Trail title: Sintilimab (PD-1 inhibitor) Combined with Ramucirumab (a VEGFR-2 antagonist) and Chemotherapy as First-line Treatment in Patients with Advanced Gastric Cancer with Liver Metastasis (RAMSINT): A Phase II, Single-center, Prospective Clinical Study

Study protocol and statistical analysis plan

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Protocol synopsis

Study Title	Phase II clinical study of Sintilimab (PD-1 Inhibitor) Combined with Ramucirumab (a VEGFR-2 antagonist) and chemotherapy as first-line treatment in patients with advanced gastric cancer with liver metastasis
Study purpose	To observe the efficacy and safety of Sintilimab combined with Ramucirumab and chemotherapy as a first-line treatment for patients with advanced gastric cancer with liver metastasis, and to explore the clinical indicators related to the efficacy, so as to guide the subsequent individualized treatment
Study design	A prospective, single-arm, single-center, phase II clinical trial
Study patients	Patients with advanced gastric cancer liver metastasis diagnosed and receiving first-line treatment at our center from August 2024 to July 2025
Total number of cases	39 patients with gastric cancer liver metastasis
Criteria for patients enrolled	**Inclusion criteria:** 1. Voluntary participation in the clinical study; full understanding and informed consent to this study by signing the Informed Consent Form (ICF); willingness to follow and ability to complete all trial procedures. 2. Gender is not restricted, and participants must be at least 18 years old when signing the ICF. 3. Patients with histologically or cytologically confirmed, unresectable, or who refuse surgical resection of locally advanced, recurrent, or metastatic gastric and gastroesophageal junction (GEJ) adenocarcinoma (including signet-ring cell carcinoma, mucinous adenocarcinoma, hepatoid adenocarcinoma, etc.). (Note: For patients who relapse after neoadjuvant/adjuvant therapy, the time from the end of neoadjuvant/adjuvant therapy to disease relapse

	must be more than 6 months.) 4. Patients, except those with recurrent disease after neoadjuvant/adjuvant therapy, must not have previously received systemic treatment. 5. Participants must be histologically confirmed as having HER2-negative gastric cancer, GEJ cancer, or esophageal adenocarcinoma. 6. There must be at least one measurable lesion in the liver assessed by computed tomography (CT) scan or magnetic resonance imaging (MRI) according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) that can undergo repeat radiological evaluation; the radiological tumor assessment should be performed within 28 days prior to randomization. 7. The Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) score of 0-2 within 7 days prior to the first dose of medication. 8. Expected survival greater than or equal to 3 months. 9. Hepatitis B surface antigen (HBsAg) negative and hepatitis B core antibody (HBcAb) negative. If HBsAg positive or HBcAb positive, hepatitis B virus deoxyribonucleic acid (HBV-DNA) must be less than 1000 copies/mL or less than 200 IU/mL or less than the upper limit of normal (ULN) at the research center for enrollment to occur. 10. HCV antibody negative. 11. Main organ functions must be normal, meeting the following criteria (no blood transfusions, albumin, recombinant human thrombopoietin, or colony-stimulating factor (CSF) treatments within 14 days prior to the first dose): a. Hematological System White Blood Cells (WBC) Normal range Neutrophils (NE) Normal range

	Platelets (PLT) ≥100 × 10^9/L Hemoglobin (Hb) ≥90 g/L b. Liver Function Total Bilirubin (TBIL) ≤1.5 × ULN Alanine Aminotransferase (ALT) ≤5.0 × ULN Aspartate Aminotransferase (AST) ≤5.0 × ULN Albumin ≥30 g/L c. Renal Function Creatinine (Cr) Normal range d. Coagulation Function Activated Partial Thromboplastin Time (APTT) ≤1.5 × ULN Prothrombin Time (PT) ≤1.5 × ULN International Normalized Ratio (INR) ≤1.5 × ULN 12. Availability of representative tumor tissue specimens, blood samples, and fecal samples for exploratory research. **Exclusion criteria:** 1. History of other active malignancies within the past 5 years or currently having other active malignancies. Treated localized tumors, such as basal cell carcinoma of the skin, squamous cell carcinoma of the skin, superficial bladder cancer, prostate carcinoma in situ, cervical carcinoma in situ, and breast carcinoma in situ, are eligible for inclusion. 2. Underwent surgery within 4 weeks prior to the start of the study treatment. 3. Presence of central nervous system diseases or symptoms of central nervous system metastasis. 4. Patients preparing for or with a history of organ or bone marrow transplantation.

	5. History or evidence of thrombosis or bleeding disorders within 6 months prior to the first study treatment. 6. Diagnosis of active pulmonary tuberculosis. 7. History or presence of interstitial pneumonia, pneumoconiosis, radiation pneumonia, drug-induced pneumonia, or other conditions that may interfere with the detection and management of suspected drug-related pulmonary toxicity. 8. Uncontrolled hypertension (SBP ≥ 150 and/or DBP ≥ 100) or history of hypertensive crisis or cardiovascular diseases (≥ II degree or worse congestive heart failure, myocardial ischemia, unstable angina, stroke, or transient ischemic attack). 9. Patients with high-risk factors for gastrointestinal perforation (history of gastrointestinal fistula, perforation, abdominal abscess, partial or complete intestinal obstruction, or requiring total parenteral nutrition). 10. Patients with moderate to severe proteinuria. 11. Patients with non-healing wounds, ulcers, or fractures. 12. Presence of known active or suspected autoimmune diseases, except those who are in a stable condition at the time of enrollment (not requiring systemic immunosuppressive treatment). 13. Known history of severe allergy to any monoclonal antibodies or excipients. 14. Known history of substance abuse or drug addiction; patients who have stopped alcohol consumption can be included. 15. Patients with conditions that may increase the risk associated with the study drugs, or other severe, acute, and chronic diseases, as judged by the investigator, are deemed unsuitable for participation in the clinical study. 16. Previous use of PD-1 inhibitors, LAG-3 inhibitors, CTLA-4

	inhibitors, or any other antibodies or drug treatments targeting T-cell co-stimulation or immune checkpoint pathways, including previous receipt of anti-tumor vaccines or other immunostimulatory anti-tumor therapies. 17. Previous exposure to VEGF (vascular endothelial growth factor) or VEGFR inhibitors or any anti-angiogenesis medications.
Study design	After enrollment, the participants will receive a combined treatment regimen of Sintilimab, Ramucirumab and chemotherapy. The chemotherapy regimen will include one of the following two combination therapies: SOX regimen (oxaliplatin and S-1) or XELOX regimen (oxalipatin and capecitabine). A maximum of 6 cycles of combination treatment will be administered, with each cycle lasting 21 days. After completing the 6 cycles of combined treatment regimen, the participants will receive Sintilimab and oral chemotherapy agents (Capecitabine or S-1) with or without Ramucirumab until disease progression. In case of disease progression or intolerable toxic reactions occur during treatment, the investigator will determine the subsequent treatment options. The specific dosages and methods for the medications mentioned above are as follows: **Immunotherapy:** Sintilimab: 200mg fixed dose, intravenous infusion on Day 1, repeated every 21 days. **Targeted Therapy:** Ramucirumab: 10mg/kg intravenous infusion on Day 1, repeated every 21 days. **Chemotherapy: Two combination regimens include:** SOX regimen: Oxaliplatin 130mg/m² intravenous infusion on Day 1 and S-1 40mg/m² orally twice daily on Days 1-14, repeated every 21

	days. XELOX regimen: Oxaliplatin 130mg/m² intravenous infusion on Day 1 and Capecitabine 1000mg/m² orally twice daily on Days 1-14, repeated every 21 days. Imaging assessments (CT/MRI) are required before the first treatment and after every two cycles of treatment. Gastric tissue specimens will be collected from patients prior to the first treatment (paraffin sections) (5-20 slices of surgical tissue (each tissue more than $1cm^2$), or 20-25 slices of puncture tissue). Blood samples and stool samples will be collected before the first treatment, after every two cycles, or at the time of disease progression to analyze the corresponding samples collected at baseline, when the best overall efficacy appears, or upon disease progression (Blood samples: approximately 5ml per instance; fecal samples: middle segment stool, the size of a soybean (approximately 50 mg), collected in three tubes). The expression levels of 92 proteins in blood samples will be dynamically monitored using the Olink-96 Immune-Oncology panel. Intratumoral microbiota in baseline tumor tissue samples will be detected using 5R 16S rRNA sequencing, and gut microbiota in stool samples will be detected using 16S rRNA sequencing.

Study Assessment	**Primary Endpoint:** Objective Response Rate (ORR): The proportion of participants whose tumor volume decreases to a predefined value and can maintain the minimum duration requirements, which is the sum of the rates of Complete Response (CR) and Partial Response (PR). **Secondary Endpoints:** a. Disease Control Rate (DCR): The proportion of participants who achieve either a response (PR + CR) or Stable Disease (SD) after treatment and can maintain the minimum duration requirements. b. Progression-Free Survival (PFS): The time from the start of treatment to disease progression or death (from any cause). c. Overall Survival (OS): The time from enrollment to death due to any cause. d. Incidence of adverse events and serious adverse events (AEs): Categorized according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE 5.0). Descriptive statistical analysis is primarily used to list and describe the AEs that occurred during this trial. **Exploratory Endpoints:** To identify potential predictive indicators of efficacy (including the distribution of intratumoral microorganisms in tumor tissue, protein expression levels in serum samples, and the distribution of gut microbiota in fecal samples, etc.).
Statistical analysis plan	Intent-to-Treat Population (ITT): Defined as all subjects who entered the trial. The ITT population will serve as the primary population for efficacy analysis in this study. Per-Protocol Population (PPS): The per-protocol population is a subset of the ITT population, consisting of participants who were randomly assigned, received at least one treatment, and underwent

	tumor assessment without significant protocol deviations that would affect the primary efficacy evaluation. Analyses based on PPS will serve as supportive analyses to complement the ITT-based analyses. Safety Analysis: Safety analysis will primarily involve descriptive statistics, listing adverse events (AE) that occurred during the trial. Laboratory test results will describe abnormalities that were normal before the trial but became abnormal after treatment, along with their relationship to the trial drug when abnormal changes occurred. Efficacy Analysis: The Objective Response Rate (ORR) will be calculated as the proportion of patients in the ITT population who achieved the best overall response of complete response (CR) or partial response (PR). The Disease Control Rate (DCR) will be calculated as the proportion of patients in the ITT population who achieved the best overall response of CR, PR, or stable disease (SD). For efficacy endpoints such as overall survival (OS) and progression-free survival (PFS), the Kaplan-Meier method will be used to estimate the median times and list the median events along with their two-sided 95% confidence intervals. Patient data and outcome parameters will be inputted into GraphPad Prism (version 9.1.2) and analyzed using the non-parametric Mann-Whitney U test (for continuous variables) to compare differences between two groups. For comparisons among more than two patient groups, the non-parametric Kruskal-Wallis test will be applied, followed by Dunn's post-hoc test with Bonferroni correction. Fisher’s exact test will be used for intergroup comparisons of categorical variables. The serum protein abundance data from patients receiving Sintilimab + Ramucirumab + chemotherapy will be analyzed using the Cox proportional hazards model and Benjamini-Hochberg

	multiple hypothesis correction (“R,” version 4.2.1). A sample will be identified as an outlier using PCA plots and median vs. interquartile range (IQR) plots. This sample will be excluded from the phase one analysis. Samples with QC warning status will also be excluded from both analyses on a per-panel basis. The results will then be adjusted for multiple testing using the Benjamini-Hochberg method. Hazard ratio (HR) will be based on 1 NPX difference. The association between baseline NPX (v1) and ΔNPX (Δv2/v3−v1) plasma protein levels and PFS will be assessed by plotting Kaplan-Meier curves and calculating log-rank p-values. Patients receiving Sintilimab + Ramucirumab + chemotherapy will be evaluated by dividing them into low or high expression "risk groups" based on the median expression values of each parameter as a threshold. For all statistical analyses, a p-value of less than 0.05 will be considered statistically significant.
Research progress	August 2024 - July 2026

1. BACKGROUND

Gastric cancer (GC) is a primary epithelial malignancy originating in the stomach and is one of the most common malignant tumors worldwide. In China, the incidence of gastric cancer ranks third, and its mortality rate also ranks third (1). There are approximately 1.2 million new cases of gastric cancer globally each year, with about 40% occurring in China (2). Even after radical surgery and adjuvant chemotherapy, the risks of local recurrence and distant metastasis remain high. For patients with unresectable locally advanced, recurrent, or metastatic gastric and gastroesophageal junction adenocarcinoma, the five-year survival rate is only about 13.9%. The incidence of liver metastasis in gastric cancer is higher than that of other organs. Once liver metastasis occurs, it typically indicates that most patients have lost the chance for radical treatment, and their long-term survival rate is extremely low. Although some cases can be managed with surgical interventions or other methods, systemic treatment remains the primary approach for patients with gastric cancer accompanied by liver metastasis. Historically, fluorouracil combined with platinum-based drugs has been the preferred treatment regimen for advanced gastric cancer. In recent years, the addition of immune checkpoint inhibitors has brought new hope for treating advanced gastric cancer with liver metastasis.

The emergence of immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) has revolutionized cancer treatment, providing robust and durable responses in GC. Numerous clinical trials have confirmed the significant role of PD-1 monoclonal antibodies combined with chemotherapy as first-line treatment for advanced gastric cancer. For example, the CheckMate-649 study showed that in the Chinese subgroup with a CPS $\geq$ 5, the combination of Nivolumab and chemotherapy had a better overall survival (OS) than chemotherapy alone (mOS 14.3 months vs. 10.2 months, HR 0.61 [0.44-0.85]); and also better progression-free survival (PFS) (mPFS 8.3 months vs. 5.6 months, HR 0.57 [0.40-0.80]) (3). The ORIENT-16 study demonstrated that in the CPS $\geq$ 5 population, the combination of Sintilimab and chemotherapy had superior OS compared to the

chemotherapy group (mOS 18.4 months vs. 12.9 months, HR 0.660 [0.505-0.864]); and better PFS (mPFS 7.7 months vs. 5.8 months, HR 0.628 [0.489-0.805]) (4).

Although ICIs have achieved significant success in the treatment of gastric cancer, not all patients with advanced gastric cancer benefit from PD-1 inhibitor therapy. This is especially true for those with CPS $<$ 5 or even CPS-negative patients, who have suboptimal responses to PD-1 inhibitors. Improving efficacy in this subset of patients has become a key concern for us. In fact, tumor growth is inevitably accompanied by abnormal tumor neovascularization (5), which induces an immunosuppressive microenvironment, helping tumor cells escape immune surveillance. Anti-angiogenic drugs are designed to counteract this effect (6,7). Based on the REGARD study (8) and the RAINBOW study (9), Ramucirumab gained FDA approval for second-line treatment of gastric cancer in 2014. Subsequently, the RAINBOW-Asia study confirmed the efficacy and safety of this regimen in Asian populations (10). At this year's American Society of Clinical Oncology Gastrointestinal Cancers Symposium (ASCO-GI), the results of the RAINBOW-Asia study, as a bridging trial for the RAINBOW study, were presented, providing strong evidence for clinical practice. Ramucirumab, a human IgG1 monoclonal antibody, specifically binds to VEGFR-2, inhibiting its activation and thereby suppressing ligand-induced endothelial cell proliferation and migration, ultimately inhibiting tumor angiogenesis. The activation of the vascular endothelial growth factor pathway is particularly important in liver metastatic lesions. Meanwhile, its ability to promote tumor vascular normalization can effectively regulate the immune microenvironment, achieving a truly "twice the result with half the effort" effect (11).

Based on the above research data, we boldly hypothesize that the golden combination of PD-1 inhibitors, anti-angiogenic drugs and chemotherapy can provide greater therapeutic benefits for patients with advanced first-line gastric cancer liver metastasis. In this prospective exploratory study, we plan to use Sintilimab, Ramucirumab and chemotherapy as first-line treatment for patients with advanced gastric cancer and liver metastasis, observing the efficacy and safety of this treatment regimen. Simultaneously, by examining relevant indicators such as the distribution of

intratumoral microorganisms, peripheral blood protein levels, and characteristics of intestinal microbiota, we aim to identify potential populations that could benefit from the treatment, providing evidence for personalized precision therapy for future patients with advanced gastric cancer liver metastasis.

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2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the efficacy of Sintilimab, Ramucirumab combined with chemotherapy in patients with liver metastasis from advanced gastric cancer who are receiving first-line treatment, with the objective response rate (ORR) as the primary study endpoint. The aim is to assess whether the combination of immunotherapy and chemotherapy with anti-angiogenic agents can improve the objective efficacy in patients with advanced gastric cancer and liver metastasis.

2.2. Secondary Objectives:

Disease Control Rate (DCR);

Progression-Free Survival (PFS);

Overall Survival (OS);

Incidence of adverse events and serious adverse events (AE);

2.3. Exploratory Research Objectives:

To identify potential predictive indicators of efficacy (including the distribution of

intratumoral microorganisms in tumor tissue, protein expression levels in serum samples, and the distribution of gut microbiota in fecal samples, etc.).

3. STUDY DESIGN, PRINCIPLES, and TRAIL PRDUCEDURES

3.1. Study Design

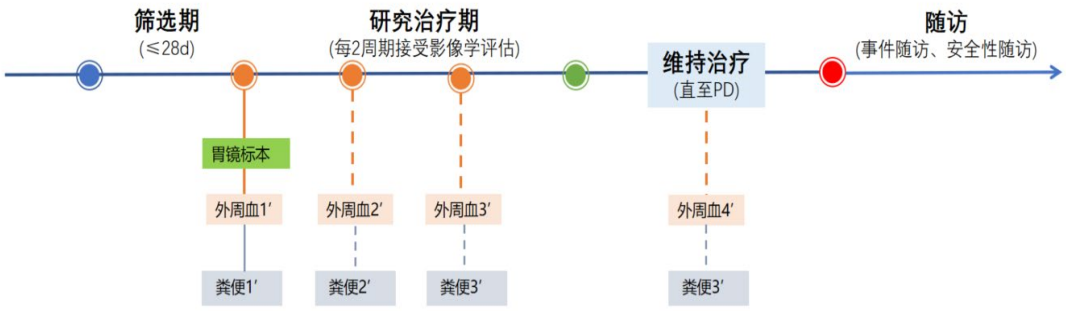
This study is a prospective, single-arm, single-center, phase II clinical trial. A total of 39 patients with advanced gastric cancer with liver metastasis, who are receiving first-line treatment, will be enrolled from August 2024 to July 2025 at our center. Participants will receive the combination treatment of Sintilimab, Ramucirumab and chemotherapy, with the chemotherapy regimen including two options: SOX regimen and XELOX regimen. A maximum of 6 cycles of combination treatment will be administered, with each cycle lasting 21 days. After completing 6 cycles of chemotherapy, patients will continue with Sintilimab and an oral chemotherapy agents (Capecitabine or S-1) with or without Ramucirumab, until disease progression. If disease progression or intolerable toxic reactions occur during treatment, the investigator will determine the subsequent treatment plan.

Radiological assessments (CT/MRI) will be performed before the first treatment and after every two cycles of treatment. Tissue samples from gastroscopy (paraffin sections) will be collected from patients before the first treatment (5-20 slices of surgical tissue (each tissue more than 1cm^2), or 20-25 slices of puncture tissue). Blood samples and stool samples will be collected before the first treatment, after every two cycles, or at the time of disease progression to analyze the corresponding samples collected at baseline, when the best overall efficacy appears, or upon disease progression (Blood samples: approximately 5ml per instance; fecal samples: middle segment stool, the size of a soybean (approximately 50 mg), collected in three tubes). The plasma protein expression levels of 92 proteins will be dynamically monitored using the Olink-96 Immune-Oncology panel. The intratumoral microorganisms in baseline tumor tissue samples will be detected by 5R 16S rRNA sequencing, while the gut microbiota in fecal samples will be analyzed using 16S rRNA sequencing. Samples

will be destroyed immediately after testing is completed.

3.2. Technical Route and Testing Process

For patients with liver metastasis from advanced gastric cancer receiving first-line treatment, screening will be completed according to the inclusion and exclusion criteria outlined in the study protocol, followed by thorough communication and signing of the informed consent form. After enrollment, patients will participate in the clinical trial of the Sintilimab, Ramucirumab and chemotherapy regimen. During treatment, tumor tissue testing, peripheral blood microprotein expression testing, and fecal gut microbiota testing will be conducted according to the protocol, with timely follow-ups. Tissue samples from gastroscopy (paraffin sections) will be collected before the first treatment for the purpose of intratumoral microorganism detection (5R 16S sequencing). Blood samples and fecal samples will be collected accordingly before the first treatment, after every two cycles, or at disease progression, and testing of blood and fecal samples collected at baseline, at the time of the best overall efficacy, or upon disease progression will be conducted. The plasma protein expression levels of 92 proteins will be dynamically monitored using the Olink-96 Immune-Oncology panel. The gut microbiota in fecal samples will be analyzed through 16S rRNA sequencing.



a. Blood Sample Microprotein Expression Level Detection Method

This study employs the Proximity Extension Assay (PEA, Olink-96 Immune-Oncology panel) technique. It involves a set of antibodies tagged with specific nucleotide sequence probes that specifically bind to the target proteins being measured. Correct and adjacent probes bind through the complementary pairing of bases at the 5' ends over 5 base pairs, forming a double-stranded template with the aid of proximity

extension enzymes. Detection is carried out using qPCR or next-generation sequencing (NGS), with the specific nucleotide sequence signals reflecting the content of the detected proteins. Olink converts the Ct values read by qPCR into relative quantification units (Normalized Protein Expression, NPX).

b. Intratumoral Microorganism Detection Method

This study utilizes the 5R 16S rRNA gene sequencing technology, which involves multiplex PCR amplification and sequencing of five regions on the 16S rRNA gene. This effectively reduces the severe interference from host DNA in the amplification of bacterial 16S rDNA from tissue samples. After quality control, OTU clustering, and species annotation of the sequencing data, a dedicated process for filtering contaminating bacteria is employed. Bioinformatics tools are then used to calculate diversity indices and perform statistical analysis to elucidate the characteristics and functions of the microbiome within the tumor tissue.

c. Fecal Sample Gut Microbiota Detection Method

This study adopts the 16S rRNA gene sequencing technology. Genomic DNA is extracted from fecal samples, and specific primers are used for PCR amplification of the 16S region, followed by high-throughput sequencing analysis. After quality control, OTU clustering, and species annotation of the sequencing data, bioinformatics tools are utilized to calculate diversity indices and statistical analysis to reveal the composition and diversity of bacterial communities in the samples.

The study aims to identify gut microbiota or proteins related to treatment efficacy and safety, exploring the relationship between these specific microbial communities or proteins and objective response rate (ORR), disease control rate (DCR), and adverse reactions. It seeks to identify potential populations that may benefit from treatment, providing evidence for personalized precision therapy for future patients with advanced gastric cancer and liver metastasis. Additionally, the study will analyze the correlation of microbiota or proteins with patients' progression-free survival (PFS) and overall survival (OS), seeking potential prognostic biomarkers.

4 STUDY POPULATION

4.1 Inclusion criteria

1. Voluntary participation in the clinical study; full understanding and informed consent to this study by signing the Informed Consent Form (ICF); willingness to follow and ability to complete all trial procedures.

2. Gender is not restricted, and participants must be at least 18 years old when signing the ICF.

3. Patients with histologically or cytologically confirmed, unresectable, or who refuse surgical resection of locally advanced, recurrent, or metastatic gastric and gastroesophageal junction (GEJ) adenocarcinoma (including signet-ring cell carcinoma, mucinous adenocarcinoma, hepatoid adenocarcinoma, etc.). (Note: For patients who relapse after neoadjuvant/adjuvant therapy, the time from the end of neoadjuvant/adjuvant therapy to disease relapse must be more than 6 months.)

4. Patients, except those with recurrent disease after neoadjuvant/adjuvant therapy, must not have previously received systemic treatment.

5. Participants must be histologically confirmed as having HER2-negative gastric cancer, GEJ cancer, or esophageal adenocarcinoma.

6. There must be at least one measurable lesion in the liver assessed by computed tomography (CT) scan or magnetic resonance imaging (MRI) according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) that can undergo repeat radiological evaluation; the radiological tumor assessment should be performed within 28 days prior to randomization.

7. An Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) score of 0-2 within 7 days prior to the first dose of medication.

8. Expected survival of greater than or equal to 3 months.

9. Hepatitis B surface antigen (HBsAg) negative and hepatitis B core antibody (HBcAb) negative. If HBsAg positive or HBcAb positive, hepatitis B virus deoxyribonucleic acid (HBV-DNA) must be less than1000 copies/mL or less than200 IU/mL or less than the

upper limit of normal (ULN) at the research center for enrollment to occur.

10. HCV antibody negative.

11. Main organ functions must be normal, meeting the following criteria (no blood transfusions, albumin, recombinant human thrombopoietin, or colony-stimulating factor (CSF) treatments within 14 days prior to the first dose):

a. Hematological System

White Blood Cells (WBC)	Normal range
Neutrophils (NE)	Normal range
Platelets (PLT)	≥100 × 10^9/L
Hemoglobin (Hb)	≥90 g/L

b. Liver Function

Total Bilirubin (TBIL)	≤1.5 × ULN
Alanine Aminotransferase (ALT)	≤5.0 × ULN
Aspartate Aminotransferase (AST)	≤5.0 × ULN
Albumin	≥30 g/L

c. Renal Function

Creatinine (Cr)	Normal range

d. Coagulation Function

Activated Partial Thromboplastin Time (APTT)	≤1.5 × ULN
Prothrombin Time (PT)	≤1.5 × ULN
International Normalized Ratio (INR)	≤1.5 × ULN

12. Availability of representative tumor tissue specimens, blood samples, and fecal samples for exploratory research.

Exclusion criteria:

1. History of other active malignancies within the past 5 years or currently having other active malignancies. Treated localized tumors, such as basal cell carcinoma of the skin, squamous cell carcinoma of the skin, superficial bladder cancer, prostate carcinoma in situ, cervical carcinoma in situ, and breast carcinoma in situ, are eligible for inclusion.

2. Underwent surgery within 4 weeks prior to the start of the study treatment.

3. Presence of central nervous system diseases or symptoms of central nervous system metastasis.

4. Patients preparing for or with a history of organ or bone marrow transplantation.

5. History or evidence of thrombosis or bleeding disorders within 6 months prior to the first study treatment.

6. Diagnosis of active pulmonary tuberculosis.

7. History or presence of interstitial pneumonia, pneumoconiosis, radiation pneumonia, drug-induced pneumonia, or other conditions that may interfere with the detection and management of suspected drug-related pulmonary toxicity.

8. Uncontrolled hypertension (SBP ≥ 150 and/or DBP ≥ 100) or history of hypertensive crisis or cardiovascular diseases (≥ II degree or worse congestive heart failure, myocardial ischemia, unstable angina, stroke, or transient ischemic attack).

9. Patients with high-risk factors for gastrointestinal perforation (history of gastrointestinal fistula, perforation, abdominal abscess, partial or complete intestinal obstruction, or requiring total parenteral nutrition).

10. Patients with moderate to severe proteinuria.

11. Patients with non-healing wounds, ulcers, or fractures.

12. Presence of known active or suspected autoimmune diseases, except those who are in a stable condition at the time of enrollment (not requiring systemic immunosuppressive treatment).

13. Known history of severe allergy to any monoclonal antibodies or excipients.

14. Known history of substance abuse or drug addiction; patients who have stopped alcohol consumption can be included.

15. Patients with conditions that may increase the risk associated with the study drugs, or other severe, acute, and chronic diseases, as judged by the investigator, are deemed unsuitable for participation in the clinical study.

16. Previous use of PD-1 inhibitors, LAG-3 inhibitors, CTLA-4 inhibitors, or any other antibodies or drug treatments targeting T-cell co-stimulation or immune checkpoint pathways, including previous receipt of anti-tumor vaccines or other immunostimulatory anti-tumor therapies.

17. Previous exposure to VEGF (vascular endothelial growth factor) or VEGFR inhibitors or any anti-angiogenesis medications.

4.3. Withdrawal Criteria

1. Voluntary Withdrawal by Participants: Participants may withdraw from the study due to poor efficacy, inability to tolerate side effects, desire to pursue other treatment options, or for any other reason.

2. Investigator's Decision for Withdrawal: The investigator may determine that a participant needs to withdraw from the study due to significant organ dysfunction, allergic reactions to the medication, poor compliance, worsening of the condition, or the occurrence of severe adverse reactions that necessitate the cessation of the investigational drug treatment or the adoption of alternative treatment methods.

4.4. Suspension/Termination Criteria

The study may be temporarily suspended due to unexpected events, high study risks, funding issues, administrative changes, or other related concerns. The study may resume once the issues affecting its conduct are resolved.

4.5. Termination Criteria

The study may be terminated due to legal or regulatory requirements, or at the request of the investigator, administrative authorities, etc., especially if there are serious safety issues, the efficacy does not meet expectations, or there is no further necessity to continue the clinical research.

5. RESEARCH METHODS and TECHNICAL ROUTE

5.1. Sample Size Calculation

Based on a clinical study conducted by Yang et al. involving 60 patients with gastric cancer liver metastasis who received chemotherapy or immunotherapy combined with chemotherapy, the experimental group (n = 30) received continuous

intravenous infusion of Endu combined with the SOX regimen, while the control group (n = 30) received only the SOX regimen. The overall response rates (ORR) were 63.3% and 43.3%, respectively (PMID: 30539861). Considering that immunotherapy combined with anti-vascular treatment and chemotherapy provides a better response for gastric cancer with liver metastasis compared to chemotherapy alone, it is expected that the ORR for patients receiving this combined treatment will increase to 65%. Assuming the original ORR is 43%, under the conditions of a type I error of 0.05 and a power of 0.8, and considering a dropout rate of 20%, the study plans to enroll a total of 39 participants.

5.2. Treatment Plan

After enrollment, participants will receive treatment according to the combination therapy of Sintilimab, Ramucirumab and chemotherapy. The chemotherapy regimens include the following two combinations: SOX regimen and XELOX regimen. Participants will receive up to 6 cycles of combination therapy, with each cycle lasting 21 days. After completing 6 cycles of chemotherapy, participants will receive Sintilimab and oral chemotherapy agents (Capecitabine or S-1) with or without Ramucirumab until disease progression. If disease progression or intolerable toxic reactions occur during treatment, the investigator will determine the subsequent treatment plans.

The specific dosages and administration methods for the aforementioned medications are as follows:

Immunotherapy:

Sintilimab: 200mg fixed dose, administered intravenously on Day 1, repeated every 21 days.

Targeted Therapy:

Ramucirumab: 10mg/kg administered intravenously on Day 1, repeated every 21 days.

Chemotherapy:

a. SOX regimen (Oxaliplatin 130mg/m² intravenously on Day 1 and S1 40 mg/m² orally, twice daily from Days 1 to 14, repeated every 21 days);

b. XELOX regimen (Oxaliplatin 130mg/m² intravenously on Day 1 and Capecitabine 1000mg/m² orally, twice daily from Days 1 to 14, repeated every 21 days).

6. OBSERVATIONAL ITEMS AND TESTING POINTS

Screening Period: Once patients are enrolled, blood tests (including complete blood count, coagulation function, tumor markers, myocardial markers, thyroid function, and microprotein expression levels) will be conducted one week before the start of the first treatment cycle, along with the collection of fecal samples, chest and abdominal imaging (plain scan or enhanced scan), and cardiac function evaluation (two-dimensional echocardiogram). Patients' age, gender, and other basic clinical data will be collected and organized. Gastric mucosal tissue specimens will be collected for intratumoral microbiome detection before the first treatment.

After Every 2 Treatment Cycles: After every 2 cycles of treatment with Sintilimab, Ramucirumab and chemotherapy, peripheral blood and fecal samples will be collected, and peripheral blood microprotein expression levels and gut microbiome analysis will be conducted during imaging assessments when the best overall efficacy is observed.

Disease Progression: If patients experience symptom deterioration or new symptoms, peripheral blood microprotein expression levels and fecal gut microbiome analyses will be performed within one week after confirming symptom deterioration or the emergence of new symptoms.

Follow-Up Period: During the treatment period, if the patient's disease does not progress, follow-ups are required every 3 months following the completion of 6 cycles of treatment, including: (1) routine follow-up assessments: clinical history, physical examination, routine hematological tests (complete blood count and chemical analysis, tumor markers CEA and CA199), Helicobacter pylori testing, nutritional evaluation (vitamin B12, iron), and enhanced CT scans of the chest, abdomen, and pelvis; (2) If patients experience symptom deterioration or new symptoms emerge, blood and fecal sample collections must be completed within one week, along with peripheral blood protein detection and gut microbiome analyses. Upon the patient's death, overall survival (OS) will be recorded, marking the clinical trial observation endpoint.

7. EFFICACY ASSESSMENT CRITERIA

Efficacy will be evaluated according to RECIST v1.1 standards, specifically as follows:

Primary Endpoint:

Objective Response Rate (ORR): The sum of the proportions of CR and PR after treatment.

Secondary Endpoints:

Disease Control Rate (DCR): The sum of proportions of participants achieving either a response (CR + PR) or SD after treatment.

Progression-Free Survival (PFS): The time from the start of treatment to disease progression or death (for any reason).

Overall Survival (OS): The time from enrollment to death due to any cause.

8. ADVERSE EVENT MONITORING

Adverse events mainly include: 1) infusion reactions (infusion-related adverse reactions, IRR) and other immune-related adverse events (irAE); 2) incidents leading to death; 3) life-threatening events (adverse events that present an immediate risk of death to the participant); 4) events necessitating hospitalization or an extension of the current hospitalization period.

9. QUALITY CONTROL AND QUALITY ASSURANCE OF THE STUDY

Data collection will be verified by at least two individuals, and statistical analyses will be performed by professional statisticians. The writing and revisions of the manuscript will undergo thorough discussion and modification within the department.

10. DATA SAFETY MONITORING

The clinical study will establish a corresponding data safety monitoring plan based on the level of risk involved. All adverse events will be documented in detail, appropriately handled, and tracked until resolved or the patient's condition is stabilized. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, sponsors, and drug supervision departments as required. The principal investigator will conduct regular cumulative reviews of all adverse events and will convene investigator meetings to assess the risks and benefits of the study when necessary. Studies with risks greater than minimum will arrange for independent data monitors to oversee the study data, and high-risk studies will establish an independent Data Safety Monitoring Board (DSMB) to review accumulated safety and efficacy data and make recommendations on whether the study should continue.

11. STATISTICAL PROCESSING

Intention-to-Treat (ITT) Population: Defined as all participants who entered the trial; the ITT population will serve as the primary analysis group for this study's efficacy evaluation.

Per-Protocol Set (PPS): The PPS is a subset of the ITT population, consisting of participants who were randomly assigned and underwent at least one treatment with tumor assessment and who had no significant protocol deviations that could materially affect primary efficacy evaluations. Analysis based on the PPS will serve as supportive analysis to complement the ITT-based analysis.

Safety Set (SS): Defined as all participants who received at least one dose of the investigational drug. The safety analysis population will be the primary analysis group for safety indicators and will be based on the treatment groups actually received.

Kaplan-Meier methods will be used to summarize Progression-Free Survival (PFS) and Overall Survival (OS); results will provide the median PFS and OS, along with 95% confidence intervals for all participants in the trial (ITT population). The Objective Response Rate (ORR) (defined as CR + PR) and Disease Control Rate (DCR)

(defined as CR + PR + SD) will be estimated with 95% credible intervals. Adverse event data for all participating patients will be summarized using descriptive statistics.

Patient data and outcome parameters will be entered into GraphPad Prism (version 9.1.2) and analyzed using the non-parametric Mann-Whitney U test (for continuous variables) to compare differences between two groups. For comparisons among more than two patient groups, the non-parametric Kruskal-Wallis test will be employed, followed by Dunn's post-hoc test with Bonferroni correction. Fisher's exact probability method will be used for between-group comparisons of categorical variables.

For all statistical analyses, a p-value of less than 0.05 will be considered statistically significant.

12. ETHIC OF CLINCICAL RESEARCH

The clinical study will adhere to the relevant regulations set forth in the Declaration of Helsinki by the World Medical Association. Clinical research will commence only after the ethical review board approves the study protocol. The investigator is responsible for providing each participant or their representative with a complete and comprehensive introduction to the study's purpose, procedures, and potential risks before enrollment, and obtaining written informed consent. Participants will be informed of their right to withdraw from the study at any time, and the informed consent document will be retained as part of the clinical research records. The privacy and data confidentiality of participants will be protected throughout the research process.

13. RESEARCH SCHEDULE

August 2024 – July 2025: Patient enrollment, treatment, and data collection

August 2025 – December 2025: Follow-up and data organization/statistics

January 2026 – June 2026: Data analysis and manuscript writing