid out, and 5×10 was added to each well^{5}Cells, i.e., a 100 μl cell suspension. After placing the PBMCs in a 37° C incubator for 1h of rest, they were stimulated with 0 g/ml AS1501,30 g/ml AS1501,60 g/ml AS1501,120 g/ml AS1501, and 240 g/ml AS1501, as well as 0.5 g/ml anti to human CD3e antibody, for 5, 6, or 24 hours.μμμμμμ

group / Excipient or preparation	0μg/ml	30μg/ml	60 μg/ml	120 μg/ml	240μg/ml	0.5 μg/ml anti-human CD3e
cell suspension (μl)	100	100	100	100	100	100
AS1501 Test sample A (μl)	0	0.30	0.60	1.21	2.46	0
normal saline (μl)	2.46	0	0	0	0	0
Anti-human CD3e (μl)	0	0	0	0	0	0.05

(3) After incubation, centrifuge at room temperature for 5min at 400g, collect the supernatant into clean centrifuge tube, and freeze it in-80℃ refrigerator.

(4) Detection of IL-10 content in the supernatant after culture.

Results: AS1501 treatment of PBMC did not cause IL-10 release

As shown in Figure 18, no IL-10 was detected in the supernatant after 0.5 h, 6 h, or 24h of treatment with different concentrations of AS1501. As a positive control, the release of IL-10 from PBMC was also minimal at 0.5 μ g/ml of anti-human CD3e treatment for 24 h, approximately 1.7 pg/ml. In summary, the experimental results indicate that AS1501 stimulation of PBMC in vitro does not lead to the release of IL-10.

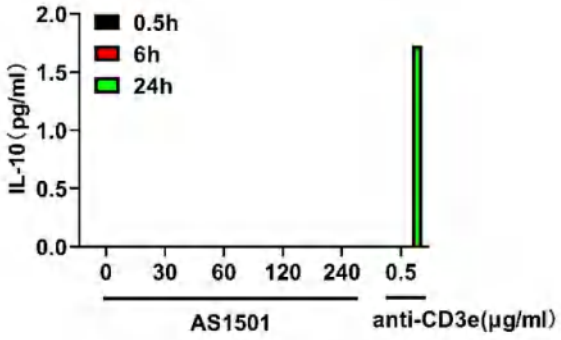


Figure 18. Detection of IL-10 release from human peripheral blood mononuclear cells induced by AS1501 treatment

The content of IL-10 released into the supernatant of human PBMC after AS1501 treatment in vitro was detected by ELISA, and the positive control was 0.5μg/ml anti-human CD3e treatment for the same time.

2.2.3. An overview of the studies conducted on the drug under study

A single-center, randomized, double-blind, placebo-controlled, single-dose, dose-escalation study of an injectable AS1501 in healthy subjects has been initiated, screening a total of 314 healthy adult participants. Thirty-two participants were randomly assigned, with 28 receiving the investigational drug and 27 completing the trial as required by the protocol; 5 withdrew early (all deemed unsuitable for continued study drug by the investigators). Eight dose groups were set up, ranging from 1mg/kg, 2mg/kg, 3mg/kg, 4.5mg/kg to 6mg/kg, 8mg/kg, 10mg/kg, and 12mg/kg. The actual single-dose dose escalation trial evaluated the incremental effects of doses ranging from 1 to 3mg/kg.

Adverse Events: Among the 28 participants who enrolled and received the study medication, 82.14% (23 cases, 76 instances) experienced AE, with most being Grade 1-2; the incidence of Grade 2 or higher AE was 28.57% (8 cases, 15 instances, involving decreased lymphocyte count); the incidence of Grade 3 or higher AE was 25.00% (7 cases, 8 instances, involving decreased lymphocyte count); 3.57% (1 case, 1 instance) of participants experienced AE leading to withdrawal (cytokine release syndrome, likely related to the study drug, resulting in discontinuation of medication); 7.14% of participants (2 cases, 2 instances) experienced SAEs before adjusting the infusion rate (infusion-related hypersensitivity reaction, Grade 3, likely related to the study drug), which were assessed as SUSARs, and no further serious AE occurred after slowing the infusion rate or dexamethasone pre-treatment; no Grade 4 or higher AE occurred. AE rates by dose group were: 100% (7 cases, 12 instances) for the placebo group, 50% (3 cases, 5 instances) for the 1 mg/kg group, 83.33% (5 cases, 16 instances) for the 2 mg/kg group, and 88.89% (8 cases, 43 instances) for the 3 mg/kg group. There was a preliminary trend toward dose-related AE rates in the single-dose escalation study, with higher adverse reaction rates in the placebo group. At least 2 of the subjects had AE mainly abnormal blood

routine indicators, mostly grade 1, including: elevated neutrophil count (32.14%), Decreased lymphocyte count (21.43%), increased interleukins (IL-6, IL-10) (14.29%), as well as headache (10.71%), dizziness (7.14%), chest discomfort (7.14%), hypersensitivity reaction (7.14%), cytokine release syndrome (7.14%), and rash (7.14%); Grade 2-3 AE include: Grade 3 hypersensitivity reaction (2 cases, 2 episodes), Grade 2 cytokine release syndrome (2 cases, 2 episodes), Grade 2 decreased lymphocyte count (3 cases, 3 episodes), all recovering quickly and completely. AE incidence by dose group in the placebo group, 1 mg/kg, 2 mg/kg, and 3 mg/kg dose groups were: 100% (7 cases, 12 episodes), 50% (3 cases, 5 episodes), 83.33% (5 cases, 16 episodes), and 88.89% (8 cases, 43 episodes), respectively. There was an initial trend of dose-related AE incidence in the single-dose escalation study, with higher adverse reaction rates in the placebo group. AEs are summarized according to system organ classification and preferred terminology as follows: 1) Common adverse events (incidence ⩾20%) in each dose group according to preferred terminology are listed as follows: 1 mg/kg dose group: all were single events, incidence <20%; 2 mg/kg dose group: decreased lymphocyte count (33.33%), oral mucositis (33.33%), hypersensitivity reaction (33.33%), rash (33.33%); 3 mg/kg dose group: decreased lymphocyte count (77.78%), neutrophil countIncreased blood glucose (77.78%), elevated interleukin levels (44.44%), headache (33.33%), increased triglycerides (22.22%), elevated monocyte count (22.22%), and cytokine release syndrome (22.22%); in the placebo group: decreased lymphocyte count (28.57%) and elevated interleukin levels (28.57%). 2) Common adverse events (incidence ⩾20%) by system organ classification for each dose group are summarized as follows: 1 mg/kg dose group: systemic diseases and various reactions at the site of administration (33.33%); 2 mg/kg dose group: various examinations (66.67%), gastrointestinal diseases (33.33%), immune system diseases (33.33%), skin and subcutaneous tissue diseases (33.33%); 3 mg/kg dose group: various examinations (88.89%), various neurological diseases (44.44%), gastrointestinal diseases (33.33%), immune system diseases (22.22%), respiratory, thoracic, and mediastinal diseases (22.22%); placebo group: various examinations (71.43%).

Adverse Reactions: Among the 28 participants who entered the study and received the investigational drug, 60.71% experienced adverse reactions (17 cases, 42 occurrences), mostly grade 1-2; the incidence of grade 2 or higher adverse reactions was 17.86% (5 cases, 8 occurrences); the incidence of grade 3 or higher adverse reactions was 10.71% (3 cases, 3 occurrences). By group, the incidence of adverse reactions in the placebo group, 1 mg/kg, 2 mg/kg, and 3 mg/kg dose groups were: 57.14% (4 cases, 6 occurrences), 33.33% (2 cases, 3 occurrences), 50.00% (3 cases, 10 occurrences), and 88.89% (8 cases, 23 occurrences). This indicates a preliminary trend of dose-related adverse reaction rates in single-dose escalation studies, with higher rates observed in the placebo group. At

least 2 cases of adverse reactions were mainly characterized by abnormal blood routine indicators, including elevated neutrophil count (32.14%), decreased lymphocyte count (21.43%), and increased interleukins (IL-6, IL-10) (14.29%). Additionally, these included headache (10.71%), dizziness (7.14%), chest discomfort (7.14%), hypersensitivity reactions (7.14%), cytokine release syndrome (7.14%), and rash (7.14%), with most being of grade 1 severity; grade 2-3 adverse reactions were: 2 cases of grade 3 hypersensitivity reactions, 2 cases of grade 2 cytokine release syndrome, and 3 cases of grade 2 decreased lymphocyte count. All these adverse reactions occurred relatively quicklyfull recovery.

Special attention to adverse events: 1) Hypersensitivity reactions: In the early stage of this trial, the total infusion time was 30 minutes, during which 2 cases of "hypersensitivity reaction" severe adverse events occurred in the 2 mg/kg dose group, with a CTCAE5.0 grade of 3. The researchers determined that these were likely related to the trial drug. After receiving both pharmacological and non-pharmacological treatments, all patients fully recovered. In subsequent trials, the total infusion time was adjusted to 2 hours, and some participants in the 3 mg/kg dose group received dexamethasone (5-10 mg) and antiallergic drugs (oral diphenhydramine) as pre-treatment. No further hypersensitivity reactions or serious adverse events occurred; 2) Cytokine release syndrome: In this trial, 2 cases of cytokine release syndrome occurred in the 3 mg/kg dose group, with a CTCAE5.0 grade of 2. The researchers determined that these were likely related to the trial drug. One participant (who did not receive pre-treatment) led to discontinuation of medication, and after receiving both pharmacological and non-pharmacological treatments, they fully recovered. Another participant (who received pre-treatment) completed the intravenous infusion of the study drug and recovered spontaneously.

The blood TRAIL (the protein targeted by this drug) concentrations in the first three dose groups showed a significant inhibitory trend after administration, and the clearance rates in the second and third groups reached 100%. The drug exposure increased significantly with the dose (1-3 mg/kg). Therefore, after discussion between the sponsor and researchers, combined with safety data from the trial, it was determined that single-dose administration at 1 mg/kg to 3 mg/kg was safe and well-tolerated. The dose escalation trials for subsequent dose groups (>3 mg/kg) were discontinued.

2.2.4. Overview of similar drug studies

There is a significant unmet clinical need for the treatment of recurrent and refractory advanced solid tumors and lymphomas. Cancer treatment strategies aim to overcome two key characteristics of cancer: excessive proliferation and anti-apoptosis. Inducing apoptosis has the potential to eliminate cancer cells, thereby offering a chance for curing cancer. The TRAIL-DR pathway plays a crucial role in the intrinsic apoptosis pathway; therefore, drug development targeting the TRAIL-DR pathway

primarily focuses on recurrent and refractory advanced solid tumors and lymphomas with excessive apoptosis resistance. Currently, there are no specific inhibitors targeting TRAIL-DR5 to inhibit the apoptosis pathway. Targeted drugs currently under clinical trial include three categories: recombinant forms of TRAIL (such as Dulanermin), agonist antibodies specifically targeting TRAIL-DR4 (mapatumumab), and agonist antibodies specifically targeting TRAIL-DR5 (Conatumumab [AMG-655]). These drugs have all undergone multiple clinical trials in recurrent and refractory advanced solid tumors and lymphomas, with generally favorable safety assessments [41]。

2.3. Risk/benefit assessment

AS1501 is a "recombinant human soluble death receptor 5-antibody fusion protein," abbreviated as sDR5-Fc. The sDR5 part of AS1501 can bind to its ligand tumor necrosis factor-related apoptosis-inducing ligand (TNF-related apoptosis-inducing ligand, TRAIL). However, due to the lack of a transmembrane region and intracellular death domain in sDR5, it cannot transmit apoptotic signals into the cell. Therefore, sDR5 can competitively bind to TRAIL on the cell membrane surface, thereby blocking TRAIL-mediated apoptosis.

Several preclinical studies have confirmed that AS1501 can specifically bind TRAIL, block TRAIL-induced apoptosis, significantly reduce ALT and AST levels of liver injury and liver failure, significantly improve the prognosis of liver injury and liver failure caused by various causes, and is expected to improve the recovery of ALT and AST and long-term survival of patients with liver injury and liver failure.

A single-center, randomized, double-blind, placebo-controlled, single-dose, dose-increasing study of AS1501 for injection in healthy subjects has been conducted. Twenty-eight subjects enrolled in the study received the study drug and were assessed as safe and tolerable doses of 1mg/kg, 2mg/kg, and 3mg/kg.

Therefore, based on the results of AS1501s nonclinical pharmacological and toxicological studies, combined with data from phase 1 healthy human clinical studies, the sponsor conducted an overall evaluation of the benefit/risk relationship and concluded that AS1501 has potential clinical benefits and acceptable risks, supporting further clinical development in ACLF subjects.

3. The purpose and endpoint of the trial

3.1. test objective

3.1.1. Objective of phase IIa study

fundamental purpose:

- To evaluate the safety and tolerability of AS1501 for injection in early ACLF subjects, and to explore a reasonable dosing regimen.

- Determine the recommended dose and frequency for stage IIb.

Secondary objective:

- The efficacy, immunogenicity and PK characteristics of AS1501 for injection in the treatment of early ACLF were evaluated.
- The changes in serum TRAIL expression levels before and after administration of AS1501 for injection in early ACLF subjects were preliminarily explored.

3.1.2. Objective of phase IIb study

fundamental purpose:

To evaluate the efficacy of AS1501 for injection in ACLF subjects.

Secondary objective:

- To evaluate the safety and PK characteristics of AS1501 for injection in ACLF subjects.
- To explore the changes in serum TRAIL expression levels before and after administration of AS1501 for injection in ACLF subjects.

3.2. End point indicators of the trial

3.2.1. Phase IIa study endpoint

Main endpoint:

1) Incidence of all types of adverse events (AE) and serious adverse events (SAE) and incidence of AE and SAE related to the study product.

Secondary endpoint:

1) AS1501 Changes in MELD score and AARC score at the end of treatment compared with baseline.
2) AS1501 Survival rate of ACLF patients at the end of treatment.
3) AS1501 The cure rate of ACLF patients at the end of treatment; Cure criteria: ① disappearance of clinical symptoms such as fatigue, poor appetite, abdominal distension, reduced urine output, tendency to bleed, and hepatic encephalopathy; ② resolution of jaundice, liver size returning to normal; ③ basic normalization of liver function indicators; ④ normalization of PTA.
4) AS1501 The improvement rate of ACLF patients at the end of treatment; Improvement criteria: ① Significant improvement in clinical symptoms such as fatigue, poor appetite, abdominal distension, and bleeding tendency, with the disappearance of hepatic encephalopathy; ② Significant improvement in physical signs such as jaundice and ascites; ③ Significant improvement in liver function indicators (TbiL <5 ULN, PTA> 40% or INR <1.5).
5) AS1501 The proportion of ACLF patients with serum biochemical response rate (ALT, AST,

Tbil, PTA are normal) at the end of treatment.

6) Incidence of switching to other treatments (artificial liver, liver transplantation, cell therapy, etc.).
7) AS1501 Pharmacokinetic parameters after single and multiple dosing, including: apparent volume of distribution (Vd/f), plasma clearance rate (CL/F), plasma elimination half-life (t1/2), plasma peak concentration (Cmax), time to peak (Tmax), plasma concentration-time curve area under the curve (AUC), steady-state plasma peak concentration (Css-max), steady-state time to peak (Tss-max), steady-state plasma trough concentration (Css-min), steady-state plasma concentration-time curve area under the curve (AUCss), and accumulation ratio (Rac).
8) Immunogenicity related indicators, such as ADA antibodies.
9) The changes in serum TRAIL expression levels before and after administration of AS1501 for injection in ACLF subjects were preliminarily explored.

3.2.2. Phase IIb study endpoint

Main endpoint:

Survival of ACLF patients at 28 days after the first dose.

Secondary endpoint:

1) Survival of ACLF patients at 8 and 12 weeks after the first dose.
2) The cure rate of ACLF patients at 4, 8, and 12 weeks after the first dose; Cure criteria: ① disappearance of clinical symptoms such as fatigue, poor appetite, abdominal distension, reduced urine output, bleeding tendency, and hepatic encephalopathy; ② resolution of jaundice, liver size returning to normal; ③ basic normalization of liver function indicators; ④ normalization of PTA.
3) The improvement rate of ALCF patients at weeks 4, 8, and 12 after the first dose; Improvement criteria: ① Significant improvement in clinical symptoms such as fatigue, poor appetite, abdominal distension, and bleeding tendency, with hepatic encephalopathy disappearing; ② Significant improvement in physical signs such as jaundice and ascites; ③ Significant improvement in liver function indicators (TbiL <5 ULN, PTA> 40%, or INR <1.5).
4) The percentage of ALCF patients with serum biochemical response (ALT, AST, Tbil, and PTA were normal) at weeks 4, 8, and 12 after the first dose.
5) Changes in the MELD (end-stage liver disease model) score and AARC (ACLF Research Alliance of the Asia Pacific Association for Research on Liver Diseases) score of ALCF patients at weeks 4, 8, and 12 after the first dose compared to baseline.
6) Incidence of switching to other treatments (artificial liver, liver transplantation, cell therapy, etc.).
7) Liver transplant rate at 90 days;

8) AS1501 Pharmacokinetic parameters after multiple dosing, including: apparent volume of distribution (Vd/f), plasma clearance rate (CL/F), plasma elimination half-life (t1/2), plasma peak concentration (Cmax), time to peak (Tmax), plasma trough concentration (Cmin), area under the plasma concentration-time curve (AUC), steady-state plasma peak concentration (Css-max), time to steady-state peak (Tss-max), steady-state plasma trough concentration (Css-min), area under the steady-state plasma concentration-time curve (AUCss), and accumulation ratio (Rac).

9) The changes in serum TRAIL expression levels before and after administration of AS1501 for injection in ACLF subjects were preliminarily explored.

4. research design

4.1. system design

This trial is a phase II clinical study, which is divided into two stages:

Phase IIa: A sentinel and single-arm design is adopted, with an expected enrollment of 12 early ACLF participants, receiving a dose of 0.5 mg/kg. The first two participants will be sentinels and sequentially enrolled for a single intravenous administration. If these two sentinels do not experience any SAEs related to the study drug within 2 weeks after the initial administration, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to further explore the study. After receiving a single intravenous administration, participants must fast for 20 days. During this 20-day fasting period, if no SAEs related to the study drug occur at grade 3 or higher, and after joint evaluation by the investigator and sponsor, the participant will enter the multiple-dose phase (once a week [D21 is the first dose of multiple-dose administration], for 4 consecutive weeks). Otherwise, the dose will be reduced to further explore the study, with the specific dose determined through discussion between the sponsor and the investigator. If any participant drops out during the fasting period after a single administration, additional participants can be added to ensure that no fewer than 12 participants enter the multiple-dose phase.

After all 12 early ACLF subjects in the 0.5mg/kg dose group have completed continuous dosing, the DMC (Data Monitoring Committee) will evaluate the safety of this dose group. If any of the following conditions occur in the 0.5mg/kg dose group, the DMC will discuss whether to continue dose escalation:

1) More than 1/3 of the subjects had a drug-related grade 3 SAE;
2) Any drug-related SAE at level 4 or above.

If the DMC evaluation confirms compliance with the dose escalation criteria, the remaining 12 early ACLF participants will receive the 1 mg/kg dose group. The inclusion rules for the 0.5 mg/kg dose group apply, with the first two participants serving as sentinel cases. They will be sequentially enrolled and receive a single intravenous dose. If these two sentinel cases do not experience any SAEs

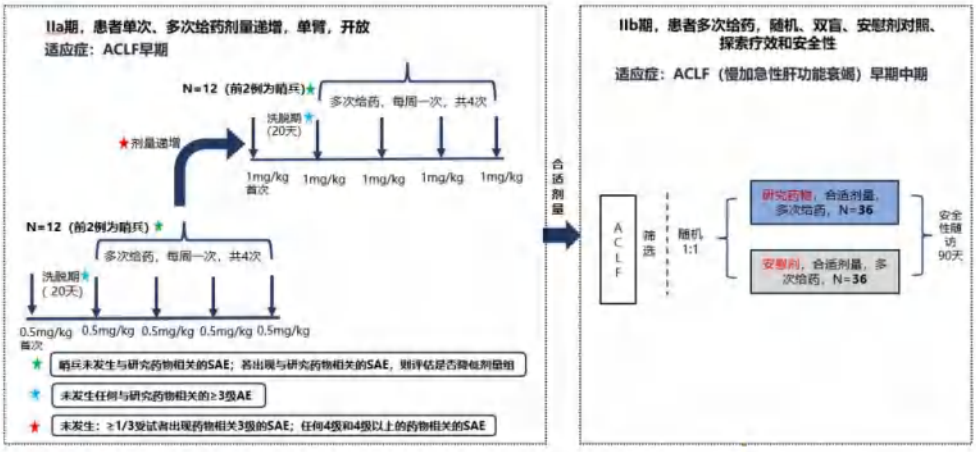
related to the study drug within 2 weeks after the initial dose, the remaining 10 participants can continue to be enrolled. Otherwise, the dose will be reduced to continue exploring the study. After receiving a single intravenous dose, participants must fast for 20 days. During this 20-day fasting period, if no grade 3 or higher AE related to the study drug occurs, and after joint assessment by the investigator and sponsor confirms safety and tolerability, participants will enter the multiple-dose phase (once a week [D21 is the first of multiple doses], for 4 consecutive weeks). If any participant drops out during the fasting period after a single dose, additional participants will be added to ensure that at least 12 participants enter the multiple-dose phase.

After completing the 0.5mg/kg and 1mg/kg dose exploration studies, the investigator and sponsor can determine the recommended appropriate dose for phase IIb studies based on cumulative safety, efficacy, and possible PK/PD results, or may explore other dose groups or other dosing frequencies.

Phase IIb: A randomized (1:1), double-blind, placebo-controlled parallel design is adopted, with an expected enrollment of 72 ACLF participants receiving appropriate doses and frequencies of AS1501 or placebo to further evaluate the efficacy and safety of injectable AS1501. The specific design will be determined by the investigators and sponsors in consultation with the results from Phase IIa.

The research process of each stage of this study was divided into screening period, treatment period and safety follow-up period.

The specific design is shown in the figure below.



4.2. Dose escalation design

4.2.1. Principle of dose escalation at termination

Phase IIa dose escalation

After the completion of continuous dosing in 12 early ACLF subjects in the first dose group (0.5mg/kg), the DMC (Data Monitoring Committee) will evaluate the safety of this dose group. If any

of the following occurs in the 0.5mg/kg dose group, the DMC will discuss whether to continue dose escalation:

[1] More than 1/3 of the subjects had a drug-related grade 3 SAE;

[2] Any drug-related SAE of grade 4 or above;

Determination of the dose for stage IIb

When all subjects at 0.5mg/kg and 1mg/kg have completed treatment, the DMC will determine the recommended dose for phase IIb based on the safety data (AE, SAE, AESI), PK, PD(TRAIL protein) and other data of the subjects.

4.3. Definition of the end of research

Phase II a: The last subject completed the final visit.

Phase IIb: The last subject completed the 4-week efficacy and safety assessment or withdrew from the study (whichever occurred first), and long-term follow-up data can be collected continuously.

In accordance with laws and regulations, regulations of the drug regulatory department or requirements of the ethics committee, the sponsor shall report to the regulatory agency upon completion of the study and the sponsor or investigator shall report to the ethics committee.

4.4. Randomization and blinding

Study IIb was a randomized, double-blind study.

The subjects random number was generated by an independent statistician who was not involved in the study. The independent random statistician used SAS 9. 4. The PLAN process involves generating 72 random numbers using the block randomization method. Participants are randomly assigned to either the AS1501 (experimental drug) or placebo group at a ratio of 1:1, with 36 participants in each group. Researchers should assign the random numbers in the order of participant eligibility, from smallest to largest.

Blinding: Double-blind refers to the situation where neither the subjects, researchers, monitors, nor data analysts know the allocation of the treatment drugs. The sponsor or its designated entity provides both the trial drug and the placebo, ensuring that the appearance and weight of the placebo are similar to those of the trial drug. The sponsors designated entity blindens the trial drugs and placebos according to a blinded randomization schedule provided by the statistician. The blinding code should be duplicated and sealed in envelopes, to be kept separately by the clinical research unit and the drug registration applicant. Additionally, the statistician prepares an emergency unblinding envelope and sends it to the research center. In cases of SAEs or other emergencies, the study physician can open the emergency unblinding

envelope corresponding to the subjects random number to determine the assigned treatment drug for rescue purposes, which is known as emergency unblinding.

Blinding level: This trial used double-blind technique, that is, the physician and the subject could not determine which drug the subject was receiving.

The specific operation is as follows:

1) According to the relevant regulations on trial drug management under GCP, the trial drugs are uniformly packaged and labeled as for clinical trials, with identical packaging for both the treatment group and the placebo. Blinding is performed by designated personnel, ensuring each participant receives a single, individually packaged drug. The entire process must be verified by a dedicated person and meticulously documented. After the randomization table used for blinding at the site is completed, it should be collected by the statistician.

2) After the trial drugs are distributed to the research unit, a special place (drug storage place) shall be set up by the research unit for special personnel to keep them. The research unit may set up non-blind personnel as needed, such as non-blind drug dispensing nurses, to ensure that the research physician and the subject are in a blind state.

Emergency Letters and Blind Bottles: Before the trial begins, an emergency envelope is prepared for each participant. The envelope is marked with the participants random number, and the letter paper inside is labeled with the participants group assignment for use in case of an emergency to break the blind. All study medications will be distributed along with the corresponding numbered emergency envelopes to the research site.

The process of blinding should be recorded in writing and signed by all personnel involved in the blinding. The blind bottom should be sealed with an official seal and stored separately by the dedicated personnel of the research institution and the sponsor; emergency blinding letters should be sent along with the investigational drug to the research institution, where they should be properly kept by the dedicated personnel of the research institution until the end of the trial.

Emergency Blindness Break: In case of emergency, when it is necessary to break the blindness, the investigator shall consult with the head of the research unit and, after obtaining the signature of the head, may read the emergency blindness break letter. Within 24 hours after breaking the blindness, the relevant personnel of the sponsor shall be notified and the reasons for breaking the blindness explained. The following situations can be considered for emergency blindness break, including but not limited to the following situations

(1) When a serious adverse event occurs in the patient, it may be considered to be related to the trial or control drug;

(2) When the patient has a serious complication.

Blinding regulations: This trial is a single blinding. After the database is closed, the research unit and the sponsor will conduct blinding, and the personnel who keep the blind bottom will hand over the trial grouping information to the statistical department for statistical analysis.

5. study population

5.1. IIa phase study population

5.1.1. II A selection criteria

[1] Age of 18 to 75 years (including the critical value) when signing the informed consent;

[2] According to the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Liver Failure and Artificial Liver Group of the Infection Disease Branch of the Chinese Medical Association and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure was diagnosed with specific indicators including:

a) Chronic liver disease (chronic hepatitis B, autoimmune hepatitis, drug-induced hepatitis, etc.) as the basis, and acute impact factor is drug;

b) Serum TBil ⩾ 10 x ULN or mean daily increase of ⩾ 17.1 μ mol/L;

c) Meets any of the following three criteria: A bleeding tendency with PTA ⩽ 40% (or INR ⩾ 1.5); B hepatic encephalopathy; C hepatorenal syndrome or ascites.

[3] The screening was performed at the early stage of liver failure and did not meet the conditions for liver transplantation;

- Early signs of liver failure:
  - Extremely weak, with obvious loss of appetite, vomiting and abdominal distension and other serious digestive symptoms;
  - ALT and/or AST continued to rise significantly, with progressive deepening of jaundice (TBil>171 μ mol/L or daily increase>17.1 μ mol/L);
  - Tenderness of bleeding, 30% <PTA⩽40% (or 1.5⩽INR<1.9);
  - No complications and other extrhepatic organ failure.

[4] The serum TRAIL increased during screening, and the TRAIL content was more than 3 times that of normal people;

[5] Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form

[6] Able to follow the protocol and complete the trial;

[7] Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration.

5.1.2. Exclusion criteria for stage II a

Subjects are not eligible for inclusion in this study if they meet one of the following conditions.

[1] Patients with a history of allergic constitution or severe allergy to protein drugs (CTCAE v5.0 points>3 levels);

[2] Patients who have undergone liver transplantation or who plan to undergo liver transplantation within 1 month.

[3] ACLF patients in the middle and late stages;

[4] Severe grade III ascites or refractory ascites with grade III-IV hepatic encephalopathy.

[5] Patients who had received artificial liver treatment within 1 week prior to screening.

[6] Patients with malignant tumors or a history of malignant tumors; those who were diagnosed with lung cancer, liver cancer, pancreatic cancer and gastrointestinal tumors by imaging (ultrasound, CT or MRI) and tumor markers (AFP, CEA, CA125 or CA199) during or within 1 month before screening.

[7] Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results indicating a risk of severe varices with bleeding during or within 1 month prior to screening.

[8] Subjects with acute kidney injury (AKI) as defined by KDIGO criteria:(1) Scr increased by 26.5 μ mol/L(0.3mg/dL or more within 48 h, 1mg/dL=88.4 μ mol/L); (2) Scr increased by 1.5 times or more than baseline value within 7 d; (3) Urine volume decreased (<0.5ml/kg/h) and lasted for more than 6 h.

[9] The following laboratory test values or abnormal test values are present: a.Blood routine: platelet (PLT) <75 x 10^9L/L, hemoglobin (HGB) <80g/L; b. PT-INR> 1.9 or PTA <30%; c.Left ventricular ejection fraction (LVEF) <50%; serum creatinine> 1.5 x ULN.

[10] Associated with severe respiratory dysfunction, dyspnea or failure.

[11] Severe infections that cannot be controlled by concomitant drugs, including infections in major organs such as the abdominal cavity, lungs, urinary tract and skin.

[12] HIV positive, or active tuberculosis or syphilis infected.

[13] Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history.

[14] Subjects with uncontrolled severe hypertension or diabetes with concomitant medications.

[15] Women who are pregnant or breastfeeding, or who have a positive pregnancy test.

[16] Participants who participated in clinical trials of other drugs or medical devices within the first 30 days of randomization or five drug half-lives.

[17] Subjects who have had trauma or major surgery (e.g., requiring general anesthesia) within

28 days prior to the first dose of the study drug. Note: Subjects who plan to undergo surgical procedures under local anesthesia may participate in the study.

[18] Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia or mental status change; or any problem that may impair the subjects ability to receive or tolerate planned treatment at the research center, understand informed consent, or the investigators belief that the subject is contraindicated from participating in the study or confused about the assessment or study results as specified in the protocol.

5.2. IIb study population

Selection criteria for stage IIb

[1] The age of signing the informed consent form is 18 to 75 years old (including the critical value);

[2] According to the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Liver Failure and Artificial Liver Group of the Infection Disease Branch and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure is diagnosed, and specific indicators include:

a) History of chronic liver disease;

b) Serum TBil≥10 times ULN or mean daily increase of more than 17.1μmol/L;

c) Meets any of the following three criteria: A bleeding tendency with PTA ≤40% (or INR ≥1.5); B hepatic encephalopathy; C hepatorenal syndrome or ascites.

[3] The screening was in the early or middle stage of liver failure and did not meet the conditions for liver transplantation;

- ◆ Early signs of liver failure:
  - Extremely weak, with obvious loss of appetite, vomiting and abdominal distension and other serious digestive symptoms;
  - ALT and/or AST continued to rise significantly, with progressive deepening of jaundice (TBil> 171μmol/L or daily increase> 17.1μmol/L);
  - Tenderness of bleeding, 30% <PTA≤40% (or 1.5 ≤INR<1.9);
  - No complications and other extrhepatic organ failure.
- ◆ Mid-stage manifestations of liver failure:
  - On the basis of early manifestations of liver failure, the disease progresses further;
  - ALT and/or AST decreased rapidly, TBil continued to rise;

- Significant bleeding (petechiae or ecchymosis), 20% <PTA≤30% (or 1.9≤INR<2.6);
- There was 1 complication and/or 1 failure of extrhepatic organ function.

[4] The serum TRAIL increased during screening, and the TRAIL content was more than 3 times that of normal people;

[5] Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form;

[6] Able to follow the protocol and complete the trial;

[7] Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration.

5.2.1. Exclusion criteria for stage IIb

Subjects are not eligible for inclusion in this study if they meet one of the following criteria.

[1] Patients with a history of allergic constitution or severe allergic history to protein drugs (CTCAE v5.0 points>3 levels);

[2] Patients who have undergone liver transplantation or are scheduled for liver transplantation within one month.

[3] Severe grade III ascites or refractory ascites.

[4] Patients with associated stage III-IV hepatic encephalopathy.

[5] Patients who had received artificial liver treatment within 1 week prior to screening.

[6] Patients with malignant tumors or a history of malignant tumors; those who were diagnosed with lung cancer, liver cancer, pancreatic cancer, gastrointestinal tumors, etc., by imaging (ultrasound, CT or MRI) and tumor markers (AFP, CEA, CA125 or CA199, etc.) during the screening period or within 1 month prior to the screening period.

[7] Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results indicating a risk of severe varices with bleeding during the screening period or within 1 month prior to screening.

[8] Subjects with acute kidney injury (AKI) as defined by KDIGO criteria:(1) Scr increased by 26.5μmol/L(0.3mg/dL or more within 48h,1mg/dL=88.4μmol/L); (2) Scr increased by more than 1.5 times of baseline value within 7d; (3) Urine output decreased (<0.5ml/kg/h) and lasted for more than 6h.

[9] Severe coagulation failure with PT-INR> 2.5 or PTA <20%.

[10] Associated with severe respiratory dysfunction, dyspnea or failure.

[11] Severe infections that cannot be controlled by concomitant drugs, including major organs such as the abdominal cavity, lungs, urinary tract and skin.

[12] HIV positive, or active tuberculosis or syphilis infected.

[13] Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history.

[14] Subjects with uncontrolled severe hypertension or diabetes with concomitant medications.

[15] Women who are pregnant or breastfeeding, or who have a positive pregnancy test.

[16] Participants who participated in clinical trials of other drugs or medical devices within the first 30 days of randomization or within five drug half-lives.

[17] Subjects who have undergone trauma or major surgery (e.g., requiring general anesthesia) within 28 days prior to the first dose of the study drug. Note: Subjects who plan to undergo surgical procedures under local anesthesia may participate in the study.

[18] Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia, or mental status change; or any problem that may impair the subjects ability to receive or tolerate planned treatment at the research center, understand informed consent, or participate in the study or confuse the assessment or study results as determined by the investigator.

[19] Other conditions that the researchers did not think were suitable for this study.

5.3. Precautions for daily life

Throughout the trial, participants were required to:

- Consumption of products containing caffeine or xanthine (e.g., coffee, tea, cola, and chocolate) is prohibited 24h prior to the start of each dosing cycle until the collection of final pharmacokinetic (PK) and/or pharmacodynamic samples;
- Alcohol is prohibited for 24 hours prior to each dosing cycle until the collection of final pharmacokinetic (PK) and/or pharmacodynamic samples;
- Smokers are not allowed to use any nicotine-containing products (including nicotine patches) at the research center;
- Intense exercise is prohibited within 8h before blood collection for each laboratory examination. Participants can participate in some light recreational activities (such as watching TV and reading) during the test;
- Avoid sexual intercourse with people who have compromised immune function.

5.4. Filter failed

Screening failure is defined as a participant signing an informed consent but failing to receive the study medication for certain reasons, such as not meeting the inclusion criteria or satisfying at least one exclusion criterion. For participants who fail screening, it is necessary to collect basic information

about their screening failure, including the date of informed consent, demographic data, reasons for screening failure, and all SAEs that occurred between signing the ICF and the screening failure.

If a subject is identified as a screening failure (based on laboratory assessment results and other assessment results), the screening can be repeated once, but if the screening time exceeds 28 days, the ICF must be re-signed. Each subject has a maximum of one re-screening opportunity.

If the subjects who failed in the randomization and screening were completed in phase IIb, they should be re-submitted.

5.5. Subject substitution

If a subject withdraws from the study before receiving drug treatment, a new subject is required. If a subject terminates the study after receiving drug treatment, no replacement is required.

5.6. Study suspension/termination, subject suspension/withdrawal from study

5.6.1. Study suspension/termination

Recruitment of subjects will be suspended in the event of any of the following:

1) The primary investigator, sponsor and statistical experts determined that the number and severity of adverse events warranted early termination of the study;
2) Major deviations or human errors are found in the implementation of the test, which seriously affect the quality of the test and make it difficult to achieve the purpose of the test;
3) The study drug was evaluated as failed by the investigator and sponsor;
4) A new malignancy was detected in the subject after infusion of AS1501, and it was considered to be possibly related to AS1501;
5) The administrative department requires the termination of the test;
6) The sponsor requests to terminate the trial under the premise of fully protecting the rights and safety of the subjects (such as financial reasons, management reasons, etc.);
7) Other situations that have been jointly assessed by the investigator and sponsor require a suspension of recruitment.

If there are sufficient reasonable reasons, this study may be temporarily suspended or terminated.

If the study is temporarily suspended or prematurely terminated, the principal investigator should promptly notify the participants and the ethics review committee, providing reasons for the termination or suspension. In appropriate circumstances, the investigator will contact the participants and inform them of any changes to the visit schedule. The study can only resume once issues related to safety, protocol compliance, and data quality have been resolved and approved by the clinical trial institution and/or regulatory authorities.

When discontinuing an intervention, subsequent study procedures should be completed according to the protocol. If a subject exhibits clinically significant changes (including but not limited to

deviations from baseline levels) after enrollment, the investigator or a qualified designated person will determine whether subject management needs to be altered. Any clinically relevant new findings will be reported as an adverse event (AE).

5.6.2. Subjects discontinued or withdrew from the study

Subjects may request to discontinue or withdraw from the study at any time. The reasons for such requests shall be recorded in the case report form (CRF), including but not limited to the following aspects:

The subject withdrew from the study

- The subject withdraws the informed consent;
- encyesis;
- Missing persons;
- die;
- other;

The subject was discontinued or withdrew from the study by the investigator

- Major protocol deviations or protocol violations occurred during the study that had a significant impact on drug tolerance, safety, or PK evaluation;
- Researchers consider it necessary to stop the trial from the perspective of medical ethics, such as if there is a suitable liver donor and an emergency liver transplant is needed;
- Those who experienced life-threatening serious adverse events (SAE) related to the study drug and were not suitable to continue the trial;
- After the investigator discontinued the study drug treatment, it was determined that the withdrawal of the study was most beneficial to the subject;
- Poor subject compliance (e.g., failure to take prescribed medications and receive examinations, use of other drugs that affect the evaluation of efficacy and safety); and serious protocol violations/biases;
- Clinical adverse events (AE), laboratory abnormalities or other medical conditions that continue to participate in the study may endanger the safety or health of the subject and will not be in the best interest of the subject;
- Subjects who meet the exclusion criteria (new or previously undetected) are not eligible to continue in the study.

Subjects who have signed an informed consent and are randomized but have not received the study intervention can be replaced. Subjects who have signed an informed consent and are randomized

and have received the study intervention but subsequently discontinued the study will not be allowed to be replaced.

5.6.3. Treatment termination criteria

The subject may request termination of treatment at any time, and all reasons should be recorded in the case report form (CRF), including but not limited to:

- The subject withdraws the informed consent;
- Unacceptable toxicity occurs;
- Poor compliance;
- The subject requests termination of treatment (e.g., consider receiving an artificial liver);
- Disappearance;
- die;
- encyesis;
- other.

5.6.4. Missing persons

If the subject fails to return to the visit plan on multiple occasions or the center cannot contact the subject, the subject will be considered lost to follow-up.

If the subject fails to return to the research center for the requested study visit, the following measures must be taken:

- The research center must try to contact the subjects, rearrange missed visits as soon as possible, explain to the subjects the importance of complying with the visit schedule, and determine whether the subjects are willing and/or should continue to participate in the study.
- Before confirming the loss to follow-up of a subject, the investigator or designator must make every effort to recontact the subject (up to three phone calls if possible; if necessary, send a registered letter to the subjects last known mailing address or use an equivalent method). All such attempts should be documented in the subjects medical record.
- If the subject cannot be contacted after the above attempts, he/she is considered to have withdrawn from the study.

6. Study intervention

6.1. research on drug

6.1.1. Study drug description

AS1501, a research drug developed by Shenzhen Zhongke Amshenn Pharmaceutical Co., Ltd., is a therapeutic biological product of class 1 drug that competitively binds tumor necrosis factor-related apoptosis-inducing ligand (Tumor necrosis factor related apoptosis inducing ligand, TRAL) to TRAIL-R2 (DR5) on the cell surface.

The detailed properties of AS1501 are shown in the figure below.

The common name of the Chinese language	Injection of AS1501
The generic name of the English word	AS1501 for Injection
active ingredient	Human recombinant AS1501 fusion protein
accessories	Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, mannitol and polysorbate 20
constitutional formula	The dimer fusion protein consists of a truncated human death receptor 5 (DeathReceptor5) extracellular region and a human IgG1Fc segment (hinge, CH2 and CH3 regions)
formula weight	About 79KD
physical characteristics	The pH value of the drug is 6.7~7.3; the osmotic pressure molar concentration is 270~330mOsmol/kg; and the water content is not greater than 3.0%.
stability	The validity period is tentatively set at 3 years
the form of a drug	Lyophilized powder for injection
shape and properties	White loose body
specifications	25mg per dose
storage procedures	Sealed, lightproof, 2℃~8℃ refrigerated
Administration method	I.V
Frequency of administration	**Phase II a: a total of 5 times. Phase II b: tentatively once a week, a total of 4 times.**

In phase IIb, the placebo was a white lyophilized powder with the same appearance and the administration method was the same as that of AS1501.

6.1.2. Study drug dosing regimens

Ⅱa designated time

Using a sentinel and single-arm design, it is expected to include 12 early ACLF participants who will receive the 0.5 mg/kg dose group. The first two participants will be sentinels, entering the study in sequence and receiving a single intravenous injection. If these two sentinels do not experience any SAEs related to the study drug within 2 weeks after the initial dose, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to continue exploring the study. After receiving a single intravenous injection, participants must be followed for 20 days. During this 20-day washout period, if no SAEs related to the study drug occur at grade 3 or higher, and after joint evaluation by the investigator and sponsor, the participant will enter the multiple-dose phase (once a week [D21 is the first dose] for 4 consecutive weeks). Otherwise, the dose will be reduced to continue exploring the study, with the specific dose determined through discussion between the sponsor and the

investigator. If any participant drops out during the washout period after a single dose, additional participants will be added to ensure that no fewer than 12 participants enter the multiple-dose phase.

After all 12 early ACLF subjects in the 0.5mg/kg dose group have completed continuous dosing, the DMC (Data Monitoring Committee) will evaluate the safety of this dose group. If any of the following conditions occur in the 0.5mg/kg dose group, the DMC will discuss whether to continue dose escalation:

1) More than 1/3 of the subjects had a drug-related grade 3 SAE;
2) Any drug-related SAE of grade 4 or above.

If the DMC evaluation confirms compliance with the dose escalation criteria, the 12 early ACLF participants who continue to be enrolled will receive the 1 mg/kg dose group. The enrollment rules for the 0.5 mg/kg dose group apply, with the first two participants serving as sentinels and subsequently enrolled for a single intravenous administration. If these two sentinel participants do not experience any SAEs related to the study drug within two weeks after the initial administration, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to continue exploring the study. After receiving a single intravenous administration, participants must be followed for 20 days. During this 20-day follow-up period, if no SAEs related to the study drug occur at or above grade 3, and after joint assessment by the investigator and sponsor, they will enter the multiple-dose phase (once a week [D21 is the first dose] for four consecutive weeks). If any participant drops out during the follow-up period after a single dose, additional participants will be added to ensure that no fewer than 12 participants enter the multiple-dose phase.

After completing the 0.5mg/kg and 1mg/kg dose exploration studies, investigators and sponsors can determine the recommended appropriate dose for phase IIb studies based on cumulative safety, efficacy, and possible PK/PD results, and may also explore other dose groups or other dosing frequencies.

Ⅱb designated time

Using a randomized (1:1), double-blind, placebo-controlled parallel design, it is expected to enroll 72 ACLF participants who will receive an appropriate dose and frequency of AS1501 or placebo to further evaluate the efficacy and safety of injectable AS1501. The specific design will be determined by the investigators and sponsors in consultation with the results from Phase IIa.

6.1.3. Principles of expected toxicity treatment measures and dose adjustment

6.1.3.1. During the elution period of single dose in phase IIa

If any study drug-related AE of grade ≥ 3 occurs during the single-dose washout period, the subject should receive active supportive care as per center protocols until the AE is reduced to <2 or

baseline levels. The investigator will thoroughly evaluate and confirm that continued dosing would be beneficial before entering the continuous dosing phase. If the subject cannot receive planned continuous dosing on D21, dosing can be deferred initially by 2 weeks, and the study drug can be administered again at n+7, n+14, and n+21 from the actual dosing date (n).

6.1.3.2. Multiple dosing phases

Phase IIa participants entering the multiple-dose phase and Phase IIb participants may undergo AS1501 dose adjustments based on the severity of toxic reactions. The toxicity of participants should be assessed using the NCI CTCAE v5.0 scale. The primary approach to managing toxicity (of any level) is dose adjustment and delayed dosing of the investigational drug. Any changes to the dosing regimen must be discussed with the sponsors medical monitor before implementation, unless there is an immediate safety risk, and should be supervised by a medically qualified research center personnel (principal investigator or assistant investigator).

a) Treatment can be paused for up to 3 weeks (from the time of the next scheduled dose) until toxicity returns to levels that allow resumption of treatment or baseline. Treatment may only be restarted after 3 weeks if clear clinical benefit is observed and approved by the sponsors medical monitor. After reducing the study drug dose, it cannot be increased to a higher level without approval from the sponsors medical monitor.
b) If the toxicity meets the criteria for treatment termination, the treatment will be terminated.
c) During the administration of the drug, if the subject suspends or delays the administration of the drug and then resumes the administration, the study drug should be continued at n+7, n+14 (if required), and n+21 (if required) from the actual date of resumption of the administration of the drug.

The principle of dose adjustment is shown in the table below:

AE class (CTCAE)	**Principles for dose adjustment**
Level 1 or level 2	
AE occurred at any frequency	According to the investigators judgment, if the subject can tolerate the toxicity, the maintenance dose remains unchanged.
Grade 3 and related to the study drug	
AE occurred any number of times	Administration was suspended until toxicity returned to baseline level, grade 1 or tolerable grade 2. The high-dose group could reduce the dose level to return to administration, and the low-dose group could be terminated or resumed by the investigator.
Grade 4, and related to the study drug	
AE occurred at any frequency	The investigator confirms whether to terminate the treatment. If it is decided to resume the administration, the high-dose group should be reduced by one dose level after the toxicity has returned to baseline level, grade 1 or tolerable grade 2. The sponsor and the

	investigator may decide whether to explore lower doses for the low-dose group.

6.1.3.3. Expected toxicological treatment measures

6.1.3.3.1. lymphocytoponia

Lymphocyte reduction can be seen in healthy human phase I studies, and the following treatment measures are recommended:

toxicity criterion	**measure**
No more than grade 2	o Regular blood routine tests until relief to less than grade 1 or baseline level; o Follow-up to remission or baseline level; o Provide active supportive treatment; o If lymphocytopenia persists, granulocyte colony-stimulating factor (G-CSF) can be considered for prophylactic treatment;
Grade 3 or higher	o Stop treatment; o Regular blood routine tests until relief to less than grade 1 or baseline level; o If there is no relief, continue to follow up; o Provide active supportive treatment; o According to the researchers judgment, one dose level of recovery treatment can be reduced after remission;

6.1.3.3.2. Hyper sensitivity reaction, infusion reaction

In the Phase I clinical study of the investigational drug in healthy individuals, two cases of grade 3 hypersensitivity reactions were observed in the 2 mg/kg dose group. If any dose group in Phase IIa observes a study drug-related hypersensitivity reaction or infusion reaction of grade 2 or higher, the subsequent dosing and subsequent enrollment of other participants can receive antiallergic treatment before the administration of the study drug, including: corticosteroids (dexamethasone 5-10mg or equivalent), antihistamines (oral or intravenous diphenhydramine 25mg to 50 mg), and antipyretic analgesics (oral acetaminophen 650mg to 1000 mg). The specific dosing regimen may be administered according to the investigators routine procedures at the center.

If hypersensitivity reaction or infusion reaction occurs, the following treatment measures are recommended:

toxicity criterion	**measure**
No more than 2 levels	o Continuous follow-up until remission to recovery or ≤1 grade; o Slow down the infusion rate or stop the infusion; o Provide active supportive treatment: glucocorticoids (dexamethasone 5-10mg or equivalent), antihistamines (oral or intravenous diphenhydramine 25mg to 50 mg), and antipyretic analgesics (oral acetaminophen 650mg to 1000 mg), while closely monitoring. If symptoms improve, reinfuse AS1501 at 50% of the original infusion rate; if a grade 3 or higher infusion reaction occurs again, permanently discontinue AS1501; o After that, antiallergic drugs were given for each subsequent administration. If infusion reaction of grade 3 or above occurred again, AS1501 was permanently discontinued;
Level 3	o Stop treatment; o Follow-up to remission or baseline level; o Provide active supportive treatment: glucocorticoids (dexamethasone 5-10mg or equivalent), antihistamines (oral or intravenous diphenhydramine 25mg to 50 mg), and antipyretic analgesics

toxicity criterion	measure
	(oral acetaminophen 650mg to 1000 mg), with close monitoring. If symptoms improve, reinfuse AS1501 at 50% of the original infusion rate; if a grade 3 or higher infusion reaction occurs again, permanently discontinue AS1501; o After that, antiallergic drugs were given for each subsequent administration. If infusion reaction grade 3 or above occurred again, AS1501 was permanently discontinued;
Grade 4 or higher	o Permanent discontinuation of medication; o Follow-up to remission or baseline level; o Provide active treatment: glucocorticoids (dexamethasone 5-10mg or equivalent), antihistamines (oral or intravenous diphenhydramine 25mg-50mg) and antipyretic analgesics (oral acetaminophen 650mg-1000mg) and close observation.

6.1.3.3.3. Cytokine release syndrome

Cytokine release syndrome (Cytokine Release Syndrome, CRS) is an exaggerated response following immunotherapy, characterized by the activation of endogenous or infused T cells and/or other immune effector cells. It manifests as a large number of lymphocytes (B cells, T cells, and/or natural killer cells) and/or bone marrow cells (macrophages, dendritic cells, and monocytes) being activated and releasing inflammatory cytokines. Cytokines such as IFN-γ, TNF-α, IL-6, and IL-10 all play a role in this process, with IL-6 being particularly associated with the severity of CRS[42].

CRS mild symptoms include fever, fatigue, headache, rash, arthralgia, and myalgia. More severe manifestations involve hypotension and high fever, which can progress to uncontrolled systemic inflammatory response, accompanied by circulatory shock requiring vasopressors, vascular leakage, disseminated intravascular coagulation, and multi-organ system failure.

If any of the following symptoms or signs that cannot be explained by other causes occur after AS1501 infusion, CRS should be highly suspected: ① fever ≥ 38℃; ② hypotension (defined as: systolic blood pressure <90mmHg, below baseline value); ③ hypoxemia (requiring oxygen to correct hypoxia).

If CRS occurs, the following treatment measures are recommended:

CRS classify	Handling recommendations
Level 1	• Slow down the infusion rate or stop the infusion; • Continuous follow-up until remission to recovery or ≤ grade 1; • Nonsteroidal anti-inflammatory drugs to control body temperature; • Exclusion of possible infectious pathogens (blood, urine, sputum culture, lung imaging, etc.); • For example, granulocytopenia is combined with broad-spectrum antibiotics; • If persistent fever (temperature> 39℃ for more than 10h or persistent fever for more than 3 days) remains after symptomatic treatment, dexamethasone 5~ 10mg iv can be considered; • According to the judgment of the investigator, treatment can be resumed at the original dose or reduced by one dose level after remission;
Level 2	• Slow down the infusion rate or stop the infusion; • Continuous follow-up until remission to recovery or ≤ 1 grade; • On the basis of the above level 1 CRS clinical treatment protocol, apply physiological saline

	10~20 ml/kg to strengthen fluid replacement therapy, and repeat fluid replacement if necessary to maintain blood pressure; • Dexamethasone 10mg iv q6h (or equivalent dose) or methylprednisolone 1mg/kg iv q6h; • Low-flow oxygen support; • According to the judgment of the investigator, treatment can be resumed at the original dose or reduced by one dose level after remission;
Level 3	• Stop treatment; • Symptomatic supportive treatment (fever reduction, fluid replacement, balance of internal environment, etc.); • On-demand intravenous rapid injection of fluids and use of pressor agents; • Dexamethasone 10~ 20mg iv q6h (or equivalent dose) or methylprednisolone 1~2mg/kg iv q6h; • If the above treatment is not effective or there are contraindications to hormone therapy, plasma exchange can be considered; • Application of high flow oxygen inhalation; • If necessary, consider transfer to ICU and perform hemodynamic monitoring; • According to the researchers judgment, one dose level of recovery treatment can be reduced after remission;
Level 4	• Permanent discontinuation of medication; • The patient should be transferred to ICU for enhanced monitoring and treatment; • Dexamethasone 20mg iv q6h (or equivalent dose) or methylprednisolone 500mg iv q12h for 3 days, and then rapidly reduced after symptom control; • Symptomatic supportive treatment (fever reduction, fluid replacement, balance of internal environment, etc.); • Mechanical ventilation as required; • On-demand intravenous rapid infusion of fluids and use of multiple pressor agents; • ICU monitoring and hemodynamic monitoring; • If the above treatment is not effective, plasma exchange may be considered.

Note: For patients receiving hormone therapy CRS, the investigator may assess the use of antifungal prophylaxis. Other hormones of equivalent dose may be considered as an alternative to dexamethasone/methylprednisolone.

6.2. Study drug preparation/process/storing/duties

6.2.1. Reception and counting of drugs

The sponsor is responsible for delivering the investigational drug (AS1501 or placebo) to the research center, and the investigator/pharmacist signs to confirm receipt.

Study drugs (AS1501 or placebo) can only be used for this study and can only be managed by a person authorized by the investigator. The investigator/pharmacist responsible for drug management distributes, recovers, destroys and accurately records the study drugs according to the research protocol.

6.2.2. Product storage and stability

Researchers or their designated personnel must ensure that all drugs used in the study are sealed, protected from light and stored at 2℃~8℃ refrigerated during transportation. If deviations occur, they must report and resolve the problems before conducting the study treatment.

Only participants who have enrolled in the study can receive the investigational treatment, and only authorized research center personnel can provide or manage the investigational drug. All investigational drugs or related items must be stored in a safe, environmentally controlled, and monitored (manual or automatic) area according to the storage conditions specified on the label, ensuring that only investigators and authorized research center personnel have the necessary access to the investigational drug.

The investigator, the head of the institution or medical institution (if applicable) is responsible for the accountability, coordination and maintenance of records (i.e., receipt, coordination and final disposition of records) for the study treatment.

6.3. Randomization and blinding

Study ii b was a randomized, double-blind study. See 4.5 for details.

6.4. Study adherence to interventions

The definition of treatment adherence is the actual amount of medication received by the participant divided by the amount that should have been received during the study period. During the study, all medication use for the investigational treatment will be recorded in the electronic data collection (EDC) system. Any deviation from the prescribed medication regimen, including the date and reason for any deviation, will be documented.

The study will assess adherence to the study treatment at each visit.

6.5. Combination medication and treatment

6.5.1. Drugs and treatments that are prohibited

During the trial, artificial liver, glucocorticoids (except for pre-treatment, treatment of infusion reactions/cytokine release syndrome and other AE), hepatocyte growth factor, and any drugs with definite hepatotoxicity (including traditional Chinese medicine, proprietary Chinese medicine, etc.) are not allowed to be used. Plasma, cold precipitate and other blood products are prohibited from being infused within 48h before AARC and MELD score assessment.

6.5.2. Allowable medications and treatments

ACLF combined treatment

ACLF subjects are allowed to receive basic therapeutic drugs, including but not limited to necessary nutritional support, plasma infusion (except within 48h prior to AARC and MELD score assessment), platelet infusion, albumin supplementation, intravenous catheterization, dynamic monitoring, fluid resuscitation, microecological regulation, analgesics, sedatives, antibiotics, oxygen inhalation, artificial ventilation, etc.

Pre-treatment

If any dose group in Phase IIa observed a study drug-related hypersensitivity reaction of grade 2 or higher, infusion reaction, or any level of CRS, the subsequent dosing and subsequent enrollment of other participants can be preceded by pre-treatment with the study drug, which should include at least glucocorticoids (dexamethasone 5-10mg or equivalent), and additional antihistamines (oral or intravenous diphenhydramine 25 mg^3 50 mg) and antipyretic analgesics (oral acetaminophen 650 mg^3 1000 mg) as per standard procedures at each center.

Treatment for AE

Participants are allowed to receive symptomatic supportive treatment for AE, including but not limited to: anti-infection, nutritional support, fluid replacement, and blood transfusions. If participants experience infusion reactions or cytokine release syndrome after receiving the study drug infusion, they may be given hormones (dexamethasone 5-10mg or equivalent), antihistamines (oral or intravenous diphenhydramine 25 mg^3 50 mg), and antipyretic analgesics (oral acetaminophen 650 mg^3 1000 mg).

7. stages of research

7.1. Screening period

7.1.1. Phase II a screening period

In principle, all subjects must sign an informed consent form approved by the ethics committee (IEC) before the study-related examination begins. However, in clinical practice, the relevant examination and evaluation during the screening period are routine clinical procedures, and the results before the informed consent form can be used to avoid repeated examination of subjects.

The investigator shall explain to the subject the medical evaluation prior to the commencement of the study, the possible risks, benefits and that participation in the study is voluntary, and provide the subject with a written informed consent and the signature and date of the investigator.

ACLF patients are required to complete the following examinations and assessments prior to enrollment:

1) Sign the informed consent form;
2) Collect demographic data;
3) Ask about medical history/surgical history, history/combined medication;
4) Complete a full physical examination;
5) Measure height (cm), weight (kg), BMI;
6) Monitor vital signs;
7) 12 lead electrocardiogram;

8) Imaging: abdominal CT (digestive system), echocardiography;
9) Clinical laboratory tests:
   - HCG blood pregnancy test (women of childbearing age);
   - Serological detection of pathogens;
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - routine urine test;
   - arterial blood gas analysis;
   - Indicators of inflammation;
10) Severe disease severity score:
   - Child-Pugh score;
   - ACLF Research Alliance (AARC) score;
   - End-stage liver disease model (MELD) score;
   - SIRS diagnose;
11) Clinical symptom assessment;
12) Criteria for selection and exclusion.
13) Special tests: TRAIL blood sample.

Participants must meet all criteria to participate in the study, and all screening work must be completed within 14 days of signing an informed consent form.

7.1.2. Phase II b screening period

In principle, all subjects must sign an informed consent form approved by the ethics committee (IEC) before the study-related examination begins. However, in clinical practice, the relevant screening examinations and assessments are routine clinical examinations, so the results before the informed consent form can be used to avoid repeated examinations of subjects.

The investigator shall explain to the subject the medical evaluation prior to the commencement of the study, the possible risks, the benefits and the voluntary participation in the study, and provide the subject with a written informed consent form signed by the investigator and dated.

ACLF patients need to complete the following examinations and assessments prior to enrollment:

1) Sign the informed consent form;
2) Collect demographic data;
3) Ask about medical history/surgical history, history/medication;

4) Complete a full physical examination;
5) Measure height (cm), weight (kg) and BMI;
6) Monitor vital signs;
7) 12 lead electrocardiogram;
8) Imaging: abdominal CT (digestive system), echocardiography;
9) Clinical laboratory tests:
   - HCG blood pregnancy test (women of childbearing age);
   - Serological detection of pathogens;
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - blood gas analysis;
   - Routine urine test;
   - Indicators of inflammation.
10) Severe disease severity score:
   - AARC grade;
   - MELD grade;
   - Child-Pugh score;
   - SIRS grade;
11) Clinical symptom assessment;
12) Criteria for selection and exclusion.
13) Special tests: TRAIL blood sample.

Participants must meet all criteria to participate in this study, and all screening procedures must be completed within 14 days of signing the informed consent form. After enrollment, patients will be randomly assigned to either the AS1501 group or the placebo treatment group, with combined therapy on top of conventional therapy.

7.2. stage of therapy

7.2.1. Stage IIa treatment period

For all inpatient days from day 1 to day 42, the subjects were required to complete the following basic items:

1) Basic treatment allowed by the programme;
2) Medication combination records;

3) Clinical symptom assessment;
4) Record adverse events.

During the study treatment period, items to be completed on the day of treatment evaluation or/and blood draw are described below.

Day 1 (first dose)

Subjects were required to complete the following items before and after the first dose of the study drug:

1) Basic treatment and combination therapy;
2) Weigh before the first dose;
3) check-up;
4) Biology: within 0.5h 5 min before administration, 1h 10 min, 2h 10 min, and 4h 20 min after infusion began;
5) 12 lead electrocardiogram: within 0.5h 10 min before administration, 0h± 10min, 1h 10 min, 2h 10min and 8 h 20 min after completion of D1 infusion;
6) Laboratory tests (results within 3 days prior to study drug administration):
   - HCG blood pregnancy test (for women of childbearing age only);
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - Routine urine test;
   - arterial blood gas analysis;
   - Indicators of inflammation;
7) Severe disease score (assessment results within 3 days prior to study drug administration):
   - Child-Pugh score;
   - ACLF Research Alliance (AARC) score;
   - End-stage liver disease model (MELD) score;
   - SIRS diagnose;
8) Cytokines: 4 ml was collected before the first dose (0.5h 5 min) and 0h after infusion completion (± 30min);
9) Immunogenicity: Blood was drawn 4ml within 0.5h 5 min before the first dose;

10) PK: 4ml was collected at 0.5h 5 min before the first dose, 1h± 5min after the start of infusion, 0h± 5min after the completion of the whole infusion, 1h± 5min, 2h± 5min and 8h± 10 min;
11) PD marker (TRAIL): 4ml blood was drawn before the first dose (0.5h 5 min) and 0h±5min after infusion;
12) Research drug therapy;
13) adverse event.

Days 2 to 14 (washout period after first dose)

During the elution period after the first dose, the subjects were required to complete examinations and assessments including:

1) Basic treatment and combination therapy;
2) Biomarkers: 144h (D7) and 312h (D14) after infusion completion;
3) 12 lead electrocardiogram: 144h (D7) and 312h (D14) after infusion completion;
4) Laboratory tests: 144h (D7) and 312h (D14) after infusion
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - Routine urine test;
5) Cytokines: collect 4ml venous blood 24h (± 30min) after infusion (D2);
6) Immunogenicity: 4ml of venous blood was collected at 144h (D7) and 312h (D14) after infusion;
7) PK: Collect 4ml venous blood at 24h±30min (D2),72h±30min (D4),144h±30min (D7) and 312h±30min (D14) after infusion;
8) PD marker (TRAIL): 4ml venous blood was collected at 144h± 30min (D7) and 312h± 30min (D14) after infusion;
9) adverse event.

Days 21 to 42 (continuous dosing period D21, D28, D35, D42)

To study the continuous drug administration period, the subject must complete the following items:

1) Basic treatment and combination therapy;
2) Body weight: D21/D28/D35/D42 before administration;

3) Physical examination: before D21/D28/D35/D42 administration;
4) Biomarkers: D21/D28/D35/D42 within 0.5h ± 10min before administration, 1h ± 10min after infusion start, 2h ± 10min, and 4h ± 20min;
5) 12-Lead ECG: Before D21/D28/D35/D42 administration (within 0.5h ± 10min), after the completion of D21/D28/D35/D42 infusion, at 0h ± 10min, 1h ± 10min, 2h ± 10min, and 8h ± 20min; if the subject exhibits abnormalities such as prolonged QTcF interval, the investigator may increase the frequency of examination based on the subjects condition; after administration, the investigator can assess and add ECG examinations;
6) Laboratory tests (to be performed within 24h prior to study drug administration at D21/D28/D35/D42):
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - Routine urine test;
7) Severe disease severity score (to be completed within 24h prior to study drug administration on D21/D28/D35/D42):
   - AARC grade;
   - MELD grade;
   - Child-Pugh score;
   - SIRS grade;
8) Cytokines: D21/D28/D35/D42 Blood was drawn 4ml before each infusion (0.5h ± 5min), 0h (± 30min) and 24h (± 30min) after each infusion;
9) Immunogenicity: 4ml of blood was drawn before D21/D28/D35/D42 administration;
10) PK: Blood samples were collected before D21/D28/D35 administration, before D42 administration and 1h±5min after infusion started, 0h±5min after infusion completion, 1h±5min, 2h±5min, 8h±10min, 24h±30min (D43),72h±30min (D45),144h±30min (D48),312h±30min (D55) respectively, with 4ml of venous blood collected;
11) PD marker (TRAIL): 4ml was collected before D21/D28/D35 administration, before D42 (the 4th continuous administration), 0h± 5min after infusion, 144h± 30min (day 48) and 312h± 30min (D55);
12) Research drug therapy;
13) adverse event.

7.2.2. Stage IIb treatment period

All inpatient days from day 1 to day 28, the subjects were required to complete the following basic items:

1) Basic treatment permitted by the programme;
2) Medication combination records;
3) vital sign;
4) Clinical symptom assessment;
5) Record adverse events.

During the study treatment period, the items to be completed on the day of treatment evaluation or/and blood collection are described below.

Day 1 to Day 28 (provisional weekly dosing, D1/D8/D15/D22,4 consecutive times)

During the study drug administration, the subject is required to complete the following items:

1) Basic treatment and combination therapy;
2) Randomization before D1 administration;
3) D1/D8/D15/D22 Body weight measurement before each dose;
4) Physical examination: before each dose at D1/D8/D15/D22;
5) Biomarkers: D1/D8/D15/D22 within 0.5h 10min before each dose, 1h 10min, 2h 10min, 4h 20min after infusion began;
6) 12-Lead ECG: D1/D8/D15/D22 within 0.5h 10min before each dose, 0h± 10min, 1h 10min, 2h 10min and 8 h 20min after each infusion;
7) Laboratory tests (to be performed before D1/D8/D15/D22 administration):
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - Routine urine test;
   - arterial blood gas analysis;
   - Blood pregnancy test (before D1 administration, limited to women of childbearing age, results can be accepted within 3 days);
   - Indicators of inflammation: before D1 administration (if the screening period is less than 3 days, it can be omitted);
7) Severe disease score (to be completed before D1/D8/D15/D22 administration):
   - Child-Pugh score;
   - AARC grade;

- MELD grade;
- SIRS grade;

8) Cytokines: Before each infusion (within 0.5h± 5min), 0h (± 30min) and 24h (± 30min) after each infusion, samples were collected from all veins with 4ml;
9) Immunogenicity: 4ml of venous blood was collected before D1/D8/D15/D22 administration;
10) PK blood collection: PK blood samples for trough concentration were collected before infusion at D8, D15 and D22, and PK blood samples for peak concentration were collected immediately after infusion at D1 and D22, with 4ml of venous blood collected for each sample;
11) Blood collection of PD markers (TRAIL blood sample): 4ml venous blood was collected before D1/D8/D15/D22 administration;
12) Study drug treatment: AS1501 and placebo were given at D1/D8/D15/D22 respectively.
13) Clinical symptom assessment;
14) adverse event.

7.3. follow-up period

7.3.1. The follow-up period of stage II a

End the treatment visit

Subjects who have withdrawn early or completed 4 consecutive doses will need to complete the end-of-treatment visit. Each subject needs to complete the following items:

1) Basic treatment and combination therapy;
2) vital sign;
3) check-up;
4) Weigh (kg);
5) 12 lead electrocardiogram;
6) Laboratory test items:
   - HCG blood pregnancy test (women of childbearing age);
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - Routine urine test;
   - arterial blood gas analysis;

- Indicators of inflammation;

6) Severe disease severity score:
   - Child-Pugh score;
   - AARC grade;
   - MELD grade;
   - SIRS grade;
7) Immunogenicity: venous blood was collected 4ml;
8) adverse event;
9) survival condition.

7.3.2. Follow-up period for stage IIb

End the treatment visit

Subjects who have withdrawn early or completed four consecutive doses will need to complete the end-of-treatment visit. Each subject needs to complete the following items:

1) Basic treatment and combination therapy;
2) weight;
3) vital sign;
4) check-up;
5) 12 lead electrocardiogram;
6) Laboratory test items:
   - Pregnancy test (for women of childbearing age only)
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - blood gas analysis;
   - Routine urine test;
7) Severe disease severity score:
   - Child-Pugh score;
   - AARC grade;
   - MELD grade;
   - SIRS grade;
7) PD (TRAIL blood sample collection): 4ml venous blood was collected;
8) adverse event;

9) Clinical symptom assessment;
10) Record of survival status and liver transplantation.

Day 60-7

Subjects who have withdrawn early or completed four consecutive doses will be required to undergo a D60 visit and complete the following items:

1) Basic treatment and combination therapy;
2) weight;
3) vital sign;
4) 12 lead electrocardiogram;
5) Laboratory test items:
   - Blood routine test;
   - Blood biochemical test;
   - Coagulation function test;
   - blood gas analysis;
   - Routine urine test;
6) Severe disease severity score:
   - Child-Pugh score;
   - AARC grade;
   - MELD grade;
   - SIRS grade;
7) Clinical symptom assessment;
8) Record of survival status and liver transplantation.

Day 90/7

Subjects who have withdrawn early or completed four consecutive doses will be required to undergo a D90 visit and complete the following items:

1) Basic treatment and combination therapy;
2) weight;
3) vital sign;
4) 12 lead electrocardiogram;
5) Laboratory test items:
   - Blood routine test;

- Blood biochemical test;
- Coagulation function test;
- blood gas analysis;
- Routine urine test;

6) Severe disease severity score:
   - Child-Pugh score;
   - AARC grade;
   - MELD grade;
   - SIRS grade;
7) Clinical symptom assessment;
8) Record of survival status and liver transplantation.

7.4. Sample collection, processing, storage and testing

7.4.1. Blood sample collection and pretreatment

P K Sample Collection: Collect about 4mL of whole venous blood into the collection tube. After collection, gently invert and mix the blood. Place the tube upright at room temperature for at least 30 to 60 min. After setting aside, centrifuge at 1500-1600 g, 2-8℃ for 10 min. Immediately after centrifugation, transfer the supernatant serum and evenly distribute it into two labeled cryovials. The volume of serum in both the test tube and backup tube should be no less than 500 μL.

PD Sample Collection: Primarily to detect serum TRAIL levels, collect about 4mL of whole venous blood into a collection tube. After collection, gently invert and mix the blood. Place the tube upright at room temperature for at least 30 to 60 minutes. After settling, centrifuge at 1500-1600 g, 2-8℃ for 10 minutes. Immediately after centrifugation, transfer the supernatant to two labeled cryovials, with each tube containing approximately 500 μL of serum.

Immunogenicity (ADA) Sample Collection: Collect about 4mL of whole venous blood into an uncoagulated collection tube. After collection, gently invert and mix the tube. Place the tube vertically at room temperature for at least 30 to 60 min. After setting, centrifuge at 1500-1600g,2-8℃ for 10 min. Immediately after centrifugation, transfer the supernatant serum into three labeled cryovials, with each vial containing approximately 500 μL of serum.

After distributing the serum, tightly seal the tube and place it with the cap facing up in the cryobox. The cryobox should be stored vertically in a-60℃ to-90℃ freezer until it is transported to the bioanalytical laboratory. Each sample has a unique sample number. The time interval between whole blood collection and storage in the freezer must not exceed 2 hours. Test samples are sent to the sample analysis laboratory in dry ice, while backup samples are stored at the clinical center.

The actual date and time of blood sample collection and the exact time of administration will be recorded in the original medical record and the PK, PD, ADA blood sample collection page of the eCRF. Problems encountered during blood sample collection will be noted in the original medical record and the eCRF.

7.4.2. Sample tube information label

Before blood sample collection, the blood collection tube and cryopreservation tube should be uniformly numbered and affixed with special labels.

The label shall include the following information: protocol number, subject number, dose group information, sample type (PK, PD, immunogenicity), collection time point, sample type (test sample, backup sample), etc.

7.4.3. Storage and transportation of samples

The biological samples used for testing are transported to the testing party by cold chain. The temperature is controlled at-60℃~-90℃ during transportation, and the whole process temperature monitoring is carried out. The transport temperature monitoring records, biological sample list and biological sample handover form are provided.

For biological samples that exhibit abnormal conditions during clinical trials, if no testing is required, the clinical institution should provide relevant explanations. When receiving biological samples, the labels, status, and quantity of the samples should be checked, and a record of the receipt should be made. The clinical trial unit should provide a transfer list containing information such as sample labels, status, quantity source, transportation method and conditions, and transport time. The testing unit should keep temperature monitoring data from the cold chain transportation process and store the biological samples in a temperature-controlled-60℃ to-90℃ refrigerator.

7.4.4. Sample testing

（1） PK sample testing:

PK blood sampling time point

Phase IIa: Blood samples were collected at the following time points: before D1 (first dose) infusion start (within 0.5h ± 5 min), 1h ± 5min after D1 infusion start, 0h ± 5min after infusion completion, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 30min (D2),72h ± 30min (D4),144h ± 30min (D7),312h ± 30min (D14), before D21/D28/D35 dosing, before D42 dosing, and 1h ± 5min after infusion start, 0h ± 5min after infusion completion, 1h ± 5 min, 2h ± 5 min, 8h ± 10 min, 24h ± 30min (D43),72h ± 30min (D45),144h ± 30min (D48), and 312h ± 30min (D55). A total of 23 PK blood sampling time points were planned, with 4ml of venous blood collected and placed in serum tubes for PK analysis.

Phase IIb: PK blood samples of the subject were collected before infusion at D8, D15 and D22, and peak concentration PK blood samples were collected immediately after infusion at D1 and D22. A total of 5 PK blood sampling time points were tentatively determined.

PK analyse

AS1501 Blood drug concentration is completed by a third-party bioanalytical laboratory and tested by enzyme-linked immunosorbent assay (ELISA). The testing method needs to be validated and the test results are reported independently. All blood samples of patients receiving treatment in phase II b should be tested in a blind manner, and 10% of the blood samples should be retested.

PK evaluating indicator

Calculate the average values at each time point based on individual blood drug concentrations, and plot the individual or mean blood drug concentration-time curves. Statistical analysis of drug-related PK parameters such as peak concentration (Cmax), time to peak (Tmax), area under the blood drug concentration-time curve (AUC), mean residence time (MRT), apparent volume of distribution (Vd), total clearance rate (CL/F), and volume of distribution (Vz/F) should be conducted.

（2） PD sample test:

The time point for PD blood collection

Stage IIa: Screening period, before infusion on D1 (within 0.5h ± 5min), 0h ± 5min after completion of infusion on D1, 144h ± 30min (on D7),312h ± 30min (on D14), before administration on D21/D28/D35, and 0h ± 5min before and after infusion on D42 (the fourth consecutive dose) and 144h ± 30min (on D48),312h ± 30min (on D55). A total of 12 PD blood sampling time points were selected, with 4ml venous blood drawn at each time point.

Phase IIb: TRAIL blood samples were collected at six time points, including screening, before D1/D8/D15/D22 administration and end of treatment visit.

PD biomarkers

The biomarkers mainly include the drug target protein TRAIL.

（3） Immunological sample testing

Phase II a: Before D1, before D7, D14, D21/D28/D35/D42, and at the end of treatment visit, immunogenic blood samples were collected at 8 sampling time points, and 4ml of venous blood was collected for each sample.

Phase IIb: 4ml of venous blood was collected before D1/D8/D15/D22. During the trial, the design of subsequent immunogenic blood sampling points may be adjusted according to the results of previous immunogenicity tests.

Immunogenicity (ADA) testing is performed by a third-party bioanalytical laboratory designated by the sponsor. The testing method is determined after validation. The test analysis results are reported independently and are not presented in the study summary report.

8. Study evaluation

8.1. Demographic and other baseline characteristics

Demographic and baseline characteristic variables will be summarized by dose group. Continuous variables (age, height, weight, etc.) will be summarized using descriptive statistics, and categorical variables (sex, race, etc.) will be summarized using frequency and percentage. Related summaries will be performed for each analysis set.

8.2. PK, PD estimate

8.2.1. Pharmacokinetic assessment

For each subject receiving the study drug, pharmacokinetic parameters were determined from the plasma concentration-time curve.

Main PK parameter: C_{max},T_{max}, $t_{1/2}$, CL, AUC_{0-t}, AUC_{0-inf}, R_{AUC}, R_{Cmax}class.

Other PK parameters: CL/F (total clearance), λ z (terminal rate constant), Vz/F (distribution volume), Vss (steady state distribution volume) and so on.

8.2.2. PD index evaluation

For each subject receiving the study drug, pharmacokinetic parameters were determined based on serum TRAIL levels before and after administration.

8.2.3. Group PK/PD assessment

According to the data characteristics and the mechanism of drug action, appropriate models were selected for population PK/PD or PPK studies to explore covariates affecting PK/PD.

8.3. Efficacy evaluation

Phase IIa efficacy analysis

The key evaluation of the effectiveness was to evaluate the biochemical, blood routine, coagulation function, and blood gas analysis test indicators collected after four consecutive doses, and to evaluate the changes in TBil, PT-INR or PTA, AST/ALT, ALB, Pre-ALB, Scr, LA, blood ammonia, MELD score, Child-Pugh score, AARC score, SIRS score, and infection-related indicators.

The effectiveness evaluation mainly includes the following indicators:

Multiple dosing periods (a total of 4 times, with D21 as the first multiple dosing period) were evaluated

- Baseline: all test results prior to D21 administration

- Changes in MELD score, AARC score and Child-Pugh score from baseline to end of treatment;
- Survival at end of treatment;
- Changes in TBil, DBil, PT-INR, ALB, Pre-ALB, AST/ALT, blood ammonia, serum creatinine and serum lactate from baseline to end of treatment;
- Baseline complications such as ascites or hepatic encephalopathy changes (stages, grades) at end of treatment;

Phase IIb efficacy analysis:

The effectiveness evaluation focuses on the efficacy data within 28 days after the first dose and collects efficacy data for the follow-up period of 90 days. Data collection methods include: mortality or liver transplantation within 28 days after randomization; complications of liver failure. Blood biochemical, blood routine, and coagulation function test indicators are collected at the screening stage, 24 hours before each dose, and on day 28, and the changes in TBil, PT-INR or PTA, AST/ALT, ALB, Pre-ALB, Scr, LA, blood ammonia, MELD score, Child-Pugh score, AARC score, SIRS score, and infection-related indicators are evaluated. At the same time, the main clinical biochemical indicators, AARC score, survival status, liver transplantation situation, and mortality are assessed at days 60 and 90 of the follow-up period.

The effectiveness evaluation mainly includes the following indicators:

- Baseline: all the scores and examination results before D1 administration;
- Change in AARC-ACLF score from baseline to day 28 after first dose;
- Survival at day 28 after the first dose;
- 90-day all-cause mortality;
- 90-day liver transplant rate;
- Baseline to 28-day changes in MELD score, AARC score, and Child-Pugh score;
- Baseline to 28,60,90 days TBil, DBil, PT-INR, ALB, Pre-ALB, AST/ALT, blood ammonia, serum creatinine and serum lactate changes;
- Baseline complications such as ascites or hepatic encephalopathy changes within 28 days (stages, grades);
- Duration of hospitalization after initial trial drug treatment.

End-stage liver disease model (MELD) score

The End Stage Liver Disease Model (modelforend-stageliverdisease, MELD) is a system primarily used to evaluate end-stageliverdisease by assessing serum bilirubin, international standardized ratio of prothrombin time, and serum creatinine levels. The MELD score can predict mortality in patients with end-stage liver disease and is widely applied in liver transplantation, severe hepatitis, and hepatocellular carcinoma assessment. This study adopted an improved formula: MELD=3.8 * ln[TBil (mg/dl)] + 11.2 * ln[INR] + 9.6 * ln[SCr (mg/dL)] + 6.4 * (cause: 0 for cholestatic or alcoholic liver disease, 1 for other causes), with results rounded to the nearest integer. For detailed criteria, see Attachment 5.

Child-Pugh classification

The Child-Pugh classification is a commonly used clinical grading system for quantitatively assessing liver reserve function in patients with cirrhosis. This standard was first proposed by Child in 1964. At that time, Child divided patients into three levels based on five indicators (including general condition, ascites, serum bilirubin, serum albumin concentration, and prothrombin time), each scored as 1 point, 2 points, or 3 points. The scores of these five indicators are added together, with the lowest total score being 5 points and the highest being 15 points. Based on this total score, liver reserve function is classified into three grades: A, B, and C, indicating three different levels of liver damage (the higher the score, the poorer the liver reserve function). For more details on the scoring criteria, see Attachment 3.

Asia Pacific Association for the Study of the Liver (AARC) score

In 2017, the Asia-Pacific Association for the Study of the Liver (APASL) included 1,402 patients with ACLF who met APASL criteria. The study found that bilirubin, hepatic encephalopathy grade, INR, lactate, and creatinine were factors influencing patient prognosis. It also introduced the ACLF-AARC score, which ranges from 5 to 15 points and is divided into three levels: Level I from 5 to 7 points, Level II from 8 to 10 points, and Level III from 11 to 15 points. The corresponding 28-day mortality rates are 12.7%,44.5%, and 85.9%, respectively. The AARC score is relatively easy to calculate, with reference indicators readily available in clinical practice, and it allows for dynamic monitoring of patient outcomes. For more details on the scoring criteria, see Attachment 4.

8.4. Safety and other assessments

Life signs, physical examination, laboratory (complete blood count, biochemical blood, routine urine, coagulation function) and 12 lead electrocardiogram examinations, adverse events and serious adverse events.

vital sign

The vital signs examination (temperature, pulse, respiration, and blood pressure) will be performed at the time points of the study flow chart and the trial flow plan.

Reference range of vital signs: systolic blood pressure 90~140mmHg, diastolic blood pressure 60~90mmHg (sitting)

Pulse 60~100 times /min

Breath 12~20 times /min

Temperature 35.8~37.2oC (forehead, including boundary value)

check-up

Physical examination (general condition, head, neck, lymph nodes, chest, abdomen, musculoskeletal, skin, nervous system and other areas) will be performed at the planned time points in the study flow chart.

12 lead ECG

For each subject, a twelve-lead electrocardiogram (ECG) will be collected at the planned time points according to the study flow chart. All ECG tests are required to be performed at least 5 minutes after the subject is at rest. The subject must be in the supine position and remain awake during the collection of ECG.

laboratory examination

The following laboratory tests will be performed at the time points planned in the study flow chart.

Table 16 Laboratory tests

Laboratory Evaluation	**parameter**
routine blood test	White blood cell count (WBC), neutrophil count (ANC), percentage of neutrophils, percentage of eosinophils, percentage of basophils, lymphocyte count (LYM), percentage of lymphocytes, monocyte count (MONO), percentage of monocytes, red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT)
Blood biochemistry	Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin (ALB), pre-albumin (Pre-ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), creatinine (CREA), urea (UREA), uric acid (UA), potassium (K), sodium (Na), chloride (Cl), calcium (Ca), magnesium (Mg), phosphorus (P), glucose, lactate dehydrogenase (LDH), creatine kinase, and uric acid.
coagulation	Prothrombin time (PT), international standardized ratio (INR), activated partial

function	thromboplastin time (APTT), fibrinogen (FIB).
Virus screening	Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), hepatitis C antibody (Anti-HCV), hepatitis B virus DNA (HBV-DNA), hepatitis C virus RNA (HCV-RNA), human immunodeficiency virus antibody (HIV), syphilis spirochete antibody.
URAN	PH Glucose, protein, occult blood, ketone bodies, bilirubin, urobilinogen, nitrite, white blood cells, specific gravity.
Indicators of inflammation	C-reactive protein (CRP), procalcitonin, etc.
arterial blood gas analysis	pH (pH), partial pressure of oxygen (PO2), arterial oxygen saturation (SaO2), partial pressure of carbon dioxide (PCO2), lactate, etc.
pregnancy tests	Blood pregnancy tests are required only in women of reproductive age during screening, prior to the first dose, and at the end of treatment visit. Additional serum pregnancy tests may be performed by the investigator as indicated clinically.
cell factor	At least TNF-α and IL-6 should be included. Researchers can add other cytokine detection items according to the feasible cytokine detection spectrum of each center based on the condition of subjects.

9. Adverse events and serious adverse events

9.1. adverse event

9.1.1. Definition of adverse events

Adverse events (AE) refer to all adverse medical events that occur in subjects after receiving the investigational drug, which can be manifested as symptoms and signs, diseases or laboratory test abnormalities, but do not necessarily have a causal relationship with the investigational drug.

AEs include, but are not limited to, the following: (1) worsening of preclinical medical conditions/diseases (including worsening of symptoms, signs, and laboratory test abnormalities); (2) any new medical conditions (including symptoms, signs, and newly diagnosed diseases); (3) abnormal clinically significant laboratory test values/results.

9.1.2. The severity of the adverse event

Grading refers to the severity of adverse events. The severity of AE is determined according to the CTCAE5.0 standard of the U.S. Department of Health and Human Services. If an AE not listed in the table appears, the severity of AE is graded according to Table 17.

Table 17 Severity of adverse events

grade	CTCAE circumstances
1	Mild: asymptomatic or mild; only clinically or diagnostically visible; no treatment required.

2	Moderate: requires small, localized or non-invasive treatment; restricted instrumental daily activities of living comparable to age*
3	Serious or medically significant but not immediately life-threatening: hospitalization or prolonged length of stay; disability; restriction of daily activities of self-care **.
4	Life-threatening: Requires urgent medical treatment.
5	Deaths associated with adverse events.

CTCAE= The Terminology Standard for Adverse Events

*: Instrumental daily activities refer to cooking, buying clothes, using the telephone, financial management, etc.

**: Self-reliant daily activities refer to bathing, dressing, eating, washing and taking medicine, etc., without being bedridden.

9.1.3. Judgment of drug-related adverse events

The causal relationship assessment between AE and study treatment in this study will be conducted using a five-category system: definitely related, probably related, possibly related, possibly unrelated, and definitely unrelated.

Table 18 Relationship between adverse events and study drugs

	Sure thing	Its probably relevant	It might be relevant	It may not matter	I dont think it matters
RESEARCH DRUGS HAVE A REASONABLE CHRONOLOGICAL ORDER	+	+	+	+	-
KNOWN TYPES OF DRUG RESPONSE	+	+	+	-	-
THE REACTION IS REDUCED OR DISAPPEARED AFTER DISCONTINUATION OF THE DRUG	+	+	±	±	-
THE REACTION OCCURRED AGAIN AFTER RE-ADMINISTRATION	+	?	?	?	-
IT CANT BE EXPLAINED BY THE SUBJECTS DISEASE	+	+	-	±	-

Note: + positive-negative ± possible? Unknown

- Causality between adverse events and investigational drugs

The causal relationship between adverse events and investigational drugs should be assessed by the investigator based on all available information when filling in the eCRF.

For all AE, researchers should separately evaluate the causal relationship between each event and each investigational drug. If the researcher determines that an event cannot be definitively attributed to a specific investigational drug (for example, suspected potential interactions), the same assessment should be recorded for each study treatment. The evaluation of causality should be conducted by an authorized clinical physician, who must not only determine whether there is a causal relationship with the investigational drug but also provide as much justification as possible for their judgment.

Important factors to be considered in assessing the causal relationship between an adverse event and a investigational drug include:

a. Temporal relationship to administration: The event should occur after administration. The length of time from drug exposure to the occurrence of the event should be evaluated in the clinical context of the event.

b. Reactions after discontinuation (de-activation) and after re-administration (re-activation): The patients response after de-activation or re-administration should be assessed in the context of the common clinical course of related events.

c. Basic diseases, combined diseases, and concurrent diseases: each event should be evaluated in combination with the current treatment of the disease and the natural history and course of other diseases that the patient may have.

d. Combination medication or treatment: Other medications or treatments that the patient is receiving at the time of an adverse event should be examined to determine whether they may have contributed to the event.

e. Known types of reactions (clinical/clinical pre) for these drugs

f. Physiological/psychological stress exposure: Stress exposure may induce adverse changes in patients and provide a more plausible explanation for the event.

g. Pharmacology and pharmacokinetics of therapeutic studies: the pharmacokinetic properties of therapeutic studies (absorption, distribution, metabolism and excretion) and pharmacodynamics in individual patients should be considered in combination.

O Judgment of drug-related adverse events

The adverse reactions of the test drugs were combined into "affirmatively related", "likely to be related" and "possibly related", and the incidence of adverse reactions was calculated accordingly.

The classification and basis of the determination results can be referred to Table 18.

special explanation:

a. The correlation between adverse events and drugs was evaluated by personnel with medical expertise.

b. Table 18 may not cover all the situations in actual work. If the judgment criteria in Table 18 cannot be fully corresponding, the professional judgment logic of adverse events and drug correlation in the table can be referred to to make as reasonable a judgment result as possible.

c. When more information and evidence about the correlation between adverse events and drugs are collected during the clinical trial, sufficient reasons should be provided if it is necessary to modify the previous correlation judgment results.

9.1.4. Collection of adverse events

When collecting adverse event information from participants, a consistent non-injurious questioning method should be used. Adverse event collection will begin when the participant signs an informed consent form and continue until the end of the safety follow-up period (28 days ± 7 days after the last dose) or until the participant starts new liver protection treatment (such as artificial liver support, liver transplantation, etc.). Researchers must monitor the health status of patients during the trial. Clinical adverse events occurring between signing the informed consent form and before the first dose are recorded as medical history/comorbidities on the CRF and are not recorded as AE unless one of the following conditions applies:

- Any injury/damage caused by clinical laboratory examination procedures;
- Adverse events caused by discontinuation of medication related to the trial protocol;
- Adverse events caused by drugs other than investigational drugs taken as part of a treatment regimen.

After the end of the safety follow-up period (28 days ± 7 days after the last dose), AE will no longer be actively collected. However, if the investigator learns that there is a reasonable causal relationship between the trial drug and an SAE, it should still be reported to the sponsor (or CRO appointed by the sponsor).

The sources of AE include:

- Patient responses to questions about his/her health status (standard non-guiding questions are asked at each visit, such as "How have you felt since your last visit?").
- Symptoms reported spontaneously by the patient.
- The investigator evaluates the findings as clinically significant changes or abnormalities of the findings or examination.
- Other information about the patients health that the researcher has learned (e.g., hospitalization).

9.1.5. Recording of adverse events

Researchers must document all adverse events in the adverse event table provided by each patient on the eCRF, which should include the following information:

- Name of adverse event
- Date and time of start (time can be omitted if not applicable)
- order of severity
- Relationship to investigational drug
- Measures taken for the test drug
- Other measures taken
- Date and time of transfer (if not applicable, the date can be omitted)
- lapse to
- Whether it is a serious adverse event and the corresponding severity criteria

➢ **Name of adverse event**

The name of an adverse event should be a medical term and prioritize the use of medical diagnoses. If a definitive diagnosis cannot be made, individual symptoms/signs should be recorded separately. When subsequent diagnoses become clear, update the diagnosis to replace the previous symptoms/signs with the new one. If the same adverse event occurs more than once in a patient and the patient recovers between episodes, the adverse event should be recorded separately according to the number of occurrences.

Note that the measures taken are not adverse events, but the reasons for taking them are. Hospitalization is not an adverse event, but the reasons for hospitalization are. Death is not an adverse event, but the causes of death are (sudden death of unknown cause can be recorded as "death of unknown cause").

➢ **Measures taken for the test drug**

Measures taken against the investigational drug to address an adverse event must be classified as one of the following:

- Permanent discontinuation of drug-the investigational drug is permanently discontinued due to a specific AE.
- No change in dose-specific AE does not require a change in the drug regimen of the investigational drug and continues to be administered.
- Dose reduction-dose reduction due to a specific AE.
- Drug suspension-temporary interruption (suspension) of the investigational drug due to a

specific AE (including voluntary discontinuation by the subject) and resumption of drug use thereafter.

- Not applicable-discontinuation of the investigational drug for reasons other than a specific AE, such as termination of the study, death of a subject, or discontinuation of the investigational drug prior to the occurrence of an AE.

➢ **Other measures taken**

Adverse events requiring treatment must be treated according to recognized medical standards to protect the health and interests of patients. Appropriate cardiopulmonary resuscitation equipment and medicines must be available to ensure that the best possible treatment is provided in an emergency.

If a medication is used to treat an adverse event, the medications used should be documented in the combined treatment record.

➢ **End date and time**

The end date of AE should be filled in accordance with the requirements of CRF and SAE report form.

➢ **lapse to**

The outcome of an adverse event must be classified as one of the following:

- Recovery/cure: indicate the (serious) end date of the adverse event.
- Improvement/relief: recovery from the condition / improvement or stabilization.
- Unrestored / Unhealed / Persistent: The event is ongoing.
- Residual effects of recovery/cure: Only when the subject has residual effects that last for a long time or for life, such as blindness caused by diabetes, hemiplegia caused by stroke. The (severe) adverse event termination date should be noted.
- Death: When the adverse event is fatal, the time of death should be recorded.
- Unknown: The investigator was unable to learn of adverse events, such as loss of follow-up.

9.1.6. Follow-up of adverse events

After the first AE report, researchers are required to proactively follow up with each participant and provide the sponsor with further information about the participants condition. The frequency of follow-ups should be determined based on the severity of the adverse event, clinical practice guidelines, and the trial protocol. If an adverse event that was not resolved during the initial visit is encountered again, it should be re-inquired about and recorded during the next visit; if there are any concomitant medications, they should be collected and documented; if treatment was received at a local hospital, efforts should be made to gather records of local hospital management and medication

information. Specific requirements should comply with the relevant SOPs of the healthcare institution.

AEs that have not fully recovered or stabilized at the end of the safety follow-up period (regardless of causality) must be followed up until recovery/cure (return to baseline level or cure) with or without sequelae, clinical stability, reasonable explanation, subject death or loss to follow-up.

9.2. Serious adverse events（ SAE）

9.2.1. Definition of serious adverse events

Serious adverse events (SAE) refer to death, life-threatening, permanent or severe disability or loss of function, hospitalization or prolonged hospital stay, congenital abnormalities or birth defects, and other important medical events that occur after the subject receives the investigational drug.

Table 19 Definition of serious adverse events

SAE definition	rudder
die	AE was the direct or major cause of death in the subject.
threat to life	The occurrence of an adverse event that immediately poses a risk of death to the subject. This does not include adverse events that may lead to death after severe progression, such as drug-induced hepatitis without liver failure.
Permanent or severe disability or loss of function	Any adverse event that results in injury, impairment or destruction of the subjects function, physiological structure or both, physical activity or quality of life.
Hospitalization or extended hospital stay is required	Events that occur in hospitalized subjects requiring expanded treatment should be recorded as SAEs. Examples of such events include transfer from routine hospital care to the intensive care unit (ICU), or if the event results in an extended planned length of stay. The following hospitalizations should not be considered as SAE: • Observation in the emergency department or other hospital departments for no more than 24 hours without resulting in hospitalization (unless it is considered an important medical event or life-threatening); • Scheduled elective surgery prior to signing the informed consent; • Hospitalization required for routine health examinations (such as routine colonoscopy); • The hospitalization is not for the purpose of disease treatment, and it is planned before enrollment, so there should be a case to check; • Admission is not related to health status and does not require medical/surgical intervention (e.g., family reasons).
Congenital abnormalities or birth defects	The offspring of the subjects have congenital abnormalities and deformities.
Other important medical events	Medical and scientific judgment must be applied to decide whether to expedite reporting for other conditions. For instance, significant medical events that may not

	immediately endanger life, death, or hospitalization can still be considered severe if medical measures are needed to prevent such occurrences. Examples include critical treatments in the emergency room, allergic bronchospasm occurring at home, cachexia or seizures not requiring hospitalization, and the development of drug dependence or addiction

9.2.2. Reporting of serious adverse events

In clinical trials, any serious adverse event, regardless of whether it is related to the investigational drug, should be promptly managed by the investigator. The investigator must complete the Sponsors provided "Severe Adverse Event (SAE) Report Form" within 24 hours of becoming aware of the event, sign it, and date it before submitting it to the Sponsor and (or) the CRO appointed by the Sponsor. The SAE should detail the description of symptoms, severity, time of occurrence, time of management, measures taken, follow-up time and method, as well as the outcome. In cases involving fatalities, the investigator should provide additional required information to the Sponsor and the ethics committee, such as autopsy reports and final medical reports.

Researchers must provide their assessment of causality when reporting serious adverse events. If the researchers assessment of causality is missing or unavailable, the sponsor or the CRO appointed by the sponsor (based on the project contract) should immediately contact the researcher and send an urgent medical inquiry request for the assessment. If the researcher is temporarily unable to provide a causality assessment, the sponsor will make a judgment until the researchers assessment can finally be obtained.

If the investigator is not sure whether an adverse event is a serious adverse event, it is considered a serious adverse event until its nature has been proven.

After receiving the relevant safety information of clinical trials provided by the sponsor, the investigator should sign for reading in time, consider whether to adjust the treatment of the subject, communicate with the subject as soon as possible if necessary, and report the suspicious and unexpected serious adverse reactions provided by the sponsor to the ethics committee.

9.2.3. Follow-up of serious adverse events

The follow-up requirements of SAE are the same as those of AE. The investigator should timely fill in the Serious Adverse Event (SAE) report form and report to the sponsor and CRO appointed by the sponsor (according to the project contract) according to the SAE reporting process.

9.2.4. Suspected unexpected serious adverse reaction

The following is based on the Standards and Procedures for Rapid Reporting of Safety Data During Drug Clinical Trials issued by the CDE.

9.2.5. Definition of suspected unexpected serious adverse reactions

Suspected and Unexpected Serious Adverse Reactions (Suspected Unexpected Serious Adverse Reaction, SUSAR) refer to serious adverse reactions whose nature and severity exceed the information provided in the study drug investigators manual, the package insert for marketed drugs, or the product characteristic summary, and are suspected and unexpected. Unexpected adverse reactions refer to adverse reactions whose nature, severity, consequences, or frequency differ from the anticipated risks described in the current relevant information about the investigational drug (such as the investigators manual). The investigators manual serves as a primary reference for determining whether an adverse reaction is expected or unexpected. For example: (1) Acute renal failure is listed as an adverse reaction in the investigators manual, but interstitial nephritis occurs during the trial, which should be judged as an unexpected adverse reaction; (2) Pneumonia is listed as an adverse reaction in the investigators manual, but acute severe pneumonia occurs during the trial, which should also be judged as an unexpected adverse reaction.

9.2.6. Report of suspected unexpected serious adverse reaction

Upon receiving any safety-related information from any source, the sponsor shall immediately analyze and evaluate it, including its severity, relevance to the investigational drug, and whether it constitutes an expected event. The sponsor should promptly report SUSAR to all investigators involved in the clinical trial, the clinical trial institution, the ethics committee, as well as the drug regulatory authority and health authorities.

（1） Start/end time of rapid report: The start date is the date of clinical trial approval or the implied start date of the national drug review agency, and the end date is the date when the last domestic subject follow-up is completed (unexpected serious adverse reactions occurring after the end of clinical trial follow-up and before the review approval conclusion should also be reported by rapid report).

（2） The deadline for the rapid report

- Deadly or life-threatening SUSAR: report the first time within 7 days, and report and improve the follow-up information within the following 8 days.
- Non-fatal or life-threatening SUSAR: first reported within 15 days.
- The day when the sponsor or CRO (signing according to the project contract) first learns of the signed version of the valid report is day 0.

9.2.7. Follow-up to the rapid report

After the first report (SUSAR), the sponsor shall continue to follow up serious adverse reactions and timely submit relevant new information or changes in the previous report in the form of follow-up

reports within 15 days from the acquisition of new information;

9.3. Reporting of other potentially serious safety risk information

（1） Other potential serious safety risks should also be reported to the national drug review agency as soon as possible, and medical and scientific judgments should be made for each case.

（2） In general, information that significantly affects the risk-benefit assessment of a drug or that may consider a change in the use of the drug, or information that affects the overall drug development process, falls into this category.

I. For known and serious adverse reactions, the incidence is increased and clinically important;

II.There is obvious harm to the exposed population, such as ineffective drugs in treating life-threatening diseases;

III.Significant safety findings (such as carcinogenicity) in recently completed animal studies.

IV.The latest global safety warning for a similar drug or the latest safety hazard disclosed in an epidemiological survey or academic conference paper recently completed.

9.4. Adverse events of particular concern (ASEI)

Adverse events of particular concern (AESI) refer to those that require close monitoring in order to enhance understanding of the safety of the investigational drug. Adverse events of particular concern in this study include:

- hypersensitivity:
- Cytokine release syndrome;

9.5. encyesis

Male and female participants of reproductive age must agree to use effective, medically approved contraception from the start of medication to six months after discontinuation. Researchers should emphasize the time limit for contraceptive use to the participants when they are informed, and advise them on the potential effects of the investigational drug on the fetus.

During the study, female participants should immediately discontinue the investigational drug and inform the investigator if they become pregnant. Female participants must withdraw from the study. Investigators should complete the "Pregnancy Report Form" within the same timeframe as for serious adverse events and report it to the sponsor and the CRO designated by the sponsor within 24 hours, followed by monitoring of pregnancy outcomes (such as termination or delivery) until the end of the pregnancy/newborn birth three months later. Male participants who have a pregnancy with their female partner during treatment should also immediately notify the investigator. The investigator will obtain a separate informed consent form from the female partner to collect pregnancy-related information and follow up on her partner until the end of the pregnancy/newborn birth three months

later.

Pregnancy is not a SAE, but abnormal pregnancy outcomes (natural miscarriage, medical reasons for abortion, fetal/newborn congenital abnormalities, malformations or death, etc.) should be recorded and reported as SAEs when the pregnancy report is completed. The subject should be monitored and cared for appropriately until the end of the pregnancy.

10. Statistical considerations

The detailed summary and statistical analysis methods for the data collected in this study will be included in the Statistical Analysis Plan (SAP). The SAP will be developed after the protocol and CRF are finalized, and it will be finalized before database locking and data unblinding. The SAP will specify and describe all statistical analyses planned according to the main characteristics of the protocol. Any changes to this protocol, if determined by the sponsor or principal investigator to have a significant impact on the statistical analysis plan, require a revision of the SAP to align with the research protocol.

10.1. Statistical hypothesis

10.1.1. Sample size estimation

This study is a multicenter phase II clinical study. Phase II a is the dose exploration stage, which will sequentially explore the safety and tolerability of 0.5mg/kg, 1mg/kg and other doses. Each dose group is planned to include 12 subjects. The sample size of the dose exploration stage does not need to be calculated based on statistical assumptions.

Phase IIb is randomized, double-blind, and placebo-controlled. The appropriate dose and primary efficacy endpoint will be selected based on the results of Phase IIa, without estimating the required sample size based on statistical assumptions. For exploratory efficacy purposes, a total sample size of 72 cases (36 in the AS1501 group, 36 in the placebo group) is provisionally set.

10.1.2. Analyze the crowd

IIa phase analysis population

Subjects will be analyzed according to the following analysis sets:

- Safety Analysis Set: All subjects who received at least one dose (or any part of a single dose) of study drug (AS1501) treatment and had safety metrics recorded. This analysis set will be used for all safety analyses, with subjects analyzed according to the actual treatment received.
- Full analysis set: All subjects who received at least one dose of study drug (AS1501) and had a compliant post-treatment efficacy assessment (liver function or coagulation function). This

analysis set will be used for all efficacy analyses and will be analyzed according to randomized treatment.

- Conforming to the protocol analysis set: all subjects who meet the inclusion criteria, do not meet the exclusion criteria, have completed at least 3 (75%) consecutive dosing periods of study drug treatment, have no major protocol deviation or violation, and have achieved 42 days of efficacy indicators. This analysis set will be used for sensitivity analysis of the FAS-based efficacy analysis.
- Pharmacokinetic Analysis Set: All participants who received at least one dose of AS1501 treatment and completed at least 60% (3/5) of the intensive blood sampling points after the first continuous dosing, with no significant deviations from the PK evaluation metrics (Cmax, AUC, etc.), and at least one evaluable PK parameter. This analysis set will be used for all pharmacokinetic analyses, with participants analyzed based on their actual treatment received.
- Pharmacokinetic Analysis Set: All subjects who received at least one dose of the study drug and had at least one pharmacokinetic (biomarker) data after treatment. Subjects will be analyzed according to treatment.
- Immunogenicity Analysis Set: All subjects who received at least one dose of study drug and had at least one post-treatment immunogenicity data. This analysis set will be used for immunogenicity analysis, and the subjects will be analyzed according to the actual treatment they received.

IIb phase analysis population

Subjects will be analyzed according to the following analysis sets:

- Safety analysis set: all subjects who received at least one dose (or any part of one dose) of the study drug (AS1501 or placebo) and had safety metrics recorded. This analysis set will be used for all safety analyses, with subjects analyzed according to the actual treatment received.
- Full analysis set: all randomized subjects who received at least one dose of the study drug (AS1501 or placebo). This analysis set will be used for all efficacy analyses, and subjects will be analyzed according to randomized treatment.
- Conforming to the protocol analysis set: all randomized subjects who meet the inclusion criteria, do not meet the exclusion criteria, have completed at least 3 doses (75%) of study drug (AS1501) treatment, and have not experienced significant protocol deviation or violation. This analysis set will be used for sensitivity analysis of the FAS-based efficacy analysis.
- Pharmacokinetic Analysis Set: All participants who received at least one dose of the study drug (AS1501) and completed at least 60% (3/5) of blood sampling points, with no significant

deviations in PK evaluation parameters (such as Cmax, AUC), and at least one evaluable PK parameter. This analysis set will be used for all pharmacokinetic analyses, with participants analyzed based on their actual treatment received.

- Pharmacokinetic Analysis Set: All randomized subjects who received at least one dose of the study drug (AS1501 or placebo) and had at least one pharmacokinetic (biomarker) data after treatment. Subjects will be analyzed according to randomized treatment.
- Immunogenicity Analysis Set: All subjects who received at least one dose of study drug (AS1501 or placebo) and had at least one post-treatment immunogenicity data. This analysis set will be used for immunogenicity analysis, and subjects will be analyzed according to randomized treatment.

10.2. statistical analysis

10.2.1. conventional method

The statistical description of categorical variables is expressed using the number of cases and percentages (%); for continuous variables, it includes the number of cases, missing values, mean, standard deviation, median, lower quartile, upper quartile, minimum value, and maximum value. Unless otherwise specified, hypothesis tests will use two-tailed tests with a significance level of 0.05, and confidence interval estimates for parameters will be based on a 95% confidence interval.

10.2.2. Subject distribution

The enrollment, medication, dropout, exclusion and data set division of each group of subjects were summarized, and the flow chart of subject distribution was used to present.

The list describes the details of the subjects who were removed.

10.2.3. Demographics and baseline characteristics

Statistical descriptions of baseline characteristics for each group, including demographic and laboratory test results, were performed. Descriptive statistics for the baseline of the program were discussed to determine whether statistical inference was performed.

10.2.4. Pharmacokinetics and pharmacodynamic analysis

Pharmacokinetic assessment

The blood drug concentrations of the subjects will be summarized by dose group and sample collection time points. Pharmacokinetic parameters will be calculated using a non-atrial model and summarized by dose group using descriptive statistics. The peak time (Tmax) statistics include the number of non-missing values, median, minimum, and maximum. Other parameter statistics include the number of non-missing values, mean, standard deviation, coefficient of variation, median, minimum, and maximum. Pharmacokinetic analysis will be based on the pharmacokinetic analysis set

and the actual treatment received by the subjects.

PD index evaluation

Efficacy kinetics (biomarkers) The measured values of each visit and their changes from baseline will be summarized by dose group using descriptive statistics. Efficacy kinetics analysis will be based on the pharmacokinetic analysis set.

Group PK/PD assessment

According to the data characteristics and the mechanism of drug action, appropriate models were selected for population PK/PD or PPK studies to explore covariates affecting PK/PD.

10.2.5. safety analysis

Adverse events and adverse reactions:

Analysis of adverse events and adverse reactions during drug administration, summary of the number, times and percentage of adverse events, adverse reactions, termination of trial due to adverse events, death due to adverse events, and serious adverse events in each group; if necessary, inter-group comparison of incidence.

According to the MedDRA Dictionary, adverse events and adverse reactions are systematically classified by organ (SOC) and preferred terms (PT), and the number, frequency and percentage of adverse events and adverse reactions are statistically calculated according to SOC/PT classification:

The number, frequency and percentage of adverse events and adverse reactions were classified according to SOC/PT and severity. A single type of adverse event in a subject was counted only once under the highest severity item of the same term (SOC or PT).

All adverse events (including adverse events during non-medication), adverse reactions, serious adverse events, termination of study due to adverse events, death due to adverse events, and special concern adverse events were listed separately.

laboratory examination:

The statistical time points of each laboratory test item include baseline, post-baseline visit time points and final visit time points. The measured values at each time point, the minimum value and maximum value of the post-baseline measurement results, and the final visit observation value were calculated separately according to dose groups and total, as well as their relative changes from baseline.

The normal and abnormal changes of each indicator before and after drug use were compared by clinical determination of the cross table before and after drug use.

List the detailed laboratory test indicators by group.

vital sign:

The statistical time points for vital signs examination items include baseline, post-baseline visit time points, and pre-exit visit time points. Descriptive statistics are calculated by group and total for baseline, post-baseline visit time points, and at the end of the study. The normal or abnormal changes in each indicator before and after medication use (if applicable) are compared using a cross-tabulation for clinical judgment before and after medication use.

Physical examination and electrocardiogram:

Physical examination items: general condition, head, neck, lymph nodes, chest, abdomen, musculoskeletal system, skin, nervous system and other parts. The normal and abnormal changes before and after the administration of each dose group were compared by clinical judgment before and after the administration of drugs.

The ECG examination items include: heart rate, PR, QRS, QTc. Baseline and post-baseline measurements at each visit time point were statistically analyzed by dose group and overall. The minimum value, maximum value, and final observation values after baseline measurement, along with their changes from baseline, were statistically analyzed. The normal and abnormal changes before and after medication use in each dose group were compared using a cross-tabulation of clinical determinations before and after medication.

List the physical and electrocardiogram examinations in detail by group.

10.2.6. efficiency analysis

The validity variables will be analyzed on an individual visit basis. For continuous variables, the measurement values and their changes from baseline will be summarized by dose group using descriptive statistics. Categorical variables will be summarized by frequency and percentage of the measurement values from baseline, also by dose group. For the changes in continuous variable measurements from baseline, a covariance analysis will be used to compare differences between dose groups. If the data do not meet the assumption of normal distribution, a Wilcoxon rank sum test will be used to compare differences between dose groups. For binary variables, a Fisher exact test will be used to compare differences between dose groups.

The effectiveness analysis is based primarily on the full analysis set and randomized treatment of subjects, with the protocol-compliant analysis set to be used for sensitivity analysis.

11. Data processing and record keeping

In accordance with GCP, ICHGCP and laws and regulations on the protection of subject privacy, each participating institution shall keep medical and research records applicable to the study.

11.1. Data collection and management responsibilities

Before conducting clinical trial data management, the data management department formulates a

Data Management Plan (Data Management Plan, DMP) based on the actual situation of the project. The Data Management Plan is a dynamic document written by data managers according to the clinical trial protocol. It details and records all data management tasks for a specific clinical trial, including personnel roles, work content, and operational standards.

11.2. Data collection and methods

In this experiment, the electronic data acquisition system (Electronic Data Capture System, EDC) was used for data acquisition. Data administrators/database programmers created accounts according to different identities and granted different permissions to access the EDC system.

The data recorded in the electronic case report form (eCRF) should come from source documents and be consistent with the source data. All source documents must remain clear and tidy to ensure that the data can be accurately identified. A permanent copy of the study visit records will be considered the source document for recording the data of enrolled subjects. Data entry personnel should promptly and accurately enter the data from source documents such as research medical records into the eCRF.

11.3. Data cleaning and question solving

The data cleaning process includes data verification (system logic check and manual logic check), triggering questions, answering questions by researchers/research assistants, updating data, until the questions are resolved.

Data administrators and monitors regularly clean data through the EDC system, while medical monitors conduct regular medical reviews via the EDC system. For questions raised in the EDC system, researchers/research assistants provide answers and/or correct erroneous data online. The question initiator confirms the answered data, and may re-post questions if necessary.

11.4. Modification and review of data

Data entry personnel or researchers can modify the data after verifying the data, and the reasons for modification should be filled in on the eCRF. Researchers have the right to review all the final data.

11.5. Data locking and export

After all the data have been reviewed without error, the data administrator will lock the data. If any modification is made after the data are locked, it must be confirmed by the sponsor, the researcher and the data manager before execution. All the data will eventually be imported into the designated database by the data manager and handed over to the statisticians for analysis.

At the end of the study, the eCRF will be printed and archived as required. The data center will keep the electronic data.

The data management related work not detailed in the programme shall be carried out in accordance with the data management plan of this trial.

11.6. Preservation of research records

To ensure the evaluation and supervision by the National Medical Products Administration and the sponsor, researchers should maintain all research data according to GCP, including all source data records of participants, such as study medical records or original record books, signed informed consent forms, detailed records of drug distribution, etc. After the trial ends, the PDF version of the eCRF for all participants at each research center should be recorded on a CD and stored at the corresponding research center. Research data must be retained for at least 5 years after the clinical trial concludes or 5 years after the investigational drug is approved for marketing. No data may be destroyed without written consent from the sponsor.

All data from this clinical study are owned by the sponsor and shall not be provided to a third party in any form by the investigator without the written consent of the sponsor, except as required by the National Medical Products Administration.

11.7. Research publication and data sharing policies

The data and results obtained from this trial, as well as the intellectual property rights of all data and results, are owned by the sponsor. Researchers may use the data obtained from this trial for scientific purposes, but must discuss with the sponsor before publication and obtain written consent from the sponsor for the proposed content.

The sponsor acknowledges the researchers right to publish results after the trial. However, the researcher must send the article or abstract manuscript to the sponsor before submission. The sponsor will review whether the manuscript is accurate (to avoid inconsistencies with materials submitted to regulatory authorities), ensure that confidential or patent information is not disclosed, and supplement relevant information as needed. If there are differing opinions between the sponsor and the researcher, they will discuss the proposed content to find a solution satisfactory to all parties.

For multicenter studies, the first publication must be based on data from all centers and must be analyzed by a biostatistical analyst designated or accredited by the sponsor according to the protocol, rather than being analyzed independently by the investigators. Investigators participating in multicenter studies may not publish data collected from one center or a few centers before the first publication of data from all centers, unless they have obtained formal consent from all other investigators and the sponsor.

The authors of the manuscripts are determined by negotiation. If the data is submitted in summary, the authors may include members of each center involved in the study and staff of the sponsor.

The publication of the results of the trial will be detailed in the clinical study protocol.

12. Ethical guidelines and informed consent

12.1. Ethical norms

The implementation of this trial adheres to GCP, the Helsinki Declaration, relevant regulations, and the review opinions of the ethics committee. Researchers must ensure that this trial is reviewed and approved by a qualified ethics committee that meets GCP requirements. During the review, sponsors and researchers should provide the ethics committee with the trial protocol, informed consent form, and other necessary materials. Sponsors can only provide trial drugs after receiving approval from the ethics committee. They must also inform the ethics committee about any additional protocols that may affect the safety of participants and their continued participation in the trial, as well as any serious adverse events that occur during the trial. Researchers are responsible for reporting the progress of the trial to the ethics committee. Additionally, researchers must promptly submit all copies of communications with the ethics committee to the sponsor. When reviewing and approving the trial protocol, the ethics committee must confirm the protocol title, protocol number, and note the reviewed protocol document and review date. During the trial, if there are any new revisions to the trial protocol or informed consent form, they must again obtain written approval from the relevant regulatory authority according to regulations.

12.2. informed consent

Participants must provide informed consent to participate in this trial before receiving the treatment, to protect their legal rights. Researchers have the responsibility to fully and comprehensively inform participants or their designated proxies about the purpose of the study, the methods used, the effects of the medication, reasonable expected benefits, potential toxic side effects, and possible risks. They should ensure that participants understand their rights, the risks they will face, and the potential benefits. Any new information regarding the investigational drug should be promptly communicated to the participant. Participants should be informed that this clinical trial is entirely voluntary, and they can withdraw from the trial at any time without penalty. The researchers and sponsors have the right to read, retain, and statistically process the participants data according to relevant regulations. The informed consent form should indicate the version number and the date of preparation or modification. The informed consent form must be signed by the participant (and the legal guardian if applicable), with a date noted. The researcher conducting the informed consent process must also sign the name and date on the informed consent form. An independent witness who can attest to the participants agreement to participate may also sign the informed consent form. One copy of the informed consent form should be retained by both the researcher and the participant.If important new information involving the investigational drug is found, the informed consent form must be modified in writing and submitted to the ethics committee for approval before obtaining the

consent of the subject again.

12.3. confidentiality and privacy

The confidentiality and privacy of participants are strictly maintained by the researchers involved, their staff, and the sponsor along with its interventions. This confidentiality extends beyond clinical information related to the participants to include testing for biological samples and genetic tests. Therefore, the research protocol, documents, data, and all other information generated during the study will be kept strictly confidential. Without the written permission of the sponsor, no research materials or data may be disclosed to unauthorized third parties.

All research activities will be conducted in a private environment whenever possible.

Research monitors, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies, or representatives of the pharmaceutical company providing the investigational product may review all documents and records that need to be retained by the investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records of participants in this study. The clinical research center should allow access to these records.

The contact information of the participants will be securely stored at each clinical research center for internal use during the study period. At the end of the study, all records will continue to be kept in a secure location for a longer period than required by IRB review, institutional regulations, or sponsor requirements.

13. Quality assurance and quality control

All parties involved in clinical trials, such as sponsors, research centers and CROs, should take appropriate quality control measures to ensure that clinical trials comply with the Helsinki Declaration, GCP and relevant laws and regulations and SOPs.

13.1. Requirements for applicants

1) The sponsor or its representative must provide the latest version of the investigators manual (IB) for each unregistered clinical trial compound. For marketed products, provide product instructions and/or the latest product information.
2) The sponsor or its representative, together with the investigator and statistical experts, develops the final version of the clinical trial protocol. After agreement is reached, the clinical trial protocol is signed and submitted to the ethics committee.
3) The sponsor or its representative provided all investigators with a sufficient number of case report forms and directed them to complete and keep them.
4) The sponsor or its representative provides all investigators with adequate trial materials to support and allow the investigator to conduct the trial according to the established protocol.

5) The sponsor or its representative reserves the right to terminate the clinical trial early due to repeated program violations, or other well-founded ethical issues. In such cases, both parties will review and negotiate to take necessary measures to ensure the rights and interests of patients.

13.2. The duties of the inspector appointed by the sponsor or its representative

Clinical trial monitors are the primary liaison between sponsors and investigators. Monitors must adhere to GCP and SOPs, regularly or as needed, visit research centers to conduct clinical monitoring, oversee the progress of clinical trials, check and confirm the accuracy and completeness of all data records and reports, eCRF entries, ensuring they match the original data, and guarantee that clinical trials are conducted according to the protocol. Investigators should actively cooperate with the monitors work. The specific responsibilities of a monitor include:

1) Before the trial, it is confirmed that the undertaking unit has appropriate conditions, including personnel allocation and training, complete laboratory equipment in good condition, various testing conditions related to the trial, sufficient number of subjects are estimated, and the participants are familiar with the requirements in the trial protocol.
2) During the trial, monitor the implementation of the protocol by the investigator, confirm that all informed consent is obtained from all subjects before the trial, understand the inclusion rate of subjects and the progress of the trial, and confirm that the selected subjects are qualified.
3) Confirm that all data records and reports are correct and complete, and that all case report forms have been entered accurately and consistent with the original data. All errors or omissions have been corrected or noted, signed by the investigator with the date. Any changes in dosage for each participant, treatment modifications, concomitant medications, incidental illnesses, loss to follow-up, or missed examinations should be confirmed and recorded.
4) Confirm that all adverse events are recorded, serious adverse events are reported and recorded within the prescribed time; verify that the trial drugs are supplied, stored, distributed and recovered in accordance with relevant regulations, and make corresponding records.
5) Assist the investigator with necessary notifications and applications, and report trial data and results to the sponsor.
6) Visits that the investigator failed to make, tests that were not performed, examinations that were not done, and whether corrections were made for errors or omissions should be clearly recorded.

7) A written monitoring report shall be completed after each visit, stating the date and time of the monitoring, the name of the monitor, and the findings of the monitoring.

13.3. Requirements for researchers

1) Researchers participating in clinical trials must have professional expertise, qualifications and ability in clinical trials, and pass the qualification examination. The personnel requirements are relatively fixed;
2) Before the patient is selected, the researcher should explain the significance of the clinical study to the patient or his/her family members, obtain their consent and sign the informed consent form;
3) To personally perform and supervise the conduct of clinical studies;
4) Fill in the case report form (eCRF) carefully as required;
5) Regular visits by clinical monitors appointed by the sponsor or its representative;
6) Complete retention of laboratory test records, clinical records and original medical records of patients;
7) In the event of a serious adverse event, the researcher must determine the cause and take appropriate action, and report to the person in charge of the study;
8) Follow up serious adverse events.

13.4. Inspections and visits

According to the needs of the trial, when the sponsor or a third party is entrusted to conduct an audit or the drug administration department inspects the study, the original documents in the trial can be directly obtained, and the data in the eCRF should be directly derived from the original data.

13.5. Program deviation

All requirements stipulated in the research protocol must be strictly enforced. Any intentional or unintentional deviation from or violation of the protocol and GCP principles can be categorized as a protocol deviation. During monitoring, if a protocol deviation is identified, it should be recorded by the investigator or monitor in a protocol deviation log, detailing the time of discovery, the occurrence time and process of the event, the cause, and corresponding measures taken. The investigator must sign the log, and the ethics committee and sponsor should be notified. In statistical analysis reports and summary reports, the impact of any protocol deviations on the final data and conclusions should be analyzed and reported.

An evaluation should be conducted when a serious protocol deviation occurs, and the sponsor may terminate the study early if necessary.

13.6. Safety supervision

The Safety Oversight Committee will consist of representatives from the sponsor and primary

investigators. The committee will review all data and detail dose-limiting toxicity according to the protocol, determining whether further dose escalation or reduction is necessary. The DRC will review all safety data, and any deaths related to treatment will also trigger a review by the DRC to ensure patient safety. The DRC will enforce the terms established and approved during the committees formation. These terms will clearly define all data elements that require evaluation by the DRC and the responsibilities of all parties involved.

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15. appendix

Appendix 1Cockcroft-Gault formula

The unit of creatinine is mg/dl:

$$\text{Male Ccr (ml/min)} = \frac{(140-\text{年龄})\times(\text{体重})}{72\times\text{血清肌酐}}$$

$$\text{Female Ccr (ml/min)} = \frac{0.85\times(140-\text{年龄})\times(\text{体重})}{72\times\text{血清肌酐}}$$

The unit of creatinine is μmol/L:

$$\text{Male Ccr (ml/min)} = \frac{(140-\text{年龄})\times(\text{体重})}{0.818\times\text{血清肌酐}}$$

$$\text{Female Ccr (ml/min)} = \frac{0.85\times(140-\text{年龄})\times(\text{体重})}{0.818\times\text{血清肌酐}}$$

Note: Age is measured in years and weight in kilograms (kg).

Appendix 2 Central laboratory information and sample destruction company information

Central laboratory: pharmacokinetics, immunogenicity testing, TRAIL expression

Name: Junke Zhengyuan (Shanghai) Biomedical Technology Co., LTD

Unified social credit code: 91310115MA7CFRF017

Address: China (Shanghai) Free Trade Zone Chenhui Road 88, Building 2, 1st Floor

Sample destruction company

Name: Shanghai Solid Waste Disposal Co., LTD

Unified social credit code: 913101147294906145

Address: No.2491 Jiaju Road, Jiading District, Shanghai

Annex 3 Child-Pugh scale

The Child-Pugh classification is a commonly used clinical grading system for quantitatively assessing liver reserve function in patients with cirrhosis. This standard was first proposed by Child in 1964. At that time, Child divided patients into three levels based on five indicators (including general condition, ascites, serum bilirubin, serum albumin concentration, and prothrombin time), each scored as 1 point, 2 points, or 3 points. The scores of these five indicators were added together, with a minimum total score of 5 points and a maximum of 15 points. Based on this total score, liver reserve function was classified into three grades: A, B, and C, indicating three different levels of liver damage (the higher the score, the poorer the liver reserve function). However, due to the difficulty in scoring patients general conditions, Pugh later proposed using the presence and severity of hepatic encephalopathy as an alternative to general condition. This led to the development of the improved Child-Pugh classification, which is now widely used in clinical practice.

The specific classification criteria are as follows:

Clinical grading criteria	1 point	2 points	3 points
Hepatic encephalopathy (stage)	not have	1－2	3－4
ascites	not have	mild	Moderate to severe
Total bilirubin (umol/L)	<34	34－51	>51
albumin (g/L)	>35	28-35	<28
Prothrombin time prolonged (s)	<4	4-6	>6

Grade A: 5-6 points, the risk of surgery is small, the prognosis is the best, and the 1-2 year survival rate is 100%~85%;

Grade B: 7-9 points for moderate surgical risk, 80% to 60% survival rate for 1 to 2 years;

Grade C: more than 10 points, the risk of surgery is greater, the prognosis is the worst, and the 1-2 year survival rate is 45%~35%.

Annex 4 AARC scoring sheet

In 2017, APASL included 1,402 patients with ACLF who met the APASL definition. The study found that bilirubin, hepatic encephalopathy grade, INR, blood lactate, and serum creatinine were factors influencing patient prognosis, and proposed the ACLF-AARC score. The AARC score is the sum of scores from five indicators, ranging from a minimum of 5 to a maximum of 15 points, divided

into three levels. Specifically, Level I scores 5 to 7 points, Level II scores 8 to 10 points, and Level III scores 11 to 15 points. The specific criteria for each indicator in the AARC score are shown in the table below:

fraction	Total bilirubin (mg/dL)	HE by stages	INR	Blood lactate (mmol/L)	serum creatinine (mg/dL)
1	<15	0	<1.8	<1.5	<0.7
2	15~25	I~II	1.8~2.5	1.5~2.5	0.7~1.5
3	>25	III~IV	>2.5	>2.5	>1.5

Appendix 5 End-stage liver disease model (MELD) score

The End Stage Liver Disease Model (modelforend-stageliverdisease, MELD) is a system primarily using serum bilirubin, international standardized ratio of prothrombin time, and serum creatinine to evaluate end-stage liver disease. As the MELD score has become increasingly mature in predicting mortality and liver transplantation for end-stage liver disease, its application has also expanded to include severe hepatitis and liver cancer.

In the year 2000, Malinchoc et al. first applied MELD to predict mortality in patients undergoing intrahepatic portosystemic shunt surgery for end-stage liver disease, and confirmed that MELD can predict both mortality and postoperative survival time in end-stage liver disease. The formula for calculating MELD is: R = 0.957 × ln(creatinine mg/dl) + 0.378 × ln(bilirubin mg/dl) + 1.120 × ln(INR) + 0.643 × (cause: 0 for cholestatic and alcoholic cirrhosis, 1 for other causes). The higher the R value, the greater the risk and the lower the survival rate.

For the convenience of calculation, Kamath et al. improved the formula to R=3.8 × ln[bilirubin (mg/dl)] + 11.2 × ln (INR)+9.6ln [creatinine (mg/dl)] + 6.4 × (cause: 0 for biliary or alcoholic liver disease, 1 for other).

Note: INR is called international standardized ratio in Chinese. INR is calculated from prothrombin time (PT) and international sensitivity index (ISI) of the test reagent. INR makes PT measured by different laboratories and different reagents comparable, so as to facilitate the unification of drug standards. INR= $(PTtest/PTnormal)^{ISI}$ISI is the superscript.

Annex 6 SIRS diagnostic criteria

Two or more of the following clinical manifestations can be diagnosed as SIRS:

temperature	<36°C or> 38°C
heart rate	> 90 times/min
breathing rate	> 20 times/min or PCO_2<32mmHg（1mmHg=0.133kPa）
leucocyte count	<4.0*10^9/L or> 12*10^9/L

Annex 7 CRS grading criteria (ASTCT consensus)

CRS symptom[1]	Level 1	Level 2	Level 3	Level 4
give out heat[2]	Body temperature ≥ 38℃	Body temperature ≥ 38℃	Body temperature ≥ 38℃	Body temperature> 38℃
		merge		
hypopiesia	not have	No pressurizing drugs are required	A pressor drug is required, with or without vasopressin	Multiple pressor drugs are required (excluding vasopressin)
		And/or[3]		
hyoxemia	not have	Oxygen needs to be administered by low-flow nasal cannula or artificial ventilation	Oxygen is required through a high-flow nasal cannula or mask, non-rebreather mask, or Venturi mask	Positive pressure oxygenation is required (e.g. CPAP, BiPAP, intubation or mechanical ventilation)

Note: CPAP: continuous airway positive pressure ventilation; BiPAP: double level airway positive pressure ventilation

1 Organ toxicity associated with CRS can be classified according to CTCAE V5.0, but does not affect CRS classification.

2 Fever is defined as a body temperature of 38°C or higher for no other reason. After the use of antipyretics or steroids, fever is no longer required to assess the severity of subsequent CRS. In this case, CRS grading is determined by hypotension and/or hypoxemia.

3 CRS grading is determined by more severe events: hypotension or hypoxia need to be ruled out for other causes. For example, a patient with a temperature of 39.5℃ requires one pressor drug for hypotension and low-flow nasal cannula oxygen for hypoxia, which is judged as grade 3 CRS.

4 Low-flow nasal oxygen administration is defined as oxygen flow rate ≤6L/min. Low-flow oxygen administration also includes artificial ventilation and is sometimes used in pediatrics. High-flow nasal oxygen administration is defined as oxygen flow rate>6L/min.

Subject screening number: |__|__|__|__|__|

A phase II clinical trial to evaluate the safety and efficacy of AS1501 for injection in patients with chronic acute liver failure (ACLF)

Informed Consent Form

Subject number _____

Name of research center: Shenzhen Third People's Hospital

Research center number: |__ |__ |

Those who intend to participate in this study

Research phase: _____

investigator:

Sponsor: Shenzhen Zhongke Amshenn Pharmaceutical Co. LTD
,

CRO: Kunling Enterprise Management Shanghai Co., LTD

The informed page

1. preface

The "Phase II Clinical Trial to Evaluate the Safety and Efficacy of AS1501 for the Treatment of Chronic-on-Acute Liver Failure (ACLF) in Patients with Injection" (Protocol Number: AS1501-CTP-II-01), initiated by Kunling Enterprise Management (Shanghai) Co., Ltd. on behalf of Shenzhen Zhongke AmshennPharmaceutical Co., Ltd., is currently underway. This research institution has the qualifications to conduct clinical trials.

You will be invited to participate in this clinical trial. Before you agree to take part, please read this informed consent form carefully and ask your doctor or researcher any questions you need until you receive satisfactory answers. This informed consent form has two parts: the Informed Page (to share research information with you) and the Signature Page (for your signature if you agree to participate). If you agree to take part in this study, please sign this informed consent form and keep it signed by both you and the researcher.

2. research background

Chronic-onset acute liver failure (ACLF) is a syndrome that develops on the basis of chronic liver disease and is characterized by acute worsening of jaundice and coagulation disorders as manifestations of liver failure. It can be accompanied by complications such as hepatic encephalopathy, ascites, electrolyte disturbances, infection, hepatorenal syndrome, hepatopulmonary syndrome, and dysfunction of extrhepatic organs. According to the 2018 edition of the Chinese "Guidelines for the Diagnosis and Treatment of Liver Failure," the diagnostic criteria are: rapid worsening of jaundice, serum TBil> 10ULN or daily increase 17.1 μmol/L; signs of bleeding, PTA 40% (or INR 1.5).

With active prevention and good control of chronic hepatitis B, the incidence of ACLF in China has decreased year by year. The main causes of ACLF are: hepatitis B virus, alcohol, drugs (including traditional Chinese medicine, anti-tuberculosis drugs, antibiotics, antitumor drugs and non-steroidal anti-inflammatory drugs) and other hepatotoxic substances.

ACLF has a rapid clinical course, with diverse manifestations and a high short-term mortality rate. The 28-day mortality rate is approximately 30% to 45% (95% CI, 41% to 48%), and the 90-day mortality rate is about 58% (95% CI, 55% to 61%). The more organ failures that occur, the more severe the disease, and the higher the mortality rate. Over the past two decades, the mortality rate for ACLF patients in Asian populations has not significantly decreased, remaining close to 50%.

The pathogenesis of ACLF is comprehensive and complex, which has not been fully elucidated. The pathogenesis of ACLF is usually described by the PIRO concept, including susceptibility, injury strike, body response and organ failure.

Currently, there is no specific targeted treatment for ACLF patients. Treatment methods mainly include comprehensive medical management, artificial liver support systems, liver transplantation, and stem cell transplantation. Comprehensive medical management is a crucial foundation for treating liver failure but lacks effective drugs and methods. Artificial livers come in three types: non-biological, biological, and hybrid. They can remove harmful substances

from the body, replenish essential nutrients, temporarily replace some functions of the failed liver, improve internal conditions, and create opportunities for hepatocyte regeneration and liver function recovery or await liver transplantation. However, non-biological artificial livers primarily focus on removing toxic substances from the blood and cannot fully perform liver metabolism, secretion, and synthesis functions. They do not fundamentally reverse pathological damage to the liver and cannot significantly improve survival rates in patients with advanced liver failure.Biological and hybrid artificial livers, while possessing liver-specific detoxification, biosynthesis, and conversion functions, hold great potential for application. However, their clinical use is limited by cell sources, cell culture, and bioreactors. Liver transplantation is the most effective treatment for moderate to late-stage liver failure that does not respond well to comprehensive medical treatments and artificial liver support. Yet, liver transplantation has significant drawbacks: (1) donor shortage, particularly prominent in China; (2) the need for long-term use of immunosuppressants, which are expensive and have many side effects.

In the progression from liver injury to liver failure, the core event is the massive death of hepatocytes. When hepatocyte death exceeds the liver's regenerative capacity, liver failure occurs. The classic pattern of hepatocyte death is divided into two categories: necrosis and apoptosis. Apoptotic defects mainly include exogenous pathways and endogenous pathways. In both apoptotic pathways, death receptors such as Fas and tumor necrosis factor play a role

(TNF)-R1 and TRAIL (TNF-related apoptosis-inducing ligand) -death receptor 4 (DR 4)/TRAIL-death receptor 5 (DR5) are all expressed on hepatocytes. They activate multiple death domains by binding to their respective ligands Fas ligand (FasL), TNF-α, or TRAIL, initiating the apoptosis program. Normal hepatocytes are tolerant of TRAIL-mediated apoptosis, whereas chronic viral infections, steatosis, and toxic substances can upregulate TRAIL expression in hepatocytes.

The research drug AS1501 is a Class 1 therapeutic biologic that competitively binds to TRAIL-R2 (DR5) on the cell surface. The SDR5 portion of AS1501 can bind to its ligand TRAIL, but due to the lack of a transmembrane region and intracellular death domain, SDR5 cannot transmit apoptotic signals into the cell. Therefore, SDR5 can competitively bind to TRAIL on the cell membrane surface, thereby blocking TRAIL-mediated apoptosis.

This study has been approved by the National Medical Products Administration (NMPA), with the clinical trial approval number CXSL1900091, in compliance with the Good Clinical Practice (GCP). The study has been approved by the ethics committee of our research center. The primary objectives of this trial are: Phase IIa to evaluate the safety and tolerability of AS1501 for injection in early chronic-on-acute liver failure (ACLF) patients, and to determine the recommended dosing and frequency for Phase IIb; Phase IIb to evaluate the efficacy of AS1501 for injection in treating ACLF patients. The Phase IIa trial is planned to be conducted at two centers nationwide, with approximately 24 early-stage ACLF patients invited to participate; the Phase IIb trial is planned to be conducted at six centers nationwide, with approximately 72 ACLF patients invited to participate.

3. purpose of research

a designated time

Main objective: To evaluate the safety and tolerability of AS1501 for injection in subjects with early chronic acute liver failure (ACLF), to explore a rational dosing regimen; to determine the recommended dose and frequency for phase IIb.

Secondary objective:

- To evaluate the efficacy, immunogenicity and pharmacokinetic (PK) characteristics of AS1501 for injection in the treatment of early ACLF subjects.
- To preliminarily explore the changes in serum TRAIL expression levels before and after administration of AS1501 for injection in early ACLF subjects.

b designated time

Main objective: To evaluate the efficacy of AS1501 injection in treating ACLF subjects.

Secondary objective:

- The safety and PK characteristics of AS1501 for injection in ACLF subjects were evaluated.
- To explore the changes in serum TRAIL (TNF-related apoptotic induction ligand) expression levels before and after administration of AS1501 to ALCF subjects.

4. study population

We sincerely invite you to participate in this study, and your condition may meet the eligibility criteria for this study.

4.1 Main inclusion criteria for phase IIa study population:

(1) Age of 18 to 75 years (including the critical value) when signing the informed consent;

(2) According to the Guidelines for the Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Hepatic Failure and Artificial Liver Group of the Infection Disease Branch of the Chinese Medical Association and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure was diagnosed, with specific indicators including:

a) Chronic liver disease (chronic hepatitis B, autoimmune hepatitis, drug-induced hepatitis, etc.) with acute impact factor as drugs;

b) Serum TBil 10 x ULN or mean daily increase of 17.1 μmol/L;

c) Meets any of the following three criteria: A bleeding tendency with PTA 40% (or INR 1.5); B combined with hepatic encephalopathy; C combined with hepatorenal syndrome or ascites.

(3) The screening was in the early stage of liver failure and did not meet the conditions for liver transplantation;

◆ Early signs of liver failure:

- Extremely weak, with obvious loss of appetite, vomiting and abdominal distension and other serious digestive symptoms;
- ALT, and/or AST continued to rise significantly, and jaundice progressively deepened (TBil> 171 μmol/L or daily increase> 17.1 μmol/L);
- Tenderness of bleeding, 30% <PTA 40% (or 1.5 INR <1.9);
- No complications and other extrhepatic organ failure.

(4) The serum TRAIL increased during screening and was more than 3 times the normal TRAIL content;

(5) Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form;

(6) Able to follow the protocol and complete the trial;

(7) Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration.

Main exclusion criteria

(1) Patients with a history of allergy or severe allergy to protein drugs (CTCAEv5.0 points>3);

(2) Patients who have undergone liver transplantation or are scheduled for liver transplantation within 1 month.

(3) ACLF patients in the middle and late stages;

(4) Severe grade III ascites or refractory ascites with stage III-IV hepatic encephalopathy.

(5) Patients who had received artificial liver treatment within one week prior to screening.

(6) Patients with malignant tumors or a history of malignant tumors; those who were diagnosed with lung, liver, pancreatic, gastrointestinal and other tumors by imaging (ultrasound, CT or MRI) and tumor markers

(AFP, CEA, CA125 or CA199, etc.) during the screening period or within 1 month prior to the screening period.

(7) Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results indicating a risk of severe varices with bleeding during the screening period or within 1 month prior to screening.

(8) KDIGO According to the standard definition, acute kidney injury (AKI) subjects: (1) Scr increased by 26.5μmol/L(0.3mg/dL or more within 48h,1 mg/dL=88.4μmol/L); (2) Scr increased by 1.5 times or more than the baseline value within 7d; (3) Urine output decreased (<0.5ml/kg/h) and lasted for more than 6h.

(9) The following laboratory test values or abnormal test values are as follows: a. Blood routine: platelet (PLT) <75x 109/L, hemoglobin (HGB) <80g/L; b. PT-INR>1.9 or PTA <30%; c. Left ventricular ejection fraction (LVEF) <50%; serum creatinine>1.5x ULN.

(10) Associated with severe respiratory dysfunction, dyspnea or failure.

(11) Severe infections that cannot be controlled by concomitant drugs, including major organs such as the abdominal cavity, lungs, urinary tract and skin.

(12) HIV positive, or active tuberculosis or syphilis infected.

(13) Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history.

(14) Subjects with uncontrolled severe hypertension or diabetes.

(15) Women who are pregnant or breastfeeding, or who have a positive pregnancy test.

(16) Participants who participated in clinical trials of other drugs or medical devices within the first 30 days of randomization or within five drug half-lives.

(17) Subjects who had trauma or major surgery (e.g., requiring general anesthesia) within 28 days prior to the first study drug administration were excluded. Note:

Participants who plan to undergo surgery under local anesthesia can participate in the study.

(18) Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia, or mental status change; or any problem that may impair the subject's ability to receive or tolerate planned treatment at the research center, understand informed consent, or participate in the study or confuse the assessment or study results as determined by the investigator.

(19) Other conditions that the researchers did not think were suitable for this study.

4.2 Main inclusion criteria for phase IIb study population:

(1) Age of 18 to 75 years (including the critical value) when signing the

informed consent form;

(2) According to the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) issued by the Hepatic Failure and Artificial Liver Group of the Infection Disease Branch of the Chinese Medical Association and the Severe Liver Disease and Artificial Liver Group of the Hepatology Branch of the Chinese Medical Association, chronic plus acute liver failure was diagnosed, and the specific indicators included:

- History of chronic liver disease;
- Serum TBil 10 x ULN or mean daily increase of 17.1 μmol/L;
- Meets any of the following three criteria: A bleeding tendency with PTA 40% (or INR 1.5); B combined with hepatic encephalopathy; C combined with hepatorenal syndrome or ascites.

(3) The screening was in the early or middle stage of liver failure and did not meet the conditions for liver transplantation;

◆ Early signs of liver failure:

- Extremely weak, with obvious anorexia, vomiting and abdominal distension and other serious digestive symptoms;
- ALT and/or AST continued to rise significantly, with progressive deepening of jaundice (TBil> 171μmol/L or daily increase> 17.1μmol/L);
- Tenderness of bleeding, 30% <PTA 40% (or 1.5 INR <1.9);
- No complications and other extrhepatic organ failure.

◆ Mid-stage manifestations of liver failure:

- On the basis of early manifestations of liver failure, the disease progresses further;
- ALT, and/or AST rapidly decreased, TBil continued to rise;
- Significant bleeding (petechiae or ecchymosis), 20% <PTA 30% (or 1.9 INR <2.6);
- There was 1 complication and/or 1 failure of extrhepatic organ function.

(4) The serum TRAIL increased during screening and was more than 3 times that of normal people;

(5) Be able to understand the informed consent form, voluntarily participate in and sign the informed consent form;

(6) Able to follow the protocol and complete the trial.

(7) Participants (including partners) were willing to take effective contraceptive measures voluntarily from screening until 6 months after the last trial drug administration.

Main exclusion criteria

(1) Patients with a history of allergy or severe allergy to protein drugs (CTCAE v5.0 points>3);

(2) Patients who have undergone liver transplantation or are scheduled for liver transplantation within 1 month.

(3) Severe grade III ascites or refractory ascites.

(4) Patients with associated stage iii-iv hepatic encephalopathy.

(5) Patients who had received artificial liver treatment within one week prior to screening.

(6) Patients with malignant tumors, or a history of malignant tumors; those who were diagnosed with lung cancer, liver cancer, pancreatic cancer and gastrointestinal tumors by imaging (ultrasound, CT or MRI) and tumor markers (AFP, CEA, CA125 or CA199, etc.) during the screening period or within 1 month before the screening period.

(7) Gastroscopy or imaging (abdominal B-ultrasound, CT or MRI) results indicating a risk of severe varices with bleeding during the screening period or within 1 month prior to screening.

(8) KDIGO Acute kidney injury (AKI) as defined by the standard: (1) Scr increases by more than 26.5μmol/L(0.3mg/dL within 48h,1mg/dL=88.4μmol/L); (2) Scr increases by more than 1.5 times the baseline value within 7d; (3) Urine output decreases (<0.5ml/kg/h) and persists for more than 6h.

(9) Severe coagulation failure with PT-INR>2.5 or PTA <20%.

(10) Associated with severe respiratory dysfunction, dyspnea or failure.

(11) Severe infections that cannot be controlled by concomitant drugs,

including major organs such as the abdominal cavity, lungs, urinary tract and skin.

(12) HIV positive, or active tuberculosis or syphilis infected.

(13) Previous or associated with unstable ischemic heart disease, congestive heart failure, myocardial infarction, stroke history, severe arrhythmia and other medical history.

(14) Subjects with uncontrolled severe hypertension or diabetes with concomitant medications.

(15) Women who are pregnant or breastfeeding, or who have a positive pregnancy test.

(16) Participants who participated in clinical trials of other drugs or medical devices within the first 30 days or five drug half-lives prior to randomization.

(17) Subjects who had trauma or major surgery (e.g., requiring general anesthesia) within 28 days prior to the first study drug administration.
Note:
Participants who plan to undergo surgery under local anesthesia can participate in the study.

(18) Any serious underlying medical or psychiatric condition (e.g., alcohol or drug abuse), dementia, or mental status change; or any problem that may impair the subject's ability to receive or tolerate planned treatment, understand informed consent at the research center, or that the investigator considers contraindicated for participation in the study or confuses the assessment or study results as specified in the protocol.

(19) Other conditions that the researchers did not think were suitable for this study.

After signing the informed consent, you will be further examined in detail under the guidance of your research doctor. Your research doctor will judge whether you meet the requirements of the protocol based on your examination results to decide whether you can continue to participate in this study.

5. research design

This study is a phase II clinical study, which is divided into two phases: phase IIa and Phase IIb.

If you have participated in Phase IIa of the study, the design of Phase IIa is as follows: It is expected to enroll 12 early-stage ACLF participants who will receive a dose of 0.5 mg/kg. The first two participants will be enrolled sequentially and receive a single intravenous administration. If these two participants do not experience any SAEs related to the study drug within 2 weeks after the initial administration, the remaining 10 participants can continue to be enrolled; otherwise, the dose will be reduced to further explore the study. After receiving a single intravenous administration, participants must fast for 20 days. During this 20-day fasting period, if no AE of grade 3 or higher related to the study drug occurs, and after joint evaluation by the investigator and sponsor confirms safety and tolerability, the participant will enter the multiple-dose phase (once a week [D21 is the first dose], for 4 consecutive weeks); otherwise, the dose will be reduced to further explore the study, with the specific dose determined through discussion between the sponsor and the investigator. If any participant drops out during the fasting period after a single administration, additional participants can be added to ensure that no fewer than 12 participants enter the multiple-dose phase.

After all 12 early ACLF subjects in the 0.5mg/kg dose group have completed continuous dosing, the DMC (Data Monitoring Committee) will evaluate the safety of this dose group. If any of the following conditions occur in the 0.5mg/kg dose group, the DMC will discuss whether to continue dose escalation:

1) More than 1/3 of the subjects had drug-related grade 3 SAE;

2) Any drug-related SAE of grade 4 or above.

If the DMC evaluation confirms compliance with the dose escalation criteria, 12 early-stage ACLF participants will continue to be enrolled and receive the 1 mg/kg dose group, with the same enrollment criteria as the 0.5 mg/kg dose group. After completing the 0.5 mg/kg and 1 mg/kg dose exploratory studies, investigators and sponsors will comprehensively determine the recommended appropriate dose for Phase IIb study, and may also explore other dose groups or different dosing

frequencies.

If you participate in phase IIb study, the design of phase IIb study is as follows: It is expected to include 72 ACLF subjects. You will be randomly assigned to receive appropriate dose and frequency of AS1501 or placebo to further evaluate the efficacy and safety of AS1501 for injection.

6. stages of research

6.1 Phase IIa study steps

6.1.1 Screening period

If you wish to participate in this study, please sign this informed consent form. After your research doctor confirms that you have participated in this study and obtained your signed informed consent form, he/she will issue a corresponding checklist according to the clinical trial protocol and conduct a series of tests on you to confirm whether you meet the eligibility criteria.During the screening period of this study, your doctor will do the following: collect detailed records of your personal information, medical history, and illness; collect your vital signs and physical examination;

Blood will be drawn for the following tests, including HCG blood pregnancy test (if you are of childbearing age, this test must be completed, requiring 4mL / about 1 teaspoon), serum pathogen testing (4mL / about 1 teaspoon), complete blood count (4mL / about 1 teaspoon), biochemical blood test (4mL / about 1 teaspoon), coagulation function (4mL / about 1 teaspoon), arterial blood gas analysis (4mL / about 1 teaspoon), inflammatory markers (4mL / about 1 teaspoon), TRAIL blood sample (4mL / about 1 teaspoon), and collection of a 4mL urine sample for routine urinalysis, electrocardiogram, abdominal CT (digestive system), and echocardiography. The research doctor will determine whether you meet the eligibility criteria based on your test results to decide if you can continue participating in this study.

6.1.2 Treatment period visit

If you have successfully enrolled in this clinical study, before the first study drug administration (Day 1) visit, your research doctor will conduct the following tests: HCG blood pregnancy test (if you are a woman of childbearing age, this test must be completed, requiring a blood sample of 4mL / about 1 teaspoon), complete blood count (4mL / about 1 teaspoon), biochemical blood test (4mL / about 1 teaspoon), coagulation function test (4mL / about 1 teaspoon), arterial blood gas analysis (4mL / about 1 teaspoon), inflammatory markers (4mL / about 1 teaspoon), cytokines (8mL / about 2 teaspoons), immunogenicity (4mL / about 1 teaspoon), PK (24mL / about 6 teaspoons), PD marker (8mL / about 2 teaspoons), and collection of 4mL urine samples for routine urinalysis and electrocardiogram.

After the first dose, you will enter the post-dose washout period (D2 to D14) visit. The study physician will perform the following tests on you at D7 and D14: complete blood count (4mL / approximately 1 teaspoon), biochemical blood test (4 mL / approximately 1 teaspoon), coagulation function (4mL / approximately 1 teaspoon), cytokines (4mL / approximately 1 teaspoon), immunogenicity (4mL / approximately 1 teaspoon), PK (a total of 4 blood draws, totaling 16mL / approximately 4 teaspoons), PD markers (4mL / approximately 1 teaspoon), and a 4mL urine sample for routine urinalysis and electrocardiogram.

After completing the initial dosing period, you will enter a continuous four-dose period (i.e., D21, D28, D35, D42). The research physician will conduct the following tests on you at D21, D28, D35, and D42: complete blood count (4mL / approximately 1 teaspoon), biochemical blood test (4mL / approximately 1 teaspoon), coagulation function (4mL / approximately 1 teaspoon), cytokine test (8 mL / approximately 2 teaspoons), immunogenicity test (4mL / approximately 1 teaspoon), PK (a total of 13 blood draws, totaling 52ml / approximately 13 teaspoons), PD markers (a total of 7 blood draws, totaling 28ml / approximately 7 teaspoons), and collection of a 4mL urine sample for routine urinalysis and electrocardiogram.

6.1.3 Follow-up visits

If you have completed 4 consecutive doses or if you have discontinued treatment early for various reasons, you will need to complete the end of treatment visit.During this visit, the research doctor will perform the following tests on you: HCG blood pregnancy test (if you are of childbearing age, this test needs to be completed, requiring 4mL/about 1 teaspoon of blood), complete blood

count (4mL/about 1 teaspoon), biochemical blood test (4mL/about 1 teaspoon), coagulation function test (4mL/about 1 teaspoon), arterial blood gas analysis (4 mL/about 1 teaspoon), inflammatory markers (4mL/about 1 teaspoon), immunogenicity (4mL/about 1 teaspoon), and 4mL urine samples for routine urinalysis and electrocardiogram.

6.1.4 Unplanned visits

Depending on your specific situation, the doctor will make a comprehensive assessment of your condition and conduct additional visits or tests, including but not limited to: blood routine, biochemical blood, coagulation function, electrocardiogram, imaging examination, cytokines, etc.

6.2 Phase IIb study steps

6.2.1 Screening period

If you are willing to participate in this study, please sign this informed consent form. After your research doctor confirms that you will participate in the study and obtains your signed informed consent, they will issue a corresponding examination order according to the clinical trial protocol to conduct a series of tests on you to determine whether you meet the eligibility criteria. During the screening period of this study, your research doctor will

Perform the following operations: collect detailed records of your personal information, medical history, and condition; gather your vital signs and physical examination data; conduct blood tests for the following items, including HCG pregnancy test (if you are a woman of childbearing age, this test must be completed, requiring 4mL approximately 1 teaspoon), serological pathogen testing (4mL approximately 1 teaspoon), complete blood count (4mL approximately 1 teaspoon), biochemical blood test (4mL approximately 1 teaspoon), coagulation function test (4mL approximately 1 teaspoon), arterial blood gas analysis (4mL approximately 1 teaspoon), inflammatory markers (4mL approximately 1 teaspoon), TRAIL blood sample (4mL approximately 1 teaspoon), and collect 4mL urine samples for routine urinalysis, electrocardiogram, abdominal CT (digestive system), and echocardiography. The research doctor will determine whether you meet the eligibility criteria based on your test results to decide if you can continue participating in this study.

6.2.2 Treatment period visit

If you have successfully enrolled in this clinical study, you will be randomly assigned to receive either the investigational drug or placebo for a total of four consecutive dosing (i.e., D1/D8/D15/D22). The research physician will conduct the following tests on you at D1, D8, D15, and D22: complete blood count (4 mL/approximately 1 teaspoon), biochemical blood tests (4 mL/approximately 1 teaspoon), coagulation function (4 mL/approximately 1 teaspoon), arterial blood gas analysis (4 mL/approximately 1 teaspoon), cytokines (3 blood draws per visit, totaling 12 mL/approximately 3 teaspoons), immunogenicity (4 mL/approximately 1 teaspoon), pharmacokinetics (5 blood draws, totaling 20 mL/approximately 5 teaspoons), PD markers (4 mL/approximately 1 teaspoon), and 4mL urine samples for routine urinalysis and electrocardiogram.

6.2.3 Follow-up visits

If you have completed four consecutive doses or if you terminate the treatment early for various reasons, you will need to undergo a treatment completion visit. The study physician will perform the following tests: HCG blood pregnancy test (if you are of childbearing age, this test must be completed, requiring a blood sample of 4 mL/approximately 1 teaspoon), complete blood count (4 mL/approximately 1 teaspoon), biochemical blood test (4 mL/approximately 1 teaspoon), coagulation function test (4 mL/approximately 1 teaspoon), arterial blood gas analysis (4 mL/approximately 1 teaspoon), PD (4 mL/approximately 1 teaspoon), and collection of 4mL urine samples for routine urinalysis and electrocardiogram.

After that, you will have a visit on day 60 (±7), and the research doctor will perform the following tests on you: complete blood count (4mL/about 1 teaspoon), biochemical blood (4mL/about 1 teaspoon), coagulation function (4mL/about 1 teaspoon), arterial blood gas analysis (4mL/about 1 teaspoon), urine routine with 4mL urine sample, and electrocardiogram.

Finally, you will be visited on day 90 (±7), and the research doctor will perform the following tests: complete blood count (4mL/about 1 teaspoon), biochemical blood (4mL/about 1 teaspoon), coagulation function (4mL/about 1 teaspoon), arterial blood gas analysis (4mL/about 1 teaspoon), urine routine with

a 4mL urine sample, and electrocardiogram.

6.2.4 Unplanned visits

Research doctors will make additional visits or tests based on your condition.

All tests conducted during the study are designed to determine whether you are suitable for continued participation and to ensure your safety. In addition, you need to work with your doctor to document any symptoms of discomfort and bring them for review during each follow-up visit. You must inform your research doctor or nurse about all medications and treatments you are taking. The doctor will ask if you have experienced any side effects after taking the medication, whether you have followed medical advice, and assess whether you can continue with the trial drug treatment.

7. Possible risks and discomfort

7.1 drug adverse reaction

During the whole study, some adverse reactions may occur. AS1501 The common adverse reactions of the drug are lymphocyte reduction, and the rare adverse reactions include hypersensitivity reaction, infusion reaction, cytokine release syndrome, etc.

During the drug administration process, you may experience hypersensitivity reactions/intravenous infusion reactions, primarily manifested as fever, chills, shivering, fatigue, generalized itching, rash, shortness of breath, and difficulty breathing. If you experience any of the above discomforts, you should promptly communicate with the researcher for feedback, and the researcher will provide you with active symptomatic treatment.

During the process of drug administration, you may develop cytokine release syndrome, which is mainly manifested as fever, chills, dizziness, fatigue, breathlessness, dyspnea, etc. If you have any of the above discomforts, you should communicate with the investigator in time and the investigator will give you active symptomatic treatment.

Your research doctor will monitor for any adverse reactions to the study medication. If you experience any side effects or discomfort during the trial, it is crucial that you report them immediately to your research doctor. The research doctor will take appropriate measures promptly. If you or your research doctor determines that you cannot tolerate these side effects, the study medication may be discontinued entirely, and you may be asked to leave the study.

7.2 Risks associated with research operations

7.2.1 draw blood

Although blood drawing is a routine procedure, it can cause discomfort and bruising at the puncture site.

7.2.2 Imaging

During the trial, you will undergo a CT scan and functional imaging. You may feel discomfort during the scans and need to remain in the confined space of the scanning equipment. Any contrast agents used during the CT scan can also cause allergic reactions. Your trial doctor or the radiologist performing the scan will discuss the examination procedures and any potential risks in more detail before the test.

7.2.3 electrocardiogram (ECG)

During an ECG, small pads are placed on certain parts of your body. You may feel a slight pain when the pads are removed.

AS1501 Does not necessarily cure or improve your disease, so during your participation in this clinical trial, your condition may remain unchanged or progress.

In addition, any drug may cause adverse reactions that have not been foreseen. If you feel unwell, please contact your doctor in time, and the doctor will give you reasonable treatment.

8. On pregnancy

For female participants: If you are breastfeeding, pregnant, believe you may be pregnant, or planning to conceive, you cannot participate in this study. If you are pregnant or breastfeeding, there may be risks to both you and your baby that are not yet fully understood. During the study screening, pregnancy status will be checked for women of childbearing age. There is no information available on whether AS1501 is safe for breastfeeding or unborn infants.

For male participants: Participating in this study may damage your sperm, potentially harming the child you conceive during the study period. This harm is

currently unpredictable. Please inform your partner about this risk to the unborn baby. She should be aware that if she becomes pregnant, you need to immediately inform your research doctor, and she should also promptly inform her own doctor.

For all subjects: You must be using contraception to participate in this study. If you are sexually active, you should use a method of contraception that is acceptable to you, your research physician, and the sponsor. You must continue to use contraception for 6 months after the last dose/usage of the study drug.

During the study, if you or your sexual partner become pregnant or believe there is a possibility of pregnancy, it is crucial to inform the research doctor immediately. If you become pregnant, the study will be terminated, and the research doctor will discuss with you what you should do. The research doctor will provide you with contact information for the program, and even after the study ends, you may be asked questions about pregnancy and your baby.

9. Possible benefits

Your participation in this study may help control or alleviate your condition, and you may benefit from it, though this is not guaranteed; it may also be ineffective or even worsen your condition. During or at the end of the trial, you may not directly benefit more from this study, but the valuable information you provide will assist future participants in their treatment.

10. Other alternative treatments

Your other options include current treatment for the disease, including comprehensive medical supportive care, non-biological artificial liver, liver transplantation, participation in other trials, or no treatment at all. You can discuss these other treatments with your doctor to determine whether you should participate in this study.

If you decide not to participate in this study, your doctor will still recommend other treatments that are appropriate for you and will not affect your participation in other clinical trials or use of other treatments.

11. Early withdrawal from the study of treatment/early withdrawal from the study/ study termination

The study physician or sponsor may discontinue your participation in this study at any time for the following reasons:

You may withdraw from the study for the following reasons

? The subject withdraws the informed consent;
? encyesis;
? Missing persons;
? die;
? other;

The investigator may decide to discontinue or withdraw you from the study for one of the following reasons

- Major protocol deviations or protocol violations occurred during the study that had a significant impact on drug tolerance, safety, or PK evaluation;
- Researchers consider it necessary to stop the trial from a medical ethics perspective, such as if there is an appropriate liver donor and an emergency liver transplant is needed;
- Those who experienced life-threatening serious adverse events related to the study drug (SAE) and were not suitable to continue the trial;
- After the investigator discontinued the study drug treatment, it was determined that withdrawal from the study was most beneficial to the subject;
- Poor subject compliance (e.g., failure to take prescribed medications and receive examinations, use of other drugs that affect the evaluat-

ion of efficacy and safety); and serious protocol violations/biases;

- Clinical adverse events (AE), laboratory abnormalities or other medical conditions that continue to participate in the study may endanger the safety or health of the subject and will not be in the best interest of the subject;
- The subject meets the exclusion criteria (new or previously undetected) and cannot continue to participate in the study.

If you revoke your consent, the agreement to process data or collect samples remains in effect; however, no new information or samples will be collected. You can request that no new analyses be conducted after revocation, and any remaining samples that have not been analyzed or are stored after analysis will be destroyed. Until your revocation, any samples and research information collected from you may still be used by the sponsor as part of the study data.

If you withdraw from the study due to side effects or related injuries caused by participating in this study, for your safety, please inform the research doctor of the side effects. The research doctor will timely treat you and arrange follow-up diagnosis, treatment and follow-up.

You can withdraw from the study at any time without penalty, discrimination or retaliation, and your medical treatment and rights will not be affected.

12. breakthrough

During the study, if there is any important information about the disease or any medication that may affect your decision to continue participating in this study, your doctor will inform you promptly so you can decide whether to continue. If you decide not to continue with the trial, your research doctor will arrange for your follow-up treatment. If you decide to continue, you will be asked to sign a new consent form. Similarly, if new information becomes available, your research doctor may discontinue your participation without your consent. In such cases, your research doctor will explain the reasons and assist in arranging your follow-up treatment. Throughout the trial, you can access relevant information at any time. After the trial, the results will be provided to you.

13. Please cooperate with us

If you choose to participate in this study, please cooperate closely with the investigator during the study and comply with the following:

(1) Follow up at the hospital on time;

(2) You need to provide your doctor or researcher with any discomfort related to your trial process during the study so that your doctor can treat it; especially any beneficial or adverse changes. As previously stated, you or your sexual partner must inform your physician immediately if you become pregnant;

(3) If you seek medical advice from other physicians, you must inform them that you are participating in this clinical trial;

(4) Inform the investigator of all other medications (including herbal medicines) other than the study drug that you have used prior to and during the study

(5) During the course of the trial, you are expected to inform us of all symptoms that occur. During the course of the trial, you are also expected to inform us of any other treatments and medications you are taking.

14. Cost of research

The cost of this trial study is explained as follows:

(1) The sponsor will bear the cost of imaging, electrocardiogram, blood test and other treatment/checks as stipulated in the study protocol during your participation in this study, as well as the registration fee, medical care fee and consumables (such as the cost of sampling tubes and blood needles that may be generated during blood drawing if the above requirements are required) during follow-up.

(2) The sponsor will provide the investigational drug (AS1501) at no cost to you.

(3) If you need to be hospitalized to complete the procedures specified in the study protocol, the sponsor will pay for the hospitalization.

（4） The sponsor shall bear the costs incurred by the investigator for unplanned examinations or follow-up visits.

During this trial, other treatments and examination costs, as well as medical care that are not related to this trial, are not covered. If you have other diseases that require treatment and examination, or if you switch to another treatment because the treatment is ineffective, these costs are not covered.

From the start of this study to its conclusion, if you experience any adverse events, whether these events are related to the study medication or the diagnostic tests required by the study protocol should be determined by the investigator. If an adverse event caused by the study medication or the diagnostic tests required by the study protocol results in harm to you, you can receive active treatment at your participating hospital. However, any costs unrelated to this study will be borne by yourself.

15. Subject compensation and damages

The compensation for this test is explained as follows:

(1) For blood samples collected for pharmacokinetics (PK) or immunogenicity testing, and pharmacodynamic (PD) markers (TRAIL), we will provide a nutritional compensation of 100 yuan per blood collection point, up to a total of 43 blood collection points, a total compensation of 4300 yuan, which will be paid according to the number of blood collection points you actually participate in.

(3) Every time you come to the research center for follow-up, you will receive a corresponding transportation subsidy of 200 yuan per visit (according to the flow chart visit time), and the fee will be paid according to the actual number of visits.

The total compensation fee is related to whether you have completed the study and at what stage of the study. The above fees will be paid to you according to the process of the research center.

If you experience any harm related to the study, please inform your research doctor. The research doctor will provide you with appropriate treatment and guidance. The sponsor has purchased insurance for this study. In addition to damages caused by the negligence of the investigator or the clinical trial institution itself, the sponsor will bear the reasonable and necessary costs associated with injuries or deaths related to the study, in accordance with current Chinese laws and regulations.

16. maintain secrecy

Your participation in the study and your personal information during the study are kept confidential. To protect your privacy, you will be assigned a participant number during the study. Your research physician will maintain your personal medical records and a list that links your personal medical records to your participant number. The hospital will keep all of your records for this study, and no one may access this information without authorization. Only the research physician retains your basic information, and your name abbreviation and code will be used in other study documents.

Your medical records may be made available to sponsors, ethics committees and regulatory authorities during or after the study so that they can verify the authenticity and accuracy of the data and information collected. However, in this case, all of your information is not publicly available and strictly confidential.

The research data will be analyzed by the sponsor or other statistical companies to determine whether and how the investigational drug works in you and other participants. Your study results may be analyzed in any country worldwide, or compared with data from other sources. These data may also be reported to

health authorities in other countries, or presented at scientific or medical conferences and academic journals. The findings of this study can serve as a reference for future medical research.

In the future, applicants may need to collect additional data from your medical records in order to put the data already collected into the proper medical environment for analysis. However, prior to collecting this additional information, approval from the hospital ethics committee will be sought.

You may withdraw from the study at any time, but as long as the law allows, the sponsor will continue to use the information collected before your withdrawal.

In all cases, any private information that can identify you is strictly confidential.

The description of this clinical study is shown in http://www.chinadrugtrials.org.cn/. This website will not include any information that can identify you.

If you withdraw your consent to participate in the study, no new information about you will be collected. However, in order to comply with regulatory requirements to protect the integrity of research science,
Shenzhen Zhongke Amshenn Pharmaceutical Co., Ltd.
will still be able to use and share any research data
about you that has been collected during the study. If during the study, the sponsor, representatives, monitors, or auditors of this study are unable to review your original medical records related to the study on-site due to special circumstances, your data will be subject to remote data verification (using copies of source documents without identifiable information sent to the monitors via secure methods for remote CRF data verification). By signing this consent form, you agree that relevant personnel may conduct remote data verification of your information.

If the test process finds that the infectious disease is positive and meets the reporting scope of the Law on the Prevention and Treatment of Infectious Diseases, your test results and personal information should be reported to the CDC system.

In addition, please be aware that according to the 2020 edition of China's "Good Clinical Practice for Drug Clinical Trials," essential documents for clinical trials refer to files used to evaluate the implementation and data quality of clinical trials. These documents are intended to demonstrate that investigators, sponsors, and monitors have adhered to this standard and relevant laws and regulations during the clinical trial process. For clinical trials applying for drug registration, essential documents must be retained for at least five years after the approval of the investigational drug; for clinical trials not applying for drug registration, essential documents must be retained for at least five years after the termination of the clinical trial.

17. Human biological samples

Your samples (including blood, urine, etc.) will be used for the purposes described in the "Purpose of the Study" section of this informed consent and only for this study.

17.1 Blood samples

➢ Laboratory tests: blood routine, blood biochemistry, coagulation function, infectious disease screening, blood pregnancy test, cytokines, arterial blood gas analysis, and the amount of blood for inflammatory indicators shall be carried out according to the laboratory requirements of the research center. The specific collection frequency is detailed in Chapter 6. The research doctor will increase your examination frequency when necessary based on your clinical indications.

➢ Pharmacokinetics testing, immunogenicity testing, cytokines, PD testing: see Chapter 6 for the frequency of testing.

17.2 Urine samples

About 5-10 ml of urine is collected for each routine urinalysis, and the frequency of collection is detailed in Chapter 6. The research doctor will increase your frequency of examination as necessary based on your clinical indications.

18. Sample preservation and processing

The collected subject samples, if to be sent to the central laboratory for testing, will be uniformly stored at the central laboratory (Junke Zhengyuan [Shanghai] Biomedical Technology Co., Ltd., address: Building 2, No.88 Chenhui Road, China [Shanghai] Pilot Free Trade Zone, 1st Floor). Discarded samples will be uniformly sent to the sample disposal company (Shanghai Solid Waste Disposal Co., Ltd., address: No.2491 Jiaju Road, Jiading District, Shanghai).

If the collected subject samples need to be tested in each research center, they will be operated in strict accordance with the sample preservation and processing specifications of each research center.

19. Principles of participation

You are participating in this study entirely on a voluntary basis. You have the right to withdraw from the study at any time without facing discrimination or retaliation, and your medical treatment and rights will not be affected. You have the right to learn about potential adverse reactions associated with the study medication. If continuing the study would harm your health, or if your health is no longer suitable for continued participation in the trial, or if you cannot comply with the requirements of the protocol, or if the investigator cannot contact you to continue the clinical trial, or if the clinical trial has ended, or if it has been canceled, your doctor may terminate your participation in the study without your consent. If you decide to withdraw from the study, please contact the physician responsible for the trial, who will arrange a follow-up visit for you to assess various indicators.

The sponsor of this study may also terminate the entire study or restrict the entire study for safety or other reasons.

During the study, the content and version of this informed consent may be updated. Please read the new version of this informed consent carefully and ask your doctor or researcher any questions you need to know until you get a satisfactory answer. Please cooperate closely with the researcher to sign the new version of this informed consent.

[For the Fair Witness]:

You have the right to withdraw your consent at any time. Withdrawing consent will not affect the legality of processing data based on consent prior to withdrawal. After withdrawing consent, your data will no longer be processed, which will terminate your participation in this study according to your role in this study.

[For the legal representative of the patient]

According to the current law of our country, withdrawal of your consent will also terminate/pause the participation of the patient you represent unless another legal representative is appointed and signs a research informed consent form and an informed consent form for data processing as the legal representative.

[For the patient's guardian, such as parents]

According to the current law of our country, after withdrawing your consent, the participation of the patient you represent will also be terminated/paused.

20. contact way

If you have any questions about this study, or if you have a disease related to the study or have suffered an injury related to the study, please contact your research physician by:

Name of research doctor: _____ Contact number: _____

If you have any questions about the rights of research subjects, please contact the Clinical Research Ethics Committee of Shenzhen Third People's Hospital at 0755-61222333-16539

Informed consent form signature page

This informed consent form is in duplicate, one for the subject and one for the research doctor, which is valid after both parties sign it.

After fully understanding all the contents of the subject's information and the possible benefits and risks of participating in this trial, I voluntarily participate in this trial and make the following declaration:

If you participate in this study, please choose whether to participate voluntarily: ☐ Yes ☐ No

1. As a subject, I have read the above information for subjects and understand the nature, purpose and possible adverse reactions of this study. My questions have been satisfactorily answered;

2. I agree to attend the follow-up visit on time during the study and accept the corresponding examination related to the study. I will abide by the requirements of the subject information, fully cooperate with the researchers, and provide the health status and related information before, during and at each follow-up period of the study truthfully and objectively;

3. I understand that I can withdraw from the study at any time without adversely affecting my subsequent treatment. I understand that the investigator has the right to terminate the study at any time based on my condition;

4. I understand that I will receive an informed consent form signed and dated;

5. I agree to the collection, use and publication of my medical health data in this medical study;

6. After careful consideration, I voluntarily participate in "a phase II clinical trial to evaluate the safety and efficacy of AS1501 injection in patients with chronic acute liver failure (ACLF)". I will not participate in other clinical studies during this study period.

The subject's regular name: _____

Subject signature:_____Date _____ Year _____ month ____ day

contact way:_____Time: _____ hours _____ minutes

I confirm that the study physician has explained to me and the participants in the study the details of the study, including their rights and possible benefits and risks, and will provide us with a signed copy of the informed consent form. I agree on behalf of the participant to participate in this study.

If the subject is unable to sign an informed consent due to lack of capacity, his/her guardian shall sign it.

Guardian's formal name: _____ (if applicable) and relationship to the subject: _____

Guardian signature:_____ Date: _____ _____ ____

contact way:_____Time: _____ hours _____ minutes

Reasons for the need of guardian signature:

_____

If the subject or his/her guardian is unable to read, a person unrelated to the study may act as an impartial witness and participate in the informed consent process by reading the informed consent form and other written materials provided to the subject.

I confirm that the information in the informed consent form has been correctly interpreted and that the subject (or his/her guardian) understands

this information. The subject (or his/her guardian) has voluntarily agreed to participate in this study.

Witness's name in regular script: (If applicable)

Signature of impartial witness:_____Date _____ Year _____ month ____ day

contact way:_____Time: _____ hours _____ minutes

Reasons for the need of witness signature: _____

Declaration and signature of the researcher

I have informed the subject (or their guardian) about the background, purpose, procedures, risks, and benefits of participating in the evaluation of "a Phase II clinical trial assessing the safety and efficacy of injectable AS1501 for patients with chronic-on-acute liver failure (ACLF)." I provided them with sufficient time to read the informed consent form, discuss it with others, and answer any questions they had about the study; I informed the subject of the contact information to be used if they encounter any issues; I informed the subject (or their guardian) that they can withdraw from the study at any time during the study period without giving any reason. I provided them with an informed consent form signed and dated.

The researcher's formal name is _____

Researcher signature:_____Date _____ Year ____ month ____ day

Time: _____ hours _____ minutes