Phase I Study to Evaluate the Safety and Preliminary Efficacy of Bortezomib Combined with Cisplatin in the Treatment of Recurrent or Metastatic Breast Cancer

Research Unit: Sun Yat-sen University Cancer Center

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Version Number: 1.2

Version date: December 18, 2024

Principle Investigator

Project Signature Page

I have read this trial protocol (version number: 1.2, version date: December 18, 2024) and agree to conduct this trial in accordance with the protocol and related annexes. I will provide the protocol to my research team and discuss it with them to ensure that they fully understand this trial. I may request to terminate this trial or stop enrollment at any time for certain pre-specified reasons; I may also stop this trial if I need to protect the rights and interests of the subjects. I agree to conduct this trial in strict accordance with all current applicable regulations and clinical trial good management standards (GCP).

At the same time, as the principal investigator of this trial, I coordinate the overall progress of the trial.

Name: _____

sign: _____

date: _____

Test Center Name: _____

Program Summary

Test title	Phase I clinical study on the safety and preliminary efficacy of bortezomib combined with cisplatin in the treatment of recurrent or metastatic advanced breast cancer
Version Number/Date	Version 1.2, December 18, 2024
Test Type	Investigator-Initiated Trials (IITs)
IIT Sponsor	Professor Shi Yanxia, Cancer Center, Sun Yat-sen University
Overall Design	Single-center, single-arm, non-randomized, open-label trial design
Indications	Recurrent or metastatic advanced breast cancer
Number of cases	This study plans to enroll 18-20 subjects
Purpose of the test	To evaluate the safety and efficacy of bortezomib combined with cisplatin in the treatment of recurrent or metastatic advanced breast cancer
Study Duration	Enrollment was completed within 18 months after trial initiation. The data were collected until the last subject completed the efficacy evaluation, and survival was followed up until 2 years after the last subject completed treatment.
Trial Drugs	**Bortezomib** This drug is divided into three dosage groups, namely 1.3mg/ m2 , 1.5mg/ m2 , and 1.7mg / m2 , and is administered on D1 , D4 , D8 , and D11 , with a course of treatment every three weeks . **Cisplatin** The drug is divided into two dosage groups, 50mg/ m2 and 70mg / m2 , which are administered from D1 to D3 , with a course of treatment every three weeks .
Experimental Design	This study plans to evaluate the safety and preliminary efficacy of cisplatin combined with bortezomib. The DLT assessment period is from day 1 to day 21 of the subject's first dose plus 24 hours after the second dose, that is, 22 days. Each dose group must first enroll 3 subjects. If no DLT occurs in the first cycle (within 28 days after the first dose), the dose will be increased to the next cohort; if 1 subject develops DLT, 3 subjects will be added to the cohort, and if no DLT occurs in the last 3 subjects, the dose will be increased to the next dose. If 2 or more subjects in 3 or 6 subjects in a dose group develop DLT, the dose escalation will be stopped, and the previous dose of the dose will be the MTD. After the dose escalation reaches the MTD, the dose escalation will be stopped. If the MTD is not reached in this trial, the researchers will discuss whether to continue the subsequent escalation trial. Subject Replacement: If a subject discontinues study treatment before completion of C1 (ie, within 21 days of dosing) other than due to DLT, an additional subject will need to be recruited

	to replace him/her.
Inclusion criteria	Patients enrolled in the study must meet the following criteria: 1. Women aged 18 years and above with pathologically confirmed recurrent or metastatic advanced breast cancer ; 2. The patient has tumor specimens (formalin-fixed, paraffin-embedded or fresh pre-treated recurrent tumor tissue); 3. Patients who have failed standard treatment in the late stage ; 4. At least one measurable lesion; 5. ECOG PS : 0-2 points; 6. Estimated survival period ≥12 weeks; 7. The function level of major organs meets the following standards: 1) The blood routine examination standards must meet: ANC ≥1.5×109/L, PLT ≥75×109/L, Hb ≥85g/L (no blood transfusion and blood products within 14 days, no use of G-CSF and other hematopoietic stimulating factors for correction) 2) Biochemical examinations must meet the following standards: TBIL <1.5×ULN, ALT, AST <2.5×ULN, ALT, AST <5×ULN for patients with liver metastasis, BUN and Cr ≤1×ULN or endogenous creatinine clearance ≥50ml/min (Cockcroft-Gault formula); 8. Women of childbearing age must have taken reliable contraceptive measures, or have undergone a pregnancy test (serum or urine) within 7 days before enrollment, with a negative result, and are willing to use appropriate contraceptive methods during the trial and 8 weeks after the last administration of the trial drug. 9. The subjects voluntarily join this study, have good compliance, and cooperate with follow-up.
Exclusion criteria	Patients who meet any of the following criteria are not eligible for inclusion: 1. Acute active hepatitis B or acute active hepatitis C. 2. Any serious underlying disease, comorbidity and active infection 3. Undergoing other anti-tumor treatment; 4. History of epilepsy or epilepsy-induced state; 5. Pregnant or breastfeeding patients; 6. Poor compliance or unable to follow up normally; 7. Allergic to study drugs; 8. Patients diagnosed with other malignant tumors within 5 years, except for the following: surgically resected non-melanoma skin cancer, adequately treated cervical carcinoma in situ, surgically radically treated ductal carcinoma in situ, or malignant tumors diagnosed 2 years ago with no current evidence of disease and untreated ≤ 2 years before randomization; 9. The researcher determines other situations that may affect the progress of the clinical study and the determination of the study results.
Concomitant medication	1. Information on all other concomitant medications during the study (generic name of the drug, purpose of administration, dosage, time of administration, etc.) must be recorded in detail in the case report form.

	2. The use of other clinical trial drugs was prohibited during the study.
Efficacy indicators	Tumor response (ORR and DCR) will be assessed based on RECIST1.1 with reference to iRECIST; duration of response (DOR) and progression-free survival (PFS) will also be evaluated.
Safety indicators	Safety endpoints included DLT, MTD or HTD, adverse events (AEs, TEAEs) during treatment, evaluation of SAEs, clinically significant abnormalities in safety laboratory tests, clinically significant changes in physical examination, ECG, LVEF and vital signs.
Statistical analysis	Sample size: This trial is an open, single-arm, dose-escalating Phase I clinical study to evaluate the safety of bortezomib combined with cisplatin in advanced breast cancer and to preliminarily explore its anti-tumor activity. In the dose-escalation phase, each dose group adopts the 3+3 method, with 3-6 cases included in each group, and ≤ 20 subjects enrolled. Statistical analysis set: Enrolled Subjects Set (ESS): defined as all enrolled subjects, regardless of whether they used the trial drug or not. This data set is used to analyze demographic and baseline data, subject allocation, and protocol violations. Full Analysis Set (FAS): defined as all enrolled subjects who used the trial drug at least once. This data set is used for efficacy analysis. Safety Analysis Set (SS): defined as all enrolled subjects who have used the trial drug at least once and have at least one safety evaluation. This data set is used for the analysis of safety data. Dose-limiting toxicity analysis set (DLTS): all subjects who received the trial drug and completed the 28-day DLT assessment or experienced DLT within 28 days. PK Concentration Analysis Set (PKCS): defined as all enrolled subjects with at least one PK concentration value after using the trial drug. PK Parameter Analysis Set (PKPS): defined as all enrolled subjects with at least one PK concentration value after using the trial drug. Statistical analysis methods: The test results are mainly presented using descriptive statistical methods. Measurement data will generally list the number of observations, mean, standard deviation, median, quartile, maximum, and minimum. Count data will list frequency and frequency (composition ratio). Statistical analysis will be performed using SAS 9.4 and above. Descriptive statistics were used to describe the number and proportion of subjects entering each analysis set, the number and proportion of subjects who completed the trial and withdrew from the trial, and the reasons for withdrawal from the trial (and their proportion); and to describe demographic and other baseline characteristics. Adverse events, adverse reactions, and serious adverse events will be summarized for each dosing group using the safety analysis set. The severity of adverse events and reactions will be graded using the NCI CTCAE version 5.0 criteria. Adverse events and reactions will also be summarized by System Organ Class and Preferred Term. The number and proportion of subjects with at least one dose-limiting toxicity (DLT) and the number of DLTs will be summarized for each dosing group. Using the safety analysis set, the baseline data, post-dose data, and post-dose change data (Change from Baseline) including laboratory tests, physical examinations, vital signs,

	electrocardiograms, LVEF, etc. will be summarized by each follow-up and by each dosing group; a shift table will be used to describe the changes from baseline to each follow-up after dosing for the normality and clinical significance of each test result as categorical data. RECIST (Evaluation of Response Criteria in Solid Tumors) version 1.1 was used with reference to iRECIST to evaluate the imaging of the patient's tumor at baseline and after treatment. The evaluation results included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The objective response rate (ORR) and disease control rate (DCR) of each dosing group and their bilateral 95% confidence intervals (Clopper-Pearson exact probability method) were calculated using the full analysis set. Objective response rate (ORR) = (number of patients assessed as CR + number of patients assessed as PR) / total number of patients assessed. Disease control rate (DCR) = (number of patients assessed as CR + number of patients assessed as PR + number of patients assessed as SD) / total number of patients assessed. The Kaplan-Meier method was used to draw the survival curves of progression-free survival (PFS) and duration of response (DOR), and the median PFS and median DOR and their bilateral 95% confidence intervals were estimated. PFS and DOR will be analyzed using the full analysis set. The treatment of censored data in survival analysis and the determination of censoring dates will be detailed in SAP. A descriptive summary of the blood drug concentrations at each planned time point was made, and for each dosing group, the concentration-time curve of each subject, the average concentration-time curve of all subjects, and the semi-logarithmic curve corresponding to the two curves were plotted. Phoenix WinNonlin software was used to estimate and analyze the non-compartmental pharmacokinetic parameters of the blood drug concentration data and calculate the pharmacokinetic parameters.

1. Research background

1.1 Background

Breast cancer ranks first in the incidence of female malignant tumors in the world, with more than 2.2 million new cases each year. Advances in diagnosis and treatment have reduced the mortality rate of early breast cancer, but there are still quite a number of breast cancers that are insensitive to treatment or resistant to drugs, namely "refractory breast cancer", which leads to the death of nearly 680,000 patients each year. The incidence of breast cancer in my country has increased rapidly in recent years, and the trend of younger patients is becoming increasingly obvious. Its incidence ranks first among female malignant tumors in my country. According to the "Analysis of Incidence and Mortality of Malignant Tumors in China" recently released by the National Cancer Center of China in 2020, the incidence of breast cancer in my country is increasing at an annual rate of 3%. The incidence of breast cancer in major cities in China has increased by 37% in the past 10 years, and the mortality rate has increased by 38.9%. The reason is that Chinese patients are mostly in the middle and late stages when breast cancer is discovered, and treatment can no longer effectively control the spread of cancer cells, eventually leading to the death of patients. It is expected that the incidence of breast cancer will continue to rise in the future, and the group of refractory breast cancer will continue to expand. Therefore, continuing to develop treatment strategies for refractory breast cancer is an important measure to improve the survival rate of patients.

Bortezomib is approved for the first-line treatment of patients with myeloma and mantle cell lymphoma. It is a cornerstone drug throughout the induction, consolidation, and maintenance treatment of myeloma. Its function is to inhibit the 26S proteasome, which is an enzyme complex involved in the degradation of multiple intracellular regulatory proteins, including the NF-κB (nuclear factor-κB) inhibitor IκBα, p53, p21, and p27. Malignant tumors with high concentrations of activated NF-κB, such as breast cancer, are reasonable targets for drugs that interrupt this pathway. Mutations in the tumor suppressor gene p53 occur in 20-40% of sporadic breast cancers and are associated with poor prognosis and adverse reactions to certain chemotherapy and hormonal drug treatments. Cyclic kinase inhibitors p21 and p27 also play an important role in breast cancer. Moreover, bortezomib has shown cytotoxic activity in in vivo breast, lung, pancreatic, prostate, and head and neck tumor models. Therefore, bortezomib has certain application prospects in the treatment of breast cancer. At the same time, we also found in the breast cancer organoid specimen library that breast cancer organoids are generally sensitive to protease inhibitors. Unfortunately, previous clinical trials have found that single-agent bortezomib treatment cannot effectively inhibit the progression of breast cancer in patients.

In preliminary in vitro and in vivo studies of a series of solid tumors, bortezomib and standard cytotoxic drugs showed synergistic anti-tumor effects. A phase I/II clinical study for refractory breast cancer found that bortezomib combined with docetaxel can achieve a median progression-free survival rate of 5.4 months , and the side effects are predictable and manageable.

Cisplatin is a commonly used drug for metastatic triple-negative breast cancer, especially for patients with BRCA 1/2 gene mutations. Our previous preclinical studies found that cisplatin combined with bortezomib can reverse the acquired multidrug resistance of breast cancer cells, and animal experiments showed that the combination regimen is safe and has the potential for clinical application. Therefore, based on the known single-agent antitumor activity of bortezomib and cisplatin, as well as their synergistic efficacy in preclinical models, different toxicity spectra, and feasibility in clinical studies of various solid tumors, we intend to explore the clinical safety and preliminary efficacy of bortezomib combined with cisplatin in patients with advanced/metastatic breast cancer, and try to develop new treatment strategies for refractory breast cancer.

1.2 Risks and benefits

The subjects will receive good medical services during the study. According to relevant clinical research reports, the subjects may benefit from the drug efficacy, such as delayed disease progression and prolonged life, but we cannot guarantee or promise that all subjects will benefit. In addition, if the subjects are successfully enrolled, they will receive good medical treatment; after the subjects are successfully enrolled, bortezomib will be provided as a gift during the treatment period , and the relevant examination costs will be borne by the subjects themselves.

2. Research objectives

2.1 . Primary End Point

Security :

(DLT) of bortezomib combined with cisplatin and confirm its maximum tolerated dose (MTD) ;

Recommend clinical doses for subsequent Phase II clinical trials;

2.2 Secondary End Points

Initial anti-tumor efficacy:

Progression- free survival (PFS)

Tumor response (ORR and DCR) ;

Pharmacokinetic parameters : AUC, C_{max} , T_{max} , $T_{1/2}$.

2.3 Exploratory research

Tumor samples (before and after chemotherapy) , including blood samples and biopsy samples before chemotherapy, were collected for exploratory research on factors that may affect or predict the efficacy (including effectiveness and safety). The main test contents are as follows:

1) Proteasome activity : Proteasome activity is detected using fresh tissue specimens or patient organoids.

2) Other biomarkers: Explore biomarkers that have predictive effects on treatment through next-generation sequencing technology .

3. Research plan

This trial is a single-arm, open-label, phase I clinical study designed to evaluate the safety and preliminary antitumor activity of bortezomib combined with cisplatin in patients with advanced or metastatic breast cancer.

Each dose group must first enroll 3 subjects. If no DLT occurs in the first cycle (within 22 days after the first dose), the dose will be increased to the next cohort; if 1 subject develops DLT, 3 subjects will be added to the cohort, and if no DLT occurs in the last 3 subjects, the dose will be increased to the next dose. If 2 or more subjects in 3 or 6 subjects in a dose group develop DLT, the dose escalation will be stopped , and the previous dose of the dose will be the MTD. If the MTD is not reached in this trial, the researchers will discuss whether to continue the subsequent escalation trial.

Cohort 1 : Bortezomib 1.3 mg/ m^2 , cisplatin 50 mg / m2

Cohort 2 : Bortezomib 1.5 mg / m2 , cisplatin 50 mg / m2

Cohort 3 : Bortezomib 1.7 mg / m2 , cisplatin 50 mg / m2

Cohort 4 : Bortezomib 1.3 mg / m2 , cisplatin 75 mg / m2

Cohort 5 : Bortezomib 1.5 mg/ m^2 , cisplatin 75 mg / m2

Cohort 6 : Bortezomib 1.7 mg / m2 , cisplatin 75 mg / m2

Every 3 weeks is a treatment cycle. All subjects will receive 22 days of treatment and follow-up (day 1 to day 21 of the first dose plus 24 hours after the second dose). The decision on whether to conduct subsequent dose escalation will be based on the toxicity assessment within 22 days of the previous dose level and the occurrence of DLT. After the dose escalation reaches the MTD, the dose escalation will be stopped. If the MTD is not found, the researchers will discuss whether to conduct subsequent dose escalation.

Toxicity assessment will be based on the NCI Common Therapy Adverse Events Criteria (CTC AE version 4.03).

Pharmacokinetics :

Cycle 1: Blood samples for PK analysis were collected within 60 minutes before dosing, at 0.5, 2, 6, 24, 72 hours, and on days 8, 15, and 22 after the start of dosing;

Cycle 4: only within 60 minutes before the first dose of the cycle, and before dosing at 0.5, 2, 6, 24, 72 hours after the start of dosing, and on the 8th and 15th days;

Other cycles: only within 60 minutes before the first dose of the cycle, 0.5 hours after the start of the dose, and EOT.

In order to explore the deep mechanism of the effectiveness/ineffectiveness of this regimen in breast cancer, one tissue pathology specimen and 10-15 paraffin slide specimens will be collected during the pathological biopsy and after surgery (if any), and about 10 ml of blood specimens will be collected before the first treatment, before the third treatment, after the treatment, and after surgery (if any) to detect proteasome activity and possible second-generation sequencing to find biomarkers . After each course of treatment, the researchers must evaluate the adverse reactions and quality of life of the subjects.

4. Study Population

4.1 Inclusion Criteria

Patients enrolled in the study must meet the following criteria:

1. Women aged 18 years and above with pathologically confirmed recurrent or metastatic advanced breast cancer ;

2. The patient has tumor specimens (formalin-fixed, paraffin-embedded or fresh pre-treated recurrent tumor tissue);

3. Patients who have failed standard treatment in the late stage;

4. At least one measurable lesion;

5. ECOG PS : 0-2 points;

6. Estimated survival period ≥12 weeks;

7. The function level of major organs meets the following standards:

1) The blood routine examination standards must meet: ANC ≥1.5×109/L, PLT ≥75×109/L, Hb ≥85g/L (no blood transfusion and blood products within 14 days, no use of G-CSF and other hematopoietic stimulating factors for correction)

2) Biochemical examinations must meet the following standards: TBIL <1.5×ULN, ALT, AST <2.5×ULN, ALT, AST <5×ULN for patients with liver metastasis, BUN and Cr ≤1×ULN or endogenous creatinine clearance ≥50ml/min (Cockcroft-Gault formula);

8. Women of childbearing age must have taken reliable contraceptive measures, or have undergone a

pregnancy test (serum or urine) within 7 days before enrollment, with a negative result, and are willing to use appropriate contraceptive methods during the trial and 8 weeks after the last administration of the trial drug.

9. The subjects voluntarily join this study, have good compliance, and cooperate with follow-up.

4.2 Exclusion criteria

Any of the following will be considered as meeting the exclusion criteria of the study:

1. Patients with acute active hepatitis B or acute active hepatitis C;
2. Any serious underlying disease, comorbidity and active infection
3. Currently receiving other anti-tumor treatments;
4. History of epilepsy or epileptic-induced condition;
5. Patients who are pregnant or breastfeeding;
6. Those with poor compliance or unable to undergo normal follow-up;
7. Allergic to study drugs;
8. Patients diagnosed with other malignant tumors within 5 years, except for the following: surgically resected non-melanoma skin cancer, adequately treated cervical carcinoma in situ, surgically radically treated ductal carcinoma in situ, or malignant tumors diagnosed 2 years ago with no current evidence of disease and untreated ≤ 2 years before randomization;
9. The researcher determines other situations that may affect the conduct of the clinical study and the determination of the study results.

5. Treatment options

Bortezomib:

1.3mg/ m^2 , 1.5mg/ m^2 , 1.7mg/ m^2 , on D 1 , D 4 , D 8 , D 11 , one course of treatment every three weeks .

Cisplatin:

50mg/ m2 , 75mg/ m2 , on D1 - D3, every three weeks .

Before the formal start of the trial, the subjects must undergo relevant examinations in our hospital, including: routine blood tests, routine coagulation tests, liver function, kidney function, tumor markers (CA199 , CE A, CA153 , CA125) , electrocardiogram , cardiac ultrasound , chest and abdominal CT, and breast MRI . Before each course of chemotherapy, routine blood tests, liver function, kidney function, and electrocardiogram must be reviewed to assess toxicity ; after the second course of treatment, chest and abdominal CT must be reviewed to assess efficacy, and the researchers will assess whether there is disease

progression and whether to continue medication.

6. Preventive, supportive and concomitant medication

6.1 Preventive medication

6.1.1 Antiemetic treatment

of cisplatin can be manifested as nausea and vomiting. Acute vomiting generally occurs 1 to 2 hours after administration and can last for about a week. It is recommended to use a standard antiemetic regimen. The antiemetic drugs that can be selected include 5-HT3 receptor antagonists alone or in combination with aprepitant or dexamethasone.

6.2 Supportive care

All patients received standard supportive care, including blood and platelet transfusions, antibiotics, and antiemetic therapy.

6.2.1 Colony stimulating factors

Granulocyte colony stimulating factor (G-CSF) and macrophage - granulocyte knockdown stimulating factor (GM-CSF) can be administered according to clinical routine for the treatment of chemotherapy-related neutropenia, and are recommended for grade III/IV neutropenia and febrile granulocytopenia. *Prophylactic use of G-CSF and GM-CSF is not recommended* .

6.2.2 Platelet transfusion and / or platelet-raising therapy

III or above thrombocytopenia occurs , treatment with interleukin -11 (IL-11), thrombopoietin (TPO) and / or platelet transfusion can be given according to clinical routine. Prophylactic use of *IL-11 , TPO and GM-CSF is not recommended* .

6.2.3 Use of antibiotics

It should be avoided during treatment. If necessary, the dosage should be adjusted according to the renal function and the renal function should be closely monitored. Other antibiotics can be used according to clinical routine.

6.3 Concomitant medication

In this study, the amount of concomitant medication should be minimized. However, if it involves the interests of the patients and does not interfere with this study, the researcher may use it according to the specific situation, such as drugs to control common chronic diseases in internal medicine. All concomitant

medications during the study (including within 4 weeks before the first dose) should be recorded in the case report form.

7. Adverse Reactions and Dosage Adjustment

7.1 Possible adverse reactions of the drugs involved in this trial

Bortezomib :

Hematology: Anemia, bone marrow suppression, thrombocytopenia, neutropenia, and neutropenia associated with fever have been reported in clinical trials of this product.

Allergic reactions: Allergic reactions have been reported in clinical trials of this product, manifested as skin itching and rash.

Nervous System: Sensory neuropathy and sensory peripheral neuropathy have been reported in clinical trials.

Myalgia/arthralgia: Arthralgia, musculoskeletal pain and myalgia have been reported in clinical trials of this product. They are usually transient, appearing 2 to 3 days after administration and recovering after a few days.

Skin Reactions: Hair loss, generalized rash, nail changes, nail pigmentation/discoloration, and itching have been reported in clinical trials of this product.

Liver function: Increased alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and bilirubin have been reported in clinical trials of this product.

Gastrointestinal: Nausea, vomiting, constipation, and diarrhea have been reported in clinical trials of this product.

Other clinical events: Metabolic/nutritional adverse reactions such as weight loss and loss of appetite, as well as systemic adverse reactions such as fatigue and injection site reactions have been reported in clinical trials of this product.

Cisplatin :

Digestive system: including diarrhea, nausea, vomiting, loss of appetite, etc.

Liver function: including increased alkaline phosphatase, increased alanine aminotransferase, increased aspartate aminotransferase and increased bilirubin.

Allergic reactions: such as increased heart rate, decreased blood pressure, difficulty breathing, facial edema, flushing, rash, etc. may occur.

Others: weight loss, upper respiratory tract and urinary tract infections, fatigue, dizziness, etc.

7.2 Dosage Adjustments

Dose adjustment is only applicable to the second cycle and subsequent dosing phases. In principle, no medical treatment is given to non-DLT adverse events that occur during the DLT observation window (first cycle) in order to observe the possible adverse reactions of the trial drug and its degree and reversibility. However, once DLT toxicity specified in the protocol occurs, treatment must be terminated

immediately and actively managed. The investigator shall negotiate to adjust the dosing method or permanently stop the drug, and the drugs used shall be recorded on the eCRF.

Body weight was measured before the first administration of each cycle. The first dose was calculated based on the baseline body weight. It was recommended to adjust the dose when the body weight changed by more than 10%, and the researcher made the decision based on the actual clinical situation.

After the subjects complete the first cycle, the dose is allowed to be adjusted according to the toxic reactions related to bortezomib /cisplatin (definitely related, possibly related). When the following adverse reactions occur, the use of bortezomib /cisplatin should be suspended until the adverse reactions are relieved, and the original dose can be continued or reduced by one dose (such as 1.5mg/ m2 to 1.3mg/ ^{m2} , which is determined by the researcher based on the overall condition of the subject) :

① Non-hematological toxicity related to the study drug is ⩾ Grade 2 (a single occurrence of Grade 2 fatigue does not require suspension of the study drug);

② Grade 4 hematological toxicity related to the study drug;

In addition to the above situations, if the patient repeatedly experiences the same serious adverse reaction, medication should be discontinued. If the drug-related adverse reaction still cannot be alleviated to grade 0-1 within 8 weeks after discontinuation of medication, termination of medication should be considered.

8. Termination of treatment

Patients need to terminate the study treatment if any of the following occurs:

1. Tumor progression occurs;

2. Serious adverse events that may be life-threatening and related to the study drug occur;

3. Any adverse events that result in the patient being unable to continue to participate in this trial;

4. Any reason causes the patient to delay study treatment for more than 21 days;

5. The researcher believes that the patient is no longer suitable for research treatment due to concurrent diseases or changes in the patient's condition;

6. Breastfeeding or pregnancy;

7. The researcher believes that the patient's compliance with the study procedures and treatment is poor;

8. The patient or his/her attending physician requests to stop the study treatment;

9. The patient requires other anti-tumor drugs or measures for any reason. In this case, the patient needs to stop the study treatment and withdraw from this trial before starting new anti-tumor treatment or

measures;

10. The researcher decides to stop the patient's research treatment or stop the entire trial for any ethical, medical or scientific reasons, taking into account the safety and benefit of the patient.

9. Exploratory Research

Tumor samples (before and after chemotherapy) were collected , including blood samples, biopsy samples before chemotherapy, and surgical samples after chemotherapy (if any), for exploratory research on factors that may affect or predict the efficacy (including effectiveness and safety). The main contents of the measurement are as follows:

1. Proteasome activity : Fresh specimens were obtained from patients to test proteasome activity and evaluate the correlation between proteasome activity and efficacy. This test was performed by the Cancer Prevention and Treatment Center of Sun Yat-sen University .

2. Other biomarkers: Biomarkers for patients who respond/do not respond to the regimen were identified through methods such as exome sequencing, DNA methylation testing, and RNA sequencing .

All sequencing experiments will be completed by Hangzhou Lianchuan Technology Co., Ltd. , and after the experiments are completed, the remaining DNA , RNA and tissue samples will be returned and kept by the Sun Yat-sen University Cancer Prevention and Treatment Center .

10. Research Evaluation

10.1 Baseline Assessment

10.1.1 Medical history

First diagnosis, TNM staging, previous treatment history, previous diseases and concomitant medications, vital signs, ECOG score, and full physical examination.

10.1.2 Laboratory tests

Routine blood tests, routine urine tests, liver and kidney function tests (biochemical routine tests), endogenous creatinine clearance, coagulation function, tumor markers, electrocardiogram (ECG) and cardiac ultrasound were performed one week before the formal start of treatment.

10.1.3 Tumor Assessment

4 weeks before the start of treatment and as close to the start of treatment as possible. For imaging examinations, chest and abdominal CT and breast MRI are recommended. The same examination methods are required for subsequent follow-up and evaluation of the same lesion. Bone scans and head CT or MRI

may be performed as baseline tumor examinations if the clinician deems it necessary . The RECIST 1.1 standard is used to evaluate the efficacy, including measurable and non-measurable lesions, target lesions and non-target lesions. The objective tumor efficacy criteria (complete remission, partial remission, stable and progressive disease) are shown in Appendix 2 .

10.2 Treatment Phase Evaluation

10.2.1 Clinical evaluation

Before each course of treatment, the patient's vital signs, ECOG score, and physical examination were performed.

10.2.2 Laboratory tests

Before each course of treatment, blood routine, liver and kidney function, electrocardiogram, and other tests should be performed as appropriate. The next course of treatment can only be carried out after all toxicity has recovered to below grade I. If the baseline is accompanied by elevated tumor markers, re-examination should be performed every 2 courses.

10.2.3 Evaluation of therapeutic efficacy

The methods for evaluating the baseline of the tumor include chest and abdominal CT , breast MRI , and whole-body bone ECT . The same examination methods should be used for follow-up and evaluation of the same lesion as much as possible. Patients suspected of brain / meningeal metastasis can undergo cranial MRI examination, and other suspicious lesions can also be examined and evaluated at baseline and follow-up. After the baseline evaluation, all patients who meet the criteria will be re-evaluated for efficacy after every 3 courses of treatment (see the evaluation schedule for details) until disease progression as defined by RECIST 1.1 occurs. If the patient withdraws from treatment (and / or receives treatment with other regimens) before disease progression , the patient should still be followed up every 6 ± 2 weeks until disease progression as defined by RECIST 1.1 .

The evaluation criteria for objective tumor response rate refer to RECIST 1.1 standard: complete response (CR) , partial response (PR) , stable disease (SD) and progression of disease (PD) . When evaluating the progression of target lesions (TL) , it should be compared with the minimum tumor burden (such as the minimum sum of the long diameters after the start of the study). In addition to disease progression, other tumor response indicators (CR, PR, and SD) are compared with the baseline level.

If the investigator or client is unsure whether the disease has progressed, especially when non-target lesions have been alleviated or new lesions have appeared, reassessment can be performed after 6-8 weeks. If reassessment confirms progression, the initial examination date is considered the date of disease

progression.

If disease progression is determined based on non-target lesions, disease progression can only be determined when non-target lesions are significantly worse and the patient's total tumor burden (including target lesions and non-target lesions) is significantly increased to the point where treatment needs to be terminated (even if the target lesions are SD or PR). Slight enlargement of one or more non-target lesions does not meet clear progression criteria.

All patients who receive the trial regimen should be re-examined after two courses of treatment to evaluate the efficacy. After the end of treatment , chest and abdominal CT or ultrasound must be re-examined every 3 months in the first 1 to 3 years; every 6 months in the third to fifth years; and every 12 months after 5 years. Survival follow-up should be performed every 2 months. The evaluation should be conducted as much as possible according to the evaluation schedule.

10.3 Safety Assessment

After each chemotherapy cycle, adverse reactions should be evaluated based on clinical and laboratory test results and in accordance with the NCICTC 5.0 common toxicity criteria, and the patient's quality of life should also be evaluated.

11. Adverse Events

11.1 Definitions

Any unexpected medical event that occurs or worsens during the study and is either related to or unrelated to treatment is an adverse event. Adverse events are not necessarily related to the drug.

11.2 Adverse Event Monitoring

Adverse events during the period from the signing of the informed consent form for the subject to 28 days after the last study dose , whether related to the study drug or not, should be filled in the case report form. Disease progression during the study and events determined to be caused by disease progression should not be reported as AEs . Deaths clearly caused by disease progression should not be reported as SAEs .

11.3 Serious Adverse Events

A serious adverse event is any of the following:

1. die;

2. Serious life-threatening medical events; (Note: In the definition of " life-threatening " , " serious " means that the patient is at risk of death when the event occurs, not that if the event is more serious, it may

cause death.)

3. Leading to hospitalization or prolonged hospitalization;

4. Permanent or severe disability;

5. A significant medical event resulting in a congenital malformation or defect.

Other situations that require an expedited reporting system are judged from a medical and scientific perspective, such as major medical events that may not immediately endanger the patient's life, death, or hospitalization, but may endanger the patient or require immediate intervention to prevent the above results. These events are generally also considered serious adverse events.

11.4 Adverse Event Grading

Whether it is an AE or a SAE, the investigator must assess the intensity of the event.

The term " intensity " is often used to describe the severity of a particular event (e.g., mild, moderate, or severe myocardial infarction); the event itself may be of minor medical significance (e.g., severe headache). This standard is different from " severity , " which is based on the consequences or circumstances of the event because it threatens the subject's life or causes loss of function. Adverse events will be classified as grades 1 to 5 according to the NCI Common Toxicity Classification (CTC) version 4.0 . If the adverse event is not listed in the NCI Toxicity Classification, it may be classified into 5 grades according to the following grades and detailed in the case report form:

Slight	It is uncomfortable but does not affect normal daily activities and usually does not require special treatment
Moderate	Feeling of discomfort that interferes with daily activities; usually relieved with basic treatment
Severe	Inability to carry out daily activities or severe clinical impairment requiring medical treatment. Hospitalization may or may not be required
Life-threatening	Immediate risk of death; hospitalization and clinical intervention required
Die	

11.5 Causation

Investigators should assess the possible relationship between adverse events and the investigational drug, referring to the following criteria:

1. Definitely related: The reaction occurs at a time that is consistent with the chronological order of medication administration, the reaction fits the known reaction pattern of the test drug, it improves after drug discontinuation, and reoccurs with repeated dosing.

2. Possibly Related: The timing of the reaction is consistent with the chronological order of drug administration, the reaction fits the known reaction pattern of the investigational drug, the patient's clinical status or other treatment modalities could have produced the reaction.

3. Likely unrelated: The timing of the reaction does not fit the chronological order of medication administration, the reaction does not fit the known reaction type of the investigational drug, and the patient's clinical condition or other treatments may also produce the reaction.

4. Unrelated: The time of occurrence of the reaction does not conform to the chronological order of medication, the reaction is consistent with the known reaction type of non-experimental drugs, the patient's clinical condition or other treatment methods may also produce the reaction, the disease status improves or other treatment methods are stopped, and the reaction disappears, and the reaction occurs when other treatment methods are repeated.

5. Unable to determine: The time of reaction has no clear relationship with the time sequence of medication, the reaction is similar to the known reaction type of the test drug, and other drugs used at the same time may also cause the same reaction.

11.6 Adverse Event Reporting

Record all adverse events during the trial and their course, degree, time of occurrence, treatment measures and outcomes in detail, and fill in the case report form (CRF). SAEs should be reported in accordance with SFDA regulations. In this study, SAEs must be reported to the hospital GCP center and hospital ethics committee within 24 hours .

12. Sample size calculation

This trial is a single-arm, open-label, phase I clinical study designed to evaluate the safety and preliminary antitumor activity of bortezomib combined with cisplatin in patients with advanced or metastatic breast cancer.

Each dose group adopts the 3+3 method, with 3-6 subjects in each group, and ⩽ 20 subjects in each group. If the subject fails to receive all the scheduled infusion doses due to reasons other than DLT during the first dose, the subject will be replaced. If the subject withdraws from the trial due to reasons other than DLT within 28 days after the first dose, the subject will be replaced. Subjects with DLT will immediately terminate treatment and enter the follow-up period and will not be replaced.

1 3. Statistical Analysis

13.1 Description of the Statistical Analysis Dataset

Enrolled Subjects Set (ESS): defined as all enrolled subjects, regardless of whether they used the trial drug. This data set is used to analyze demographic and baseline data, subject allocation, and protocol violations.

Full Analysis Set (FAS): defined as all enrolled subjects who have used the trial drug at least once. This dataset is used for efficacy analysis.

Safety Analysis Set (SS): defined as all enrolled subjects who have used the trial drug at least once and have at least one safety evaluation. This data set is used for the analysis of safety data.

Dose-limiting toxicity analysis set (DLTS): All subjects who received trial drug and completed the 28-day DLT assessment or developed a DLT within 28 days.

13.2 Statistical methods

The test results are mainly presented using descriptive statistical methods. Measurement data will generally list the number of observations, mean, standard deviation, median, quartile, maximum, and minimum. Count data will list frequency and frequency (composition ratio). Statistical analysis will be performed using SAS 9.4 and above.

Descriptive statistics were used to describe the number and proportion of subjects entering each analysis set, the number and proportion of subjects who completed the trial and withdrew from the trial, and the reasons for withdrawal from the trial (and their proportion); and to describe demographic and other baseline characteristics.

Adverse events, adverse reactions, and serious adverse events will be summarized for each dosing group using the safety analysis set. The severity of adverse events and reactions will be graded using the

NCI CTCAE version 5.0 criteria. Adverse events and reactions will also be summarized by System Organ Class and Preferred Term. The number and proportion of subjects with at least one dose-limiting toxicity (DLT) and the number of DLTs will be summarized for each dosing group.

Using the safety analysis set, the baseline data, post-dose data, and post-dose change data (Change from Baseline) of laboratory tests, physical examinations, vital signs, electrocardiograms, LVEF, and other safety data will be summarized by each follow-up and by each dosing group; for the normality and clinical significance of each test result as categorical data, a shift table will be used to describe the changes from baseline to each follow-up after dosing.

RECIST (Evaluation of Response Criteria in Solid Tumors) version 1.1 was used with reference to iRECIST for baseline and post-treatment imaging assessments of patients' tumors. The assessment results included complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) and disease control rate (DCR) of each dosing group and their bilateral 95% confidence intervals (Clopper-Pearson exact probability method) were calculated using the full analysis set. Objective response rate (ORR) = (number of people assessed as CR + number of people assessed as PR) / total number of people assessed. Disease control rate (DCR) = (number of people assessed as CR + number of people assessed as PR + number of people assessed as SD) / total number of people assessed.

The Kaplan-Meier method was used to draw the survival curves of progression-free survival (PFS) and duration of response (DOR), and the median PFS and median DOR and their bilateral 95% confidence intervals were estimated. PFS and DOR will be analyzed using the full analysis set. The treatment of censored data in survival analysis and the determination of censoring dates will be detailed in SAP.

A descriptive summary of the blood drug concentration at each planned time point was made, and for each dosing group, the concentration-time curve of each subject, the average concentration-time curve of all subjects, and the semi-logarithmic curve corresponding to the two curves were plotted respectively; Phoenix WinNonlin software was used to estimate and analyze the non-compartmental pharmacokinetic parameters of the blood drug concentration data and calculate the pharmacokinetic parameters.

14. Ethics and Informed Consent

14.1 Ethics

This study should be based on the ethical principles derived from the Declaration of Helsinki and be consistent with the International Conference on Harmonization (ICH) / Good Clinical Practice (GCP), its regulatory provisions and the policy of the Affiliated Cancer Hospital of Fudan University on ethics and human biospecimens.

14.2 Informed Consent

Consent Form (ICF) must include information about data privacy protection (or in some cases, a separate document). The principal investigator is not allowed to disclose the genetic results to patients, insurance companies, employers, family members, doctors, and other third parties, unless required by law.

14.3 Ethics and Supervision Unit

The IRB/IEC should review the final research procedures including the final version of the ICF and other written information and / or materials that should be provided to patients. The researcher or agent must ensure that these documents are provided to the IRB/IEC and all relevant staff. The opinions of the IRB/IEC should be recorded in writing. According to the regulations of each research center, the research plan may be reviewed by the IRB/IEC once a year if necessary . Each principal investigator must provide the ethics committee /IRB with reports of serious adverse reactions and unexpected adverse drug reactions in the study. Drug gene research and its related ICF must be approved by the ethics committee, and clear written proof must be issued at the same time. Before the patient participates in this study, the researcher / agent must obtain his or her written informed consent.

14.4 Notes on the Informed Consent Process

The principal investigator should pay attention to the following points:

1. Ensure that each patient is informed in detail, both orally and in writing, of the nature, purpose, potential risks, and benefits of the study;

2. Ensure that every patient understands their right to withdraw from the trial at any time;

3. Ensure that every patient has the right to ask questions and time to consider the information provided;

4. Ensure that each patient signs and dates the informed consent form before participating in the study;

5. Ensure that the original, signed ICF is kept in the researcher's folder;

6. Ensure that each patient has a signed ICF ;

7. Measures to ensure benefits to patients entering the study and to prevent harm arising from the trial are described in the ICF approved by the ethics committee.

15. Publish your plan

This research team promises that regardless of whether this trial obtains positive results, a research paper will be written based on the true conclusions and published in domestic/international journals for reference by clinical workers.

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Appendix 1 Study flow chart (excluding surgery)

Research content	Screening period	Per cycle	After 2 cycles	End of treatment	Follow up
Informed	✓				Follow-up observation was performed according to the NCCN guidelines for breast cancer.
Entry criteria	✓				
Past medical	✓				
Physical	✓	✓		✓	
ECOG scoring	✓	✓		✓	
Tumor	✓				
Blood routine	✓	✓			
Kidney	✓	✓			
CB4 [4]	✓				
Tumor markers	✓		✓	✓	
Electrocardiogra	✓	✓			
Cardiac	✓				
Imaging [4]	✓		✓	✓	
Bortezomib		✓			
Cisplatin		✓			
Concomitant	✓	✓	✓	✓	
Adverse Events	✓	✓	✓	✓	
Quality of life	✓	✓		✓	

1. Collect tumor samples during the screening period;

2. A routine blood test is required before each chemotherapy, and the frequency can be increased as needed;

3. Liver and kidney function (including total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, white/total protein, urea nitrogen, and serum creatinine) should be checked within one week before the first day of each course of treatment. The frequency of the check can be increased as needed;

4. Examination during the screening period, and increase the number of examinations as needed during subsequent treatment;

5. Imaging examinations mainly include chest and abdominal CT and breast MRI. The same examination method must be used throughout the study to maintain the comparability of the results.

Annex II Response Evaluation Criteria for Solid Tumors , version 1.1 Evaluation Criteriain Solid Tumors RECIST Version 1.1)

1. Tumor measurability at baseline

1.1 Definition

At baseline, tumor lesions/nodes were categorized as measurable or non-measurable based on the following definitions:

Measurable lesions

Tumor lesions: There is at least one diameter that can be accurately measured (recorded as the maximum diameter), and its minimum length is as follows:

CT scan 10 mm (CT scan layer thickness is no more than 5 mm)

10 mm for routine clinical examination instruments (tumor lesions that cannot be accurately measured with caliper instruments should be recorded as unmeasurable)

Chest X-ray 20 mm

Malignant lymph nodes: pathologically enlarged and measurable, the short diameter of a single lymph node on CT scan must be ≥15 mm (the CT scan layer thickness is recommended not to exceed 5 mm). During baseline and follow-up, only the short diameter is measured and followed up.

Non-measurable lesions

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph node short diameter ≥10 mm to <15 mm) and lesions that cannot be measured. Lesions that cannot be measured include: meningeal disease, ascites, pleural or pericardial effusion, inflammatory breast cancer, skin/lung lymphangitic carcinomatosis, abdominal masses that cannot be diagnosed and followed up by imaging, and cystic lesions.

Special considerations regarding lesion measurements

Bone lesions, cystic lesions, and lesions previously treated with local therapy require special mention:

Bone lesions:

Bone scans, PET scans or plain films are not suitable for measuring bone lesions, but can be used to confirm the presence or absence of bone lesions;

Osteolytic or mixed lytic/osteoblastic lesions with a definite soft tissue component that meets the above definition of measurability can be considered measurable lesions if they can be evaluated using cross-sectional imaging techniques such as CT or MRI.

Osteogenic lesions are non-measurable lesions.

Cystic lesions:

Lesions that meet the definition of simple cysts on radiographic imaging should not be considered malignant lesions because they are simple cysts by definition. They are neither measurable lesions nor non-measurable lesions;

If the metastatic lesion is cystic and meets the above definition of measurability, it can be considered a measurable lesion. However, if non-cystic lesions exist in the same patient, non-cystic lesions should be preferred as target lesions.

Topically treated lesions:

Lesions located in areas that have been irradiated or treated with other locoregional therapies are generally considered non-measurable unless there is clear progression of the lesion. The study protocol should describe in detail the conditions under which these lesions are considered measurable.

1.2 Description of measurement method

Lesion measurement

During clinical evaluation, all tumor measurements should be recorded in metric units. All baseline assessments of tumor lesion size should be completed as close to the start of treatment as possible and must be completed within 28 days (4 weeks) before the start of treatment.

Evaluation Method

The same techniques and methods should be used for baseline assessment and subsequent measurements of lesions. All lesions must be evaluated using imaging, except for lesions that cannot be evaluated by

imaging but can only be evaluated by clinical examination.

Clinical lesions: Clinical lesions are considered measurable only when they are superficial and have a diameter ≥10 mm (such as skin nodules). For patients with skin lesions, it is recommended to use color photos with a ruler to measure the size of the lesion for archiving.

When lesions are evaluated using both imaging and clinical examination, imaging should be used as much as possible because it is more objective and can be reviewed again at the end of the study.

Chest X-ray: When tumor progression is an important research endpoint, chest CT should be used first because CT is more sensitive than X-ray, especially for new lesions. Chest X-ray is only applicable when the measured lesion has clear boundaries and the lungs are well ventilated.

CT, MRI: CT is currently the best available and repeatable method for evaluating efficacy. The definition of measurability in this guideline is based on CT scan slice thickness ≤ 5 mm. If the CT slice thickness is greater than 5 mm, the minimum measurable lesion should be twice the slice thickness. MRI is also acceptable in some cases (such as whole body scans).

Ultrasound: Ultrasound should not be used as a measurement method to measure lesion size. Ultrasound examinations are not repeatable after the measurement is completed due to their operation dependence, and the consistency of technology and measurement between different measurements cannot be guaranteed. If new lesions are found using ultrasound during the trial, CT or MRI should be used for confirmation. If the radiation exposure of CT is considered, MRI can be used instead.

Endoscopy, laparoscopy: These techniques are not recommended for objective tumor evaluation but can be used to confirm CR when biopsy specimens are obtained and to confirm recurrence in trials where the endpoint is recurrence after CR or surgical resection.

Tumor markers: Tumor markers cannot be used alone to evaluate objective tumor response. However, if the marker level exceeds the upper limit of normal at baseline , it must return to normal levels to evaluate complete response. Because tumor markers vary from disease to disease, this factor must be taken into account when writing measurement standards into the protocol. Specific criteria for CA-125 response (recurrent ovarian cancer) and PSA (recurrent prostate cancer) response have been published. In addition, the International Gynecologic Cancer Organization has developed CA-125 progression criteria, which will soon be added to the objective tumor evaluation criteria for first-line ovarian cancer treatment.

Cytology/histology techniques: These techniques can be used to identify PR and CR in specific circumstances specified in the protocol (e.g., residual benign tumor tissue is often present in lesions of germ cell tumors). When effusions may be a potential side effect of a therapy (e.g., treatment with taxane compounds or angiogenesis inhibitors) and the measurable tumor meets the criteria for response or stable disease, the appearance or worsening of tumor-related effusions during treatment can be confirmed by cytology techniques to distinguish between response (or stable disease) and progressive disease.

2 Evaluation of Tumor Response

2.1 Target lesion assessment

Complete remission (CR): All target lesions disappear and the short diameter of all pathological lymph nodes (including target nodules and non-target nodules) must be reduced to <10 mm.

Partial response (PR): The sum of the target lesion diameters decreased by at least 30% compared with the baseline level.

Disease progression (PD): The minimum value of the sum of all target lesion diameters measured during the entire experimental study is used as a reference, and the relative increase in the sum of diameters is at least 20% (if the baseline measurement value is the smallest, the baseline value is used as a reference); in addition, the absolute value of the sum of diameters must increase by at least 5 mm (the appearance of one or more new lesions is also considered disease progression).

Stable disease (SD): The reduction of target lesions has not reached the level of PR, nor has the increase reached the level of PD, but is somewhere in between. The minimum value of the sum of diameters can be used as a reference for research.

2.2 Precautions for target lesion assessment

Lymph nodes: Even if the lymph nodes identified as target lesions are reduced to less than 10 mm, the actual short diameter value corresponding to the baseline should be recorded for each measurement (consistent with the anatomical plane at the baseline measurement). This means that if the lymph nodes are target lesions, even if the criteria for complete remission are met, it cannot be said that the lesions have disappeared, because the short diameter of normal lymph nodes is defined as <10 mm. Target lymph node lesions must be specifically recorded in a specific location on the CRF form or other recording method: for CR, all lymph node short diameters must be <10 mm; for PR, SD, and PD, the

actual measurement of the target lymph node short diameter will be included in the sum of the target lesion diameters.

Target lesions too small to measure: In clinical studies, all lesions (nodular or non-nodular) recorded at baseline should have their actual measurements recorded again at subsequent evaluations, even if the lesions are very small (e.g., 2 mm). However, sometimes they may be so small that the CT scan image is very blurry, and the radiologist may have difficulty defining the exact value, so they may be reported as "too small to measure." In this case, it is very important to record the previous value on the CRF. If the radiologist believes that the lesion may have disappeared, it should also be recorded as 0 mm. If the lesion is indeed present but is too blurry to give an accurate measurement, a default of 5 mm can be used. (Note: This is unlikely to occur with lymph nodes, as they are generally of measurable size under normal circumstances or are often surrounded by fat tissue as in the retroperitoneum; however, if this situation occurs and a measurement cannot be given, a default of 5 mm is also used.) The default value of 5 mm is derived from the cut thickness of the CT scan (this value does not change with different cut thickness values of CT). Since the chance of the same measurement being repeated is unlikely, providing this default value will reduce the risk of incorrect assessment. However, it needs to be reiterated that if the radiologist can give an exact numerical value for the lesion size, the actual value must be recorded even if the lesion diameter is less than 5 mm.

Separate or coalesced lesions: When a non-nodular lesion is fragmented, the longest diameters of the separate parts are added together to calculate the sum of the diameters of the lesion. Similarly, for coalesced lesions, the planes between the coalesced parts can be distinguished and the maximum diameters of each are calculated. However, if the lesions are inseparable, the longest diameter should be the longest diameter of the entire coalesced lesion.

2.3 Evaluation of non-target lesions

This section defines the response criteria for non-target lesions. Although some non-target lesions are actually measurable, they do not need to be measured and only need to be qualitatively assessed at the time points specified in the protocol.

Complete remission (CR): All non-target lesions disappear and tumor markers return to normal levels. All lymph nodes are non-pathological in size (short diameter <10 mm).

Non-complete response/non-progressive disease: Presence of one or more non-target lesions and/or

persistent tumor marker levels above normal levels.

Disease progression: Definite progression of existing non-target lesions. Note: The appearance of one or more new lesions is also considered disease progression.

2.4 Special Considerations Regarding the Assessment of Non-target Lesion Progression

The following is a supplementary explanation of the definition of progression of non-target lesions:

When patients have measurable non-target lesions, even if the target lesions are assessed as stable or partially remitted, in order to make a clear definition of progression based on the non-target lesions, the overall deterioration of the non-target lesions must be met to the extent that treatment must be terminated. The general increase in the size of one or more non-target lesions is often not enough to meet the progression criteria. Therefore, when the target lesions are stable or partially remitted, it is almost rare to define overall tumor progression based solely on changes in non-target lesions.

When all of a patient's non-target lesions are unmeasurable: This situation occurs in some phase III trials when the inclusion criteria do not stipulate that measurable lesions must be present. The overall assessment is still based on the above criteria, but because there is no measurable data on lesions in this case. The deterioration of non-target lesions is not easy to assess (by definition: all non-target lesions must be truly unmeasurable), so when changes in non-target lesions result in an increase in overall disease burden equivalent to disease progression in target lesions, a clear definition of progression based on non-target lesions is required, and an effective detection method needs to be established for assessment. For example, it is described as an increase in tumor burden equivalent to an additional 73% increase in volume (equivalent to a 20% increase in the diameter of measurable lesions). Another example is peritoneal effusion from "trace" to "large"; lymphatic lesions from "local" to "widely disseminated"; or described in the protocol as "sufficient to change treatment." Examples include pleural effusion from trace to large, lymphatic involvement from the primary site to distant sites, or it may be described in the protocol as "necessary for a change in treatment." If clear progression is found, the patient should be considered to have progressive disease overall at that point. It would be desirable to have objective criteria applicable to the assessment of non-measurable disease, but the added criteria must be reliable.

2.5 New Lesions

The appearance of new malignant lesions indicates disease progression; therefore, some evaluation of

new lesions is important. There are no specific criteria for detecting lesions on imaging, however, the finding of a new lesion should be unambiguous. For example, progression cannot be attributed to differences in imaging techniques, changes in imaging morphology, or other pathologies other than the tumor (e.g., some so-called new bone lesions are simply the healing of the original lesion or the recurrence of the original lesion). This is particularly important when a patient has a partial or complete response to a baseline lesion; for example, a necrotic liver lesion may be reported on the CT report as a new cystic lesion when it is not.

Lesions detected during follow-up that were not found during the baseline examination were considered new lesions and indicated disease progression.

For example, a patient who was found to have visceral lesions during the baseline examination

If metastases are found during cranial examination with CT or MRI, the patient will be considered to have evidence of progressive disease even if the patient did not undergo a cranial examination at baseline.

If a new lesion is ambiguous, for example because of its small size, further treatment and follow-up evaluation are needed to confirm whether it is a new lesion. If repeat examination confirms that it is a new lesion, the time of disease progression should be calculated from the time of its initial discovery.

FDG-PET evaluation of lesions generally requires additional testing for confirmation. It is reasonable to combine FDG-PET and supplementary CT results to evaluate progression (especially new suspected disease). New lesions can be confirmed by FDG-PET examination, according to the following procedures:

The baseline FDG-PET scan result was negative, and the subsequent follow-up FDG-PET scan was positive, indicating disease progression.

No baseline FDG-PET was performed and the follow-up FDG-PET result was positive:

If the new lesions found in the follow-up FDG-PET positive test are consistent with the results of the CT examination, it proves that the disease has progressed.

If the new lesions found in the positive results of the follow-up FDG-PET are not confirmed by the CT examination results, another CT examination is required for confirmation (if confirmed, the disease progression time is calculated from the abnormality found in the previous FDG-PET examination).

If the positive follow-up FDG-PET result is consistent with a pre-existing lesion on CT, and the lesion

has not progressed on imaging, then the disease has not progressed.

2.6 Description of missing and non-evaluable assessments

If a lesion could not be imaged or measured at a particular time point, the patient was not evaluable at that time point. If only some lesions could be evaluated at an evaluation, this would generally be considered not evaluable at that time point unless there was evidence that the missing lesions would not affect the evaluation of the efficacy response at the specified time point.

2.7 Special tips for efficacy evaluation

When nodular lesions are included in the total target lesion assessment and nodules decrease in size to a "normal" size (<10 mm), they will still have a lesion size scan reported. To avoid overestimation based on increased nodule size, measurements will be recorded even if the nodule is normal. As mentioned previously, this means that a subject who has a complete response will not have a score of 0 on the CRF.

If efficacy confirmation is required during the trial, repeated "unmeasurable" time points will complicate the optimal assessment of efficacy. The analysis plan for the trial must state that these missing data/assessments can be accounted for when determining efficacy. For example, in most trials, a subject's response of PR-NE-PR can be considered to have efficacy confirmation.

When a subject experiences an overall deterioration in health that requires discontinuation of treatment, but there is no objective evidence, this should be reported as symptomatic progression. Every effort should be made to assess objective progression even after treatment has been discontinued. Symptomatic deterioration is not an assessment of objective response: it is a reason to discontinue treatment. The objective response of such subjects will be assessed by the target and non-target lesions shown in Tables 1 to 3.

Cases defined as early progression, early death and inevaluable are study specific and should be clearly described in each protocol (depending on the treatment interval and duration).

In some cases, it is difficult to distinguish localized disease from normal tissue. When the evaluation of complete response is based on such a definition, we recommend that a biopsy be performed before the evaluation of complete response in localized disease. When some subjects have abnormal imaging results of localized disease that are thought to represent fibrosis or scarring, FDG-PET is used to confirm the efficacy of complete response with similar criteria to biopsy. In such cases, the use of FDG-PET should be prospectively described in the protocol and supported by reports in the specialized medical

literature for this situation. However, it must be recognized that due to the limitations of FDG-PET and biopsy (including the resolution and sensitivity of each), false-positive results will occur during the evaluation of complete response.

Table **1** Time point response: subjects with target lesions (including or excluding non-target lesions)

Target lesion	Non- target lesions	New lesions	Overall
CR	CR	No	CR
CR	No CR / Non PD	No	PR
CR	Cannot evaluate	No	PR
PR	Non-progressive or incompletely assessable	No	PR
SD	Non-progressive or incompletely assessable	No	SD
Cannot be fully evaluated	Non-progressive	No	NE
PD	Any situation	Yes or No	PD
Any situation	PD	Yes or	PD
Any situation	Any situation	Yes	PD
CR=complete remission	PR = partial response	SD=stable disease	PD=progressive disease

Table **2** Time point response **-** subjects with only non-target lesions

Non-target lesions	New lesions	Overall remission
CR	No	CR
Non-CR or non-PD	No	Non-CR or non-PD
Cannotbefully evaluated	No	Cannot evaluate
Not clear PD	Yes or No	PD
Any situation	yes	PD

Note: For non-target lesions, "non-CR/non-PD" refers to an efficacy that is better than SD. As SD is increasingly used as an endpoint for evaluating efficacy, the efficacy of non-CR/non-PD is formulated to address situations where no lesions can be measured.

For ambiguous progression findings (e.g., very small indeterminate new lesions; cystic or necrotic lesions of existing lesions), treatment can be continued until the next evaluation. If disease progression is confirmed at the next evaluation, the progression date should be the date of the previous suspected progression.

Table 3 Best overall response required for confirmation of **CR** and **PR efficacy**

Total remission at the first time point	Total remission at subsequent time points	Best overall response
CR	CR	CR
CR	PR	SD，PD or PR [a]
CR	SD	If SD lasts long enough , SD, otherwise it should be PD
CR	PD	If SD lasts long enough , SD, otherwise it should be PD
CR	NE	If SD lasts long enough , SD, otherwise it should be NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	If SD lasts long enough , SD, otherwise it should be PD
PR	NE	If SD lasts long enough , SD, otherwise it should be NE
NE	NE	NE

Note: CR means complete remission, PR means partial remission, SD means stable disease, PD means progressive disease, and NE means not evaluable. Superscript "a": If CR actually occurs at the first time point, and any disease appears at the subsequent time points, then even if the subject's efficacy reaches the PR standard relative to the baseline, the efficacy evaluation at the subsequent time points will still be PD (because the disease will reappear after CR). The best response depends on whether SD occurs within the shortest treatment interval. However, sometimes the first evaluation is CR, but the scans at subsequent time points seem to still appear, so the subject's efficacy at the first time point should actually be PR rather than CR. In this case, the first CR judgment should be modified to PR, and the best response is PR.

2.8. Efficacy evaluation / confirmation of remission period

Confirmation of efficacy

For non-randomized clinical studies with tumor remission efficacy as the primary endpoint, the efficacy of PR and CR must be confirmed to ensure that the efficacy is not the result of evaluation errors. In studies with disease stabilization or disease progression as the primary endpoint, efficacy confirmation is no longer required because it has no value for the interpretation of the test results. In the case of SD, at least one measurement meets the SD criteria specified in the protocol within the shortest time interval

after the start of the trial (generally no less than 6 to 8 weeks).

Total remission period

The duration of overall remission is the time from the first measurement of meeting the CR or PR (whichever is measured first) criteria to the first real recorded disease relapse or progression (the minimum measurement recorded in the trial is used as a reference for disease progression). The duration of overall complete remission is the time from the first measurement of meeting the CR criteria to the first real recorded disease relapse or progression.

Stable disease

It is the time from the start of treatment to disease progression (in randomized trials, from the time of randomization), with the smallest sum in the trial as the reference (if the baseline sum is the smallest, it is used as the reference for PD calculation). The clinical relevance of disease stability varies from study to study and disease to disease. If the proportion of patients maintaining a minimum stable disease period is used as the study endpoint in a particular trial, the protocol should specifically state the minimum time interval between the two measurements in the definition of SD.

Note: The duration of remission, stable phase, and PFS are affected by the frequency of follow-up after baseline evaluation. Defining a standard follow-up frequency is beyond the scope of this guideline. The frequency of follow-up should take into account many factors, such as disease type and stage, treatment duration, and standard practices. However, if comparisons between trials are required, the limitations of the accuracy of these measurement endpoints should be considered.