Biomedical ethics research protocol (Interventional clinical study)

An Exploratory Clinical Study for Neoadjuvant Treatment of HER2-low Expressing, Stage II-III Breast Cancer with Vedolizumab in Combination with Pembrolizumab

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Study Years: January 2023 —— December 2024

Version Number: V2.0

Version Date: 08th December, 2022

Principal Investigator Protocol Signature Page (Team LeaderUnit)

I will diligently fulfill my responsibilities as an investigator and will participate in or directly oversee this clinical study. We have reviewed and confirmed this protocol (version number: V2.0; dated December 8, 2022). I commit to performing my duties in accordance with Chinese law, the Declaration of Helsinki, Good Clinical Practice (GCP) in China, and this study protocol. I confirm that this study protocol and any subsequent amendments cannot be implemented following the approval of the Ethics Committee unless necessary measures are taken to safeguard the safety, rights, and interests of the subjects. I will maintain the confidentiality of the study protocol, as well as all documents and information related to this study.

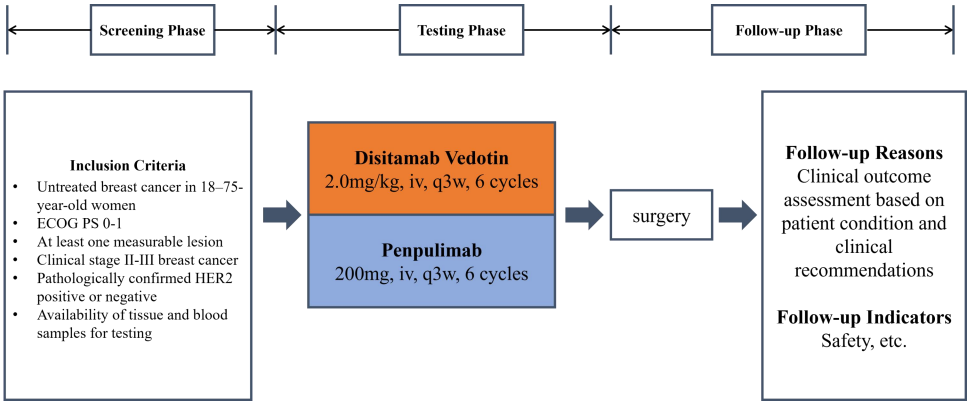
Catalogue

Study Summary

Study Design	☐ Case-control studies ☐ Cohort studies ☐ Cross-sectional studies ☐ Randomized controlled study ☐ Blinded method ■ Other: single-arm exploratory study
Type of Study	**(Class A: High risk)** □ Class III New clinical technology (accurate safety, effectiveness, technical difficulty, high risk) □ Special population studies (children, pregnant women, people with low intelligence, subjects with mental disorders, etc.) ■ Off-label study (■ off-indication □ overdose route □ overdose □ Overage □ Supernormal contraindications □ superpopulation □ Others, please specify:) □ Over-device specification study (□ off indications □ range of use □ super contraindications □ overpopulation □ Others, please specify:) □ Others (for the investigator's judgment, please specify:) **(Class B: Medium risk)** □ Post-marketing biologic study (preventive and treatment) □ Post-marketing therapeutic vaccine study □ Post-marketing drug research for rare diseases □ Class II New clinical technologies (definite safety and effectiveness, certain technical difficulty, certain medical risks and ethical risks) □ Others (for the investigator's judgment, please specify:) **(Class C: Low-risk)** □ 5 years of marketed drug research (including chemical drugs, generic drugs, etc.) □ Listed device study (including AI, imaging software) □ Class I new clinical technology (medical technology with precise safety, efficacy, low technical difficulty, and almost no ethical risk) □ Others (for the investigator's judgment, please specify:)
Total Number of Cases	20
Risk /benefit analysis	/

Risk Judgment	☐ No greater than minimum risk ■ Greater than minimum risk *Minimal risk: The likelihood and magnitude of the expected risk in the trial is no greater than the risk of daily life, or of performing a routine physical examination or psychological test*
Research Topic	An Exploratory Clinical Study for Neoadjuvant Treatment of HER2-low Expressing, Stage II-III Breast Cancer with Vedolizumab in Combination with Pembrolizumab
Research Number	RCVDBCIIR005
Version Number	2.0
Version Date	On December 08,2022
Research Unit	The West China Hospital of Sichuan University
Principal Investigator	Luo Ting
Research on Drug	Disitamab Vedotin (RC48), Penpulimab (AK105)
Research Basis	Breast cancer represents the most frequently diagnosed malignancy among women in China, with its incidence increasing, thereby presenting a considerable risk to women's health. HER2-positive breast cancer is distinguished by its aggressive tumor invasion, resistance to standard chemotherapy, and unfavorable prognosis. The introduction of anti-HER2 targeted therapies has markedly enhanced the survival rates of patients diagnosed with HER2-positive breast cancer. A range of treatment modalities, including monoclonal antibodies (mAbs), small molecule tyrosine kinase inhibitors (TKIs), and antibody-drug conjugates (ADCs), have received approval for the management of this condition. However, the benefits of traditional anti-HER2 therapy for individuals with low HER2 expression are quite limited. Achieving precise treatment for this population has become a significant research focus both domestically and internationally. Breast cancer characterized by low HER2 expression can be classified into two subtypes based on hormone receptor (HR) status: HR-positive HER2 low expression and triple-negative breast cancer (TNBC) with low HER2 expression. Among patients with low expression of HR-positive HER2, treatment recommendations for HR-positive breast cancer are primarily referenced. The current guidelines recommend neoadjuvant therapy, which mainly includes a combination of anthracyclines and taxane chemotherapy or endocrine therapy. However, the overall pathologic complete response (pCR) rate remains below 10%, indicating an urgent need for new treatment options. For patients with low expression of TNBC-HER2, treatment recommendations primarily align with those for triple-negative breast cancer (TNBC). Currently, the neoadjuvant therapy recommended by the National Comprehensive Cancer Network (NCCN) guidelines primarily involves a combination of anthracyclines and taxanes. Previous studies indicate that the pathologic complete response (pCR) rate for triple-negative breast cancer (TNBC) patients undergoing neoadjuvant chemotherapy with anthracycline and taxane ranges from 28% to 35%. This rate is superior to that of anthracycline monotherapy, which has an approximate pCR rate of 20%, or taxane monotherapy, which has an approximate pCR rate of 12%. However, it is noteworthy that the overall response rate remains

	relatively low. However, the GeparSixto study and the CALGB40603 study demonstrated that the addition of platinum to the anthracycline-paclitaxel regimen significantly increased the pathologic complete response (pCR) rate to approximately 50%. However, this also resulted in increased toxicity and reduced patient tolerance. In contrast, the Keynote-522 study and the IMpassion031 study indicate that the addition of immunotherapy to anthracycline and taxane regimens can significantly enhance the pCR rate and extend the event-free survival (EFS) benefit. Consequently, the immuno-combination chemotherapy regimen is included in the neoadjuvant therapy recommendations for triple-negative breast cancer (TNBC) in the 2021 version of the CSCO guidelines. However, the new indications for immunotherapy agents in the adjuvant setting have yet to receive approval in China. As a result, domestic TNBC patients remain in urgent need of safer and more effective neoadjuvant therapy options. Antibody-drug conjugates (ADCs) consist of monoclonal antibodies (mAbs), linkers, and small molecule toxins. ADCs uniquely combine the targeting capabilities of mAbs with the cytotoxic effects of small molecule toxins, effectively merging targeted therapy with chemotherapy. In recent years, studies have yielded promising results indicating that breast cancer patients with low HER2 expression may benefit from ADCs, as evidenced by trials such as DESTINY-A-J101, DESTINY-Breast04, and RC48-C001/C003. Furthermore, preclinical studies have shown that treatment with T-DXd combined with PD-1 mAbs in mice with HER2-expressing tumors results in significant anticancer effects. Additionally, the combination of RC48 has demonstrated significant anticancer effects. RC48 combined with PD-1/PD-L1 has shown synergistic antitumor effects in mouse models of the HER2-positive breast cancer cell line. In addition, chemotherapy combined with immunotherapy can enhance anti-tumor efficacy, and chemotherapy also activates tumor immune circulation. The IMpassion130 and IMpassion131 studies demonstrated that chemotherapy combined with Atezolizumab or Pembrolizumab exhibited significant efficacy and a controllable safety profile in the first-line treatment of metastatic triple-negative breast cancer (mTNBC). Additionally, the KEYNOTE522 study reported a 13.6% increase in the pathologic complete response (pCR) rate (ypT0/Tis ypN0) with Pembrolizumab in combination with a neoadjuvant chemotherapy regimen that included carboplatin and albumin-bound paclitaxel (51.2% vs. 64.8%). Furthermore, the IMpassion031 study indicated that in neoadjuvant therapy for TNBC patients, Atezolizumab combined with chemotherapy achieved a statistically significant pCR rate compared to placebo plus chemotherapy, with an increase of 16.5% in the pCR rate (57.6% vs. 41.1%, p=0.0044). The aforementioned studies provide a clinical rationale for the neoadjuvant treatment of HER2 low-expressing breast cancer using antibody-drug conjugates (ADCs) in combination with immune checkpoint inhibitors. To achieve precision treatment for patients with low HER2 expression in breast cancer, this study aims to investigate the efficacy and safety of the ADC drug combined with the immune checkpoint inhibitor penpulimab in the neoadjuvant setting for the treatment of HER2 low-expressing breast cancer.
Objectives of the Study	**Main Research Objective** The primary aim of this study is to evaluate the efficacy of Disitamab Vedotin in combination with Penpulimab as neoadjuvant therapy for patients diagnosed with stage II or locally advanced breast cancer exhibiting low HER2 expression. **Secondary Study Objectives** This study will evaluate the efficacy and safety of the treatment in patients with stage II or locally advanced breast cancer. **Exploratory Study Objectives** We will explore biomarkers associated with efficacy in peripheral blood and/or tumor tissue, including, but not limited to, PD-L1 expression, levels of tumor-infiltrating lymphocytes (TILs), and residual cancer burden (RCB).

End Points	**Primary End Point** The pathologic complete response rate (pCR) (ypT0/is ypN0) refers to the absence of residual invasive cancer cells in breast specimens following neoadjuvant therapy. Pathological examination of primary tumors and regional lymph nodes may reveal the presence of ductal carcinoma in situ, but no invasive cancer cells should be detected. **Secondary End Points** Effectiveness Indices: Breast Pathological Complete Response Rate (bpCR, ypT0/is), Residual Tumor Load (RCB Score), Objective Response Rate (ORR), Disease Control Rate (DCR), Invasive Disease-Free Survival (iDFS), Event-Free Survival (EFS). Safety indices include the ECOG score, vital signs, physical examinations, laboratory tests, adverse events (AEs), serious adverse events (SAEs), and quality of life assessments. These evaluations are conducted in accordance with the NCI-CTCAE 5.0 standard, among others. **Exploratory End Points (including but not limited to)** PD-L1 expression Tumor-infiltrating Lymphocytes (TILs) Circulating Tumor Cells (CTCs) Immune-related cells, such as CD4/CD8
Study Population	Patients with histologically confirmed stage II-III breast cancer who exhibit low HER2 expression. Low HER2 expression is defined as either IHC 1+ or 2+ accompanied by negative ISH results, or IHC 1+ or 2+ with an unknown ISH status.
Study Design	This study is a single-arm, exploratory Phase II clinical trial with a planned enrollment of 20 eligible patients. Participants will receive six cycles of disitamab vedotin in combination with penpulimab as neoadjuvant therapy.  In this study, the screening period was limited to a maximum of 28 days. Subjects who successfully completed the screening examination and evaluation were subsequently entered into the neoadjuvant treatment phase. Following enrollment, subjects received the trial medication every three weeks until the conclusion of treatment, disease progression, intolerable toxicity, withdrawal of consent, or at the discretion of the investigator.

Stages of Research	The study is structured into three distinct phases, which are divided into three parts: the screening period, the trial period, and the follow-up period. **Screening Period:** Patients will enter the screening phase after signing the informed consent form. Comprehensive baseline information will be collected, including demographic characteristics, medical history, concomitant medications, vital signs, physical examinations, electrocardiograms, and laboratory tests such as blood, urine, and stool analyses. Verification of compliance with the entry criteria will facilitate progression to the trial phase. **Trial Period:** Subjects will receive the drug as specified in the protocol. Following six cycles of preoperative treatment with Disitamab Vedotin (administered every three weeks) and Penpulimab (also administered every three weeks), all patients deemed suitable for surgery will undergo surgical intervention and will be evaluated for the rate of pathological remission. After surgery, patients will select their subsequent treatment regimen based on the recommendations of their clinician. For patients with estrogen receptor (ER) and/or progesterone receptor (PR) positive tumors, endocrine therapy may be initiated upon completion of adjuvant chemotherapy. Additionally, radiotherapy may be administered at the conclusion of adjuvant chemotherapy if clinically indicated. **Follow-up period:** Safety monitoring will continue for four weeks following the last dose.
Dosage regimen	**Disitamab Vedotin**: 2.0 mg/kg administered via intravenous infusion every three weeks, starting on the first day of the cycle for a total of six cycles. **Penpulimab:** 200 mg administered via intravenous infusion every three weeks on the first day of each cycle for a total of six cycles.
Inclusion Criteria	1. ECOG performance status of 0-1. 2. An estimated survival time of no less than three months. 3. The presence of at least one measurable lesion as defined by the RECIST 1.1 criteria. 4. Low HER2 expression was confirmed through pathological examination, indicating clinical stage II-III breast cancer [cT1N1-2, cT2 and any N, cT3 and any N, cT4 and any N] according to the American Joint Committee on Cancer (AJCC) criteria. *Note: Low HER2 expression is defined as IHC 1+ or 2+ with negative ISH, or IHC 1+ or 2+ with unknown ISH status.* 5. Organ function meeting the following requirements: a) Blood routine tests: – ANC ≥ 1.5×10^9/L – PLT ≥ 90×10^9/L – Hb ≥ 90 g/L b) Blood biochemistry: – TBIL ≤ 1.5×ULN – ALT and AST ≤ 2×ULN – BUN and Cr ≤ 1.5×ULN and creatinine clearance ≥ 50 mL/min (Cockcroft-Gault formula)

	c) Cardiac color Doppler ultrasound: – LVEF ≥ 50% d) 12-lead ECG: – QT interval corrected with Fridericia's method (QTcF) < 450 ms in men and < 470 ms in women 6. Known hormone receptor status 7. Availability of tissue samples for biomarker testing 8. A negative serum pregnancy test is required. Patients with fertility potential must consent to use effective non-hormonal contraception during treatment and for at least six months after the last use of the study medication. 9. Participation in the study is voluntary and requires signed informed consent, good compliance, and a willingness to cooperate with follow-up procedures.
Exclusion Criteria	Patients will be excluded from the study if any of the following conditions are met: 1. Inflammatory breast cancer 2. Metastatic breast cancer (stage IV) 3. Any anti-tumor treatment administered within four weeks prior to enrollment, including radiotherapy, chemotherapy, surgical interventions for breast cancer, endocrine therapy, molecular targeted therapy, immunotherapy, biological therapy, and others. 4. Participation in any other clinical trial within four weeks prior to enrollment. 5. Receipt of a live vaccine within four weeks prior to the initiation of the study dose, or the planned receipt of any vaccine during the study, is not permitted. 6. Prior or current use of monoclonal antibodies targeting HER2 (trastuzumab, pertuzumab), tyrosine kinase inhibitors (lapatinib, pyrotinib), or antibody-drug conjugates (T-DM1, DS8201a). 7. Prior administration of PD-1, PD-L1, or PD-L2 inhibitors, or other agents that directly inhibit T cell surface receptors (e.g., CTLA-4, OX-40, CD137). 8. History of other malignant tumors within the past five years, excluding treated cervical carcinoma in situ, basal cell carcinoma of the skin, and squamous cell carcinoma of the skin. 9. Concurrent administration of any other antineoplastic therapy. 10. The presence of third-space effusion, which includes significant amounts of pleural effusion or ascites, that cannot be managed through drainage or other interventions. 11. Known hypersensitivity or delayed hypersensitivity reactions to the study regimen, its components, or related medications. 12. Active autoimmune disease necessitating systemic treatment (e.g., the use of disease-modifying drugs, corticosteroids, or immunosuppressive therapies) within the past two years. 13. A diagnosis of immunodeficiency or the receipt of study treatment within 7 days prior to the first dose (with a daily dose exceeding 10 mg of prednisone equivalent) or any form of immunosuppressive therapy is contraindicated.

	14. Individuals with a documented history of active tuberculosis, encompassing all forms of the disease, were excluded. 15. A history of immunodeficiency, whether acquired or congenital, including a positive HIV test or a history of organ transplantation, disqualifies patients from participation. 16. Patients with a hepatitis B surface antigen (HBsAg) and a hepatitis B virus DNA copy number exceeding 2000 IU/mL were excluded. Those with HBsAg positivity and a DNA copy number below 2000 IU/mL were required to undergo at least two weeks of anti-HBV therapy before receiving the first dose. Additionally, patients who tested positive for hepatitis C virus (HCV) antibodies and had positive HCV RNA were also excluded. 17. Individuals with a history of non-infectious pneumonia that required steroid therapy or those currently experiencing pneumonia. 18. Patients who have a history of any cardiac disease, including: (1) angina; (2) pharmacological or clinically significant arrhythmia; (3) myocardial infarction; (4) heart failure; and (5) any other cardiac condition deemed unsuitable for the trial by the investigator. 19. Women who are pregnant or lactating, women of childbearing age who tested positive on baseline pregnancy tests, and women who are unwilling to use effective contraception throughout the trial. 20. At the investigator's discretion, individuals who pose serious risks to patient safety or have concomitant diseases that may hinder the completion of the study (including, but not limited to, severe hypertension, severe diabetes, active infections, etc.) may be excluded. 21. A prior history of significant neurological or psychiatric disorders, including epilepsy and dementia, is noted. 22. The investigator did not deem the patient suitable to participate under any other circumstances of the study.
Data Analysis and Statistical Methods	**Sample capacity** Exploratory study, 20 planned enrollment. **Efficiency analysis** The validity endpoint analyses for this study will focus primarily on both the Full Analysis Set (FAS) and the Per Protocol Set (PPS), with an emphasis on the results from the FAS. For descriptive statistical analyses, unless otherwise specified, we will present the number of subjects (n) along with their corresponding percentages (%) for each category of subtyped data, as well as the number and percentages of missing subjects. For continuous data, we will report the number of non-missing subjects (n), the arithmetic mean, standard deviation, median, minimum, and maximum values, along with the percentages of missing data for each level of categorization. For dichotomous validity endpoints (including pathological Complete Response [pCR], Residual Cancer Burden 0/I [RCB-0/I], and Overall Response Rate [ORR]), we will summarize the number of subject cases and percentages under each classification, and calculate 95% confidence intervals for the rates using the Clopper-Pearson method. **Safety analysis** The safety analysis available in this study will be based on the SS set. All adverse events will be graded according to NCI-CTCAE 5.0 with descriptive statistics: AE, SAE, AE Grade 3, SAE, AE related, drug / treatment related AE, AE leading to dose adjustment, AE leading to termination. Laboratory test results, vital signs, electrocardiogram and other data will be analyzed from baseline and after baseline. **Exploratory analysis**

	Correlation analysis was conducted based on biomarker status and efficacy measures, including pCR, ORR, RCB, and others.
Research progress	Anticipated first subject enrollment: September 2022 The estimated date of enrollment for the last subject is August 2023. The follow-up plan includes a safety assessment 28 days after the last dose, as well as efficacy and survival evaluations extending up to 5 years.

Map of the Data Collection Plan

Time / Items	Screening PeriodD-28-D-1	Testing Period						End of Treatment/Study /Withdrawal Surgery	Follow-up Period		
		Weekly D1, ±3d							±7d		
		C1	C2	C3	C4	C5	C6		Safety Visit	Efficacy Visit	Survival Visit
Informed Consent Form	X	-									
Demographic Data[1]	X	-									
Medical/Treatment History[2]	X	-									
ECOG Assessment[3]	X	X	X	X	X	X	X	X	X		
Vital Signs[4]	X	X	X	X	X	X	X	X	X		
Physical Examination[5]	X	X	X	X	X	X	X	X	X		
Hematology[6]	X(D-7)	X	X	X	X	X	X		X		
Blood Chemistry[7]	X(D-7)	X	X	X	X	X	X		X		
Urinalysis[8]	X(D-7)	X	X	X	X	X	X		X		
Stool Analysis[9]	X(D-7)	X	X	X	X	X	X		X		
Coagulation Function[10]	X(D-7)	-	-	-	-	-	-				
Thyroid Function[11]	X(D-7)	X	X	X	X	X	X				
Cardiac Enzymes[12]	X(D-7)	-	-	-	-	-	-				
Infectious Disease Screening[13]	X(D-7)	-	-	-	-	-	-				
Pregnancy Test[14]	X(D-7)	-	-	-	-	-	-				
12-Lead ECG[15]	X(D-7)	X	X	X	X	X	X		X		
Echocardiography[16]	X	-	-	-	-	-	X				
Imaging Assessment	X	Evaluation once every 2 cycles						X			
Imaging Assessment (Non-	X	-						X(Post-surgery)			
Original Tumor Location/Metal	X	-									
Inclusion/Exclusion Criteria[20]	X	-									
Vinorelbine Administration[21]		X	X	X	X	X	X				
Paclitaxel Administration[22]		X	X	X	X	X	X				
Tumor Tissue Biomarkers[23]	X							X(Post-surgery)			
Peripheral Blood Biomarkers[24]	X	Collection at Cycle 2, Cycle 4, and End of Cycle 6						X			
Patient Diary[25]		X	X	X	X	X	X				
Concomitant Medications/Proce	X										
Adverse Event Monitoring[27]	X										
Progression/Death Time[28]	X										

Note: The following inspection items should be completed according to the time window listed in the trial process. In case of legal holidays, the reason for the exceeding window can be recorded in the CRF. Each cycle all inspection items shall be completed within three days before the dosing, assessed by the researchers rear can use. ▲ Indicates the same examination as in the previous cycle.

Assessment Details

1. **Demographic data**: Initials, sex, ethnicity, marital status, date of birth, height, weight, and based on which body surface area and Body mass index (BMI).

2. **Past medical history/treatment history**: Including previous diseases or concomitant diseases/symptoms (name of disease/symptoms, name, dose, method of use, outcome of combined drugs); Autoimmune diseases with onset at any time; History of smoking and drinking (frequency, amount, duration), drug allergy (medicine name of drugs, allergic symptoms), bowel habits (frequency).

3. **ECOG score**: Screening, within 3 days before each cycle of dosing, at the end of treatment or before surgery, and safety follow-up visits were required. It can be added at any time if there are any related symptoms of discomfort.

4. **Vital signs**: Including temperature, blood pressure, respiratory rate, heart rate; Screening, the day of each dose, the end of treatment or before surgery, and safety follow-up visits were required. During the treatment period, one vital sign check was required within 30min before each drug infusion and 30min to no more than 60 min after the completion of all study drug infusions. Healing/exit check 1 time (if not before 7 days). If you have any related symptoms, can at any time.

5. **Physical examination**: Including general condition, skin and mucosa, lymph nodes, head and neck, chest, abdomen, musculoskeletal, nerve reflex, respiratory system, cardiovascular system, genitourinary system, mental status, etc. Screening and trial final (D21 + 3) check 1 times a week, end treatment/once at withdrawal (if not performed within the previous 7 days); If you have any related symptoms, can at any time.

6. **Blood routine**: Including absolute counts of WBC, ANC, LC, RBC, Hb, PLT; At the start of the trial period, for each cycle is three days before testing (except for the first time to give medicine), postoperative chemotherapy final supervision inspection once a week, end treatment/exit check 1 time (if before 7 days before).

7. **Blood biochemical**: Including Glucose, TP, A/G, ALT, AST, ALP, gamma GT, propagated, TBIL, DBIL, IBIL, TG, CHOL, UA, BUN, Cr, K+, Na+, magnesium 2+, CL-, Ca2+, P; Screening stage for the first time within 7 days prior to the treatment, trial period at the end of a week (D21 + 3) check 1 time, postoperative chemotherapy final supervision inspection once a week, end of treatment/exit check 1 time (if not performed within the previous 7 days); Myocardial enzymes or additional tests could be performed if necessary, at the discretion of the investigator.

8. **Routine urine**: Including the urine protein, urine routine shows the urine protein +++, please check for 24h urine protein quantitative; Screening stage and final inspection once a week, a trial period of treatment/exit check 1 time (if not before 7 days).

9. **Stool routine**: Including stool character and fecal occult blood; During the screening and trial periods, they were checked at the end of each week and at the end of treatment/withdrawal 1 time (if not within 7 days before).

10. **Blood coagulation function**: Including international standardization ratio (INR), partial thromboplastin time (APTT), fibrinogen (FIB) and thrombin time (TT); If international standardization INR unavailable, the prothrombin time (PT) instead. Screening stage for the first time within 7 days before dosing, drug delivery within 3 days before each cycle (except the first course for the first time to give medicine), end or preoperative, and complete follow-up treatment one test, the researchers can increase additional testing if necessary.

11. **Thyroid function**: Including serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4), if FT3, FT4 unavailable, usable T3, T4 instead. Within 7 days before the screening period for the first time to give medicine. During the study period dosing detection within 3 days before each cycle (except for the first time to give medicine). Treatment ends or preoperative, security, all needs to conduct a follow-up.

12. **Myocardial enzymes**: Creatine kinase, lactate dehydrogenase. Screening stage for the first time within 7 days prior to the treatment, the study period as a significant area pain before, can be combined with ECG results and measured.

13. **Infectious disease screening**: Including five hepatitis B tests, HIV antibody and HCV antibody testing; One test during the screening period; Hepatitis carriers were required to receive antiviral treatment at the discretion of the investigators, and their viral load (HBV-DNA or HCV-RNA) was rechecked every 6 weeks during the study, and additional testing was performed if clinically indicated.

14. **Pregnancy test**: Female subjects of childbearing age need to take blood HCG test during the screening period to exclude pregnancy; Tests were performed during the screening period and at the end of treatment/withdrawal 1 time.

15. **ECG**: QT, QTc, and P-R times were measured 3 times (at least 10 minutes apart) during the screening period. The average of the 3 QTcF was used as the baseline QTcF. The QTcF was measured weekly at the end of the study period and at the end of treatment/withdrawal (if not performed within the previous 7 days). Two additional ECG examinations (at least 10 minutes apart) were required if the QTc interval increased by more than 30 msec from baseline during the trial period or if an absolute value of the QTc interval was 450 msec or more on any of the specified ECG measurements.

16. **Echocardiography**: Could be performed if reported within 28 days before the treatment period. If chest pain or palpitations occurred during the study period, additional echocardiography could be performed as appropriate. One examination at the end of treatment/withdrawal (if not performed within the previous 4 weeks).

17. **Tumor imaging examination (breast)**: Breast physical examination, mammography, breast ultrasound and breast MRI should be performed during screening and before surgery. Breast enhanced MRI combined with breast ultrasound should be used every two cycles during neoadjuvant therapy. Additional imaging could be required if other lesions were suspected except for the primary tumor, which could be reported within the first 21 days of the treatment period. Tumor imaging during the dosing period was performed at the time of the initiation of study treatment, regardless of interruptions in the treatment period because of toxic effects.

18. **Tumor imaging (outside the breast)**: Clinical tumor imaging was performed during the screening period and at regular posttreatment intervals according to RECIST v1.1 criteria. During the screening period, the subject's tumor disease should be evaluated as comprehensively as possible, and the head, neck, chest, abdomen, pelvic and bone tissue imaging examinations (such as head CT/MRI, chest, upper abdomen, pelvic CT scan, whole body bone scan, etc.) should be completed according to the principles of diagnosis and treatment of the research center. If a routine tumor imaging assessment was performed before the signing of the informed consent form and within 28 days before the first dose of medication (including a bone scan within 42 days before the first dose of medication), it was not necessary to repeat it during screening. During study treatment, investigators could perform imaging studies as needed according to the patient's specific conditions.

19. **Tumor marking**: Before neoadjuvant therapy, tumors were labeled with the use of standard center methods (e.g., skin tattooing or surgical clips). All subjects in West China Hospital of Sichuan University were required to locate the tumor before neoadjuvant therapy (baseline). Markers such as metal clips and metal rings were placed during core needle biopsy Markers such as metal clips and metal rings were placed during core needle biopsy before neoadjuvant therapy. If the sub-center (if any) places markers unconditionally, the surface skin of the tumor can be marked according to the size of the tumor and imaging before neoadjuvant therapy, such as tattoo location.

20. **Eligibility assessment**: Eligibility was assessed at the investigator's discretion according to protocol.

21. **Disitamab Vedotin administration**: 2.0 mg/kg every 3 weeks, intravenous drip, each cycle on the first day of medication, medication before should be assessed by the researchers. According to the safety and tolerance of the subjects, drug dose adjustment was allowed, including drug suspension, dose reduction and discontinuation, etc. The specific adjustment plan was according to the drug instructions.

22. **Penpulimab administration**: A fixed dose of 200mg, every 3 weeks, IV infusion, on the first day of each cycle, subject to investigator evaluation before medication. According to the individual patient's safety and tolerability, may need to stop to medicine or permanent discontinuation. It is not recommended to increase or decrease doses.

Specific adjustment protocols regarding dose suspension and permanent discontinuation are according to the drug package insert.

23. **Tumor tissue samples**: At baseline, ultrasound-guided puncture was used to detect lymph node metastasis, and core needle puncture was performed on the primary tumor (including one piece of fresh tissue) to obtain tumor tissue samples. Postoperative tumor tissue and adjacent normal tissue samples were collected, and paraffin samples and fresh frozen samples were made respectively (one sample included cancer and adjacent tumor components, about 5mm in length and width).

24. **Peripheral blood samples**: Collected at baseline and after the 2nd, 4th and 6th cycles of neoadjuvant chemotherapy.

25. **Subject log**: The information of the previous cycle was collected during each cycle of treatment.

26. **Concomitant medications/concomitant therapies**: Concomitant medications for the 28 days prior to the treatment period and during the trial were recorded; Once a subject discontinues the trial treatment, only concomitant medications and concomitant therapies for new or unresolved adverse events related to the trial treatment should be recorded.

27. **Adverse events**: Adverse events were recorded from the date of signing the informed consent until at least 28 days after the last dose of medication. Adverse events, concomitant medications/treatments, and unscheduled examinations were recorded in detail.

28. **Progression/death**: Disease progression and death were recorded throughout the trial.

Table of abbreviations

Abbreviations	Explanations
ADL	Activities of daily living
AE	adverse events
AKP/ALP	Alkaline phosphatase
ALB	albumin
ALT	Alanine aminotransferase
ANC	Neutrophil count
AST	aspartate aminotransferase
AUC	Area under the curve of drug-time
BMI	Body mass index
BUN	Urea nitrogen
CFDA	China Food and Drug Administration
CHOL	cholesterol
CI	Confidence interval
CL/F	apparent oral clearance
Cmax	Peak concentration
Cr	Creatinine
CR	Complete remission
CRF	case report form
DBIL	direct bilirubin
dL	deciliter
DLT	Dose limiting toxicity
DoR	Duration of efficacy
DPD	Dihydropyrimidine dehydrogenase
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group (ECOG)
EDC	Electronic data capture system
EGF	epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	estrogen receptor
FAS	Full Analysis set
FISH	Fluorescence in situ hybridization
g	Gram
GCP	Clinical trial specification
h	hours
Hb	Hemoglobin
HCV	Hepatitis C virus
HER2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HR	HR
IBIL	Indirect bilirubin
IC50	half inhibitory concentration

Abbreviations	Explanations
ITT	therapeutic intent
IU	International units
IV	Iv (drip)
kg	kg
L	l
LC	Lymphocyte count
LDH	lactate dehydrogenase
LVEF	Left ventricular ejection fraction
m	meters
min	Minutes
mg	milligram
ml	milliliters
mm	millimeter
ms	millisecond
MTD	Maximum tolerated drug dose
NCCN	National Comprehensive Cancer Network
NCI-CTC	National Cancer Institute Common Toxicity Criteria
NE	could not be assessed
ORR	Objective response rate
PD	PHARMACOKINETICS
PFS	Progression-free survival
PK	Pharmacokinetic
PLT	platelets
PPS	Per-protocol analysis set
PR	partial response
PR	Progesterone receptor
R	accumulation factor
RBC	Red-cell count
RTK	Receptor tyrosine kinase
SAE	Serious adverse events
SAP	Statistical analysis Plan
SBP	Systolic blood pressure
SD	Stable disease
SS	Safety analysis set
T1/2	half-life
TBIL	Total bilirubin
TG	triglycerides
Tmax	tmax
TP	total protein
TTP	Time to disease progression
μmol	micromol
ULN	Upper limit of normal

Abbreviations	Explanations
VEGF	Vascular endothelial growth factor
WBC	White blood cell count

1 Research Background

Breast cancer is the most prevalent cancer among women in China, with an increasing incidence rate. The prognosis varies significantly among different molecular subtypes of breast cancer; notably, the HER2 overexpression subtype accounts for approximately 20-25% of cases. This subtype is characterized by strong invasiveness, insensitivity to conventional chemotherapy, and poses significant challenges in clinical practice[12]. The advent of HER2-targeted therapies has markedly improved the survival rates for patients with HER2-positive breast cancer. A variety of treatments, including macromolecular single-agent tyrosine kinase inhibitors (TKIs), small molecules, and antibody-drug conjugates (ADCs), have been approved for this patient population[34]. However, the therapeutic benefits for patients with low HER2 expression remain limited. Addressing the challenge of accurately treating individuals with low HER2 expression has become a focal point of research both domestically and internationally.

Breast cancer with lower expression of HER2 can be classified based on hormone receptor (HR) status into two categories: low HR-positive HER2 expression and triple-negative breast cancer (TNBC) with HER2 expression. For patients exhibiting HR-positive status with low HER2 expression, treatment recommendations primarily align with those for HR-positive breast cancer. Current guidelines suggest neoadjuvant treatment regimens predominantly comprising a combination of anthracycline and taxane chemotherapy or endocrine therapy; however, the overall pathologic complete response (pCR) rate remains below 10%. Conversely, for patients with low HER2 expression in TNBC, treatment recommendations follow those established for TNBC. The National Comprehensive Cancer Network (NCCN) guidelines currently advocate for a neoadjuvant regimen primarily involving anthracycline and taxane. Previous studies have indicated that the pCR rate ranges from 28% to 35%, which is superior to that observed with anthracycline-only chemotherapy (approximately 20%) or taxane monotherapy (around 12%). Nonetheless, the overall response rate continues to be low.

The GeparSixto study[5] and the CALGB40603 study[6] demonstrated that the addition of platinum to an anthracycline-paclitaxel regimen significantly improved the pathologic complete response (pCR) rate to approximately 50%. However, this improvement was accompanied by increased toxicity and reduced patient tolerance. Furthermore, the Keynote-522[7] and IMpassion031[8] studies indicated that the incorporation of immunotherapy into the anthracycline-taxane regimen can substantially enhance the pCR rate and prolong event-free survival (EFS). Consequently, the combination of immunotherapy and chemotherapy has been incorporated into the recommendations for neoadjuvant therapy for triple-negative breast cancer (TNBC) in the 2021 edition of the CSCO guidelines. Nonetheless, the indications for neoadjuvant immunotherapy have yet to be approved in China. Therefore, domestic TNBC patients are still in urgent need of safer and more effective neoadjuvant treatments.

An antibody-drug conjugate (ADC) consists of three components: a monoclonal antibody, a linker, and a small-molecule toxin. ADCs possess a dual mechanism of action, targeting specific cells while leveraging the cytotoxic properties of small-molecule toxins, thereby integrating both targeted therapy and chemotherapy. In recent years, ADCs have demonstrated promising results in clinical research, indicating that breast cancer with low HER2 expression may benefit from ADC therapies, as evidenced by studies such as DESTINY-A-J101, DESTINY Breast 04, and RC48C001/C003[9]. Preclinical investigations have revealed that in breast cancer with HER2 expression, T-DXd combined with PD-1 resistance treatment exhibits a significant anticancer effect. Furthermore, in a mouse model of HER2-positive breast cancer cell lines, RC48 combined with PD-1/PD-L1 has shown a synergistic anti-tumor effect.

Meanwhile, the phase IB multicenter study further evaluated the efficacy and safety of TDXd in combination with nivolumab for the late-line treatment of HER2-low advanced or metastatic breast cancer[10]. The study enrolled 16 patients diagnosed with HER2-low breast cancer, without restrictions on hormone receptor (HR) status. Of these patients, 75% had received four or more lines of prior systemic therapy, and 75% had been treated with TDXd plus nivolumab. The overall objective response rate (ORR) was found to be 37.5%, with a median progression-free survival (PFS) of 6.3 months. Additionally, the incidence of grade 3-4 treatment-emergent adverse events (TEAEs) was reported at 43.8%. The most frequently observed adverse events included nausea, fatigue, and alopecia.

Combined immunotherapy can enhance the antitumor effects of chemotherapy by activating tumor immune functions. According to the IMpassion130/131 and KEYNOTE-355 studies, both treatment approaches have demonstrated significant therapeutic effects and manageable safety profiles for metastatic triple-negative breast cancer (mTNBC) in first-line therapy. The KEYNOTE522 study indicated that the addition of pembrolizumab to carboplatin and albumin-bound paclitaxel in neoadjuvant chemotherapy increased the pathologic complete response (pCR) rate (ypT0/Tis ypN0) by 13.6% (51.2% vs. 64.8%)[11]. Similarly, the IMpassion031 study revealed that for patients with TNBC receiving neoadjuvant therapy, the combination of atezolizumab and chemotherapy resulted in a statistically significant and

clinically meaningful increase in pCR rates compared to placebo plus chemotherapy, with an increase of 16.5% (57.6% vs. 41.1%, p = 0.0044)[12].

Disitamab vedotin (RC48) is a novel HER2-targeted antibody-drug conjugate (ADC) that consists of a recombinant humanized HER2 monoclonal antibody linked to the microtubule inhibitor Monomethyl Auristatin E (MMAE). This ADC comprises the HER2 antibody moiety, commonly referred to as the RC48 naked antibody, a linker, and the cytotoxic pentapeptide MMAE small molecule moiety. Unlike T-DM1, the vc-MAE linker in RC48 is cleaved, facilitating the rapid release of the small molecule toxin. Additionally, RC48 exhibits a bystander killing effect, wherein small molecule toxins released from the cells can continue to damage adjacent tumor cells[13]. Currently, phase I clinical trials of RC48 have demonstrated a favorable safety profile in the treatment of HER2-positive metastatic breast cancer (MBC), with the 2.0 mg/kg every two weeks (q2w) dose group exhibiting a superior benefit/risk ratio. The interim analysis of the phase II clinical trial indicates that RC48 is both effective and safe for patients with locally advanced or metastatic HER2-positive breast cancer[14]. Ongoing phase III clinical trials are investigating the efficacy of RC48 in treating HER2-positive liver metastases in advanced breast cancer, as well as its application in advanced breast cancer with low HER2 expression.

Penpulimab (AK105) is a fully human IgG1 monoclonal antibody that targets PD-1. Its first indication was approved for the treatment of relapsed and refractory classical Hodgkin lymphoma[15]. At the time of its approval, clinical trial data indicated an overall response rate (ORR) of 89.4% for penpulimab in this patient population, along with a complete response (CR) rate of 47.1% and a one-year progression-free survival rate of 74.9%. In addition to its efficacy in lymphoma, penpulimab has also demonstrated significant clinical effectiveness across various tumors, including non-small cell lung cancer, liver cancer, pancreatic cancer, nasopharyngeal cancer, gastric cancer, and cholangiocarcinoma[16].

The RC48-C014 study, a Phase Ib/II trial investigating the combination of Disitamab Vedotin and Toripalimab in patients with locally advanced or metastatic urothelial cancer (LA/mUC), presented preliminary results from 39 patients at the 2022 ASCO meeting. The overall response rate (ORR) among all patients was 71.8% (95% CI: 55.1, 85), which included three complete responses (CR), accounting for 7.7%. The disease control rate (DCR) was 92.3% (95% CI: 79.1, 98.4), and the ORR for first-line treatment (excluding those treated with GC) was also reported. The median progression-free survival (PFS) was 9.2 months (95% CI: 6.41, 11.17), while the median overall survival (OS) data remain immature. The combination therapy was well tolerated, with most adverse events classified as grade 1-2; the most common adverse events included anorexia and hypertriglyceridemia. The RC48-C014 study is currently the only reported research on the combination of checkpoint inhibitors with RC48, and further investigations into the efficacy and safety of RC48 in conjunction with immune checkpoint inhibitors are ongoing, including studies RC48-C013, RC48-C016, and RC48-C017.

The aforementioned studies establish a clinical foundation for the combination of antibody-drug conjugate (ADC) therapies with immune checkpoint inhibitors in the neoadjuvant treatment of HER2-low breast cancer. To ensure precise treatment for patients with low HER2 expression, this study aims to utilize the ADC drug Disitamab Vedotin in conjunction with the immune checkpoint inhibitor Penpulimab for neoadjuvant therapy. This research seeks to explore both the efficacy and safety of this combined approach in treating breast cancer characterized by low HER2 expression.

2 Research Purpose

2.1 Objectives of the study

Main Research Objective

The primary aim of this study is to evaluate the efficacy of Disitamab Vedotin in combination with Penpulimab as neoadjuvant therapy for patients diagnosed with stage II or locally advanced breast cancer exhibiting low HER2 expression.

Secondary Study Objectives

This study will evaluate the efficacy and safety of the treatment in patients with stage II or locally advanced breast cancer.

Exploratory Study Objectives

We will explore biomarkers associated with efficacy in peripheral blood and/or tumor tissue, including, but not limited to, PD-L1 expression, levels of tumor-infiltrating lymphocytes (TILs), and residual cancer burden (RCB).

2.2 The Endpoints

Primary End Point

The pathologic complete response rate (pCR) (ypT0/is ypN0) refers to the absence of residual invasive cancer cells in breast specimens following neoadjuvant therapy. Pathological examination of primary tumors and regional lymph nodes may reveal the presence of ductal carcinoma in situ, but no invasive cancer cells should be detected.

Secondary End Points

Effectiveness Indices: Breast Pathological Complete Response Rate (bpCR, ypT0/is), Residual Tumor Load (RCB Score), Objective Response Rate (ORR), Disease Control Rate (DCR), Invasive Disease-Free Survival (iDFS), Event-Free Survival (EFS).

Safety indices include the ECOG score, vital signs, physical examinations, laboratory tests, adverse events (AEs), serious adverse events (SAEs), and quality of life assessments. These evaluations are conducted in accordance with the NCI-CTCAE 5.0 standard, among others.

Exploratory End Points (including but not limited to)

PD-L1 expression

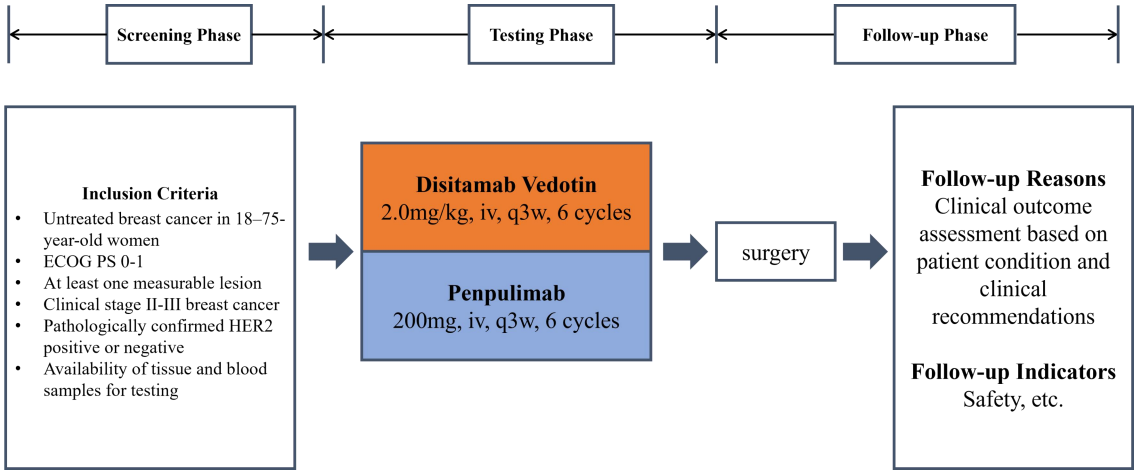
Tumor-infiltrating Lymphocytes (TILs)

Circulating Tumor Cells (CTCs)

Immune-related cells, such as CD4/CD8

3 Study Design

This study is a single-arm, exploratory Phase II clinical trial with a planned enrollment of 20 eligible patients. Participants will receive six cycles of disitamab vedotin in combination with penpulimab as neoadjuvant therapy. The study design was as follows:



In this study, the screening period was limited to a maximum of 28 days. Subjects who successfully completed the screening examination and evaluation were subsequently entered into the neoadjuvant treatment phase. Following enrollment, subjects received the trial medication every three weeks until the conclusion of treatment, disease progression, intolerable toxicity, withdrawal of consent, or at the discretion of the investigator.

The study is structured into three distinct phases, which are divided into three parts: the screening period, the trial period, and the follow-up period.

Screening Period: Patients will enter the screening phase after signing the informed consent form. Comprehensive baseline information will be collected, including demographic characteristics, medical history, concomitant medications, vital signs, physical examinations, electrocardiograms, and laboratory tests such as blood, urine, and stool analyses. Verification of compliance with the entry criteria will facilitate progression to the trial phase.

Trial Period: Subjects will receive the drug as specified in the protocol. Following six cycles of preoperative treatment with Disitamab Vedotin (administered every three weeks) and Penpulimab (also administered every three weeks), all patients deemed suitable for surgery will undergo surgical intervention and will be evaluated for the rate of pathological remission. After surgery, patients will select their subsequent treatment regimen based on the recommendations of their clinician. For patients with estrogen receptor (ER) and/or progesterone receptor (PR) positive tumors, endocrine therapy may be initiated upon completion of adjuvant chemotherapy. Additionally, radiotherapy may be administered at the conclusion of adjuvant chemotherapy if clinically indicated.

Follow-up period: Safety monitoring will continue for four weeks following the last dose.

4 Case Selection

4.1 Eligibility Criteria

1. ECOG performance status of 0-1.

2. An estimated survival time of no less than three months.

3. The presence of at least one measurable lesion as defined by the RECIST 1.1 criteria.

4. Low HER2 expression was confirmed through pathological examination, indicating clinical stage II-III breast cancer [cT1N1-2, cT2 and any N, cT3 and any N, cT4 and any N] according to the American Joint Committee on Cancer (AJCC) criteria.

Note: Low HER2 expression is defined as IHC 1+ or 2+ with negative ISH, or IHC 1+ or 2+ with unknown ISH status.

5. Organ function meeting the following requirements:

a) Blood routine tests:

– ANC ≥ 1.5×10^9/L

– PLT ≥ 90×10^9/L

– Hb ≥ 90 g/L

b) Blood biochemistry:

– TBIL ≤ 1.5×ULN

– ALT and AST ≤ 2×ULN

– BUN and Cr ≤ 1.5×ULN and creatinine clearance ≥ 50 mL/min (Cockcroft-Gault formula)

c) Cardiac color Doppler ultrasound:

– LVEF ≥ 50%

d) 12-lead ECG:

– QT interval corrected with Fridericia's method (QTcF) < 450 ms in men and < 470 ms in women

6. Known hormone receptor status

7. Availability of tissue samples for biomarker testing

8. A negative serum pregnancy test is required. Patients with fertility potential must consent to use effective non-hormonal contraception during treatment and for at least six months after the last use of the study medication.

9. Participation in the study is voluntary and requires signed informed consent, good compliance, and a willingness to cooperate with follow-up procedures.

4.2 Exclusion Criteria

Patients will be excluded from the study if any of the following conditions are met:

1. Inflammatory breast cancer

2. Metastatic breast cancer (stage IV)

3. Any anti-tumor treatment administered within four weeks prior to enrollment, including radiotherapy, chemotherapy, surgical interventions for breast cancer, endocrine therapy, molecular targeted therapy, immunotherapy, biological therapy, and others.

4. Participation in any other clinical trial within four weeks prior to enrollment.

5. Receipt of a live vaccine within four weeks prior to the initiation of the study dose, or the planned receipt of any vaccine during the study, is not permitted.

6. Prior or current use of monoclonal antibodies targeting HER2 (trastuzumab, pertuzumab), tyrosine kinase inhibitors (lapatinib, pyrotinib), or antibody-drug conjugates (T-DM1, DS8201a).

7. Prior administration of PD-1, PD-L1, or PD-L2 inhibitors, or other agents that directly inhibit T cell surface receptors (e.g., CTLA-4, OX-40, CD137).

8. History of other malignant tumors within the past five years, excluding treated cervical carcinoma in situ, basal cell carcinoma of the skin, and squamous cell carcinoma of the skin.

9. Concurrent administration of any other antineoplastic therapy.

10. The presence of third-space effusion, which includes significant amounts of pleural effusion or ascites, that cannot be managed through drainage or other interventions.

11. Known hypersensitivity or delayed hypersensitivity reactions to the study regimen, its components, or related medications.

12. Active autoimmune disease necessitating systemic treatment (e.g., the use of disease-modifying drugs, corticosteroids, or immunosuppressive therapies) within the past two years.

13. A diagnosis of immunodeficiency or the receipt of study treatment within 7 days prior to the first dose (with a daily dose exceeding 10 mg of prednisone equivalent) or any form of immunosuppressive therapy is contraindicated.

14. Individuals with a documented history of active tuberculosis, encompassing all forms of the disease, were excluded.

15. A history of immunodeficiency, whether acquired or congenital, including a positive HIV test or a history of organ transplantation, disqualifies patients from participation.

16. Patients with a hepatitis B surface antigen (HBsAg) and a hepatitis B virus DNA copy number exceeding 2000 IU/mL were excluded. Those with HBsAg positivity and a DNA copy number below 2000 IU/mL were required to undergo at least two weeks of anti-HBV therapy before receiving the first dose. Additionally, patients who tested positive for hepatitis C virus (HCV) antibodies and had positive HCV RNA were also excluded.

17. Individuals with a history of non-infectious pneumonia that required steroid therapy or those currently experiencing pneumonia.

18. Patients who have a history of any cardiac disease, including: (1) angina; (2) pharmacological or clinically significant arrhythmia; (3) myocardial infarction; (4) heart failure; and (5) any other cardiac condition deemed unsuitable for the trial by the investigator.

19. Women who are pregnant or lactating, women of childbearing age who tested positive on baseline pregnancy tests, and women who are unwilling to use effective contraception throughout the trial.

20. At the investigator's discretion, individuals who pose serious risks to patient safety or have concomitant diseases that may hinder the completion of the study (including, but not limited to, severe hypertension, severe diabetes, active infections, etc.) may be excluded.

21. A prior history of significant neurological or psychiatric disorders, including epilepsy and dementia, is noted.

22. The investigator did not deem the patient suitable to participate under any other circumstances of the study.

4.3 Study Withdrawal Criteria

4.3.1 Subject Withdrawal Criteria

To withdraw from this clinical study:

1. All subjects had the right to voluntarily withdraw their informed consent at any time.

2. After randomization, it was discovered that the subjects had significantly violated the enrollment criteria.

Discontinuation of study treatment but continued follow-up as required by the study:

1. Progression of Medical Imaging or Clinical Progression

2. If the subject experiences any adverse clinical events, laboratory abnormalities, or other medical conditions, they may no longer benefit from continued medication.

3. Pregnancy Events That Occurred During the Study

4. For other reasons deemed by the investigator to make the continuation of the study medication impossible.

4.3.2 Disposal of withdrawn subjects

Please ensure that we make every effort to complete the study treatment and exit supervision protocols as outlined in the plan. Upon the conclusion of treatment for participants, follow-up procedures must be conducted in accordance with established guidelines. This includes monitoring therapeutic effects, patient safety, and survival outcomes, if necessary.

Researchers are encouraged to provide guidance or alternative treatment options tailored to the individual circumstances of patients.

4.4 Weed out the standard

1. Does not meet the inclusion criteria, meets the exclusion criteria
2. All data does not affect curative effect and safety of judgment
3. Not following the provisions of this scheme regarding dose, method and course of medication, drug efficacy judgment
4. Use of banned drugs

4.5 Termination of the standard

In case there are good reasons, this study may terminate or suspend. In the event of early termination or suspension of the study, the sponsor will provide written notice of the reasons to the appropriate authorities. The principal investigator is required to immediately report to the ethics committee and provide justification.

Termination criteria include but are not limited to:

5. Finding an unexpected, significant, or unacceptable risk to the subject
6. A significant error found during the execution of a test plan
7. The study drug/trial treatment is ineffective, or continuation of the trial is pointless
8. Serious lag in subject inclusion or frequent plan deviation making completion of the test extremely difficult

5 Medication Regimen

5.1 Summary of the study drug

5.1.1 Drug name and source

1. RC48: known generically as Disitamab Vedotin, is available in a lyophilized dosage form with a concentration of 60 mg per branch. This product is manufactured by Rongchang Biopharmaceutical (Yantai) Co., LTD.
2. AK105: generic name for injection is Penpulimab, 100 mg (10 ml)/bottle, injection, produced by Kanfen Biomedical Science and Technology (Shanghai) Co., LTD.

5.1.2 Preservation conditions

1. Disitamab Vedotin Storage: 2-8°C, avoid light during preservation and transportation. Packing: 1/box. Validity: 24 months.
2. Penpulimab: Storage: Store and transport in a cold storage environment protected from light at 2-8°C. Packing: 1 bottle/box. Validity: 24 months.

5.2 Dosage regimen

1. Disitamab Vedotin

- single dose and cycle:

  2.0 mg/kg, administered once every three weeks via intravenous drip,Each cycle on the first day of use, with 6 cycles per treatment

- Use, treatment and disposal:

  This product is for intravenous administration, using aseptic technique to dissolve and diluteBan push intravenous injection or rapid static noteInfusion time should be within 30-90 minutes (usually suggested 60 minutes)During the drip, if infusion reaction or hypersensitivity reactions occur, slow down or interrupt drip, and/or administer appropriate medical treatmentFor life-threatening reactions, immediately stop drug use

- After dissolved:

  – Use sterile syringes, 6 ml sterilizing water for injection, slow injection into 60 mg bottles, after dissolved concentration of 10 mg/ml

  – Gently rotate bottles until completely dissolved. Do not shake hard

  – Usually dissolves within 10 minutes after sterilization with injection water

  – After dissolving, solution is colorless to pale yellow transparent liquid

  – If not used immediately, store dissolved solution at 2-8°C. Discard unused product solution after 24 hours

  – Inspect appearance of dissolved solution before using to ensure no particulate matter or discoloration

  – Normally, no visible particles should be present; color should be colorless to light yellow transparent liquid

  – If dissolved solution contains visible particles, is turbid or discolored, it should be discarded

- Dilution:

  Based on the dosage adjustment plan and reducing dose proposed, calculate a single dose as follows:

  Volume (ml) = [weight (kg) * dose (mg/kg)] / 10 mg/ml (dissolved solution concentration)

  With sterile syringes, extract computed volume of product, dilute to 250 ml containing 0.9% sodium chloride or 5% glucose infusion bag,Infusion should use 0.2 μm or 0.22 μm filter,Once prepared for infusion, use immediately. If not used immediately, infusion liquid can be stored for no more than 6 hours at room temperature (including infusion time), or in cold storage (2-8°C) for no more than 24 hours,If infusion liquid is stored under cold conditions, return to room temperature before use,This product does not contain preservatives and is for one-time use only. Discard remaining drugs after a single use.

5.2.1 Penpulimab

- dose and cycle:

  200 mg, administered once every three weeks via intravenous drip,Each cycle on the first day of use, with 6 cycles per treatment.

- Drug delivery methods:

Should be administered under guidance of professional doctors using aseptic technique,Infusion should be completed within 60 minutes; can be extended to 120 minutes for patient tolerance,This product should not be pushed, injected intravenously, or administered rapidly.

- Preparation and infusion solution:
  - Do not shake bottle
  - Remove bottles from refrigerator; can be diluted at room temperature (25°C or below) for up to 24 hours
  - Visually inspect drug before use for suspended particles and discoloration. Product is colorless to light yellow transparent liquid with no foreign bodies. Discard if particles are observed
  - Extract 2 bottles (200 mg), transfer to intravenous infusion bag containing 9 mg/ml (0.9%) sodium chloride solution to prepare final concentration of 1.0-5.0 mg/ml. Gently flip to blend
  - From microbiological perspective, once diluted, product must be used within specified time, shall not be frozen (<0°C)
  - Diluted product stability studies show preparation can be stored at 2-8°C, protected from light, for 24 hours, including up to 6 hours at 20-25°C under room illumination (including delivery time)
  - After cold storage, medicine bottles and/or intravenous infusion bags must return to room temperature before use
  - Infusion must use sterile, non-heat source, low-protein IV filter (aperture 0.22 or 0.2 microns)
  - Do not use same IV line with other drugs
  - This product is for one-time use only. Discard any unused medication

5.3 Common adverse drug reactions and processing

5.3.1 Disitamab Vedotin by common adverse reactions and processing

According to clinical research data from experiments C005, C009, C008, as well as phase I clinical trials for solid tumors C002, and two clinical studies C001 and C006 involving breast cancer patients, the safety of Disitamab Vedotin (RC48) was evaluated in a total of 414 participants with malignant tumors. Among these, 394 patients received RC48 at dosages of 2.0 mg/kg Q2W and 2.5 mg/kg Q2W monotherapy.The most common adverse reactions include:Elevated aminotransferase (55.8%),Lower white blood cell count (53.8%),Alopecia (51.8%),Low neutrophil count (49.5%),Fatigue (45.9%),Hypesthesia (41.9%),Anemia (32.5%),Nausea (32.5%),Loss of appetite (25.1%),Lower weight (20.8%),Most adverse reactions were severity level 1 to 2. 14.5% of patients receiving RC48 monotherapy reported serious adverse events, with the most common being:Abnormal liver function (2.0%),Low neutrophil count (2.0%),Intestinal obstruction (1.5%),Fatigue (1.5%),Hypesthesia (1.3%),Lower white blood cell count (1.0%).Adverse Reactions by System Organ Class (SOC),The following adverse reactions were reported in patients receiving Disitamab Vedotin monotherapy:

Neurological System Diseases	
Very Common	Sensory reduction, peripheral neuropathy
Common	Neurotoxicity, dizziness, headache, sensory disturbance
Uncommon	Taste disturbance
Gastrointestinal System Diseases	

Very Common	Nausea, vomiting, constipation, abdominal pain, diarrhea
Common	Abdominal distention, abdominal discomfort
Uncommon	Oral ulceration, digestive tract edema
Systemic Diseases and Administration Site Reactions	
Very Common	Fatigue, fever
Common	Pain, peripheral edema, swelling, influenza-like illness, chest discomfort, injection site
Uncommon	Chills
Metabolic and Nutritional Diseases	
Very Common	Decreased appetite, hypertriglyceridemia, hyperglycemia
Common	Hypoalbuminemia, hypercholesterolemia, hypokalemia, hyponatremia, decreased food
Uncommon	Hypomagnesemia, hypoglycemia, hyperphosphatemia
Hematological and Lymphatic System Diseases	
Very Common	Anemia, decreased white blood cell count, decreased neutrophil count, decreased
Common	Decreased lymphocyte count, decreased neutrophil percentage
Uncommon	Bone marrow suppression, febrile neutropenia
Musculoskeletal and Connective Tissue Diseases	
Common	Limb disorders, joint pain, musculoskeletal pain, back pain, muscle weakness
Infectious and Infective Diseases	
Common	Urinary tract infection, upper respiratory tract infection, herpes zoster
Uncommon	Infectious pneumonia, conjunctivitis, pharyngitis, eye infection, nasopharyngitis, biliary
Cardiac Disorders	
Common	Cardiac arrhythmia, palpitations
Uncommon	Cardiomyopathy
Psychiatric Disorders	
Common	Insomnia
Uncommon	Anxiety, mental disorders

Hepatobiliary System Diseases	
Very Common	Hyperbilirubinemia
Common	Abnormal liver function
Uncommon	Hepatic steatosis
Respiratory System, Thoracic and Mediastinal Diseases	
Common	Dysphonia, cough
Uncommon	Pneumonitis
Renal and Urinary System Diseases	
Very Common	Proteinuria
Common	Hematuria
Uncommon	Renal function impairment
Vascular and Lymphatic Diseases	
Common	Hypertension
Uncommon	Hypotension
Various Injuries, Poisoning and Procedural Complications	
Common	Infusion-related reaction
Uncommon	Nerve damage
Immune System Diseases	
Common	Hypersensitivity reaction
Ear and Labyrinth Disorders	
Common	Tinnitus
Uncommon	Vertigo
Eye Disorders	
Uncommon	Increased lacrimation, blurred vision, periorbital edema
Various Laboratory Tests	
Very Common	Elevated transaminases, weight decrease, elevated γ-glutamyl transferase

Common	Elevated blood alkaline phosphatase, elevated blood creatine phosphokinase, elevated
Uncommon	Coagulation test abnormality, decreased prealbumin, elevated α-hydroxybutyrate

Note: The frequencies are defined as follows: very common (10% or higher), common (1-10%), occasional (0.1-1%), rare (0.01-0.1%), very rare (<0.01%), and unknown (inability to estimate frequency from the available data).

In the study of Disitamab Vedotin (RC48) in combination with an immune checkpoint inhibitor, Disitamab Vedotin in combination with treprostinil was well tolerated with no dose-limiting toxicities (DLTs) observed.Among the 41 patients evaluated for safety, the most common treatment-related adverse events (TRAEs) were elevated AST (65.9%), elevated ALT (63.4%), peripheral sensory neuropathy (63.4%) malaise (58.5%), decreased appetite (56.1%), hypertriglyceridemia (56.1%), and elevated gamma-glutamyl transferase (51.2%), grade 3 TRAEs were predominantly elevated gamma- glutamyl transferase (12.2%), elevated ALT (7.3%), malaise (7.3%), hypertriglyceridemia (7.3%), and immune AEs including immune-associated pneumonitis, hepatitis, myositis, hyperglycemia, and rash occurred in 16 patients (39.0%).

In case of adverse reactions, the investigator should provide medical treatment according to the clinical situation, and the following treatments are for reference:

Hematologic Abnormalities: Cases of hematologic abnormalities, primarily lower white blood cell counts, lower neutrophil counts, lower platelet counts, or anemia, have been reported in patients treated with Disitamab Vedotin and may result in Grade 3 or 4 hematologic abnormalities.

Grade 3 or 4 hematologic abnormalities can occur in a small number of patients potentially leading to secondary infections during treatment. It is essential to monitor patient blood counts prior to each dose of Disitamab Vedotin or when clinically indicated. If Grade 3 or 4 hematologic toxicity it may be necessary to consider withholding administration, reducing the dose, or discontinuation of therapy, and administer symptomatic treatment. Refer to the [Disitamab Vedotin Instructions for Use] section for specific dose adjustments. Monitor for signs and symptoms of infection during treatment and discontinue Disitamab Vedotin treatment and treat symptomatically if a serious infection occurs.

Elevated Transaminases: Elevated transaminases, including elevated aspartate aminotransferase and elevated alanine aminotransferase, may occur in patients treated with Disitamab Vedotin, and cases of grade 3 or 4 transaminase elevations have been reported. Patients should be monitored for liver function prior to each dose of Disitamab Vedotin or when clinically indicated. Dose adjustments should be made according to the severity of transaminase elevations and symptomatic treatment should be provided. Refer to the Disitamab Vedotin dosage and administration section for specific dose adjustments.

Sensory Abnormalities: Cases of sensory abnormalities have been reported in patients treated with Disitamab Vedotin, mainly characterized by hyperalgesia (numbness), mostly in the hands and feet; peripheral neuropathies, mainly sensory peripheral neuropathies. Cases of Grade 3 or 4 adverse reactions have been reported in patients treated with Disitamab Vedotin. During treatment, patients should be

Monitor patients for new or worsening signs and symptoms of sensory abnormalities. If Grade 2 or Grade 3 new or worsened dysesthesias occur, consider withholding administration, reducing the dose, or discontinuing treatment with this product and provide symptomatic treatment. Refer to the [Disitamab Vedotin Instructions for Use] section for specific dose adjustments. If necessary, a neurologist should be consulted for differential diagnosis and treatment.

Infusion-Related Reactions and Hypersensitivity Reactions: Studies have not been performed in patients who discontinued Disitamab Vedotin therapy due to infusion-related reactions or hypersensitivity reactions; therefore, Disitamab Vedotin therapy is not recommended for such patients. Infusion-related reactions and hypersensitivity reactions may be characterized by one or more of the following symptoms: flushing, chills, fever, dyspnea, hypotension, croup, crepitations, and tachycardia. Infusion-related reactions, hypersensitivity reactions may occur in patients receiving Disitamab Vedotin. Prior to dosing, patients should be asked about relevant medical history, prior allergic diseases, atopic sensitivities, and comorbid therapies to assess the risk of infusion-related reactions and hypersensitivity reactions. Monitor patients closely during administration, especially during and 1 hour after infusion. When administering this product, ensure that medications and resuscitation equipment are available to

treat such reactions and to ensure that the patient receives prompt and adaptive treatment. If an infusion-related reaction occurs, the infusion should be immediately interrupted and appropriate treatment instituted, and treatment with this product should be permanently discontinued in the event of an infusion-related reaction of Grade 3 or less, especially a life-threatening one. In the event of a rapid-onset allergic reaction, the use of this product should be immediately and permanently discontinued and adapted therapy instituted.

REPRODUCTIVE TOXICITY: Based on the results of animal studies, this product may be potentially toxic to the male reproductive system, embryo-fetal development. Female patients should undergo a pregnancy test before starting treatment with this product. Female patients of childbearing potential are advised to use appropriate methods of contraception during treatment and for at least 180 days after completion of treatment. Male patients whose partners are of childbearing potential are advised to use appropriate methods of contraception during treatment and for at least 180 days after completion of treatment.

5.3.2 Common Adverse Reactions and Management of Penpulimab

Information on the overall efficacy of Penpulimab was obtained from six clinical trials involving 465 patients receiving monotherapy or combination therapy. The incidence of adverse reactions of all grades was 73.8%, and those occurring in ≥10% of cases included: hypothyroidism, elevated alanine aminotransferase, rash, elevated aspartate aminotransferase, and anemia. The incidence of adverse reactions of grade 3 or higher was 19.6%, and those occurring in ≥1% of cases included: decreased platelet count, anemia, neutropenia, elevated aspartate aminotransferase, rash, and pulmonary infection. The incidence of grade 3 or higher adverse reactions was 19.6%. Other immune-related adverse reactions include: immune-related pneumonia (1.5%), immune-related diarrhea and colitis (0.6%), immune-related hepatitis (0.6%), immune-related nephritis (0.4%), and immune-related dermatitis (5.8%).

If adverse reactions occur, researchers should treat them according to the actual clinical situation. The following treatment methods are for reference:

- Immune-Related Adverse Events

Immune-related adverse events, including severe cases, can occur in patients treated with Penpulimab. These reactions can occur during treatment and after drug withdrawal, potentially involving any tissues and organs.

Patients receiving Penpulimab should be regularly monitored through tests of related indicators or organ function during and after treatment to detect immune-related adverse reactions that may appear at different time points.

For suspected immune-related adverse reactions, thorough assessment should be performed to rule out other causes. Most immune-related adverse effects are reversible and can be managed by discontinuing the drug, administering corticosteroids, and/or providing supportive care.

Generally, treatment should be suspended for most Grade 2 and certain Grade 3 and 4 immune-related reactions. For Grade 4 and selected Grade 3 immune-related adverse events, treatment should be permanently discontinued (see "Usage and Dosage" section).

For Grade 3 and 4 and selected Grade 2 immune-related adverse events, corticosteroids (1-2 mg/kg/day prednisone equivalent) should be administered until improvement to Grade 1 or less. Corticosteroids should be gradually reduced over at least one month to avoid worsening or relapse of adverse reactions. If adverse effects worsen or do not improve after corticosteroid therapy, non-corticosteroid immunosuppressive agents should be added.

Treatment should be permanently discontinued in the following cases:

  - Recurrent Grade 3 immune-related adverse reactions after treatment
  - Grade 2 or 3 immune-related adverse reactions that do not improve to Grade 0-1 within 12 weeks after the last dose (except for endocrine diseases)
  - Corticosteroid dose cannot be reduced to ≤10 mg/day prednisone equivalent within 12 weeks after the last dose

- Immune-related Pneumonitis

Immune-related pneumonitis has been reported in patients treated with Penpulimab. Patients should be monitored for clinical signs and symptoms such as cough, fever, chest pain, dyspnea, hypoxia, and imaging changes (e.g., ground-glass opacities, infiltrates).

Patients with pre-existing lung diseases (e.g., COPD, nonspecific interstitial pneumonia, pulmonary fibrosis) should have regular lung function assessment. For suspected immune-related pneumonitis, follow-up should include imaging, pulmonary function tests, SpO2 monitoring, and exclusion of infection and other causes.

Treatment should be suspended for Grade 2 immune-related pneumonitis and permanently discontinued for Grade 3 or 4 immune-related pneumonitis or recurrent Grade 2 pneumonitis.

- Immune-related diarrhea and colitis

Immune-related diarrhea and colitis have been reported in patients treated with Penpulimab. Patients should be monitored for diarrhea and other gastrointestinal symptoms such as abdominal pain and mucus or blood in stool, with exclusion of infectious causes.

The risk of intestinal perforation should be considered, with imaging and/or endoscopic evaluation when necessary. Treatment should be suspended for Grade 2 or 3 immune-related diarrhea or colitis and permanently discontinued for Grade 4 or recurrent Grade 3 immune-related diarrhea or colitis.

- Immune-related hepatitis

Immune-related hepatitis has been reported in patients treated with Penpulimab. Liver function should be evaluated before treatment and monitored regularly during treatment, with observation for hepatitis symptoms and exclusion of infectious causes and underlying diseases.

If immune-related hepatitis occurs, the frequency of liver function testing should be increased. Treatment should be suspended for Grade 2 immune-related hepatitis and permanently discontinued for Grade 3 or 4 hepatitis.

- Immune-Related Endocrine Diseases

Thyroid Disease:

Thyroid dysfunction, including hyperthyroidism, hypothyroidism, and thyroiditis, has been reported in patients treated with Penpulimab. Thyroid function changes and clinical symptoms should be closely monitored, with consideration of anti-thyroid autoantibody testing.

For symptomatic Grade 2 or 3 hypothyroidism (e.g., unexplained fatigue, weight gain, hair loss, chills, constipation, depression), treatment should be withheld and thyroid hormone replacement therapy initiated as needed.

For symptomatic Grade 2 or 3 hyperthyroidism (e.g., unexplained palpitations, sweating, increased appetite, weight loss), treatment should be withheld and antithyroid medications given as needed.

If acute thyroiditis is suspected, discontinuation of treatment and hormone therapy may be considered. Treatment can be restarted when symptoms improve and thyroid function tests recover. For Grade 4 hyperthyroidism or hypothyroidism, treatment should be permanently discontinued.

Hypophysitis:

Hypophysitis has been reported in patients treated with Penpulimab. Patients should be monitored for signs and symptoms of hypophysitis (including hypopituitarism and secondary adrenal insufficiency), with exclusion of other causes.

Pituitary hormone levels should be monitored and evaluated, with functional tests performed if necessary. Pituitary MRI and autoimmune antibody tests should be considered.

For symptomatic Grade 2 or 3 hypophysitis, treatment should be withheld and hormone replacement therapy provided as clinically indicated. Corticosteroids may be given if acute pituitary inflammation is suspected. For Grade 4 hypophysitis, treatment must be permanently discontinued.

Hyperglycemia and Type 1 Diabetes Mellitus:

Hyperglycemia has been reported in patients treated with Penpulimab. Blood glucose levels and diabetes symptoms should be closely monitored, with insulin replacement therapy provided as clinically indicated.

For Type 1 diabetes with Grade 3 hyperglycemia, treatment should be withheld. For Type 1 diabetes with Grade 4 hyperglycemia, treatment should be permanently discontinued.

Adrenal Insufficiency:

Patients should be monitored for symptoms and signs of adrenal insufficiency, with exclusion of other causes. Adrenal function-related hormone levels should be monitored and evaluated, with functional tests performed if necessary.

For symptomatic Grade 2 adrenal insufficiency, treatment should be withheld and corticosteroid replacement therapy provided until symptoms resolve. For Grade 3 or 4 adrenal insufficiency, treatment should be permanently discontinued.
Immune-Related Glomerulonephritis

Immune-related glomerulonephritis has been reported in patients treated with this drug. Patients should be monitored regularly for changes in renal function and for signs and symptoms associated with nephritis. The majority of asymptomatic patients have elevated serum creatinine. If immune-related glomerulonephritis occurs, the frequency of renal function testing should be increased. Other causes of renal impairment should be ruled out. For grade 2 or 3 elevations in serum creatinine, this drug should be withheld and corticosteroids administered. Grade 4 elevations in serum creatinine should be discontinued permanently (see the section on Usage and dosage of Penpulimab).

- Immune-Related Cutaneous Adverse Reactions

Immune-related cutaneous adverse reactions have been reported in patients treated with Penpulimab. Patients should be monitored for strong or extensive skin adverse reactions. The type, characteristics, and degree changes of lesions should be recorded promptly, and other causes should be excluded. For grade 1 or 2 skin rashes, treatment may be continued under physician guidance with topical corticosteroids and symptomatic treatment. In case of grade 3 rash, treatment should be stopped and symptomatic treatment or topical corticosteroid treatment should be given. Treatment with Penpulimab should be discontinued permanently in the presence of grade 4 rash, confirmed Stevens-Johnson Syndrome (SJS), or Toxic Epidermal Necrolysis (TEN) (see the section on Directions and Dosage of Penpulimab).

- Immune-Related Myocarditis

Patients should be closely monitored for clinical signs and symptoms of myocarditis. In suspected immune-related myocarditis, adequate evaluation should be performed to confirm the cause and rule out other causes, and relevant tests such as myocardial enzyme markers should be performed. In the case of grade 2 myocarditis, Penpulimab therapy should be withheld and corticosteroids should be given. The safety of Penpulimab therapy and whether it can be restarted after the myocarditis has recovered to grade 0 to 1 is unclear. In patients with grade 3 or 4 myocarditis, the drug should be discontinued permanently and corticosteroids should be given. Myocardial enzymes and cardiac function should be closely monitored (see the section of Penpulimab instructions, usage and dosage).

- Immune-Related Pancreatitis

Patients should be closely monitored for clinical signs and symptoms of pancreatitis. Blood amylase or lipase tests and pancreatic imaging should be performed at the beginning of treatment, periodically during treatment, and when indicated on the basis of clinical assessment. For grade 3 or 4 elevations in blood amylase or lipase, or grade 2 or 3 pancreatitis, treatment shall be suspended. In the case of grade 4 pancreatitis or recurrent pancreatitis of any grade, treatment should be discontinued permanently (see the section on Directions and dosage of Penpulimab).

- Immune-Related Thrombocytopenia

Patients should be closely monitored for platelet levels and symptoms and signs of bleeding tendency (such as gingival bleeding, ecchymosis, hematuria, etc.), and other causes and combined medication factors should be excluded. When grade 3 thrombocytopenia occurs, treatment should be stopped, and symptomatic and supportive treatment should be given until it improves to grade 0-1. Whether corticosteroid treatment should be given and whether treatment can be

restarted should be determined according to clinical judgment. When grade 4 thrombocytopenia occurs, treatment should be discontinued permanently and symptomatic treatment should be actively pursued. Corticosteroids should be given if necessary (see the section of instruction, usage and dosage of Penpulimab).

- Immune-Related Nervous System Adverse Reactions

Peripheral Neurotoxicity

Immune-related neurological damage has been reported in patients treated with this product (see Adverse Effects). Patients should be closely monitored for signs and symptoms of abnormalities in the motor and sensory nervous systems. Treatment should be discontinued in patients with grade 2 peripheral neurotoxicity, and should be discontinued permanently in patients with grade 3 or 4 peripheral neurotoxicity (see section for Directions and dosage of Penpulimab).

Myasthenia Gravis

Patients should be closely monitored for signs and symptoms of altered muscle tone, and other causes should be excluded. Electromyography and acetylcholine receptor antibody titers should be performed when necessary. In patients with grade 2 myasthenia gravis, treatment with pyridostigmine should be discontinued and oral pyridostigmine should be given. The dose can be increased according to symptoms, and corticosteroid treatment should be considered. For grade 3 or 4 myasthenia gravis, treatment must be discontinued permanently and corticosteroid therapy initiated. Therapies such as plasma exchange or intravenous gamma globulin can be administered as clinically indicated (see the section on Directions and Dosage of Penpulimab).

- Other Immune-Related Adverse Events

For other suspected immune-related adverse events, adequate evaluation should be performed to confirm the cause and to rule out other causes. According to the severity of adverse reactions, treatment should be suspended and corticosteroids should be given when needed for the first occurrence of grade 2 or grade 3 immune-related adverse reactions. Once the patient's condition has improved, treatment can be restarted after a reduction of the corticosteroid dose under physician guidance. For any recurrent level 3 immune-related reactions (except endocrine disease) and any level 4 immune-related reactions, treatment must be permanently discontinued. Corticosteroid therapy should be given according to clinical indications (see chapter on Usage and Dosage).

- Typical Hodgkin's Lymphoma Complications of Allogeneic Stem Cell Transplantation

In similar antibodies against PD-1 products, fatal and severe complications have been reported in patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) before the start of treatment or after its termination. Transplant-related complications include super-acute graft versus host disease (GVHD), acute GVHD, chronic GVHD, hepatic venous occlusive disease (VOD) occurring after reduced-intensity pretreatment, and febrile syndromes requiring corticosteroid therapy. Patients should be closely monitored for transplant-related complications, and timely intervention is needed. The benefit and risk of using PD-1 antibody treatment before or after allogeneic HSCT should be evaluated.

- Infusion-Related Reactions

Infusion-related reactions have been observed in patients receiving this drug. During infusion, closely observe for clinical signs and symptoms, including fever, chills, rigidity, itching, low blood pressure, chest discomfort, skin rash, urticaria, angioedema, breathing difficulty, or tachycardia. Rare life-threatening reactions can occur. In patients with grade 1 infusion-related reactions, treatment may continue under close monitoring. For grade 2 infusion-related reactions, the infusion rate can be reduced or paused, and antipyretic analgesics, anti-inflammatory medications, and antihistamines may be considered. When symptoms resolve, treatment may resume under close observation. For grade 3 or 4 infusion-related reactions, infusion should be immediately stopped and treatment permanently discontinued. Appropriate medication should be administered (see section on Usage and Dosage).

5.4 Dose Adjustment Protocol

The researchers will first determine whether adverse events may be drug-related, and make decisions regarding drug use based on the most serious adverse events in the previous drug delivery cycle. Before each return to study medication, subjects' hematology and liver and kidney function must conform to the requirements, and all other toxic

effects must be reduced to CTCAE grade 0-1 or baseline (except for hair loss, fatigue, special provisions in the plan, or cases determined by researchers to have no clinical significance). If subjects do not meet the standard drug dosing cycle interval due to adverse events, the next dosing time may be postponed.

The following circumstances should be recorded in detail:

If the treatment cycle is extended, the original scheduled drug administration time and reasons for extension should be recorded; In case of dose reduction, this should be recorded in the subject's diary card, original records, and CRF; When adverse events occur during the study, researchers should provide active symptomatic treatment and make detailed records of combined treatment and medication in the course of disease and CRF.

5.4.1 Disitamab Vedotin Dose Adjustment Scheme

During treatment with Disitamab Vedotin, the drug dose can be adjusted according to the patient's tolerance, including drug suspension, dose reduction, and discontinuation. Disitamab Vedotin dose adjustment principles:

1. During the study period, dose adjustments are not recommended if patients can tolerate the treatment and adverse events resolve. Each cycle's dose should be calculated according to the subject's baseline (first dosing) weight and remain unchanged throughout the study, unless the patient's weight changes by 10% or more.

2. Prior to scheduled medication, the subject's hematology, liver, and kidney function must conform to drug delivery requirements.

3. It is recommended that all drug-related toxicities be resolved to grade 1 or less (CTCAE 4.03) or baseline (except alopecia, fatigue, etc.) before continuing medication. Researchers may decide whether to continue medication based on the subject's condition.

4. If subjects experience significant clinically relevant drug-related adverse events that have not returned to CTCAE grade 1 or less, or baseline, before the next dosing, dosing can be delayed for up to 28 days (calculated from the scheduled date). "Significant clinical relevance" and "related" are determined by the researcher's judgment. For example, hair loss may be judged to be drug-related but may not be evaluated as having significant clinical significance.

5. If subjects have significant clinically relevant drug-related adverse events that have not resolved to a level that allows continued medication within 28 days (from the scheduled date), termination of treatment is suggested. Follow-up evaluation should continue until disease progression and survival assessment.

6. Dose adjustment should follow the order of 0.5 mg/kg reduction increments.

7. Dosage increases are not recommended, but if researchers believe increasing the dose would benefit the patient, ethics committee approval should be obtained before implementing the increase.

More detailed descriptions of dose adjustment can be found in the [Dicetuximab Manual] dose adjustment section. Dosage adjustments are described in Table 1.

Table 1: Disitamab Vedotin Dosage Adjustment Suggestions

Adverse Reaction	Severity*	Occurrence	Dose Adjustment	Suggestions for Handling
Hematology Abnormality	Grade 3	First time	No adjustment	Suspend medication, provide symptomatic treatment until return to grade 0-1 or pre-treatment level; Twice-weekly hematologic testing is

				recommended.
		Second time	First reduction	
		Third time	Secondary reduction	
		Fourth reduction	Discretionary	If treatment is stopped, doctors may continue if better for the patient, with suspension and symptomatic treatment until symptoms ease to grade 0-1 or pre-treatment level; Twice-weekly blood tests are suggested.
	Grade 4	First time	First reduction	Suspend medication, provide symptomatic treatment until return to grade 0-1 or pre-treatment level; Twice-weekly blood tests are recommended.
		Second time	Discretionary	If treatment is stopped, doctors may continue if better for the patient, with suspension and symptomatic treatment until symptoms ease to grade 0-1 or pre-treatment level; Twice-weekly blood tests are suggested.
Transaminase Rise (High)	Level 3	First time	First reduction	Suspend medication, provide symptomatic treatment until return to level 0-2 or pre-treatment level; Twice-weekly biochemical examination recommended.
		Second time	Second reduction	
		Third time	Act accordingly	f treatment is stopped, doctors may continue if better for the patient, with suspension and symptomatic treatment until symptoms ease to level 0-1 or pre-treatment levels; Twice-weekly biochemical examination recommended.
	Level 4	First time	Stop treatment	
Sensory Abnormalities	Level 2		First reduction	Suspend medication, provide symptomatic treatment until return to grade 0-1 or pre-treatment level for subsequent treatment; Continue treatment.
	Level 3	First time	Secondary reduction	
		Second time	Act accordingly	If treatment is stopped, doctors may continue if better for the patient, with suspension and symptomatic treatment until symptoms ease to level 0-1 or pre-treatment levels.

*Severity classification standard references Common Terminology Criteria for Adverse Events (CTCAE) classification.

When drug administration is suspended, if the patient does not respond within 28 days to a level that allows continued treatment (hematology abnormality: grade 0-1; elevated transaminases: grade 0-2; paresthesia: NA) or to pre-therapy levels, discontinuation of therapy is recommended.

If other adverse events associated with Disitamab Vedotin have significant clinical significance and have not returned to CTCAE ≤ grade 1 before the next dosing, the doctor may pause drug use or reduce the dosage. If recovery does not occur within 28 days (from the scheduled date), treatment should be discontinued.

5.4.2 Penpulimab Dose Adjustment Scheme

During treatment with Penpulimab, individual patient safety and tolerability may necessitate stopping medication temporarily or permanently discontinuing treatment.

Dose increases or decreases are not recommended. Detailed guidelines on managing immune-related reactions can be found in section 5.3.2 or the Penpulimab Specification note section. For specific adjustment schemes regarding suspension of dosing and permanent withdrawal, please see Table 2.

Table 2: Recommended Adjustment Scheme for Penpulimab Treatment

Immune-Related Reactions	Severity	Treatment Adjustment Scheme
Pneumonia	Grade 2	Pause until adverse reactions return to grade 0-1
	Grade 3 or 4, or recurrent grade 2	Permanent withdrawal
Diarrhea and Colitis	Grade 2 or 3	Drug administration withheld until adverse effects recover to grade 0-1
	Grade 4	Permanent withdrawal
Hepatitis	Grade 2, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) between 3 and 5 times the upper limit of normal (ULN) or total bilirubin (TBIL) between 1.5 and 3 times ULN	Pause until adverse reactions return to grade 0-1
	Grade 3 or 4, AST and ALT > 5x ULN, or TBIL > 3 times ULN	Permanently discontinue the drug
Nephritis	Grade 2 or 3 with elevated serum creatinine	Administration of the drug withheld until adverse effects return to grade 0-1
	Elevated serum creatinine level 4	Permanent discontinuation of medication
Endocrine Disorders	Symptomatic grade 2 or 3 hypothyroidism Symptomatic grade 2 or 3 thyroid hyperfunction Grade 2 or 3 hypophysitis Grade 2 adrenal insufficiency Type 1 diabetes mellitus with grade 3 hyperglycemia	Administration of the drug withheld until adverse effects return to grade 0-1
	Grade 4 hypothyroidism Grade 4 thyroid hyperfunction Grade 4 hypophysitis Grade 3 or 4 adrenal insufficiency Type 1 diabetes with grade 4 hyperglycemia	Permanent withdrawal
Adverse Skin Reactions	Grade 3	Drug administration withheld until adverse effect returns to grade 0-1
	Grade 4, Stevens Johnson syndrome (SJS) or Toxic epidermal necrosis (TEN)	Permanent withdrawal
Thrombocytopenia	Grade 3	Pause until adverse reactions return to

		grade 0-1
	Grade 4	Permanent withdrawal
Other Immune-Related Adverse Reactions	Grade 3 or 4 elevated blood amylase and lipase Grade 2 or 3 pancreatitis Grade 2 myocarditis* Grade 2 or 3 other related immune adverse reactions occurring for the first time	Pause until adverse reactions return to grade 0-1
	Grade 4 pancreatitis or recurrent pancreatitis Grade 3 or 4 myocarditis* Grade 3 or 4 encephalitis Grade 4 other immune-related adverse events occurring for the first time	Permanent drug discontinuation
Recurrent or Persistent Adverse Effects	Recurrent grade 3 or 4 (excluding endocrine disease) Grade 2 or 3 adverse events that do not improve to grade 0-1 (excluding endocrine disease) within 12 weeks after the last dose of corticosteroid ≤10mg/day prednisone equivalent dose	Permanently discontinue the drug
Infusion-Related Reactions	Grade 2	Reduce the drip rate or suspend administration, and test when symptoms are relieved. Consider resuming medication and observe closely
	Grade 3 or 4	Permanent discontinuation of medication

Note: The severity of adverse events was based on the National Cancer Institute Common Terminology Evaluation Criteria for Adverse Events, version 4.03 (NCI-CTCAE v4.03).

* The safety of resuming treatment after myocarditis improves to grade 0-1 is unclear.

5.5 Drug Management, Distribution, and Recovery

Disitamab Vedotin should be preserved and transported at 2-8°C, avoiding light. It should be stored and transported in a cold storage environment at 2-8°C, avoiding light, and saved at dedicated counters. The study should maintain relevant records of drug supply, storage, distribution, and the original data.

Researchers should appoint personnel responsible for drug storage, delivery, recycling, counting, and record-keeping to ensure all drugs comply with storage conditions, are kept in secure controlled areas, and storage complies with applicable regulatory requirements.

5.6 Associated Drug Use

5.6.1 Drugs and Treatments Permitted During the Study Period

Patients may use necessary supportive treatments and safe combination drugs. According to clinical practice, researchers can use treatments including but not limited to anti-nausea drugs, antibiotics, antifungals, antivirals, rehydration, antihistamines, analgesics, G-CSF, EPO and platelet growth factors, as well as blood component transfusions to improve anemia symptoms or control adverse reactions to chemotherapy. Researchers should provide all necessary treatments for patients' health based on community health standards.

All drug combinations will be recorded on the case report form (CRF), including all prescriptions, over-the-counter (OTC) medications, herbal supplements, intravenous dosing, and rehydration. Any changes in drug dosage, frequency, methods, and dates during the study will be recorded in the CRF. All drug combinations accepted within 28 days prior to the first study dose and within 30 days after the final drug infusion should be recorded.

5.6.2 Prohibited Drugs and Treatments During the Study Period

During treatment, other anticancer drugs and cancer-related ancillary drugs not specified in this protocol should be discontinued, such as anti-tumor and immune preparations.

Active vaccines should not be administered within 28 days before the first study treatment and during the study period. Live vaccines include but are not limited to: measles vaccine, mumps vaccine, rubella vaccine, varicella vaccine, yellow fever vaccine, seasonal influenza vaccine, H1N1 flu vaccine, rabies vaccine, and typhoid vaccine. Inactivated influenza vaccines may be used.

5.6.3 Drugs to Use with Caution During the Study Period

Drug interactions between Disitamab Vedotin and other medications have not been formally studied in patients. To characterize potential interactions with free MMAE, results from drug interaction studies with another ADC drug coupled with the same cytotoxic single methyl auristatin E (MAE) are provided below.

Effects of other drugs on injectable Disitamab Vedotin:

> **CYP3A4 potent inhibitors:** When other MMAE-coupled ADC drugs are combined with ketoconazole (a potent CYP3A4 inhibitor), free MMAE exposure increases, showing 25% higher Cmax and 34% higher AUC. This product is expected to have similar effects when combined with CYP3A4 potent inhibitors.
>
> **CYP3A4 potent inducers:** When other MMAE-coupled ADC drugs are combined with rifampicin (a potent CYP3A4 inducer), free MMAE exposure decreases, showing 44% lower Cmax and 46% lower AUC. This product is expected to have similar effects when combined with CYP3A4 potent inducers.

Effects of injectable Disitamab Vedotin on other drugs:

> **CYP3A4 substrates:** Other MMAE-coupled ADC drugs do not affect midazolam (a sensitive CYP3A4 substrate) exposure when used in combination. This product is not expected to affect the exposure of drugs metabolized by CYP3A4 enzymes.

Penpulimab is a humanized monoclonal antibody whose pharmacokinetic interactions with other drugs have not been studied. Since monoclonal antibodies are not metabolized by cytochrome P450 (CYP) enzymes or other drug metabolic enzymes, combination drugs that inhibit or induce these enzymes are not expected to affect the pharmacokinetics of this product.

To avoid potential interference with the pharmacodynamic efficacy of Penpulimab, systemic corticosteroids and other immunosuppressive agents should be avoided before starting treatment. Systemic corticosteroids and other immunosuppressive agents may be used after treatment initiation for immune-related adverse effects (see the Labeling of Penpulimab).

6 Research Steps

Before starting the study, subjects must read and sign the informed consent form approved by the Biomedical Ethics Committee (EC) of West China Hospital, Sichuan University. All study procedures should be performed within the time window specified in the study schedule.

6.1 Screening Period

After signing the informed consent form, subjects enter the screening period. Unless otherwise noted, the following screening procedures must be completed within 28 days before starting study drug treatment (see the "Data Collection Plan"):

- **Demographic data collection:** Initials, gender, nationality, marital status, birth date, height, weight, and calculation of body surface area and BMI

- **Medical history:** Including current diseases or associated diseases/symptoms (name/symptoms, medication names, doses, methods, outcomes); autoimmune diseases with onset at any time; history of smoking and drinking (frequency, amount, duration), drug allergies (drug name, allergic symptoms), stool habits (frequency)
- **ECOG score;**
- **Vital signs:** Temperature, blood pressure, breathing rate, heart rate
- **Physical examination:** General condition, skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, musculoskeletal system, nervous reflexes, respiratory system, cardiovascular system, urogenital system, mental state, etc.
- **Blood routine:** Including WBC, ANC, LC, RBC, Hb, and absolute PLT count
- **Blood biochemistry:** Including glucose, TP, A/G, ALT, AST, ALP, gamma GT, TBIL, DBIL, IBIL, TG, CHOL, UA, BUN, Cr, K+, Na+, Mg2+, Cl-, Ca2+, P
- **Urine routine:** Including urine protein; if urine routine shows protein +++, 24-hour urine protein quantitative test should be performed
- **Stool routine:** Including stool character and fecal occult blood
- **Blood coagulation function:** Including international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen (FIB), and thrombin time (TT); prothrombin time (PT) may be used if INR is unavailable
- **Thyroid function:** Including serum thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4); T3 and T4 may be used if FT3 and FT4 are unavailable
- **Myocardial enzyme spectrum:** Including creatine kinase and lactate dehydrogenase
- **Infectious disease screening:** Including hepatitis B tests, HIV antibody, and HCV antibody testing; tests completed within 28 days before the treatment period (including those completed before signing ICF) can be used
- **Pregnancy test:** Female subjects of childbearing age should have pregnancy excluded by blood HCG test
- **12-lead electrocardiogram:** Pay attention to QT, QTc, and P-R intervals; check 3 times during screening period (at least 10 minutes apart), with the average of 3 QTcF measurements used as baseline QTcF
- **Echocardiography:** Reports completed within 28 days before the treatment period (including qualified echocardiography completed before signing ICF) can be used
- **Tumor imaging examination (breast):** Screening period requires physical examination, breast mammography, breast ultrasound, and breast MRI; reports within 21 days before the treatment period (including qualified tumor imaging completed before signing ICF) are acceptable
- **Comprehensive tumor imaging:** To assess tumor disease comprehensively, according to the center's diagnosis and treatment principles, complete head, neck, chest, pelvis, and bone tissue imaging examinations (such as head CT/MRI, chest, upper abdomen, pelvic CT scan, whole-body bone scan, etc.); if completed within 28 days before first drug use (including bone scan results within 42 days) after signing the informed consent form, repetition during screening is unnecessary
- **Primary tumor location and/or surgery:** Before neoadjuvant therapy, tag tumors using the research center's standard methods (such as skin tattoo or surgical metal clip)
- **Determine all eligibility criteria**

- **Tumor tissue specimens:** During baseline period, use ultrasound-guided puncture to detect lymph node metastasis and perform coarse needle biopsy of the primary tumor focus (including fresh tissue sample), collect postoperative tumor tissue and adjacent normal tissue samples, prepare paraffin sections, and collect fresh frozen samples (including tumor and normal components, approximately 5 mm wide)
- **Peripheral blood collection**
- **Concomitant medications/therapies:** Record concomitant medications for the 28 days prior to the treatment period and during the trial
- **Adverse events:** Record from the day subjects sign informed consent until 28 days after the last medication

6.2 Trial Period

The treatment period begins when subjects receive medication for the first time. The first dose should be administered as close as possible to the completion of screening tests confirming eligibility. After subjects start study drug treatment, the following tests should be completed according to the protocol's time windows. All tests and assessments (except imaging studies) should be completed within 3 days before drug administration.

Laboratory tests (blood routine, blood biochemistry, urine routine, stool tests, and coagulation function) and electrocardiography performed within 7 days before dosing may not need to be repeated. For legal holidays, reasons for out-of-window timing can be recorded in the CRF.

Before using the study drug in each cycle, subjects should visit the study center for examination, and can use the drug after passing the investigator's evaluation:

- **ECOG score:** Required at each cycle follow-up
- **Vital signs:** Required at each cycle follow-up and before each dose of Disitamab Vedotin and Penpulimab
- **Blood routine:** Required at each cycle follow-up and before each dose of Disitamab Vedotin and Penpulimab
- **Blood biochemistry:** Required at each cycle follow-up; researchers can add additional testing when necessary; required before each dose of Disitamab Vedotin and Penpulimab
- **Urine routine:** Required at each cycle follow-up; if urine routine shows protein ++ or above, add 24-hour urine protein quantification
- **Stool routine:** Required at each cycle follow-up
- **Thyroid function:** Test within 3 days before administration of each cycle during the study period (except the first dose); required at end-of-treatment or preoperative and safety follow-up visits
- **12-lead electrocardiogram:** Required at each cycle follow-up; repeat if necessary (at least 10-minute interval)
- **Cardiac color Doppler ultrasound:** May be performed as appropriate if abnormal electrocardiogram, chest pain, palpitation, or other symptoms occur during the study
- **Adverse events:** Recorded continuously
- **Concomitant medications and therapies:** Recorded continuously
    - **Tumor imaging examination:** Timing determined after study treatment begins, regardless of any medication suspensions due to toxic reactions during this period
    - **Blood samples**: Collect after neoadjuvant therapy cycles 2, 4, and 6,Each time collect at least 10 ml of peripheral blood

6.3 Follow-up Procedures

Participants require follow-up on the day after the last follow-up, including:

- **Safety follow-up**: For adverse events not recovered after stopping the treatment, follow up and make final evaluation,All participants should have safety follow-up to 28 days after the last dose,Record adverse events, concomitant medications/treatments, and unscheduled examinations.
- **Effect follow-up**: For subjects who did not exit the study due to death or PD,Continue tumor imaging evaluations at stipulated times until subject experiences PD, starts other antitumor drugs, or death,Record detailed information about tumor imaging evaluation results and other anti-tumor treatments.
- **Disease progression/survival follow-up**: All enrolled subjects should be followed for five years after treatment ends,Record disease progression and time of death,Follow-up frequency: every 3 months (±28 days) for the first year after trial period, then every 6 months (±28 days).

6.4 Early Termination Study Visits

Subjects continue drug use until disease progression, toxicity, intolerance, withdrawal of informed consent, or researcher determination. If termination checks were not performed within 7 days before the end of study, the following should be completed:

- ECOG score
- Vital signs
- Physical examination
- Blood routine: if not done within 7 days
- Urine routine: if not done within 7 days
- Stool routine: if not performed within the previous 7 days
- Blood biochemistry: if not performed within the previous 7 days
- Pregnancy tests
- 12-lead ECG: if not performed within the previous 7 days
- Echocardiography: if not performed within the previous 4 weeks
- Tumor imaging: if not performed within the previous 4 weeks
- Concomitant medications/treatments: real-time records
- Adverse events: recorded in real time

6.5 Unscheduled Visits

If a subject needs an unscheduled follow-up due to an adverse event, the following should be recorded:

- Concomitant medications/concomitant therapies
- Adverse events
- Records of related inspections (including imaging examination, if any)

7 Effectiveness Evaluation

7.1 Imaging Evaluation

Tumor imaging was evaluated preoperatively with breast-enhanced MRI combined with breast ultrasound and postoperatively with high-resolution enhanced CT or enhanced MRI. Subjects with a history of contrast allergy are managed according to the guidelines for the prevention of allergic reactions to contrast agents at their trial center in order to perform enhanced CT or enhanced MRI whenever possible, and CT plains are permitted if the subject has strict contraindications to contrast agents.

The screening period should include breast enhancement MRI, chest, abdominal, cranial and bone scans by CT, MRI or ultrasound, with the addition of neck and pelvic exams if needed based on clinical indications. The investigator may add additional scanning sites to the tumor assessment at baseline or later as clinically indicated. MRI enhancement scans obtained prior to signed informed consent may be used for screening tumor evaluations as long as they meet the requirements (and within 21 days prior to the first dose of study drug).

Follow-up imaging evaluations should be under the same conditions as the baseline exam (layer thickness of the scan, use of contrast, etc.). The time point for tumor imaging during dosing is determined from the start of study treatment; suspension of dosing does not change the timing of the evaluation. Tumor imaging allowed a time window of ±7 days and was evaluated every 2 cycles during neoadjuvant therapy using breast-enhanced MRI combined with breast ultrasound.

According to RECIST 1.1, bone scans and PET are not suitable for target lesion efficacy assessment. If necessary, the frequency of evaluation of non-target lesions may be reduced if these examinations are used to evaluate these non-target lesions. For example, bone scans may be reviewed only when the target lesion is confirmed to have reached CR or when the presence of bone lesion progression is suspected.

Imaging evaluations for this trial will be performed at the study center and will be based on the RECIST 1.1 criteria for tumor imaging efficacy.

7.2 Primary Endpoint Indicators

- **Pathologic complete response rate (pCR, ypT0/is ypN0)**: Defined as no residual invasive tumor cells or only carcinoma in situ in primary focal specimens and axillary lymph nodes after neoadjuvant therapy

7.3 Secondary Endpoint Indicators

- **Mammary gland pathological complete response rate (bpCR)**: (ypT0/is) Defined as no residual invasive tumor cells or only carcinoma in situ in primary focal surgical specimens after neoadjuvant therapy.

- **RCB classification**: After neoadjuvant therapy, evaluation of:Mammary gland residual tumor primary focal range (mm x mm);Density of residual tumor cells (%);Carcinoma in situ (%);Proportion of positive lymph nodes;Maximum diameter of lymph node metastases (mm);Above parameters are input into calculator network (www.mdanderson.org/breastcancer_RCB) to obtain RCB index and corresponding RCB grading.

- **Objective response rate (ORR)**: Percentage of subjects with CR or PR as best response before disease progression or completion of preoperative chemotherapy,Assessment uses RECIST 1.1 standard;Subjects must have measurable tumor lesions at baseline;CR and PR require confirmation after 4 weeks.

- **Disease control rates (DCR)**: Percentage of subjects with confirmed CR, PR, and SD (≥4 weeks) per RECIST 1.1 standard [(CR+PR+SD)/total number of subjects].

- **Invasive disease-free survival (iDFS)**: Time from postoperative auxiliary treatment to invasive breast cancer recurrence or death from any cause,Invasive recurrence includes ipsilateral or contralateral recurrent breast cancer, local or regional recurrence, distant recurrence; Observation time: at least 5 years from time of receiving adjuvant therapy after surgery.

- **Event-Free Survival (EFS)**: Time from enrollment to any event, including:Death;Disease progression;Change in chemotherapy regimens;Replacement of chemotherapy;Addition of other therapies;Fatal or intolerable side effects; Observation time: at least 5 years from enrollment

7.4 Exploratory Measures

Potential predictive biomarker parameters related to efficacy in peripheral blood and tumor samples (e.g., pCR, RCB-0/I, EFS, iDFS), including but not limited to:PD-L1,CTCs,TILs,CD4/CD8,Tregs,BRCA1/2,PI3K/AKT/mTOR inhibitors.

8 Safety Evaluation

8.1 Safety Indicators

Safety evaluation indicators include:ECOG score,Vital signs,Physical examination,Laboratory examination,Adverse events (AE),Serious adverse event (SAE),Quality of life,Evaluated according to NCI-CTCAE 5.0 standard, including clinical characteristics, severity, occurrence time, duration, drug relationship, treatment, and prognosis.

8.2 Adverse Events (AE) Definition

Adverse events refer to any adverse medical events after subjects sign informed consent, regardless of causal relationship with the study drug. An adverse event can be any unexpected adverse symptom, sign, disease, or abnormal test result.

Adverse events include:

1. Pre-existing medical conditions/diseases that worsen after drug treatment begins
2. New adverse events
3. Clinically significant abnormal laboratory test results

Adverse Event Classification

Reference: NCI-CTCAE version 5.0

If adverse events are not listed in NCI-CTCAE version 5.0, refer to these standards:

- **Grade I**: Mild; No or mild clinical symptoms; Only clinical or diagnostic findings; No treatment required
- **Grade II**: Moderate; Minimal, partial, or non-invasive treatment needed; Limits instrumental activities of daily living (ADL) consistent with age (cooking, shopping, phone calls, financial management, etc.)
- **Grade III**: Severe or has important medical significance, but not immediately life-threatening; May lead to prolonged hospitalization; May lead to disability; Limits self-care ADL (bathing, dressing, eating, toileting, medication, etc.)
- **Grade IV**: Life-threatening consequences; Emergency treatment needed
- **Grade V**: Death related to adverse event

Adverse Events Record

Detailed records during the test should include:AE name,Time,Duration,Severity,Treatment measures,Outcome,Information should be truthfully recorded in the case report form (CRF). Abnormal laboratory data should be recorded on the CRF form and repeated at least once a week until recovery or end of study.Adverse events appearing within 4 weeks after the last dose should be reported and recorded.

Relationship Between Adverse Events and Study Drug

Possible associations are assessed on a five-point scale:Definitely related,Probably related,Possibly related,Probably unrelated,Unrelated,The first three levels are judged to be related to the trial drug. The incidence of adverse reactions is calculated using the sum of these three as the numerator and the total number of subjects used to evaluate safety as the denominator.

Table 3: Criteria for determining the relationship between AE and drug:

Criteria	Definitely relevant	Is likely to be related	Probably related	Probably unrelated	Has nothing to do
Reasonable chronological order	is	is	is	is	no
Type of reaction to known medication	is	is	is	no	no
Removal of the cause can improve	is	is	Yes or No	Yes or no	no
Re-administration may be repeated	is	?	?	?	no
There may be another explanation for the reaction	no	no	no	is	is

8.3 Serious Adverse Event (SAE) Definition

Definition of serious adverse events :

Serious adverse event (SAE) refers to the occurrence of medical events that require hospitalization or prolong hospitalization, disability, affect work ability, endanger life or death, and lead to congenital malformation during the course of clinical trials. The SAE includes the following unexpected medical events:

- Events leading to death
- Life-threatening events (defined as subjects at risk of death)
- Events requiring or prolonging hospital stay
- Events that can lead to permanent or severe disability/incomplete function
- Congenital anomalies or birth defects
- Drug overdose
- Pregnancy

Note on Pregnancy
Pregnancy occurring during a clinical trial should be reported as a serious adverse event.

- Disease Progression

Note on Disease Progression:
Disease progression (including signs and symptoms progress) should not be reported as a serious adverse event, but if the disease causes death during the test or safety report period, it should be reported as a serious adverse event. Hospitalization due to signs and symptoms of disease progression should not be reported as a serious adverse event. If cancer leads to death during the test or safety report period, the death must be reported as a serious adverse event.

- Antineoplastic Therapies

Note on Other Antineoplastic Therapies:
If subjects begin other anti-tumor treatment, the reporting period for adverse events ends when the new anticancer therapy begins. If death occurs at the end of the research and treatment serious adverse event reporting period, regardless of whether they accept other treatment, it must be reported.

- Hospitalization

Note on Hospitalization:
In clinical research, events leading to prolonged hospitalization or hospital admission shall be deemed serious adverse events. Any initial hospitalization (even shorter than 24 hours) complies with this standard.

Hospitalization does not include the following:

- Rehabilitation facility
- Nursing home
- Conventional emergency room visit
- Same-day surgery (outpatient/day/ambulatory surgery)
- Hospitalization unrelated to adverse event deterioration
- Hospital management reasons (e.g., annual check-up)
- Protocol-specified hospitalizations
- Elective hospitalizations unrelated to adverse event deterioration
- Scheduled treatments or surgical procedures documented in the protocol
- Hospital admission solely for blood product use

- Medical Procedures

Note on Medical Procedures:
Invasive diagnostic or therapeutic procedures (such as surgery) should not be reported as adverse events. However, the disease condition leading to the operation that meets the definition of adverse events must be reported. For example, acute appendicitis should be reported as an adverse event, and the resulting appendectomy should be recorded as treatment for the adverse event.

- Overdose Definition

Note on Overdose Definition:
Overdose is defined as excess use of a trial medication over a period of 24 hours that is higher than the dose prescribed by the investigator's order. All test drug overdoses, regardless of whether they are associated with adverse events/serious adverse events, should be reported according to serious adverse event reporting procedures.

Procedures for Reporting Serious Adverse Events

Serious adverse events will be reported from the time participants provide written informed consent until 30 calendar days inclusive after the last dose of a study drug is administered. During the test, in case of serious adverse events, whether it is the first time report or follow-up reports, researchers must immediately complete the serious adverse event (SAE) report, sign and date it, and within 24 hours notify the ethics committee, the main research unit and the study drug source security department:

- Rongchang Biological PV Department: remegenpv@remengen.cn
- Zhengda Tianyan PV Department: cttqpv@163.com

The occurrence of serious adverse events up to 4 weeks after the last dose will not generally be reported unless suspected to be connected with the drug.

Serious adverse events should include detailed records of symptoms, severity, occurrence time, processing time, measures taken, follow-up time and method, and outcome. If researchers believe that a serious adverse event has nothing to do with the experimental drug but is related to study conditions (such as termination of original treatment or complications), this relationship should be detailed in the narrative part of the serious adverse events report. All serious adverse events will be followed until recovery or stabilization.

9 Sampling

9.1 Sampling Procedures

Baseline: Prior to neoadjuvant therapy, core needle biopsy of the primary tumor focus (including one fresh tissue specimen) will be obtained.

Post-Surgery: Tumor tissue and adjacent normal tissue samples will be collected, with multiple paraffin samples and fresh frozen samples made respectively (one sample including cancer and adjacent tumor components, approximately 5mm in length and width).

Blood Samples: At baseline and after 2, 4, and 6 cycles of neoadjuvant therapy, 10 ml of peripheral blood will be taken, with plasma separated by centrifugation and preserved at -80 degrees.

9.2 HER2 Testing

Tumor tissue:

- Immunohistochemical detection of tumor tissue HER2 protein expression intensity
- Fluorescence in situ hybridization (FISH) detection of tumor tissue HER2 gene amplification

Sub-centers without FISH detection capability must send samples to the Department of Pathology, First Hospital of Sichuan University Huaxi for testing or use third-party test results.

10 Quality Control and Assurance

- Researchers must undergo physician training and work under the guidance of senior professionals.
- Clinical wards must conform to standardization requirements with complete rescue equipment.
- Professional nurses must provide detailed guidance about drug use to participants to ensure compliance.
- Research centers must strictly follow case report form procedures and record data truthfully.
- Trial monitoring will be conducted according to standard operating procedures to ensure data accuracy and protocol adherence.
- In the event of an SAE, timely reporting to each study unit is required, with study suspension if necessary.
- All participating sites will be subject to inspections by the sponsor and drug regulatory authorities.

11 Data Safety Monitoring

- All subject data should be recorded in research medical records promptly and accurately.
- Case report forms should be completed by authorized personnel within prescribed timeframes.
- Subject privacy will be protected through the use of coded identifiers rather than names.
- Data security measures include restricted access for data entry, access, and modification.
- Changes to data require approval from both the principal investigator and data manager.
- Adverse events will be recorded in detail, managed appropriately, and tracked until resolution or stabilization.
- Regular cumulative review of all adverse events will be conducted by the principal investigator.

12 Statistical Methods

12.1 Sample Size

This is an exploratory study with a planned enrollment of 20 subjects who meet the inclusion criteria.

12.2 Statistical Analysis Sets

Full Analysis Set (FAS):
Following the intention-to-treat (ITT) principle, all randomized subjects who used at least one dose of study medication will be included. For incomplete observations, the last observation carried forward (LOCF) method will be used.

Per Protocol Set (PPS):
All subjects who comply with the test plan, have good compliance, have not used prohibited drugs during the test period, and have complete case report forms. Missing data will not be filled in.

Safety Analysis Set:
All enrolled subjects who used at least one trial drug and have safety records after drug use.

12.3 Statistical Analysis Plan

The results of this trial were mainly statistically descriptive. Measurement information listed mean, standard deviation, median, maximum and minimum values, and the frequency (constitutive ratio), rate and confidence interval are listed for count data and rank data. All statistical analyses will be performed using SPSS 22.0 statistical analysis software.All statistical tests will be two-sided, with a p-value of less than or equal to 0.05 being considered statistically significant, and confidence intervals of 95% will be used.

Patient Characteristics:
Age, height, weight will be reported as mean, standard deviation, median, maximum, minimum. Qualitative data such as gender and ECOG score will be reported as frequency and percentage.

Efficacy Analysis:

1. Disease control rate (DCR = CR+PR+SD) and objective response rate (ORR = CR+PR) with 95% CI
2. Quality of life scores compared to baseline using paired t-test or signed rank sum test

3. iDFS survival curve estimated by Kaplan-Meier method

Safety Evaluation:

1. Descriptive statistical analysis of adverse events and adverse reactions
2. Laboratory results showing transitions from normal to abnormal with relationship to study drug
3. Vital signs and laboratory indicators before and after treatment compared by paired t-test

13 Ethical Principals and Requirements

This clinical research will follow:

1. The Declaration of Helsinki of the World Medical Assembly
2. Ethical Review Measures for Biomedical Research Involving Human Subjects (National Health and Family Planning Commission of China)
3. Other relevant regulations

The principles of informed consent, privacy protection, free research and compensation, risk control, protection of special subjects, and compensation for research-related damages will be implemented.

All drugs have the potential to cause side effects. The most common adverse reactions and treatment recommendations for Disitamab Vedotin and Penpulimab used in this study are described in Sections 5.3 and 5.4 of this protocol. The study co-sponsor has purchased insurance for the subjects and will bear treatment costs and financial compensation for serious adverse events related to the trial drug as required by law.

Our department has extensive experience with neoadjuvant therapy for breast tumors, with generally low toxic side effects and adverse reactions. The following emergency plan has been established:

When a patient experiences an adverse reaction:

1. Stop the infusion and replace the pipeline
2. Keep the infusion channel smooth
3. Change to normal saline
4. Retain drugs and containers
5. Report to doctors and head nurses
6. Begin observation and rescue procedures

For general reactions:

1. Provide symptomatic treatment according to doctor's advice
2. Maintain close observation
3. Record details promptly
4. Provide appropriate reassurance and explanation

For serious reactions:

1. Administer immediate oxygen
2. Cooperate with rescue measures
3. Send blood transfusion bag and remaining blood for testing
4. Monitor vital signs
5. Strengthen basic nursing care
6. Record the course of events
7. Report to the director and expert group
8. Report to relevant departments promptly

Prior to study initiation, the ethical review board must approve the trial protocol. Before enrollment, investigators must provide subjects with comprehensive information about the study purpose, procedures, and risks, and obtain written informed consent. Participation is voluntary, and subjects may withdraw at any time without affecting their medical treatment or rights.

Protocol Revisions:
Only the lead research unit may modify the protocol. Changes require approval from the ethics committee after obtaining investigator signatures.

Protocol Violations:
All study protocol requirements must be strictly enforced. Any deviations must be recorded with detailed documentation of timing, causes, and corrective measures, signed, dated, and reported to the ethics committee.

14 Publication

All articles and reports related to this trial must be approved by the principal investigators and research funders (Rongchang Bio, Chia Da Tianjing) before publication.

15 Study Timeline

April 2022 - August 2022: Preparation phase (project design, documentation, ethical approval, agreements, personnel training)

September 2022 - August 2023: Subject recruitment and enrollment

September 2023 - April 2024: Completion of neoadjuvant therapy and surgery for the last subject

May 2024 - June 2024: Data analysis, statistical analysis, paper writing and submission

July 2024 - June 2029: Complete 5-year efficacy and survival follow-up, data collation, reporting of subsequent research results

16 Participants

Name	Job title	Major	Assignments	GCP Training Certificate
Ting Luo	Associate Professor	Oncology	Program leader	have
Xiaorong Zhong	Associate Professor	Oncology	Subjects screening, enrollment, assessment	have
Ping He	Attending physician	Oncology	Subjects screening, enrollment, evaluation	have
Xi Yan	Associate Professor	Oncology	Subjects screening, enrollment, evaluation	have
Ting Lun Tian	Attending physician	Oncology	Subjects screening, enrollment, evaluation	have
Bing Wei	Chief physician	Pathology	Pathological testing	have
Zhang Zhang	Chief Physician	Pathology	Pathological testing	have
Xuemei Zhang	Nurse Supervisor	Nursing	Subject medication and care	have
Yanqi Wu	Resident	Oncology	Subject follow-up, data collection	have
Jiaojiao Suo	Resident	Oncology	Subject follow-up, data collection	have
Yuxin Xie	Lecturer	Oncology	Statistical analysis	have
Kunrui Zhu	Lecturer	Oncology	Statistical analysis	have

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