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STUDY PROTOCOL

Efficacy and Safety of Chidamide in Combination with PD-1 Inhibitor and Radiotherapy for HER2-Negative Advanced Breast Cancer: Study Protocol of a Single Arm Prospective Study

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Purpose: As one of the most important breakthroughs in cancer therapy, immune checkpoint inhibitors have greatly prolonged survival of patients with breast cancer. However, their application and efficacy are limited, especially for advanced HER2-negative breast cancer. It has been reported that epigenetic modulation of the histone deacetylase (HDAC) inhibitor chidamide, as well as immune microenvironment modulation of radiotherapy are potentially synergistic with immunotherapy. Thus, the combination of chidamide, radiotherapy and immunotherapy is expected to improve prognosis of patients with advanced HER2-negative breast cancer.

Patients and Methods: This is a single-arm, open, prospective clinical trial investigating the efficacy and safety of the combination of HDAC inhibitor chidamide, anti-PD-1 antibody sintilimab, and the novel immuno-radiotherapy, which aims to enhance efficacy of immunotherapy, in subsequent lines of therapy of HER2-negative breast cancer. Our study will include 35 patients with advanced breast cancer that has failed endocrine therapy and first-line chemotherapy. Participants will receive 30 mg of chidamide twice a week, 200 mg of sintilimab once every 3 weeks, combined with immuno-radiotherapy. Radiotherapy will be centrally 8 Gy for at least one lesion, and at least 1 Gy for the other lesions. We will complete three fractions of radiotherapy in one cycle. The primary endpoint is progression-free survival, and secondary endpoints are objective response rate, disease control rate and safety. Moreover, biomarkers including cytokines and lymphocyte subgroups will be explored.

Conclusion: As a single-arm clinical trial, the analysis of the influence of each single treatment is limited. Besides, our study is an open study, which involves neither randomization nor blinding. In spite of the abovementioned limitations, this prospective clinical trial will give an insight into subsequent lines of therapy of HER2-negative advanced breast cancer, prolong the survival or achieve long remission for these participants, and identify potential responders.

Keywords: HDAC inhibitor, sintilimab, radiation, HER2-negative breast cancer

Introduction

Background and Rationale

The number of newly arising cancer patients was about 19.29 million worldwide in 2020, with breast cancer accounting for 11.7%, making it the most common malignant tumor.[1] Comprehensive treatment including surgery, medical treatment and radiotherapy has improved five-year survival rate of patients with early-stage breast cancer to 80–90%, but advanced breast cancer is still considered incurable in some cases. Besides, although survival of patients with hormone receptor (HR)-positive human epidermal growth factor receptor-2 (HER2)-negative breast cancer has been prolonged significantly owing to endocrine therapy, advanced breast cancer that has failed endocrine therapy and first-line chemotherapy exhibits similar sensitivity to subsequent drug therapy and survival as triple-negative breast cancer (TNBC) that has failed first-line therapy, with only an average of about 3–5 months of disease control time.[2,3] Thus, development of effective treatments is vital.

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From the perspective of tumor occurrence and development, traditional treatments such as radiotherapy and chemotherapy can promote the clonal evolution of tumors as a selective pressure, when eliminating tumor cells. Resistant lesions are thus promoted after multi-line therapy. Immunotherapy including immune checkpoint inhibitors (ICIs) is an important breakthrough and has become the mainstream in cancer treatment in recent years, but application of ICIs in breast cancer is currently restricted to the first-line and neoadjuvant therapy of advanced triple negative breast cancer (TNBC).[4,5] Most breast cancers except for TNBCs have low mutation load and weak immunogenicity. Tumor infiltrating lymphocytes (TILs) of HR-positive/HER2-negative subtypes are significantly lower than those of HR-negative/HER2-negative subtypes. In particular, the increase of TNBC lymphocyte infiltration and PD-L1 expression level is significantly correlated with better responses to immunotherapy and better prognosis.[6,7] How to improve efficacy of immunotherapy and achieve the long-term survival for HER2-negative breast cancer patients are the current difficulties.

Epigenetic alternations including histone acetylases play important roles in cell differentiation, transcriptional regulation, cell cycle regulation, and apoptosis. Chidamide, a selective histone deacetylase inhibitor (HDACi), has been approved in China for combined treatment with aromatase inhibitors such as exemestane in HR-positive HER2-negative advanced breast cancer. Chidamide is able to induce and activate cellular immunity mediated by NK cells and cytotoxic T cells, upregulate expression of tumor antigen, co-stimulatory molecules and receptors including MHC-I/II, PRAME, MIC A/B and PD-L1, and inhibit immunosuppressive cells such as regulatory T cells and myeloid-derived inhibitory cells. Inhibition of HDAC2 will block the nuclear translocation of PD-L1, and thus exert anti-tumor effect in combination with PD-1/PD-L1 inhibitors.[8,9] In patients with peripheral T-cell lymphoma, chidamide can reverse the defects of circulating PD-1+ T cells by upregulating adaptive immune-related genes.[10] Therefore, chidamide potentially has a synergistic effect with PD-1/PD-L1 inhibitors. The efficacy of such a combination has been proved in treatment of refractory diffuse large B-cell lymphoma[11] and NK/T-cell lymphoma.[12] In an observational study carried out by Que et al[13] the combination of chidamide and PD-1 inhibitor triprilimab was applied in patients with advanced soft tissue sarcoma, and ORR in the patients was preliminarily evaluated to be about 45%. Chidamide can reverse the endocrine resistance mechanism by downregulating the estrogen receptor (ER) pathway in the treatment of breast cancer, and is approved for second-line endocrine therapy of HR-positive/HER2-negative advanced breast cancer in combination with exemestane.[14] In vitro studies have confirmed that chidamide can upregulate PD-L1, MHC I, and MHC II of TNBC cells to promote T-cell recognition and thus improve the efficacy of anti-PD-1/PD-L1 inhibitors.[12] Both preclinical and clinical studies have confirmed that epigenetic modulation of chidamide can improve efficacy of ICIs.

Radiation therapy not only directly induces cell death by causing DNA damage, affecting signal transduction regulation and tumor microenvironment, and inflammatory changes, but also plays an anti-tumor role by activating the immune microenvironment. The dose-segmentation model of radiation therapy has been continuously explored in recent years: higher dose radiotherapy can produce the effect of in situ vaccine through the release of tumor-associated antigens;[15] activate dendritic cells to promote antigen presentation; release danger signals, mainly the up-regulation of cytokines and chemokines;[16] and normalize the tumor vascular system. In addition, local radiotherapy can activate DNA sensing pathways in individuals and tumor cells, and the phenotype of tumor cells changes after radiotherapy, which can enhance the sensitivity of immune effector cells to radiotherapy. For example, the expression of tumor cell death receptors, MHC class I molecules,[17] adhesion molecules and stress-inducing ligand are enhanced, and the recognition and killing effect of T cells are enhanced. It promotes type I IFN production[18] and mobilizes innate and adaptive immunity.[19] However, it should not be ignored that high-dose radiotherapy can bring irreversible damage to normal tissues, inhibit or even kill tumor infiltrating lymphocytes,[20] and also contributes to the recruitment of immunosuppressive cells[21] and increased immune-regulatory cytokine secretion.[22] Radiotherapy plays the role of both accelerator and brake of the anti-tumor immune system, so selecting the correct radiotherapy is particularly important for disease control. Furthermore, radiotherapy is often insufficient to induce a systemic antitumor immune response, so combining with ICIs or multimodal combination therapy is important. Among them, low-dose radiotherapy can significantly improve the immune microenvironment. For tumor lesions with lower levels of TILs, 1 Gy once every two weeks with a total dose of 6–13 Gy can reverse resistance to immunotherapy and achieve long-term efficacy.[23] The scheme of radiotherapy working synergistically with immunotherapy to enhance the efficacy of immunotherapy is defined as immuno-radiotherapy.[24]

Due to the synergistic mechanisms of HDAC inhibitors and radiotherapy, a number of studies delivered HDAC inhibitors combining with radiation and confirmed the anti-tumor efficacy of this scheme. Blattmann et al[25] found in a xenograft model that the combination of HDACi and heavy ion radiotherapy could cause apoptosis of cancer cells, destruction of angiogenesis, and significantly delay the growth of osteosarcoma. Wang Y et al[26] showed that inhibition of HDAC in SW579 thyroid cancer cells could enhance their sensitivity to radiotherapy. Other preclinical studies have also confirmed the sensitizing effect of HDAC inhibition on radiotherapy.[27]

Therefore, in the condition that immobilization of the immune system is released by the binding of PD-1/PD-L1 inhibitors, immunity is ignited by high-dose radiotherapy, and focal immune microenvironment is regulated by low-dose radiotherapy and epigenetic modulation, tumor elimination by the immune system can be expected to be more effective. In an effort to provide better therapeutic effect and prolong the survival for patients with advanced HER2-negative breast cancer who have failed endocrine therapy and first-line chemotherapy, to tackle the problem of resistance and relapse after multi-line therapy, as well as to improve the efficacy of immunotherapy in these patients, we conducted this single-arm, open, prospective clinical trial.

Objectives

It is expected to evaluate the efficacy and safety of immuno-radiotherapy combined with chidamide and sintilimab, a PD-1 inhibitor, in the treatment of patients with HER2-negative advanced breast cancer who have failed endocrine therapy and first-line chemotherapy in this single-arm, open, prospective clinical trial.

Trial Design

The study will be executed from 31 July 2022 to 31 December 2024. It will be divided into screening, treatment, and follow-up periods (Figure 1). Among them, the screening period is from participants signing informed consent to

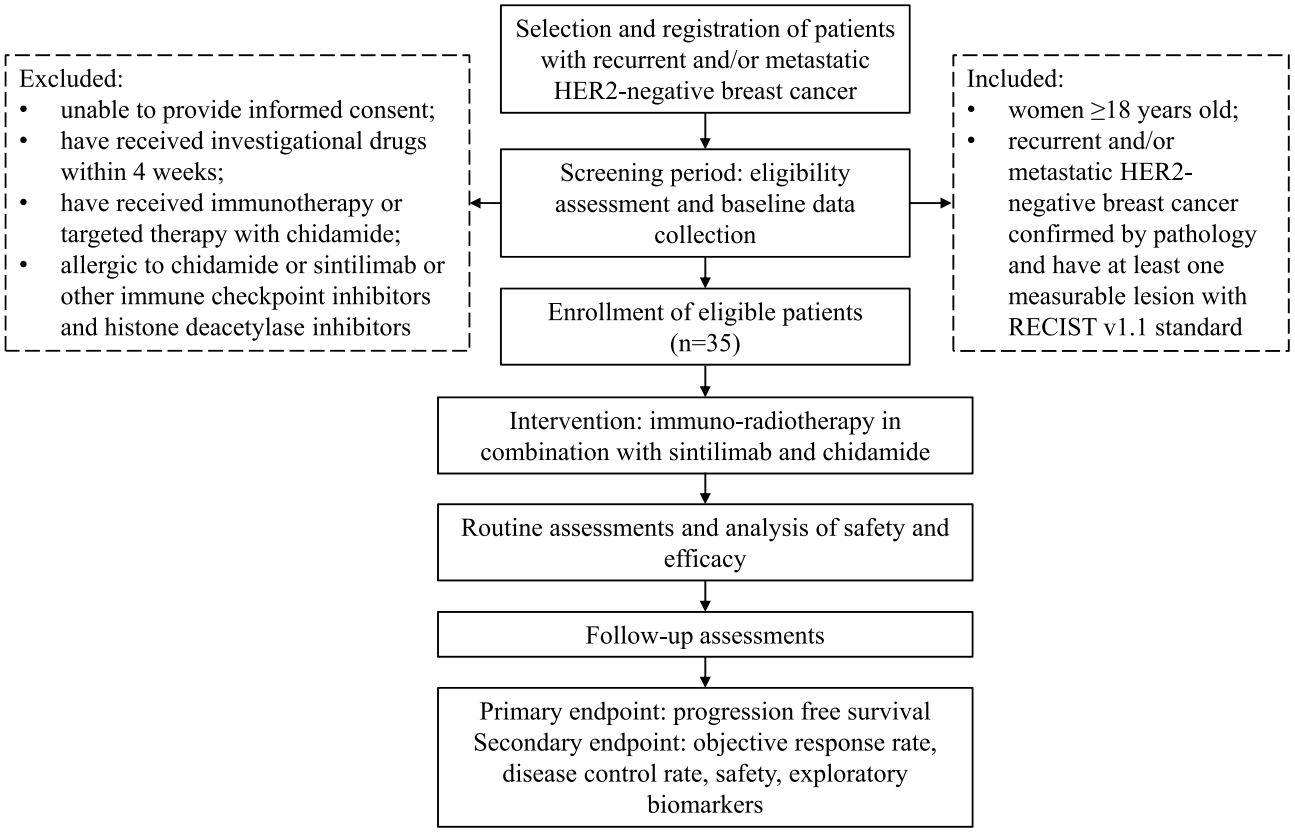

Figure 1 Study design.

receiving study drug. The treatment period is the period during which subjects receive study medication. The follow-up period is from the end of study medication (discontinuation of study treatment) to disease progression or death or the end of the study. The proposed sample size is 35 cases. We present the protocol in accordance with the SPIRIT reporting checklist.[28]

Methods: Assignment of Interventions

This study is a single arm clinical study and does not involve a randomization procedure.

Methods: Participants, Interventions, and Outcomes

Study Setting

This study was conducted by the Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School. All participants are those routinely receiving treatment at Nanjing Drum Tower Hospital.

Eligibility Criteria

The eligible participants include those with pathologically confirmed recurrent metastatic HER2-negative breast cancer through immunohistochemistry (IHC) staining and in situ hybridization (ISH), namely HER2(-) IHC-/+ or IHC++ but FISH/CISH- who have at least one measurable lesion meeting the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Major inclusion criteria also include the requirement of participants to have received ≥ one line of chemotherapy of anthracycline and/or taxane (including neoadjuvant, adjuvant, or after metastasis) in relapse and metastasis stage, or have received ≥ one line of endocrine therapy, ie patients who have recurred and metastasized during or within 12 months after adjuvant endocrine therapy or who have progressed on endocrine therapy for recurrent and metastatic breast cancer, for HR-positive [ER positive (IHC ER(+)≥1%) and PR positive (IHC PR(+) ≥1%] participants. Besides, participants should also have ideal performance status, with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–1, and an estimated life expectancy of at least 12 weeks. Excluded were participants who have received immunotherapy or chidamide previously, or other kinds of therapy within 4 weeks, as well as those with unstable or untreated central nervous system metastases, active or history of serious infection, cardiovascular disease, or autoimmune diseases (Table 1).

Interventions

Description of Interventions

Subjects who meet the requirements will be treated according to the dosing schedule specified in the protocol (Figure 2). Subjects will receive 30 mg of chidamide orally twice a week and 200 mg of intravenous sintilimab once every 3 weeks, while radiation therapy is to be completed 3 times every 2 days during the 5 days before cycle 2 (Day 22). Radiotherapy will include as many measurable lesions on computed tomography (CT) or positron emission tomography (PET) /CT scans as possible, with centrally 8 Gy each single dose for at least one primary lesion, and at least 1 Gy each single dose for the other lesions, in a total of 3 fractions of radiotherapy in one cycle. The treatment will be continued till progression.

Routine examinations, records of needed medication and adverse events (AEs), as well as collection of blood sample and tumor tissue sample will be completed at the end of each treatment cycle. Imaging including CT or magnetic resonance imaging (MRI) of the chest, abdomen, head as well as other suspicious lesions will be carried out and evaluated at the end of every two cycles of treatment. Additional tests may be done if the investigator deems it necessary.

Criteria for Discontinuing or Modifying Allocated Interventions

The participants are encouraged to be retained in the trial whenever possible. Treatment and relevant assessments will be continued unless the participant reaches treatment termination, which is defined as termination of treatment for any reason, such as disease progression as defined in RECIST v1.1 or intolerance, or early withdrawal for any reason. Results of routine examinations, imaging and key records will be checked, and a final visit will also be completed to make sure that the effectiveness and safety checks specified in the protocol, safety follow-up, and AEs are completed and fully

Table 1 Eligibility Criteria of Participants Enrolled

Inclusion Criteria	Exclusion Criteria
1. Subjects participate in this clinical trial voluntarily, and can provide written informed consent; 2. Women aged at least 18 years old; 3. Have a pathologically confirmed recurrent metastatic HER2-negative breast cancer (HER2-negative IHC-/+ or IHC++ but FISH/CISH-), with at least 1 measurable lesion meeting RECIST v1.1; 4. Have received at least one line of chemotherapy of anthracycline and/or taxane drugs in the past (including neoadjuvant, adjuvant, relapse and metastatic stage); 5. Participants with hormone receptor positive [ER positive (IHC ER positive percentage ≥1%) and PR positive (IHC PR positive percentage ≥1%)] have previously received at least one line of endocrine therapy and is resistant to endocrine therapy; Participants with metastatic breast cancer relapse in the process of adjuvant endocrine therapy or within 12 months after endocrine therapy, and participants experiencing progress after endocrine therapy targeting at relapse metastatic breast cancer; 1. Have the ability to swallow pills; 2. ECOG performance status score of 0 to 1; 3. Have an estimated life expectancy of at least 12 weeks; 4. Have at least one lesion suitable for radiation therapy estimated by experts in radiotherapy; 5. Functions of important organs conform to the following requirements (the usage of any blood components or cell growth factors in the screening period is not included): • Absolute neutrophil count ≥1.5×10^9/L; • Platelets ≥ 90×10^9/L; • Hemoglobin ≥ 9g/dL; • Serum albumin ≥ 3g/dL; • Thyroid stimulating hormone (TSH) ≤ upper limits of normal (ULN) (if the TSH level is abnormal, levels of T_3 and T_4 should be monitored, the subject can be enrolled if T_3 and T_4 levels are normal); • Bilirubin ≤ 1.5 ULN; • Alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 ULN; • Alkaline phosphatase (AKP) ≤ 2.5 ULN; • Serum creatinine ≤ 1.5 ULN or creatinine clearance rate ≥ 60 mL/min (standard Cockcroft -Gault formula is applied)	1. Pregnant or lactating women (women of childbearing potential should have a negative pregnancy test within 14 days before the first dose, if tested to be positive, possibility of pregnancy should be excluded through ultrasound), women of childbearing potential unwilling to take effective contraceptive measures during the trial; 2. Have the history of any other tumors (except for non-melanoma skin cancer or cervical carcinoma in situ), unless the tumors have achieved complete remission and have not received and treatment 5 years ahead of enrollment; 3. Have received any investigational drug within 4 weeks ahead of treatment; 4. Have received any anti-PD-1 antibody, anti-PD-L1/L2 antibody, anti-CTLA4 antibody or other immunotherapy; have received chidamide targeted therapy; 5. Have central nervous system metastases with clinical symptoms; participants with stable and asymptomatic brain metastases who have received radiotherapy, and have at least 1 measurable lesion other than the brain metastases can be enrolled, and the last time of radiotherapy should be over 4 weeks ahead of enrollment; 6. Have received major operation, or the operational wounds have not been healed within 4 weeks ahead of enrollment; 7. Have experienced embolism or bleeding within four weeks ahead of enrollment; 8. With serious cardiovascular disease, including hypertension (blood pressure ≥ 160/95 mmHg) that cannot be controlled through medical treatment, unstable angina, history of myocardial infarction within the past 6 months, congestive heart failure > NYHA grade II, serious arrhythmia and pericardial effusion, etc.; 9. Serious infection that needs intravenous antibiotic, antimycotic and antiviral treatment; 10. Have active autoimmune diseases that needs systemic treatment including disease modifying antirheumatic drug, corticosteroid and immunosuppressive agents within two years ahead of the first dosing. Replacement therapy including thyroxine, insulin or physiological corticosteroids in the treatment of adrenal or pituitary insufficiency is allowed. Have the history of known primary immunodeficiency. It relies on the investigators to judge whether there exist autoimmune diseases in participants with positive autoimmune antibody solely; 11. Have received immunosuppressive agents within 4 weeks ahead of first dosing. Local glucocorticoid given through intranasal spray, respirable drugs or other approaches, and systemic glucocorticoid of physiological dose (ie 10 mg of prednisone or other glucocorticoid with equivalent dose per day at most) will be allowed. Temporary usage of glucocorticoid in the treatment of dyspnea caused by asthma, chronic obstructive pulmonary disease, etc. will also be allowed; 12. Allergic to any sintilimab or chidamide, or have the history of serious allergy to any other immune checkpoint inhibitors or histone deacetylase inhibitors; 13. Have mental disorders with poor obedience; 14. Considered unsuitable for enrollment by the investigators.

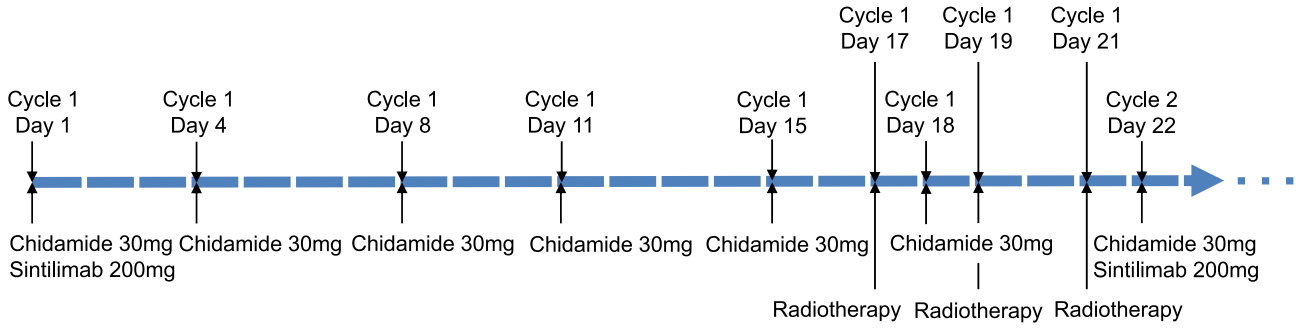

Figure 2 Schematic diagram of treatment regimen.

documented at the time of treatment termination. The investigator may suggest or offer new or alternative treatments to the patient withdrawn from the trial, depending on the patient's condition.

Strategies to Improve Adherence to Intervention Protocols

Concerns raised by the patients will be addressed thoroughly and promptly, followed by detailed instructions from the investigators. The number of cycles of treatment each patient takes will be recorded and monitored during the study to improve their adherence to intervention protocols.

Relevant Concomitant Care and Interventions That are Permitted or Prohibited During the Trial

Symptomatic or supportive interventions without antitumor effect such as administration of vitamins and antiemetic or pain-relieving drugs will be allowed and recorded. Other drugs with antitumor effects, including proprietary Chinese medicines, are prohibited during the treatment period.

Outcomes

The primary endpoint is progression-free survival (PFS), which is defined as the time from baseline tumor evaluation to disease progression as defined by RECIST v1.1 or death. The secondary endpoints include objective response rate (ORR), defined as the proportion of participants with complete response (CR) or partial response (PR) assessed by RECIST v1.1, disease control rate (DCR), defined as the ratio of participants with CR, PR or stable disease (SD) based on RECIST v1.1 and safety evaluation. Exploratory analysis will help to figure out relevant biological markers including specific cytokine levels of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, IL-17A, IL-1B, IL-5, IL-12p70, IFN-α, IL-8 and lymphocyte subgroups of CD3+ T cells, CD4+ T cells, CD8+ T cells, NK cells and DC cells that may help identify potential responders of such combined therapy. They will be measured before treatment, at the first response evaluation, and at discharge. Exploratory testing could be performed as appropriate.

Baseline information including demographic data, tumor history or other medical history, tumor baseline assessment, echocardiography, drug allergy history, combined drug use, and adverse event documentation will be collected in screening period within 28 days prior to treatment initiation. Baseline imaging evaluation includes CT or MRI of the chest, abdomen, head and other suspicious lesions. Examination of concomitant diseases and abnormalities, other routine examinations, and blood sample collection will be completed within 7 days prior to medication (Table 2).

All participants will be evaluated for efficacy every two cycles until PFS is achieved. Survival follow-up will be performed every 3 months after the PFS endpoint. Management of patients will be based on RECIST v1.1, with continued collection of all relevant imaging results. If a patient is withdrawn from treatment and/or treated with another regimen prior to disease progression, that patient will still be followed up every 6 weeks until disease progression.

In cases of uncertainty in identifying disease progression, particularly when encountering remission of non-target tumors and appearance of new tumors, treatment may be continued until the next evaluation, or re-evaluate as soon as possible. If the re-evaluation confirms disease progression, date of the initial examination is considered the date of disease progression. If disease progression is observed in a non-target tumor, disease progression will only be determined when there is significant worsening of the non-target tumor and a significant increase in the patient's total tumor load in

Table 2 Assessments in the Trial

Screening Period	Treatment Period	Treatment Termination	Safety Visits During the Follow-Up Period
1. Informed consent, demographic data collection, tumor history/ other medical history collection, tumor imaging (including CT or MRI of the chest, abdomen, and pelvis; baseline assessment of screening tumors may be relaxed to within 4 weeks prior to treatment; CT/MRI scans obtained within 28 days prior to the first study dose may be used for screening tumor assessment as long as they meet the requirements), echocardiography, drug allergy history collection, combined drug use collection, and adverse event documentation are required within 28 days prior to treatment initiation. 2. Concomitant diseases and abnormalities, ECOG score and other scores, vital signs, physical examination, blood routine, urine routine, blood biochemistry, stool routine, coagulation function, electrocardiogram, thyroid function test, pituitary adrenal axis test, pregnancy test, myocardial enzyme profile test, HIV, hepatitis B and hepatitis C test, and blood sample collection should be completed within 7 days prior to medication. 3. At the end of each treatment cycle, ECOG score was combined with other scores, physical examination, vital signs, blood routine, blood biochemistry, 12-lead electrocardiogram, echocardiography, urine routine, coagulation function, stool routine, thyroid function test, pituitary adrenal axis test, pregnancy test, myocardial enzyme profile test, HIV, hepatitis B, hepatitis C test, imaging examination, and records of needed medication, recorded adverse events, blood sample collection, tumor tissue sample collection, etc. Additional tests may be done if the investigator deems it necessary.	At the end of each treatment cycle, ECOG score was combined with other scores, physical examination, vital signs, blood routine, blood biochemistry, 12-lead electrocardiogram, echocardiography, urine routine, coagulation function, stool routine, thyroid function test, pituitary adrenal axis test, pregnancy test, myocardial enzyme profile test, HIV, hepatitis B, hepatitis C test, imaging examination, and records of needed medication, recorded adverse events, blood sample collection, tumor tissue sample collection, etc. Additional tests may be done if the investigator deems it necessary.	1. ECOG score and other scores, vital signs, physical examination, routine blood, urine, blood biochemistry, stool routine, coagulation, ECG, echocardiography, adverse events, combined medications, medication use, and imaging were checked at the time of treatment termination; 2. Fully document adverse events (AEs) and regression; 3. Participants with non-disease progression will continue to be followed for imaging evaluation every three months until the subject starts new antitumor therapy or disease progression.	1. Subjects are seen for follow-up visits from 30 days after the end of study drug, or 90 days after sintilimab administration, whichever is longer. Follow-up visits are recommended every 30 ± 7 days, with outpatient follow-up required for the first visit, after which telephone follow-up is possible until adverse events reach grade 0–1 or are stable, or return to baseline phase levels, or new antitumor therapy is initiated. ECOG score with other scores, vital signs, physical examination, blood routine, urine routine, blood biochemistry, stool routine, coagulation function, electrocardiogram, echocardiogram, adverse events, and combined medications were checked at safety follow-up; 2. Survival follow-up: every 3 months ± 14 days to collect information on survival and follow-up treatment arrangement. If subjects terminate without disease progression, imaging is required at survival follow-up and followed until new anti-cancer treatment is started or disease progression is recorded (every 3 months ± 14 days according to the frequency of clinical oncology imaging evaluation).

both target and non-target lesions to the extent that treatment needs to be suspended even if the target tumor is SD or PR. A slight increase in one or more non-target tumors will not be defined as progression.

Subjects are visited for follow-ups of 30 days after the end of drug administration, or 90 days after sintilimab administration, whichever is longer. Follow-up visits will be implemented every 30 ± 7 days, until adverse events reach grade 0 to 1 or are stable, or return to baseline levels, or new antitumor therapy is initiated. Results of routine examinations, adverse events, and combined medications were checked at safety follow-up.

Survival follow-ups are carried out every 3 months ± 14 days to collect information on survival and follow-up treatment arrangement. If subjects terminate without disease progression, imaging is still required at follow-ups. These patients will be visited every 3 months ± 14 days according to the frequency of required imaging evaluation, until new anti-cancer treatment is started or disease progression is recorded.

For safety assessment, NCI CTC AE v5.0 is used, and any AEs and serious adverse events (SAEs) of all subjects will be monitored and documented in detail. Appropriate medical care and follow-ups will be provided to participants with SAEs or AEs that lead to incompletion of treatment. Participants will be followed up until AEs recover or are reasonably explained.

Participant Timeline

Figures 1 and 2 shows the trial timeline from screening to follow-up periods.

Sample Size

In this exploratory clinical trial, PFS of patients with HER2-negative breast cancer who have failed endocrine therapy and first-line chemotherapy in subsequent lines of chemotherapy is estimated to be 3.7 months, and PFS of the experimental group is expected to be 6 months. Based on a type I error rate of 0.05 in one-tailed test and a power of 0.8 with the estimated drop-out rate of 10%, a total of 35 participants will be recruited in this study.

Recruitment

Study recruitment started on 01 March, 2023 and is expected to be completed on 31 December, 2023. From March 2023 onwards, eligible participants from Nanjing Drum Tower Hospital will be enrolled in the study until the scheduled 35 women are achieved. Written informed consent will be obtained.

Methods: Data Collection, Management, and Analysis

Data Collection Methods

Plans for Assessment and Collection of Data

With descriptive statistical analysis, our study uses SAS 9.2 for statistical processing. For description of enrolled participants and analyzed population, the number of enrolled participants, the number of participants in the full analysis set, the per-protocol set, the safety set and their exclusion, as well as the drop out cases will be analyzed. In terms of statistical description and inference of baseline data, index attained before treatment will be described. Among them, for qualitative data, the number and proportion of cases in each category including nominal data and sequential data were calculated. For quantitative data, the number of cases, mean, standard deviation, median, maximum and minimum were calculated.

Plans to Promote Participant Retention and Complete Follow-Up

Patients are encouraged to report any difficulties they come across to investigators, and the investigators will help to tackle the problems to promote participant retention. For follow-up of participant, except for the first visit when outpatient follow-up is required, it can be completed with simply a phone call or E-mail.

Data Management

Data collected will be recorded and documented in the paper case record form and kept in locked cabinets. Access to the study data will be restricted.

Statistical Methods

Analysis of Primary and Secondary outcomes and Additional Analyses

When it comes to the analysis of efficacy, changes in disease will be judged according to RECIST v1.1. PFS, ORR, DCR, and 95% confidence interval will be calculated. The Kaplan–Meier curve will be used to describe the PFS, and the median PFS will be calculated. For safety analysis, detailed description of the type and severity of AEs will be recorded during the trial, which includes time of occurrence and regression, severity, correlation with drug administration, and treatment measures. Incidence of AEs will also be calculated.

Analysis of Non-Adherence Population, and Missing Data

Patients who withdraw from or unable to continue the trial will be documented and excluded from our cohort, but imaging results will continue to be collected in follow-ups. Missing data will not be imputed in this trial.

Methods: Monitoring

Data Monitoring

As the participants of our study will be exposed to minimal risks, a data monitoring committee is not required in this trial.

Harms

AEs and SAEs will be assessed according to NCI CTC AE v5.0, and will be monitored and documented in detail. Any of the AEs that meet the criteria for SAE will be promptly notified to our clinical monitor and will be reported to the principal investigator, institutional review board, State Food and Drug Administration and local pharmacovigilance authorities within 24 hours.

Auditing

Auditing is not planned in this trial.

Ethics and Dissemination

Research Ethics Approval

The study will be conducted in accordance with the Declaration of Helsinki, and has been approved by Nanjing Drum Tower Hospital's Medical Ethics Committee, an independent organization set up to protect subjects' rights. (2022-296-02 on 07 July, 2022).

Protocol Amendments

Any substantive amendments to the protocol will be documented then acknowledged and approved by the relating authorities including the sponsor, principal investigator, institutional review board, State Food and Drug Administration and local pharmacovigilance authorities.

Consent or Assent

Every patient will be fully informed of the purposes, procedures, and potential risks of this clinical trial before enrollment. Written informed consent will be obtained from all subjects involved in the study. Additional written informed consent will also be attained for necessary collection of samples including peripheral venous blood and tumor tissue.

Biological Specimens

In order to identify potential responders of this combined therapy, peripheral venous blood samples and tumor tissues will be collected to detect specific cytokine levels of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, IL-17A, IL-1B, IL-5, IL-12p70, IFN-α, IL-8 and lymphocyte subgroups of CD3+ T cells, CD4+ T cells, CD8+ T cells, NK cells and DC cells.

Confidentiality

Privacy and confidentiality of data will be protected with caution.

Ancillary and Post-Trial Care

Study participants are accessible to new or alternative treatments that will be beneficial to their conditions after the endpoint of this trial.

Dissemination Policy

Findings of this study will be published in peer-reviewed journals and presented at national and international conferences.

Trial Status

The current protocol version is version 1.1, updated on 17 June, 2022. Study recruitment started on 01 March, 2023 and is expected to be completed on 31 December, 2023.

Clinical Trial Registration Number

Chinese Clinical Trial Registry ChiCTR2300068737, registered on 28 February, 2023.

Data Sharing Statement

The trial is ongoing and no data are available.

Ethical Approval Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study will be conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study has been reviewed and approved by the Institutional Review Board of Nanjing Drum Tower Hospital, Affiliated Hospital of Nanjing University Medical School (approval number 2022-296-02) on 07 July, 2022. Written informed consent will be obtained from all subjects involved in the study. Additional written informed consent will also be attained for necessary collection of samples including peripheral venous blood and tumor tissue.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interests.

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