Protocol

Protocol for: Mei Q, Zhang Y, et al. Manganese primed immunochemotherapy in platinum-resistant/refractory ovarian cancer: a randomized, single-blind, placebo-controlled, phase 2 trial

This trial protocol has been provided by the authors to give readers additional information about the work.

Manganese primed immunochemotherapy in platinum-resistant/refractory ovarian cancer: a randomized, single-blind, placebo-controlled, phase 2 trial

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes
2. Original statistical analysis plan (no changes were made to the original statistical analysis plan throughout the study)

Protocol

A Single-Blind, Placebo-Controlled, Randomized, Phase 2 study of Manganese, ICB plus Nab-paclitaxel and Platinum *vs.* Placebo, ICB plus Nab-paclitaxel and Platinum in Platinum-Resistant/Refractory Ovarian Cancer

Clinical Research Organization： Chinese PLA General Hospital

Medical Monitor： Weidong Han

Development Phase: 2

Date of Original Protocol: 10 June 2019

Version of Protocol: 1.0

INVESTIGATOR SIGNATURE PAGE

Declaration of the Principal Investigator

Title: A Single-Blind, Placebo-Controlled, Randomized, Phase 2 study of Manganese, ICB plus Nab-paclitaxel and Platinum vs. Placebo, ICB plus Nab-paclitaxel and Platinum in Platinum-Resistant/Refractory Ovarian Cancer

I have read this study protocol, including all appendices. By signing this protocol, I agree to conduct the clinical study, following approval by an Independent Ethics Committee (IEC)/Institutional Review Board (IRB), in accordance with the study protocol, the current International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), and applicable regulatory requirements. I will ensure that all personnel involved in the study under my direction will be informed about the contents of this study protocol and will receive all necessary instructions for performing the study according to the study protocol.

Principal Investigator

_____ _____

Name: Date

Title:

Institution:

SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor/Company: Chinese PLA General Hospital</td></tr>
<tr><td colspan="2">Title of Study:</td></tr>
<tr><td colspan="2">Name of Investigational Product: Manganese Chloride, nab-paclitaxel, Platinum chemotherapy, anti-PD-1 antibody</td></tr>
<tr><td>Study Period (years):

Estimated date first patient enrolled: June 2019

Estimated date last patient completed: October 2021</td><td>Phase of Development:

2</td></tr>
<tr><td colspan="2">Objectives:

Primary objectives:

The primary objective of the study is investigator-assessed ORR per RECIST v1.1, defined as the percentage of patients with at least 1 efficacy assessment who achieved a complete or partial response (CR/PR).

The primary safety endpoint is the incidence of treatment-related AEs per CTCAE v5.0 in patients who received at least one dose of study treatment.

Secondary objectives:

To evaluate additional measures of clinical benefit as assessed by the Investigators,

the secondary objectives of the study are as follows:

1) Progression-free survival (PFS) by RECIST v1.1

2) Overall survival (OS)

3) Disease control rate (DCR) by RECIST v1.1

4) Duration of response (DOR) by RECIST v1.1

5) Gynecological Cancer InterGroup CA-125 response rate

6) Quality of life score by EORTC QLQ-OV28

Exploratory objectives：

1) Concentration of blood Mn^{2+} was detected by the inductively coupled plasma-</td></tr>
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atomic emission spectroscopy (ICP-AES) recommended by ATSDR

2) serum cytokines and chemokines were analyzed by LEGEND^{plex} Bead-based Immunoassays (BioLegend) according to the manufacturer's instructions.

3) To correlate Concentration of blood $Mn^{2+}$with other immune-related biomarkers and with efficacy outcomes.

4) To identify the biomarker-based patient population that would derive benefit from the combination treatment based on the cytokine profile of blood.

Methodology:

Overall

This phase 2, randomized, single-blind, placebo-controlled study evaluating the efficacy and safety of combination treatment with manganese chloride or placebo plus immunochemotherapy in patients with platinum-resistant/refractory OC. The study is detailed as follows:

1) recurrent epithelial high-grade serous ovarian, fallopian tube, or primary peritoneal cancer who are currently platinum-resistant (defined as progression within 6 months following last dose of most recent platinum) or platinum-refractory (defined as lack of response or progression within 1 month since the last dose)；

2) Participants were randomly assigned in a 2:1 ratio to the test and control groups, and stratified by platinum-free interval；

—Test group:

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

anti-PD-1 antibody (200 mg, administered intravenously on day 3 in a 3-week cycle)

Manganese chloride was nebulized inhaled at 0.4mg/kg/d once daily in a 3-week cycle.

—Control group:

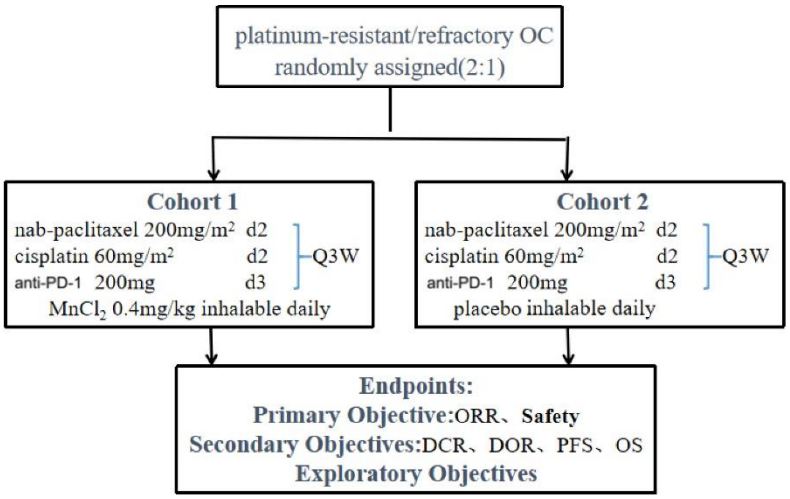
nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

anti-PD-1 antibody (200 mg, administered intravenously on day 3 in a 3-week cycle)

Placebo was nebulized inhaled once daily in a 3-week cycle.

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.



General Study Conduct

In this study, the screening period did not exceed 21 days, and qualified subjects who completed the screening examination and evaluation were randomly divided into blind groups and entered the study treatment period (every 21 days was a treatment cycle), and they were treated and visited in accordance with the protocol.

Following informed consent, all patients will undergo screening procedures within 21 days prior to the first dose of study treatment to determine eligibility for study entry.

Screening procedures include medical, surgical, cancer, and medication history; complete physical examination, including vital signs, height, and weight; Eastern Cooperative Oncology Group (ECOG) performance status; clinical laboratory tests (complete blood count [CBC], coagulation, chemistry, thyroid-stimulating hormone [TSH], triiodothyronine [T3] or free T3 [FT3], free thyroxine [FT4], serum CA-125), blood Mn^{2+} parameters and electrocardiogram (ECG). see Study Manual for details on sample collection and preparation. Radiographic evaluations (magnetic resonance imaging [MRI, preferred method]) or computed tomography [CT] of abdomen, and pelvis must be conducted at screening to determine extent of disease and confirm presence of measurable disease. The chest must be examined by computed tomography [CT]. Head scan will be conducted if clinically indicated; bone scans will be conducted per standard of care. Scans performed prior to the signing of the informed consent form (ICF) as part of routine clinical management are acceptable

for use as initial tumor imaging if they are of diagnostic quality and are performed within 21 days prior to first dose date.

Safety assessments conducted throughout the treatment period include symptom-directed physical examination, vital signs, ECGs, ECOG performance status, and clinical laboratory assessments (CBC, coagulation [Phase 1 only], chemistry, TSH, T3 or FT3, FT4, urinalysis, CA-125, blood Mn^{2+} parameters). Radiographic evaluations all patients to assess extent of disease will be conducted every 6 weeks (42 days ± 7 days) after Cycle 1/Day 1 while on study treatment independent of cycle delays and/or dose interruptions and/or at any time when progression of disease is suspected. All radiographic images/scans will be sent to a central imaging vendor upon acquisition and archived for future evaluation if needed. Per RECIST v1.1, patients who achieved complete response (CR) or partial response (PR) should have the response confirmed; tumor imaging for confirmation of response may be performed at the earliest 3 weeks after the first indication of response, or at the next scheduled scan, whichever is clinically indicated.

Safety and tolerability will be evaluated throughout the study by recording adverse events, monitoring vital signs and physical examination, laboratory evaluation of safety, and 12-lead electrocardiogram (ECG).

All AE and SAEs experienced by a patient, regardless of suspected causation, will be monitored until the AE or SAE is resolved, until abnormal laboratory values are returned to baseline or normalized, until there is a satisfactory explanation for observed changes, until the patient loses follow-up, or until the patient dies.

Number of Patients (Planned): 48

Criteria for Inclusion:

1. Patients with histologically or cytologically confirmed epithelial ovarian cancer, primary fallopian tube cancer, or peritoneal cancer.
2. Patients who have received at least one chemotherapy regimen based on platinum compounds.
3. Patients who experienced disease progression during treatment or within 6 months after completion of the above treatment regimen.
4. Patients with at least one measurable lesion.
5. Patients who have received immunotherapy (anti-PD-1 antibodies, anti-PD-L1 antibodies), or bevacizumab, are not restricted from enrollment.
6. Expected survival of more than 6 months.
7. Age over 18 years.

8. Patient weight > 40 kg.

9. ECOG performance status ≤ 2.

10. Peripheral blood white blood cell count > 3.5×10^9 /L.

11. Bone marrow reserve and liver and kidney function (the following laboratory tests should be performed before initial treatment to prove):

- Absolute neutrophil count (ANC) ≥ 1,000/mm^3;

- Hemoglobin > 80g/dL;

- Platelet count > 80,000/mm^3;

- ALT/AST < 3×ULN;

- Serum creatinine < 3×ULN;

- Total bilirubin level < 3×ULN.

12. No significant hereditary diseases. Normal liver and kidney biochemical indicators.

13. At least 4 weeks since the end of previous ovarian cancer treatment, and substantial recovery from adverse reactions of previous treatment (graded according to CTCAE5.0, excluding alopecia).

14. No treatment with paclitaxel (albumin-bound) plus platinum in the past 3 months.

15. Voluntary participation and signing of informed consent, compliance with the trial treatment plan and visit schedule, and cooperation in observing adverse events and efficacy.

Criteria for Exclusion:

1. Absolute neutrophil count (ANC) < 1×10^9/L or platelets < 80×10^9/L or hemoglobin < 80g/dL (according to the normal values of the clinical trial center).

2. Serum total bilirubin levels higher than 3 times the upper limit of the reference range.

3. ALT, AST, or ALP levels higher than 3 times the upper limit of the reference range when there are no liver metastases; ALT, AST, or ALP levels higher than 5 times the upper limit of the reference range when there are liver metastases.

4. Patients receiving known strong inhibitors (e.g., ketoconazole) or inducers (e.g., rifampicin or St. John's Wort) of CYP3A4, or strong inhibitors (e.g., gemfibrozil) or inducers of CYP2C8; patients receiving treatment with potent P-gp inhibitors or inducers, or patients who cannot stop treatment with these agents for 2 weeks

before the start of the study.

5. Toxic reactions to previous antitumor therapy that have not recovered to Grade 0 or 1 (excluding alopecia).

6. Organ failure:

- Heart: Grade III or IV.

- Liver: Grade C according to Child-Pugh B liver function classification.

- Kidney: Renal failure and uremia.

- Lung: Severe respiratory failure symptoms.

- Brain: Consciousness disorders.

7. T-cell tumors such as T-cell lymphoma or T-cell leukemia.

8. Major surgery within 4 weeks before enrollment, or planned major surgery during the study (excluding diagnostic surgery).

9. HIV antibody positive, or other acquired, congenital immunodeficiency diseases, or patients with a history of organ transplantation.

10. History of Parkinson's disease or epilepsy, or occurrence of diseases that can induce seizures within 12 months before the study (including a history of transient ischemic attack, stroke, traumatic brain injury with consciousness disorders).

11. CTCAE grade 2 infections that do not respond to treatment or active clinically serious infections with CTCAE > grade 2.

12. Patients with active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection requiring treatment.

13. Patients with a history of allergic reactions to study drug components or suspected allergies.

14. Chronic diseases requiring immunotherapy or hormone therapy.

15. Patients who have received any investigational drug treatment within 30 days before the first dose of the trial drug.

16. Subjects with symptoms of brain or leptomeningeal metastasis.

17. History of the following diseases: interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases including diabetes, hypertension, pulmonary fibrosis, acute lung disease, abdominal infection, etc.

18. Subjects with clinical symptoms of intestinal obstruction persisting before the first dose of the experimental drug; subjects with incomplete intestinal obstruction

who have returned to normal within 1 month before the first dose of the experimental drug can be included in the study after discussion by the researchers.

Investigational Product:

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

anti-PD-1 antibody (200 mg, administered intravenously on day 3 in a 3-week cycle)

Manganese chloride was nebulized inhaled at 0.4mg/kg/d once daily in a 3-week cycle.

Statistical Methods:

Sample Size Estimation

Primary endpoint is ORR per Investigator assessment. The study is designed to test the null hypothesis that the objective response is the same between the manganese plus immunochemotherapy arm and the immunochemotherapy alone arm *vs.* the alternative hypothesis that manganese promotes the objective response. The reference ORR was calculated based on the prior clinical trials with ICB plus chemotherapy in platinum resistant ovarian cancer, including the JAVELIN 200 study with a 13.3% ORR in ICB plus chemotherapy group. Approximately 45 patients will be randomized 2:1 (approximately 30 and 15 patients in the Mn^{2+} and placebo group, respectively) to provide an 80% power at the 5% level of statistical significance.

General Methods

For continuous variables such as the number of patients, mean, standard deviation, median, minimum, and maximum values in clinical study protocols, statistical analysis will be performed using two-sided 95% confidence intervals (CIs) based on the Clopper-Pearson method. Time-to-event analyses will utilize the Kaplan-Meier (KM) method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model. Comparisons between two groups of laboratories tests were performed by paired *t* test.

Efficacy

ORR and DCR will be summarized using descriptive statistics including number, percentage and 2-sided 95% CI.

Duration of response, PFS, and OS will be summarized using the Kaplan-Meier method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

Interim Analysis:

To minimize the risk of exposing patients to ineffective treatment, an assessment of the benefit comparison between the trial and control groups will be conducted during the study, a formal interim analysis of Overall Survival (OS) is planned when a pre-specified number of ORR events has been reached for the final ORR analysis.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	The absolute neutrophil count
APTT	Activated partial thromboplatin time
AST	Aspartate transaminase
AUC	Area under curve
BIL	Bilirubin
BLD	Urine latent blood
BMI	Body mass index
BOR	Best overall response
CK	Creatine kinase
CL	Clearance
Cmax	Peak concentration
CMH	Cochran mantel haenszel
CNS	Central nervous system

CR	Complete response
Cr	Serum creatinine
CRA	Clinical research associte
CRF	Case report form
eCRF	Electronic case report form
CRO	Contract research organization
CT	Computed tomography
CL	Clearance
DBIL	Direct bilirubin
DCR	Disease control rate
DOR	Duration of response
ECG	Electrocardiogram
ECOG	Eastern cooperative oncology group
EOS	Eosinophil
EPO	Erythropoietin
EORTC QLQ-OV28	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Ovarian Cancer Module 28
FIB	Fibrinogen
GCP	Good clinical practice
G-CSF	Granulocyte colony stimulating factor

GLP	Good laboratory practice of drug
GLU	Urine glucose
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCT	Hematocrit
HCV	Hepatitis C virus
HGB	Hemoglobin
HIV	Human immunodeficiency virus
HR	Hazard ratio
5-HT3	5-Hydroxytryptamine
IB	Investigator's brochure
IDMC	Independent data monitoring committee
IEC	Independent ethics committee
IHC	Immunohistochemistry
INR	International normalized ratio
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
ITT	Intention-to-treat

IV	Intravenous injection
KET	Ketone
LD	Lactic dehydrogenase
LEU	Leucocytes in urine
LYMPH	Lymphocyte percentage and counting
MedDRA	Medical dictionary for regulatory activities
MON	Monocytes percentage and counting
mPFS	Median progression free survival
MRI	Magnetic resonance imaging
NCI-CTCAE	National cancer institute common terminology criteria for adverse events
NEUT	Neutrophils percentage and counting
NIT	Nitrite
NMPA	National medical products administration
QA	Quality assurance
QC	Quality control
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease

PD-L1	Programmed cell death-ligand 1
PFS	Progress-free survival
PK	Pharmacokinetics
PLT	Platelet count
PPS	Per protocol set
PR	Partial response
PRO	Protein in urine
PT	Prothrombin time
Q3W	3 weeks using a
RBC	Red blood cell count
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Standard analysis software
SD	Stable disease
SG	Specific gravity
SMP	Standard management procedure
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction

TBIL	Total bilirubin
TEAE	Treatment emergent adverse events
TESAE	Treatment of serious adverse events
TNF	Tumor necrosis factor
TP	Total protein
TT	Thrombin time
TTP	Time-to-progress
WBC	White blood cell count
WHO-Drug	World health organization drug dictionaries

1. BACKGROUND

Ovarian cancer is a common malignant tumor of the female reproductive system, with the incidence rate occupying the 3rd place and the mortality rate ranking the first among gynecological malignant tumors. Every year,52,000 women are diagnosed with ovarian cancer in China, and about 22,000 people die as a result of the disease. 75% of the patients diagnosed for the first time are already in the advanced stage (Stage III/IV), and after receiving ideal tumor cytoreduction and standardized chemotherapy, 85% of them still suffer from relapses[1-3], and the emergence of Platinum-resistant response, and at present there is no effective standard treatment plan for platinum-resistant relapsed/refractory ovarian cancer worldwide.

Studies have shown that ovarian cancer expresses tumor-specific antigens, which can induce spontaneous anti-tumor immune responses from immunotherapy[4-5], providing some theoretical support for the application of PD-1/L1 inhibitors and other drugs that modulate acquired immune responses in ovarian cancer. However, clinical studies have confirmed that monotherapy with immune checkpoint inhibitors in recurrent/refractory ovarian cancer yields low objective response rates (ORR) ranging from 8.0% to 15.0% [6-9] , with a median PFS of 2 months; the ORR of immune checkpoint inhibitors combining with chemotherapy or anti-angiogenic drugs is also only 15%-20%, and the study of JAVELIN-200 has shown that the median PFS and OS of doxorubicin liposomal combined with avelumab are 3.7 months and 15.7 months[10] , which did not significantly improve the clinical efficacy, in response to the study, Lancet Oncol published a review "Immunotherapy in ovarian cancer: we are not there yet" in 2021[11] , suggesting that the JAVELIN-200 study is a failure, for the immunotherapy in ovarian cancer. The failure of the JAVELIN-200 study brought to a close the key question of immunotherapy in ovarian cancer and opened up new hypotheses and hopes. Therefore, further exploration of combination therapy strategies to improve clinical efficacy, prognosis and survival in relapsed/refractory ovarian cancer is necessary.

The reason for the poor efficacy of immune-detection point inhibitors in ovarian cancer may be related to the "cold tumor" property, the low frequency of pro-inflammatory immune cells and immunosuppressive signals in the tumor microenvironment of ovarian cancer[12] . Recent studies have shown that the STING (Stimulator of Interferon Genes) signaling pathway plays a key role in stimulating anti-

tumor immunity and converting "cold tumors" into "hot tumors"[13-16]. STING signaling pathway is one of the major natural immune pathways triggered by the body during antiviral processes. The cGAS-STING signaling pathway senses intracellular viral DNA, activates the transcription factors IRF3/7 and NF-κB, and produces cytokines such as type I interferon (IFN), which induces an immune response. Several studies have confirmed that the cGAS-STING pathway also has an anti-tumor effect, and the mechanisms include: 1) inducing tumor cell senescence; 2) promoting tumor cell autophagy; 3) promoting tumor cell apoptosis; 4) enhancing acquired anti-tumor response. DNA fragments of tumor cells in the tumor microenvironment, which are recognized and absorbed into the intracellular compartment by DC cells, activate the cGAS-STING pathway, induce type I interferon, promote antigen presentation and the activation of $CD8^+$ T cells, and activate acquired immunity; at the same time, locally increased mature DC cells can enhance the tumor-specific T cell response, thus playing an important role in tumor immunosurveillance and antitumor process[17-23] .

At present, many research institutions and pharmaceutical companies around the world have invested heavily in the research and development of STING agonists, including CDNs (synthetic cyclic dinucleotides), non-CDNs, and bacterial vector drugs, of which CDNs are the first-generation STING agonists, such as ADU-S100, MK-1454 and so on. Nearly 14 clinical studies of STING agonists combined with Immune checkpoint inhibitors for the treatment of solid tumors and lymphomas have been conducted internationally, and nearly 20 preclinical drug trials are under development. Among them, the clinical study data of ADU-S100 and MK-1454 combination therapy were reported in ASCO 2019 and ESMO 2018[24-25], respectively, and the four clinical studies containing the two drugs did not have significant breakthroughs in clinical efficacy, and the results of other STING agonist-based drugs have still not been published, and it has been reported that many of the clinical studies have been called off. Analyzing of treatment failure may be due to unstable drug metabolism and poor cell permeability; New STING agonists with non-CDN structures, such as liposomes, polymers, and nanoparticles, are currently being developed. These face challenges related to complex preparation methods and high production costs [26-27].

Studies have shown that metal ions also play an important role in immune regulation, such as Ca^{2+} is involved in the activation of T cells; K^+ can maintain T cell stemness and enhance T cell function; K^+, Ca^{2+} and Na^+ are involved in the occurrence of inflammation[28-31]. Therefore, the immunomodulatory function of metal ions can be utilized for the treatment of diseases, and "metal immunotherapy" has been proposed in the journal Nature 2021.

Prof. Jiang Zhengfan's team at Peking University found that manganese ions (Mn^{2+}) play an important role in activating the cGAS-STING pathway: Virus infection of cells resulted in the release of Mn^{2+} from manganese-storing organelles such as mitochondria and Golgi apparatus into the cytoplasm and extracellular space. The significantly increased concentration of Mn^{2+} in the cytoplasm has a dual activation effect on the cGAS-STING pathway: Mn^{2+} enhances the sensitivity of cGAS to DNA and promotes its synthesis of the second messenger, cGAMP; Mn^{2+} improves the binding capacity of STING and cGAMP, and Mn^{2+} released into the cytoplasm puts the cGAS-STING pathway in a state of hyper-activation, which greatly enhances its ability to respond to cytoplasmic DNA and even activating it at DNA levels that were initially non-responsive. And when mice were deficient in Mn^{2+}, the anti-DNA viral ability was significantly inhibited[32] . This study is the first to show that the trace element Mn^{2+} can play a dual role as an alarm and agonist in the body's natural immunity against infections. Mn^{2+} -STING agonist, unlike many agonists that have been developed so far, is an essential trace element that is naturally occurring inside the cell; it is more easily absorbed by the body as a trace element supplement.

To further confirm the anti-tumor effects of Mn^{2+}-STING agonists, our team collaborated with Professor Zhengfan Jiang's team to conduct both basic research and Phase I clinical studies. The basic study showed that the growth of tumor cells and metastatic lesions in manganese-deficient mice was significantly accelerated and increased, confirming that under physiological conditions, Mn^{2+} as an essential trace element plays an indispensable role in tumor immunosurveillance. Exogenous administration of Mn^{2+} can effectively activate the intracellular cGAS-STING pathway in mice, significantly promote the ability of antigen-presenting cells, such as DCs and macrophages, to present tumor-specific antigens, and enhance the infiltration and specific killing function of acquired immune cells in tumor tissues. The combination of Mn^{2+} and anti-PD-1 antibody (manganese immunotherapy) has been shown to significantly enhance the anti-tumor effect of anti-PD-1 antibody in several animal models. In the phase I clinical study of manganese immunotherapy for relapsed refractory solid tumors, the objective remission rate was 45.5%, the disease control rate reached 81.8%, and no serious adverse events were observed, which preliminarily confirms the efficacy and safety of Mn^{2+}-based therapy[33] .

Our team conducted a phase I clinical study in patients with advanced metastatic solid tumor, and the preliminary results showed that the objective remission rate of the manganese immunotherapy group was as high as 45.5%, the disease control rate was 90.9%, and the type I interferon in peripheral blood was significantly increased.

Manganese immunotherapy might significantly improve the clinical efficacy of relapsed and refractory ovarian cancer, which is considered to be related to the unique tumor microenvironment of ovarian cancer. However, the specific mechanism needs to be further analyzed. Moreover, further expansion of clinical samples is needed to confirm the efficacy and safety in relapsed refractory ovarian cancer.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is investigator-assessed ORR per RECIST v1.1, defined as the percentage of patients with at least 1 efficacy assessment who achieved a complete or partial response (CR/PR).

The primary safety endpoint is the incidence of treatment-related AEs per CTCAE v5.0 in patients who received at least one dose of study treatment.

2.2. Secondary Objectives

To evaluate additional measures of clinical benefit as assessed by the Investigators, the secondary objectives of the study are as follows:

1) Progression-free survival (PFS) by RECIST v1.1

2) Overall survival (OS) by RECIST v1.1

3) Disease control rate (DCR) by RECIST v1.1

4) Duration of response (DOR) by RECIST v1.1

5) Gynecological Cancer InterGroup CA-125 response rate

6) Quality of life score by EORTC QLQ-OV28

2.3. Exploratory Objectives

The exploratory objectives of the study are as follows:

1) Concentration of blood Mn^{2+} was detected by the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) recommended by ATSDR

2) serum cytokines and chemokines were analyzed by LEGEND^{plex} Bead-based Immunoassays (BioLegend) according to the manufacturer's instructions

3) To correlate concentration of blood Mn^{2+}, serum cytokines and chemokines with efficacy outcomes

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This phase 2, randomized, single-blind, placebo-controlled study evaluating the efficacy and safety of combination treatment with manganese chloride or placebo plus immunochemotherapy in patients with platinum-resistant/refractory OC. The study is detailed as follows:

1) recurrent epithelial high-grade serous ovarian, fallopian tube, or primary peritoneal cancer who are currently platinum-resistant (defined as progression within 6 months following last dose of most recent platinum) or platinum-refractory (defined as lack of response or progression within 1 month since the last dose);

2) Participants were randomly assigned in a 2:1 ratio to the test and control groups, and stratified by platinum-free interval;

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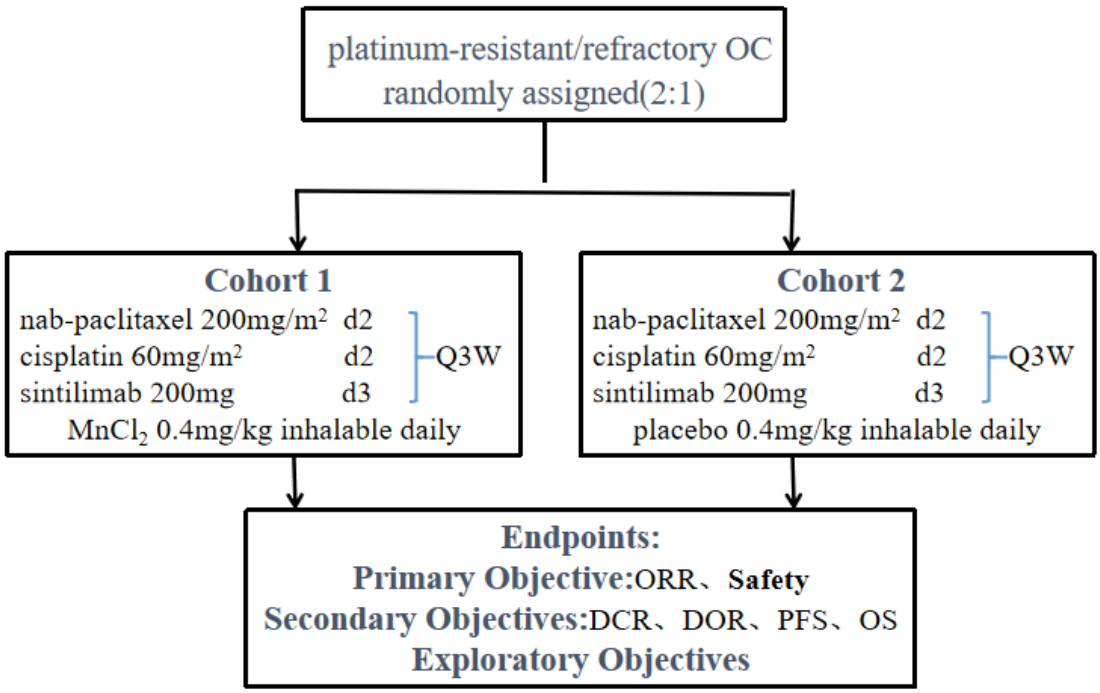
placebo was nebulized inhaled once daily in a 3-week cycle.

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.

Figure 1. Study Schema



Cohort 1：Test group (Mn^{2+} plus immunochemotherapy); Cohort 2：Control group (); n=3,4,5…

The study will be conducted in conformance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of International Council for Harmonisation.

3.2 General Study Conduct

In this study, the screening period did not exceed 21 days, and qualified subjects who completed the screening examination and evaluation were randomly divided into blind groups and entered the study treatment period (every 21 days was a treatment cycle), and they were treated and visited in accordance with the protocol.

Following informed consent, all patients will undergo screening procedures within 21 days prior to the first dose of study treatment to determine eligibility for study entry.

Screening procedures include medical, surgical, cancer, and medication history; complete physical examination, including vital signs, height, and weight; Eastern

Cooperative Oncology Group (ECOG) performance status; clinical laboratory tests (complete blood count [CBC], coagulation, chemistry, thyroid-stimulating hormone [TSH], triiodothyronine [T3] or free T3 [FT3], free thyroxine [FT4], serum CA-125), blood Mn^{2+} parameters and electrocardiogram (ECG). see Study Manual for details on sample collection and preparation) and will be sent to a centralized laboratory for biomarker testing. Radiographic evaluations (magnetic resonance imaging [MRI, preferred method]) or computed tomography [CT] of abdomen, and pelvis must be conducted at screening to determine extent of disease and confirm presence of measurable disease. The chest must be examined by computed tomography [CT]. Head scan will be conducted if clinically indicated; bone scans will be conducted per standard of care. Scans performed prior to the signing of the informed consent form (ICF) as part of routine clinical management are acceptable for use as initial tumor imaging if they are of diagnostic quality and are performed within 21 days prior to first dose date.

Safety assessments were conducted throughout the treatment period include symptom-directed physical examination, vital signs, ECGs, ECOG performance status, and clinical laboratory assessments (CBC, coagulation, chemistry, TSH, T3 or FT3, FT4, urinalysis, CA-125, blood Mn^{2+} parameters). Radiographic evaluations all patients to assess extent of disease will be conducted every 6 weeks (42 days ±7 days) after Cycle 1/Day 1 while on study treatment independent of cycle delays and/or dose interruptions and/or at any time when progression of disease is suspected. All radiographic images/scans will be sent to a central imaging vendor upon acquisition and archived for future evaluation if needed. Per RECIST v1.1, patients who achieved complete response (CR) or partial response (PR) should have the response confirmed; tumor imaging for confirmation of response may be performed at the earliest 3 weeks after the first indication of response, or at the next scheduled scan, whichever is clinically indicated.

Safety and tolerability will be evaluated throughout the study by recording adverse events, monitoring vital signs and physical examination, laboratory evaluation of safety, and 12-lead electrocardiogram (ECG).

All AEs and SAEs experienced by a patient, regardless of suspected causation, will be monitored until the AE or SAE is resolved, until abnormal laboratory values are returned to baseline or normalized, until there is a satisfactory explanation for observed changes, until the patient loses follow-up, or until the patient dies.

3.3 Randomization and Blind Method

This is a prospective, randomized, single-blind clinical investigation. Participants will be blinded to their treatment assignment and the study site personnel will be trained not to disclose the treatment assignment to the subject. Participants will be given a treatment drug or placebo with similar physical properties to reduce the possibility of unblinding. Participants blinding must be maintained until the completion of the trial.

The physician performing the treatment will not be blinded to the assigned treatment. Thus, the efficacy evaluations were performed by blinded radiologists and a different physician in order to reduce bias and maintain participants blinding. If clinical follow-up with a study physician is deemed necessary at the protocol required follow-up time points, the different physician (or designee) must conduct the follow-up clinical visits. Site personnel will be adequately trained such that the physician (or designee) conducting the clinical follow-up is adequately blinded to the treatment received by the participants. For unscheduled visits, subjects may see the physician who performed the treatment. The treating physician should prevent unblinding of the subject when they conduct any non-protocol related visits.

4. STUDY POPULATION

4.1 Inclusion Criteria:

1) Patients with histologically or cytologically confirmed epithelial ovarian cancer, primary fallopian tube cancer, or peritoneal cancer.

2) Patients who have received at least one chemotherapy regimen based on platinum compounds.

3) Patients who experienced disease progression during treatment or within 6 months after completion of the above treatment regimen.

4) Patients with at least one measurable lesion.

5) Patients who have received immunotherapy (anti-PD1 antibodies, anti-PD-L1 antibodies), or bevacizumab, are not restricted from enrollment.

6) Expected survival of more than 6 months.

7) Age over 18 years.

8) Patient weight > 40 kg.

9) ECOG performance status ≤ 2.

10) Peripheral blood white blood cell count > 3.5×10^9/L.

11) Bone marrow reserve and liver and kidney function (the following laboratory tests should be performed before initial treatment to prove):

- Absolute neutrophil count (ANC) ≥ 1,000/mm^3.
- Hemoglobin > 80 g/dL.
- Platelet count > 80,000/mm^3.
- ALT/AST < 3×ULN

- Serum creatinine < 3×ULN.

- Total bilirubin level < 3×ULN.

12) No significant hereditary diseases.

13) Normal liver and kidney biochemical indicators.

14) At least 4 weeks since the end of previous ovarian cancer treatment, and substantial recovery from adverse reactions of previous treatment (graded according to CTCAE5.0, excluding alopecia).

15) No treatment with paclitaxel (albumin-bound) plus platinum in the past 3 months.

16) Voluntary participation and signing of informed consent, compliance with the trial treatment plan and visit schedule, and cooperation in observing adverse events and efficacy.

4.2 Exclusion Criteria:

Subjects meeting any of the following conditions cannot be selected as subjects:

1) Absolute neutrophil count (ANC) < 1×10^9/L or platelets < 80×10^9/L or hemoglobin < 80 g/dL (according to the normal values of the clinical trial center).

2) Serum total bilirubin levels higher than 3 times the upper limit of the reference range.

3) ALT, AST, or ALP levels higher than 3 times the upper limit of the reference range when there are no liver metastases; ALT, AST, or ALP levels higher than 5 times the upper limit of the reference range when there are liver metastases.

4) Patients receiving known strong inhibitors (e.g., ketoconazole) or inducers (e.g., rifampicin or St. John's Wort) of CYP3A4, or strong inhibitors (e.g., gemfibrozil) or inducers of CYP2C8; patients receiving treatment with potent P-gp inhibitors or inducers, or patients who cannot stop treatment with these agents for 2 weeks before the start of the study.

5) Toxic reactions to previous antitumor therapy that have not recovered to Grade 0 or 1 (excluding alopecia).

6) Organ failure:

- Heart: Grade III or IV.

- Liver: Grade C according to Child-Pugh B liver function classification.

- Kidney: Renal failure and uremia.

- Lung: Severe respiratory failure symptoms.

- Brain: Consciousness disorders.

7) T-cell tumors such as T-cell lymphoma or T-cell leukemia.

8) Major surgery within 4 weeks before enrollment, or planned major surgery during the study (excluding diagnostic surgery).

9) HIV antibody positive, or other acquired, congenital immunodeficiency diseases, or patients with a history of organ transplantation.

10) History of Parkinson's disease or epilepsy, or occurrence of diseases that can induce seizures within 12 months before the study (including a history of transient ischemic attack, stroke, traumatic brain injury with consciousness disorders).

11) CTCAE grade 2 infections that do not respond to treatment or active clinically serious infections with CTCAE > grade 2.

12) Patients with active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection requiring treatment.

13) Patients with a history of allergic reactions to study drug components or suspected allergies.

14) Chronic diseases requiring immunotherapy or hormone therapy.

15) Patients who have received any investigational drug treatment within 30 days

before the first dose of the trial drug.

16) Subjects with symptoms of brain or leptomeningeal metastasis.

17) History of the following diseases: interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases including diabetes, hypertension, pulmonary fibrosis, acute lung disease, abdominal infection, etc.

18) Subjects with clinical symptoms of intestinal obstruction persisting before the first dose of the experimental drug; subjects with incomplete intestinal obstruction who have returned to normal within 1 month before the first dose of the experimental drug can be included in the study after discussion by the researchers.

4.3 Discontinuation from Treatment

Termination of study treatment does not mean withdrawal from the study. Subjects who discontinue study treatment must continue to complete the remaining follow up as required by the protocol. The investigator should consider arranging for the subject to terminate study treatment if any of the following conditions occur:

1) The second confirmed complete response.

2) The subject is unwilling to continue:

- The subject discontinues the drug on their own and withdraws from the trial.
- Loss to follow-up due to work, changes in living environment, or accidental accidents.
- The subject withdraws informed consent.

2) Intolerable adverse events:

- Due to significant abnormal laboratory indicators or adverse events related to safety (such as severe allergic reactions), the investigator believes it is not appropriate to continue the use of the investigational drug based on ethical

considerations.

- Requires rescue due to severe adverse events.

- Other serious illnesses occurred during the trial period, and the investigator judged it inappropriate to continue treatment.

3) Disease progression or the need for other treatments:

- Imaging studies define disease progression.

- It is judged by the investigator that new antitumor treatment or other treatments not allowed by the protocol are needed.

- If the subject experiences tumor progression during the trial, study treatment should be terminated. However, if the investigator believes that continuing study treatment may have clinical benefits and the subject does not receive other tumor treatment measures, the subject can continue study treatment after discussion by the investigator.

4) Other reasons that the investigator believes to be impossible to continue study treatment:

- Significant protocol deviations discovered after enrollment.

- If the delay in treatment exceeds 21 days (the time of one treatment cycle) from the next planned treatment time, the investigator should consider whether to withdraw from the clinical trial based on the actual benefits to the subject.

4.4 Study Withdrawal

Subjects can freely withdraw from the study at any time, and the investigator or sponsor may arrange for the subject to withdraw from the study due to safety concerns, inability to comply with the trial protocol, or other reasons. Reasons for subjects' withdrawal may include:

- The subject withdraws informed consent and refuses to participate in the study further.

- Other necessary reasons for withdrawal from the study as determined by the investigator.

- At any time, patients are found not to meet the enrollment criteria.

- Loss to follow-up.

- Subject death.

- The sponsor terminates the study.

The reasons for subjects' withdrawal must be recorded in the case report form and the subject's medical record.

5. STUDY MEDICATION

5.1 Investigational Drugs

5.1.1 Paclitaxel (Albumin-bound)

a. Mechanism:

1) In the process of cell division, microtubule proteins that make up the spindle apparatus (comprising α-tubulin and β-tubulin) can bind to specific sites on microtubule proteins in cancer cells, preventing their depolymerization. This leads to the accumulation of microtubule proteins within the cell. And microtubule proteins and microtubule dimers can interconvert, during cell division, spindle microtubules cannot be rapidly synthesized by microtubule proteins, causing the cell division to arrest in the G2 and M phases. This prevents replication and ultimately leads to the death of cancer cells, thereby acting to kill cancer cells.

2) Albumin-bound paclitaxel (nab-paclitaxel): Utilizing unique nanotechnology to bind

hydrophobic paclitaxel with albumin, it takes advantage of the natural transport mechanism of albumin (gp60-albumin-SPARC) to distribute paclitaxel more in tumor tissues, achieving higher concentrations within tumor cells.

3) Immunogenicity: Enhancing the release of tumor antigens and improving the anti-tumor response of immune checkpoint inhibition. In addition, albumin-bound paclitaxel can activate Toll-like receptors and enhance dendritic cell activity.

5.1.2 Cisplatin

Cisplatin is a heavy metal complex formed by combining divalent platinum with two chlorine atoms and two ammonia molecules. Similar to bifunctional alkylating agents, it can inhibit the DNA replication process. DDP cells are the most sensitive, and at high concentrations, it can also inhibit RNA and protein synthesis. This substance has an effect on anaerobic cells and can diffuse through charged cell membranes after entering the human body. Currently, it is believed that the primary site of action for DDP is in the purine and pyrimidine bases of DNA.

Cisplatin belongs to the category of cell cycle non-specific drugs. It possesses cytotoxicity and can inhibit the DNA replication process in cancer cells while damaging structures on their cell membranes. As a result, it exhibits a strong broad-spectrum anticancer effect.

5.1.3 anti-PD-1 antibody

anti-PD-1 antibody is the potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. anti-PD-1 antibody injection is a sterile, non-pyrogenic lyophilized powder for IV infusion supplied in single-use Type I glass vial containing 100 mg of anti-PD-1 antibody. The product is preservative-free, white to off-white powder and free from visible foreign matter.

5.1.4 Manganese Chloride

Manganese chloride with the molecular formula $MnCl_2$ and a molecular mass of 125.84, is also known as chloromanganese, dichloromanganese, tetrahydrate manganese chloride, and so on. Hydrated manganese chloride appears as rose-colored monoclinic crystals, while anhydrous manganese chloride appears as pink crystals. In clinical settings, $MnCl_2$ is often used for intravenous nutrition to supplement the body's required manganese metal element.

5.2 Administration

5.2.1 Manganese Administration

Participants were randomly assigned to the test group and the control group in a 2:1 ratio.

The administration method of $MnCl_2$ in the experimental group was as follows:

1) Manganese chloride was nebulized inhaled at 0.4mg/kg/d once daily in a 3-week cycle.

2) nebulized inhaled

- Body Position: Prior to treatment, cough up phlegm to prevent hindrance to the penetration of aerosol droplets. During nebulized inhalation, it is recommended to sit, semi-sit, or lie on one's side, while avoiding the supine position as much as possible. If a supine position is necessary, the head of the bed should be raised by 30°. During treatment, patients should take slow and deep inhalations, pause briefly at the end of inhalation to allow for deeper penetration of the aerosol droplets.

- For patients undergoing long-term nebulized inhalation therapy, the amount of aerosol used must be moderate. Over-humidification can lead to increased phlegm production. In critically ill patients with altered mental status or reduced cough reflex, the inability to expectorate phlegm promptly can worsen their condition or even lead to death. If the nebulization is insufficient, it can be challenging to achieve the treatment goals.

- During nebulized inhalation, water vapor can effectively moisten the airways. However, the expelled aerosol has some pressure, which displaces the surrounding air from entering the respiratory tract and reduces oxygen intake. Therefore, for patients with asthma, breathing difficulties, severe hypoxia, and those with pneumonia complicated by heart failure, it is necessary to improve the above symptoms, increase oxygen intake before proceeding with nebulized inhalation. Inhalation time should be short, around 5 minutes per session, to prevent worsening of hypoxia. Pay close attention to changes in the patient's condition during nebulized inhalation. If symptoms such as coughing and shortness of breath occur, stop nebulized inhalation immediately, increase oxygen intake, perform back percussion, drink water, and consider the next nebulized inhalation treatment only when the symptoms have eased. Additionally, check the temperature, dosage, and body position during nebulization, and make necessary adjustments.
- To prevent cross-infections in the respiratory tract during nebulized inhalation therapy, the following measures should be taken: the nebulizer must be thoroughly disinfected before use and replaced daily; when not in use, there should be no liquid residue in the entire system to prevent bacterial growth; sterile solution should be used during nebulization therapy.

The placebo administration method for the control group was as follows:

1) Placebo was nebulized inhaled once daily in a 3-week cycle.

2) The nebulization inhalation method was the same as that of the test group.

5.2.2 Anti-PD-1 antibody plus chemotherapy treatment

Enrolled patients will receive intravenous chemotherapy plus anit-PD-1 Antibody. The immunochemotherapy consisted of nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle), cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle) and anti-PD-1 antibody (200 mg, administered intravenously on day 3 in a 3-week cycle).

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.

5.3. Dose Modification

According to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE version 5.0), if any grade 3 or 4 adverse events occur during treatment and the investigator determines that they are related to the research protocol, the treatment must be interrupted. If the toxicity returns to normal or grade 1 （or lower） within 21 days, the patient can resume treatment with the investigational drug. However, if grade 3 or 4 adverse events recur despite preventive measures, the dose level may be reduced as needed. If the adverse events occur again, treatment should be interrupted again, and after resolution, the dose must be further reduced. Dose reduction should not exceed two times. If a patient has already experienced a maximum of two dose reductions and the toxicity has not returned to normal or grade 1 adverse events within 21 days, treatment must be permanently discontinued.

To maintain the dose intensity and cumulative dose of this study, a reasonable dose adjustment plan will be implemented to minimize the occurrence of dose reductions and treatment delays. Any subject with a treatment delay must be assessed weekly until adequate hematological and non-hematological parameters are met. Then, the treatment plan will proceed in the normal sequence.

All treatment interruptions and dose reductions (including any missed doses) and the reasons for reduction/interruption should be documented in the electronic Case Report Form (eCRF).

5.3.1. Dose Reductions or Delays for cisplatin and nab-paclitaxel

5.3.1.1 Guidelines for Hematologic Toxicity

Initial therapy modifications will consist of cycle delay and/or dose reduction as directed. Treatment decisions will be based on the ANC rather than the total white cell count.

Before each treatment cycle, blood routine tests will be conducted. Medication can be administered if the absolute neutrophil count (ANC) is ≥ 1000/μL and the platelet count is ≥ 75,000/μL. Subjects with an ANC between 1000-1500/μL or a platelet count between 75,000-100,000/μL will be able to continue treatment, with dose modifications as shown in Table 1. If the specified values are not reached, treatment may be postponed for up to three weeks until these values are achieved. For subjects who do not recover their counts within 3 weeks as shown in Table 1, the investigator should be notified.

Table 1. Dose Modifications for Dose Limiting Hematologic Toxicity or Reduced ANC (1000–1500/μL) or Reduced Platelets (75,000–100,000/μL)

ANC	PLT	Regimen –1 Level	Regimen –2 Level	Regimen –3 Level
Yes	No	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue
Yes	Yes	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue
No	Yes	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue

*Considering that hematological toxicity is primarily associated with nab-paclitaxel, dose adjustments should primarily focus on adjusting the dose of nab-paclitaxel.

Guidelines for the Use of Hematopoietic Cytokines

The use of hematopoietic growth factors is restricted as follows:

a) Generally, subjects should not receive prophylactic filgrastim (G-CSF), PEG-filgrastim (Neulasta), or sargramostim (GM-CSF) unless they experience treatment delays, dose omissions, or complications of recurrent neutropenia. Specifically, hematopoietic growth factors should not be used to avoid modifications of the initial treatment dose as specified in the protocol. However, subjects may also receive growth factor treatment for complications of neutropenia based on institutional treatment guidelines.

b) If dose adjustments are required per dosing modification guidelines, it is recommended to initiate daily subcutaneous administration within 24-72 hours after the last chemotherapy.

c) Subjects will not receive prophylactic platelet stimulants.

d) Subjects may receive erythropoiesis-stimulating agents, iron supplements, and/or blood transfusions to correct treatment-related anemia. The treating physician should be aware of the potential risks of erythropoiesis-stimulating agents, including the potential risk of shortening tumor progression or disease-free survival. These agents are used solely to avoid red blood cell transfusions and should not be used in patients with uncontrolled hypertension, as they may increase the risk of thrombotic events in cancer patients.

e) Subjects should not receive amifostine or other protective agents.

5.3.1.2 Guidelines for Non-Hematologic Toxicity

The management of grade 3 or 4 non-hematological toxicities related to treatment (excluding alopecia, fatigue, allergic reactions, nausea, vomiting, constipation, diarrhea, hypokalemia, hypomagnesemia, hypocalcemia, hyponatremia, and hypophosphatemia) should follow the specific dose level adjustments outlined in this section. The table 2 below is applicable to dose level modifications related to non-hematological toxicities:

Table 2. Modifications for Non-Hematologic Toxicity

Drug	Regimen –3 Level (Continued treatment-related CTCAE Grade 3 or 4 AE or SAE ≥ 28 days)	Regimen –2 Level (2nd dose reduction for treatment-related CTCAE Grade 3 or 4 AE or SAE where prophylaxis is not considered feasible)	Regimen –1 Level (1st dose reduction for treatment-related CTCAE Grade 3 or 4 AE or SAE where prophylaxis is not considered feasible)	Regimen Starting Dose
nab-paclitaxel	Discontinue	140mg/m^2	170 mg/m^2	200 mg/m^2
cisplatin	Discontinue	40 mg/m^2	50 mg/m^2	60 mg/m^2

Abbreviations: AE = adverse event; CTCAE = Common Terminology for Adverse Events; SAE = serious adverse event.

Peripheral Neuropathy

Peripheral neuropathy Grade 2 (or greater) requires reduction of one dose level in nab-paclitaxel (both dosing schedules) and delay in all subsequent therapy for a maximum of three weeks until recovered to Grade 1. If peripheral neuropathy fails to recover to Grade 1 by a maximum delay of three weeks from time therapy is due, the medical monitor should be contacted. If neuropathy of grade 2 (or higher) recurs after two dose reductions of nab-paclitaxel, the medical monitoring device should be contacted. If grade 3 or higher neuropathy occurs, the use of nab-paclitaxel should be discontinued.

Renal Toxicity

Renal toxicity is primarily caused by cisplatin. If creatinine levels increase to grade 3 or higher during the treatment process, it is necessary to reduce the dose level of cisplatin and delay treatment for up to 3 weeks until it recovers to grade 1 or lower. If grade 3 (or higher) elevation in creatinine cannot be restored within three weeks or does not recover despite dose adjustments, treatment with cisplatin should be discontinued, and subsequent treatment cycles should not include cisplatin but should be replaced

with carboplatin.

Hepatic Toxicity

If there is a Grade 3 (or higher) increase in SGOT (AST), SGPT (ALT), alkaline phosphatase, or bilirubin, it is necessary to reduce the dose of nab-paclitaxel by one level and delay treatment for up to 3 weeks until it recovers to Grade 1 or lower. If the Grade 3 (or higher) increase does not recover within three weeks or does not recover despite dose adjustment, treatment should be discontinued, and the investigator should be contacted.

Hypersensitivity Reaction

In general, the occurrence of allergic reactions to cisplatin or albumin-bound paclitaxel is not considered a dose-limiting toxicity. With measures such as adjusting infusion rates and strict drug management to prevent allergic reactions, full doses can still be administered. However, if despite these safety measures, repeated attempts at infusion still result in severe hypersensitivity reactions, the drug should be discontinued for the participant. Severe allergic reactions to albumin-bound paclitaxel do not need to be rechallenged, and after an allergic reaction to cisplatin, it can be replaced with carboplatin for treatment.

Other Toxicity

For alopecia, nausea, constipation, diarrhea, hypokalemia, hypocalcemia, hypomagnesemia, hyponatremia, or hypophosphatemia, dose adjustments are not required. It is recommended to manage and correct nausea, constipation, diarrhea, and electrolyte abnormalities using standard medical measures.

Nausea, Vomiting, or Fatigue

For ≥ 3-grade nausea, vomiting, or fatigue mainly caused by cisplatin, prophylactic antiemetic therapy should be administered before dosing until symptoms are relieved

to ≤1-grade. If active prophylactic antiemetic therapy is given, and the patient still cannot be relieved, experiencing severe electrolyte imbalances, shock, or other life-threatening events, cisplatin should not be used in subsequent treatment cycles, and it should be replaced with carboplatin.

Other non-hematologic toxicity dose adjustments are as follows:

a) For any 3-grade non-hematologic adverse events (except manageable nausea/vomiting, constipation, diarrhea, hypokalemia, hypomagnesemia, hypophosphatemia, or hyponatremia) considered related to the study treatment, treatment should be initiated, and the dose of the drug most likely to cause toxicity should be reduced by one dose level until symptoms are relieved to ≤1-grade or return to normal. If >3-grade adverse events persist for 3 weeks or recur after recovery, the subject may discontinue treatment after discussing with the investigator.

b) Any 4-grade non-hematologic adverse events related to treatment (except manageable nausea/vomiting, constipation, diarrhea, hypokalemia, hypocalcemia, hypomagnesemia, hypophosphatemia, or hyponatremia) should be discussed with the investigator, and the subject may stop treatment or reduce the dose.

5.3.2 Dose Reductions or Delays for anti-PD-1 antibody

Adverse events (both non-serious and serious) related to exposure to anti-PD-1 antibody may be indicative of an immune-related condition. These AEs can occur shortly after the first dose or several months after the last dose of treatment. According to Table 3, if drug-related toxicities and serious or life-threatening adverse events occur, the use of anti-PD-1 antibody must be discontinued. Please also refer to Clinical Event of Interest (ECIs).

Table 3 provides detailed information on dose interruptions and discontinuations related to anti-PD-1 antibody toxicity. Reducing the dose of anti-PD-1 antibody is not allowed.

Table 3 anti-PD-1 antibody Dose Modifications for Non-hematologic Toxicities

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hypothyroidism		Continued while thyroid replacement therapy is instituted	Continued while thyroid replacement therapy is instituted
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue.
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with anti-PD-1 antibody can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Infusion reaction	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue.
AST, ALT, or increased bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue	Permanently discontinue.
Diarrhea/colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or

			less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue.
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4 or Recurrent 2	Permanently discontinue	Permanently discontinue.
Renal failure or nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All other drug related toxicity	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Abbreviations: AE = adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase; T1DM=type 1 diabetes mellitus

The reasons for interrupting or discontinuing the administration of anti-PD-1 antibody should be documented in the eCRF (electronic case report form).

5.3.3 Dose Reductions or Delays for Manganese chloride or placebo

Exposure to Manganese chloride primarily causes manganese ion poisoning. Since

manganese poisoning is rare in everyday life and there is limited treatment experience, it is recommended to carefully observe patients and reduce their exposure risk. If a case of manganese ion poisoning occurs, usage should be stopped, and reducing the dose for further use is not allowed.

5.4. Packaging, Labeling, and Storage

5.4.1 Basic Information on Drugs

1) Storage and Preservation of Manganese Chloride:

   Drug Specification: 100mg (10ml) per bottle.

   Drug Storage: Keep in dark place

   The appearance of the placebo is the same as that of manganese chloride, and drug storage follows the same guidelines as for manganese chloride.

2) anti-PD-1 antibody injection is packaged in small boxes, with each box containing 1 vial, and each vial has a capacity of 10ml: 100mg, stored in a light-protected manner at 2-8°C.

3) Nab-paclitaxel (albumin-bound) is available in vials with a capacity of 100mg each.

4) Cisplatin is available in vials with a capacity of 10mg each.

The label text of the study treatments will comply with Good Manufacturing Practice and national legislation to meet the requirements of the participating countries. The study treatment will be open-label and non-patient-specific.

All study treatment supplies must be stored in accordance with the Pharmacy Manual instructions and package labeling. Until dispensed or administered to the patients, the study treatment will be stored in a securely locked area, accessible to authorized personnel only.

5.4.2 Drug Packaging and Labels

Each package box is affixed with a drug label. The drug label contains information such as protocol number, drug name, drug code, specification, production batch number, expiration date, storage conditions, usage instructions, precautions, and sponsor information, etc.

The vials are also affixed with drug labels containing the same information as the package box labels. The drug labels include the protocol number, drug name, drug code, specification, production batch number, expiration date, storage conditions, and sponsor information, etc.

All packaging and drug labels for investigational drugs are clearly marked with 'For Clinical Trial Use Only.

5.5. Drug Accountability

5.5.1 Drug Dispensing

The Investigator or designee is responsible for maintaining accurate dispensing records of the study treatments throughout the clinical study.

Details of maintaining drug accountability, including information on the accountability log, will be provided in the Pharmacy Manual.

All dispensation and accountability records will be available for Sponsor review. The study monitor will assume the responsibility to reconcile the study treatment accountability log. The pharmacist will dispense study treatment for each patient according to the protocol and Pharmacy Manual, if applicable.

5.5.2 Drug Dispensing, Retrieval, and Disposal

The management, dispensing, and retrieval of investigational drugs are the responsibility of designated personnel at each research center. Researchers must ensure

that all investigational drugs are used exclusively for participants in this study, and their dosage and usage should follow the research protocol. Any remaining or expired investigational drugs must be returned to the sponsor or disposed of in accordance with medical waste standards.

If there are quality issues such as turbidity or precipitation with the investigational drug, another vial of the drug may be used, and the sponsor should be promptly contacted for replacement.

Investigational drugs should be stored as per label requirements, and the dispensing and retrieval of each unit of investigational drug should be promptly recorded on a dedicated record sheet.

Monitors are responsible for overseeing the supply, use, storage, and handling of remaining drugs in the investigational drug process.

5.6 Past and Concurrent Drug Therapy

Throughout the entire research process, medications can be taken for the treatment of pre-existing conditions present before the subject's enrollment or for new conditions that may arise during the study. In addition to excluding medications prohibited by the criteria and protocol, researchers may decide to administer other medications that are necessary for the safety and health of the participants. Researchers can provide any necessary concomitant medication or treatment to provide comprehensive therapeutic support.

Apart from the investigational treatment, any medications taken by patients during the study, including herbal and other non-traditional medicines, as well as blood products or vaccines, are considered concurrent medications. All concurrent medications must be documented in the eCRF (electronic Case Report Form). Each concurrent medication must include the following information in the eCRF: generic name, route of administration, start date, end date, dosage, and indication. Any changes

in the dosage or regimen of concurrent medications must also be recorded in the eCRF.

During screening, patients will be asked about any medications they have taken in the past 30 days. During subsequent study visits, patients will be asked about the concurrent medications they are currently taking or have taken.

5.6.1 Permitted Concomitant Medication or Treatment

The following medications are allowed at the discretion during the study period:

1) Long-term medications required for underlying conditions such as hypertension and diabetes.

2) Medications determined by the investigator to be in compliance with the protocol requirements (such as concomitant medications for treating various adverse events).

— The short or long-acting granulocyte colony stimulating factor (G-CSF) can be given if the patients suffer severe neutropenia (above grade 3) and stopped 24 hours before treatment.

— Hydration and alkalization as routine treatment during chemotherapy will be allowed.

— Appropriate auxiliary drug, such as liver-protective, heart-protective and stomach-protective, are permitted to perform concomitant with investigational drugs

— Prophylactic Antiemetics: Prophylactic antiemetic drugs may be administered based on the common gastrointestinal adverse reactions observed in previous studies (such as nausea and vomiting). Depending on the actual condition of the subject, prophylactic antiemetic drugs may be given before the study treatment. Recommended prophylactic antiemetic drugs include, but are not limited to, 5-HT3 receptor antagonists, NK-1 receptor antagonists, gastric mucosal protectants, etc. Prophylactic antiemetic treatment should be administered according to the standard treatment protocols of medical institutions, published guidelines, and the respective drug labels.

3) Supportive Treatment for Alleviating Tumor-Related Symptoms, such as bisphosphonate therapy for bone metastases.

4) Researchers, prioritizing the well-being and safety of the subjects, may provide symptomatic supportive treatment for drug toxicity reactions or to manage tumor symptoms.

5.6.2 Prohibited Concomitant Medication or Treatment

During the trial, the use of any other antitumor treatments not specified in the protocol is prohibited. This includes cytotoxic drugs, radiotherapy, biologic therapy, immunosuppressive agents, or any other investigational drugs with anticancer properties. Traditional Chinese medicines with anticancer effects are also not allowed to be used in this study. Hormone replacement therapy (e.g., oral contraceptives) or local corticosteroid therapy (e.g., topical or inhaled) not intended for antitumor treatment is prohibited. The use of any other clinical research drugs besides the investigational drug specified in this protocol is also not allowed. If a subject requires such medications, they should be excluded from the trial.

5.7 Treatment Compliance

The dosage and administration method of the investigational drug for each subject will be recorded on the corresponding eCRF (electronic Case Report Form) for each cycle. Reasons for delayed or missed administrations should be documented. Compliance with treatment will be assessed using this information. Subjects will self-administer nebulized inhalation of manganese chloride/placebo and keep records on a log card. They will be required to return any unused empty packages for inventory during the next scheduled visit.

6. ENDPOINTS AND METHODS OF ASSESSMENT

6.1 Tumor Response Evaluation

The efficacy endpoints of this study's combined treatment regimen will be evaluated based on the RECIST v1.1 criteria.Tumor marker data (CA-125) will not be used to define objective response or disease progression but may serve as reference data for clinical decision-making. Clinical criteria, such as the GCIG criteria detailed in Appendix F, will also be considered for patients with ovarian cancer presenting clinical events (e.g., bowel obstruction) without radiological evidence of disease progression.

Efficacy assessments will primarily be conducted through imaging data, with imaging examinations completed at the times specified in Table 6 and archived for future evaluation.

Tumor imaging for pelvic, abdominal, and skull lesions should be performed using magnetic resonance imaging (preferred) or CT, and chest imaging should be performed using CT. The same imaging technique should be used throughout the trial.

If the chest is normal at the time of screening, repeat imaging of the area is not necessary in the absence of clinical indications that require follow-up.Bone scans should be conducted based on clinical necessity.

6.1.1 Timing of Imaging Evaluations

Initial imaging at screening must be performed within 15 days prior to the first dose study treatment date.

Scans performed prior to ICF sign-off as part of routine clinical management may be used as initial tumor imaging if they are of diagnostic quality and performed within 15 days prior to the date of the first dose.

The first study imaging assessment should occur 6 weeks after the initial study treatment; for Progressive Disease (PD), a confirmation is required 3 weeks later. Subsequent imaging should be conducted every 6 weeks, or more frequently if clinically indicated and suspected of disease progression. After 1 year of imaging

assessments, patients will undergo imaging every 12 weeks. Imaging should not be delayed by delays in cycle initiation or longer intervals between cycles of combination therapy.

According to RECIST v1.1, Complete Response (CR) or Partial Response (PR) should be confirmed through repeated tumor imaging assessments. Imaging to confirm responses can be performed 3 weeks after the first response or at the next scheduled examination (i.e., 6 weeks later), as clinically necessary.

Imaging will continue until any of the following occurs:

- Initiation of a new anti-tumor treatment,

- Withdrawal of consent by the patient,

- Death,

- End of the study.

Patients who discontinue study treatment for reasons other than disease progression will continue to have imaging assessments at the same frequency as their original follow-up.

6.1.2 Response Assessment by RECIST

RECIST v1.1 will be used by investigators as the primary measure for assessing tumor response, disease progression, and as the basis for all protocol guidelines related to disease status.

Detailed information on RECIST v1.1, including the assessment of target and non-target lesions and definitions of response, can be found in Appendix 3.

6.2 Primary Endpoint

6.2.1 Objective Response Rate (ORR)

Within the entire intention-to-treat population, the proportion of subjects

achieving a Complete Response (CR) or Partial Response (PR) as assessed by RECIST from enrollment until the end of treatment for any reason.

Proportion of subjects achieving complete response (CR) or partial response (PR) as assessed by the investigator according to RECIST from the start of enrollment to the end of treatment for any reason in all INTENTION-TO-TREAT populations.

Data obtained prior to progression, or last assessed in the absence of progression, will be included in the objective response assessment. CR assessments should be confirmed through CT/MRI evaluations. Any CR or PR occurring after further anti-cancer treatment will not be included in the ORR analysis.

6.2.2 Treatment-Emergent Adverse Events (TEAEs) and Severity

This includes the incidence of treatment emergent serious adverse events (TESAEs), abnormalities in clinical laboratory markers, and other examination results.

6.3 Secondary Endpoints

6.3.1 Progression-Free Survival (PFS)

PFS is defined from enrollment to the time of objective tumor progression or death from any cause, whichever comes first. For subjects who die without reported progression, progression is assumed to have occurred on the date of death. Patients who did not have disease progression but withdrew from the study due to toxicity or other reasons, PFS was calculated as if they had been last identified.

6.3.2 Overall Survival (OS)

Overall survival will be assessed as a secondary endpoint and is defined as the time from date of first dose of study treatment to the date of death by any cause. New malignancy information will also be collected as part of this assessment.

6.3.3 Disease Control Rate (DCR)

Disease control rate will be assessed as a secondary endpoint and is defined as the proportion of patients achieving CR, PR, or stable disease (SD) as assessed by the Investigator per RECIST v1.1.

6.3.4 Duration of Response (DOR)

For participants confirmed to have achieved a complete response (CR) or partial response (PR), the DOR is defined as the time from the first documented objective response to the first occurrence of tumor progression or death due to any cause, whichever comes first.

6.3.5 CA-125 response rate

A response according to GCIG CA125 Criteria has occurred if there is at least a 50% reduction in CA125 levels from the pre-treatment (baseline) sample. The response must be confirmed and maintained for at least 28 days.

6.4. Pharmacokinetics

PK samples will be carried out according to samples schedule specified in the program and the checks will be carried out in the central laboratory. Sampling methods, sample storage, transportation and analysis are detailed in the Laboratory Manual provided by the Sponsor's designated central laboratory.

6.5. Biomarkers

Tumor tissue and peripheral blood samples will be collected according to the protocol requirements, and the changes of blood manganese concentration, immune cells and tumor microenvironment before and after treatment will be analyzed.Sampling methods, sample storage, transportation and analysis are detailed in the Laboratory Manual provided by the Sponsor's designated central laboratory.

6.6 Health status assessment

The health status of the subjects will be assessed using the FACT-G (Version 4) questionnaire. Five main areas are covered (mobility, self-care, daily activities, pain/discomfort, and anxiety/depression) as well as an overall visual analog scale of health status.

7. ADVERSE EVENTS

7.1 Definition of Adverse Events

Investigators and any designated compliant personnel are responsible for investigating, documenting, evaluating, and tracking safety events, including Adverse Events (AEs), Serious Adverse Events (SAEs), and medication overdoses.

The AE is defined as any unfavorable medical occurrence in a clinical trial subject after administration of the trial medication, which can manifest as symptoms, signs, illness, or laboratory abnormalities, regardless of causality with the trial medication.

The AE could be the exacerbation of preexisting symptoms, signs, laboratory abnormalities, newly diagnosed diseases, and clinically significant laboratory abnormalities that were temporally related to drug use, regardless of whether they were causally related to the drug. Events caused by research procedures, collected after informed consent, must be recorded as AEs.

7.1.1 Serious Adverse Events

A Serious Adverse Event (SAE) refers to an AE that meet any of the following criteria at any stage of the clinical study:

1) Results in death;
2) Life threatening (where "life-threatening" means immediate risk of death at the time of the event, not hypothetical future severity);

3) Leads to hospitalization or prolongation of hospitalization;

Generally, hospitalization implies that the subject, due to the severity of their condition, cannot be observed and/or treated in an outpatient setting or doctor's office and requires overnight stay at a hospital or emergency room for observation and/or treatment. Complications during hospitalization are considered AEs. An AE that complicates or prolongs hospital stay or meets other serious criteria is deemed "serious." When it is uncertain if hospitalization occurred or was necessary, the AE should be considered "serious."

4) Results in persistent or significant disability/incapacity (significantly impairs normal life functions);

5) Other significant medical events that could result in the conditions listed above if not treated. Medical and scientific judgment must be applied to decide whether other situations require rapid reporting, such as important medical events that may not immediately result in life-threatening situations, death, or hospitalization but could jeopardize the subject or require medical intervention to prevent one of the listed outcomes, and are generally considered serious.

Note: The following hospitalizations do not count as SAEs:

1) Stays in the emergency room or other hospital departments for less than 24 hours (unless deemed a significant medical event or life-threatening by the researcher based on facts);

2) Hospitalizations scheduled as part of the clinical study protocol;

3) Treatments or surgeries scheduled before study enrollment (should be recorded in the trial's original documentation and subject CRF);

4) Elective or planned treatments for pre-existing conditions not related to the study disease and not worsened;

5) Hospitalizations for selective treatments of pre-existing conditions that have not

worsened compared to baseline;

6) Hospitalizations for medical management, insurance, or social reasons (e.g., check-up hospitalizations, rehabilitation/nursing home stays, or if the subject has no caregiver).

7.1.2 Suspected and Unexpected Serious Adverse Reactions (SUSARs)

SUSARs refer to serious adverse reactions that are both unexpected and suspicious, exceeding the severity and nature anticipated in the Brochure, the marketed product's label, or the Summary of Product Characteristics.

7.2 Collection and Recording of Adverse Events

7.2.1 Collection of Adverse Events During Follow-up Period

The collection and reporting period for AEs begins after the participant signs the informed consent form (if not caused by the study procedure, it's recorded as medical history with specific dates and times) and continues until the end of the safety follow-up visit. AEs deemed unrelated to study treatment by the investigator will no longer be collected after this point. However, all AEs must be followed up until any of the following occurs:

1) Death or loss to follow-up;

2) The participant withdraws consent for further data collection;

3) Study termination.

7.2.2 Recording of Adverse Events

Researchers should record AEs or SAEs using medical terminology/concepts, avoiding colloquialisms and abbreviations. All AEs, including SAEs, must be recorded on an adverse event form. Researchers must comprehensively record every adverse event, including diagnosis (or symptoms, signs including laboratory abnormalities if no diagnosis), onset and end dates and times (if applicable), CTCAE severity grade and

changes, whether it is a serious adverse event, actions taken regarding the investigational product, treatments given for the AE, and the outcome of the event, the relationship between the adverse event and the investigational product, and the causality assessment with the study procedure.

For serious adverse events, researchers must also provide the date on which the AE met SAE criteria, the date on which the researcher became aware of the SAE, the criteria for SAE severity, admission and discharge dates (if applicable), possible causes of death (if applicable), the date of death (if applicable), and whether an autopsy was conducted (if applicable), along with the rationale for the relevance judgment and a description of the SAE.

Notes on AE Recording:

1) If a diagnosis is made, the diagnosis rather than individual symptoms and signs is recorded. If symptoms and signs cannot be determined to be caused by the diagnosis at reporting, they are recorded as separate AEs/SAEs. If symptoms and signs are determined to be caused by the diagnosis, only the diagnosis is reported, including the symptoms and signs. AEs are removed from the symptoms and signs record, and SAEs are updated with follow-up reports.

2) AEs resulting from other events or clinical sequelae should trace back to the primary event, unless the secondary event is more severe or constitutes an SAE. Significantly clinical secondary events occurring at different times from the primary event should be recorded separately.

3) Recurrent AEs are those that had resolved but reoccurred between two assessment points and should be recorded upon each occurrence.

4) Symptoms/signs present during the trial screening period are recorded and reported as adverse events only if they worsen in severity, frequency, or nature after trial entry, excluding condition worsening under study.

5) Symptoms and signs resulting from disease progression are not reported as AEs or

SAEs unless the progression or symptoms change more severely than expected, or the researcher considers them related to the trial drug or procedure. Deaths should be reported as SAEs only if they result from an AE or if the cause of death is undetermined, reported as "death of unknown cause."

7.2.3 Assessment of Adverse Event Severity

CTCAE terms and grades: Researchers will use descriptions and grading scales from the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 5.0 for AE reporting.

For events without assigned CTCAE grades, severity levels ranging from mild to life-threatening, corresponding to Grades 1 through 5, are defined as follows:

- Grade 1, Mild: asymptomatic or mild symptoms; only clinical or diagnostic observations required; no intervention indicated.
- Grade 2, Moderate: minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).
- Grade 3, Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4, Life-threatening consequences; urgent intervention indicated.
- Grade 5, Death related to AE.

7.2.4 Assessment of Causality Between Adverse Events and the Investigational Product.

Researchers should assess the reasonable possibility between the adverse event and the investigational product. "Reasonable possibility" implies that there are facts, evidence, and/or arguments suggesting a causal relationship rather than the inability to exclude causality.

Researchers will use clinical judgment and refer to the product information of marketed products to determine causality. The determination of causality will take full account of other causes, such as underlying conditions, concomitant treatments, and other risk factors, as well as other events that are causally related to the study drug.

For each case of AE/SAE, Researchers had to document in the clinical record that they had reviewed the AE/SAE and made a causal assessment. In some cases, Researchers may not be able to provide sufficient additional information about SAEs that have occurred in the initial report, and Researchers are required to make a causal assessment of each event based on the available information. Researchers may update their view of the causal assessment as a result of subsequent follow-up information, but the SAE follow-up report needs to be supplemented with this updated causal assessment.

If researchers does not provide a causal assessment of an AE, including an SAE, the AE is considered to have a possible association with the trial drug that cannot be ruled out until the Researchers provides clarification.

The determination of whether an adverse event (AE) is attributed to the trial drug or other causes (e.g., natural history of the underlying disease, concomitant treatments) depends on the understanding of several aspects:

1) The known pharmacology of the trial drug.

2) The clinical and/or pathophysiological rationale.

3) Reactions observed in similar products in the past or reported in literature as related to the product.

4) The reasonableness of the temporal relationship between the event and drug administration or discontinuation.

AEs are categorized into five levels of association with the trial drug: definite, probable, possible, unlikely, and unrelated, with "definite, probable, possible" being considered related to the trial drug.

Criteria for determination include:

a. Definite – The AE *is clearly related* to the study treatment.

Evidence of drug use, reasonable temporal sequence, more plausible than other causes, positive dechallenge, positive rechallenge if feasible.

b. Probable – The AE *is likely related* to the study treatment.

Evidence of drug use, reasonable temporal sequence, more plausible than other causes, positive dechallenge.

c. Possible – The AE *may be related* to the study treatment.

Evidence of drug use, reasonable temporal association, can be explained by other causes.

d. Unlikely – The AE *is doubtfully related* to the study treatment.

Evidence of drug use, other causes may explain the AE better, negative or unclear dechallenge.

e. Unrelated – The AE *is clearly NOT related* to the study treatment.

No drug use, no temporal association with drug use, or another definite cause for the AE.

7.3 Adverse Reactions to Treatment Drugs

7.3.1 Chemotherapy Drug Adverse Reactions:

1) Hematological system: anemia, bone marrow suppression, thrombocytopenia, neutropenia and neutropenia with fever were reported in the clinical trials of this product. Neutropenia was dose-related and returned to normal. In a randomized controlled clinical trial involving patients with metastatic breast cancer, paclitaxel for injection (albumin-bound) was administered at a dose of 260mg/m^2 every 3 weeks; The dose of paclitaxel injection group was 175mg/m^2, once every 3 weeks.

The incidence of neutropenia less than 500/mm^2 (grade 4) was 9% in European and American patients. The incidence of grade 4 neutropenia was 7% in Chinese patients.

2) Allergic reactions: Allergic reactions have been reported in clinical trials of this product. In randomized controlled clinical trials conducted in European and American patients, grade 1 or 2 allergic reactions occurred on the day of administration of this drug, manifested as dyspnea (1% of the patients), skin flushing, hypotension, chest pain, and arrhythmia (<1% of the patients). In a randomized controlled clinical trial conducted in China, 3% of patients had grade 1 or 2 skin reactions on the day of administration, which were manifested as skin itching and rash. There are no data on the use of this product in patients with a history of allergy to paclitaxel or human serum albumin.

3) Cardiovascular system: arrhythmia, bradycardia, cardiac arrest, congestive heart failure, edema, left ventricular dysfunction, supraventricular tachycardia, tachycardia, flushing, hypotension, hypertension and atrioventricular block have been reported in clinical trials. In randomized controlled clinical trials of patients with metastatic breast cancer in Europe and the United States, 5% of patients had a decrease in blood pressure during the 30-minute dosing period, and <1% of patients had bradycardia. In Chinese patients, blood pressure decreased in 7% patients, and bradycardia occurred in less than 1% patients. These changes in vital signs are usually asymptomatic and require neither special management nor treatment discontinuation.

4) Respiratory system: cough, dyspnea, interstitial pneumonia, pleural effusion, pulmonary embolism, pulmonary thromboembolism, and radiation pneumonitis have been reported in clinical trials. In the randomized controlled clinical trials of metastatic breast cancer patients in Europe and the United States, 12% reported dyspnea, 7% reported cough, and less than 1% had pneumothorax. In a randomized controlled clinical trial of patients with metastatic breast cancer in China, cough or dyspnea occurred in 2% of patients after treatment.

5) Nervous system: Sensory neuropathy and sensory peripheral neuropathy have been reported in clinical trials. The frequency of sensory neuropathy was positively correlated with the cumulative dose administered. Discontinuation of treatment due to sensory neurotoxicity accounted for 3% (7/229) of all patients. Of the 24 patients (10%) with grade 3 peripheral sensory neurotoxicity, 14 had symptomatic improvement after 22 days（the median）, with 10 patients continuing treatment after dose reduction and 2 withdrawing from treatment. Of the 10 patients with no documented improvement, 4 discontinued treatments because of sensory neuropathy.

6) Gastrointestinal reactions: severe nausea and vomiting were the main limiting toxicities. Acute vomiting usually occurs 1 to 2 hours after administration and can last for about a week. Therefore, it is necessary to use this product with potent antiemetic agents to control acute vomiting, such as 5-hydroxytryptamine 3 (5-HT3), receptor antagonist antiemetic agent ondansetron, etc.

7) Nephrotoxicity: Cumulative and dose-related renal dysfunction are the main limiting toxicities of cisplatin. Generally, a dose of more than 90mg/m2 per day is a risk factor for nephrotoxicity,mainly renal tubular injury. Acute renal damage usually occurs 10-15 days after treatment. The increase of blood urea nitrogen (BUN) and creatinine (Cr) and the decrease of creatinine clearance rate are reversible in most cases, repeated high-dose therapy can cause persistent mild to moderate kidney damage at present, there is no effective means to prevent nephrotoxicity caused by this product except hydration.

7.3.2 Immune-mediated AE

Anti-PD-1 antibody can result in some immune-mediated adverse events probably due to T cell activation and proliferation. The immune-mediated AE is defined as an adverse event of unknown etiology associated with drug exposure and consistent with an immune phenomenon, and after ruling out neoplastic, infectious, metabolic, toxin or other etiologic causes.

Once the immune-mediated adverse reaction is noted, appropriate work-up should be performed, and steroid therapy may be considered if clinically necessary.

However, the patient must be informed that the clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from anti-PD-1 antibody. The skin rash/toxicity should be treatment as ESMO Clinical Practice Guidelines, 2017.

7.3.3 Manganese-related AE

Manganese is suggested as a bridge between the innate and adaptive immune reaction. The immune-mediated AE may be also observed after the manganese administration. Once the immune-mediated adverse reaction is noted, appropriate work-up should be performed, and the usage of Manganese Chloride should be discontinued. The overdose of manganese has been reported to cause a neurologic disorder, just like the Parkinsonian Syndrome. The blood concentration of Manganese must be monitored on time. Once the Manganese is overdosed and/or the neurologic AE appears, manganese chloride must be discontinued.

7.4 Management of Treatment-related Toxicity

1) Unacceptable toxicity requires study withdrawal and monitoring until resolution.
2) Administration will be interrupted and supportive care will be administered according to guidelines for ≥Grade 3 toxicities that believed to be drug-related
3) If the toxicity subsides or recovers to ≤ grade 2 within 21 days, treatment can be resumed with the test drug at the same dose.

7.5 Reporting of Serious Adverse Events (SAEs)

All initial and follow-up SAEs during the study, regardless of causality to the study drug, must be reported to the sponsor or designated person within 24 hours of discovery. SAEs post safe follow-up period won’t be reported unless deemed drug-related.

Any non-serious AE escalating to SAE criteria should be reported as a new SAE. If an SAE occurs, the investigator should complete the SAE report form as much detail as possible. Detailed SAE reports should include symptoms, severity, occurrence and treatment timing, actions taken, time of follow-up, outcomes, Source of the report, basic information about the subject, name of the test drug, name of the SAE, and drug causality, etc.

7.6 The report of deaths

All deaths occurring during the study or follow-up period must be reported. If death is directly due to disease progression, it should be informed to the study monitor at the next monitoring visit and recorded in the Case Report Form (CRF), but not reported as an SAE within the study; if death is not due to disease progression or the study disease, it should be reported as an SAE to the study monitor within 24 hours. The report should specify the primary cause of death and any related reasons.

7.7 Identification and Assessment of Risk Factors

By summarizing safety data from clinical trials, analyze the presentation and pattern of adverse reactions/events, such as time-dependency, demographic factors (age, gender, race, etc.), correlation with dose, drug concentration and administration method, and underlying diseases (e.g., renal impairment), to identify factors that might affect the frequency of adverse reactions/events. Clarify drug-related adverse reactions and adverse events that cannot be excluded from drug correlation to assess the causes of drug safety risks (high-risk factors and populations at high risk).

In safety evaluation, propose risk control/minimization measures based on all safety data from clinical trials, providing a basis for the drug's risk/benefit assessment. These measures usually include describing conditions for safe and effective use of the drug in the proposed labeling, with attention to clinically relevant information to minimize potential risks during post-marketing clinical use.

8.Research Processes

Before study, subjects must read and sign the latest version of the informed consent form approved by the Ethics Committee. The study steps required by the program shall be meticulously planned to ensure timely completion within the specified time frame, to the best of their ability. The investigator shall document the cause and promptly notify the research team sponsor of any unforeseen circumstances. Additional tests may be added if the investigator deems it necessary.

8.1 Screening (Day -21 to Day -1)

The screening evaluation should be completed within 28 days prior to study commencement, unless otherwise specified. The examination and evaluation conducted prior to the signing of the informed consent shall not be repeated within the specified time window, in order to safeguard the rights and interests of the subjects. 1. The screening period (-28 to -1 days) including the baseline period of -7 to -1 days, requires completion of the following study procedures to ensure patient eligibility for inclusion in the study.

1) Informed consent, signed informed consent and dated;

   The signing of a single research ICF will precede any research program.

2) Review the inclusion/exclusion criteria: If the screening period exceeds 7 days from the initial dosing, a second assessment must be conducted at baseline

3) Demographic information should be collected, including the subject's full name, gender, age (date of birth), ethnicity, and other relevant details.

Table 4 Schedule of Events

Cycle/Visit:	Screening[1]	Cycle 1	Subsequent Cycles	EOT[3]	Safety Follow-up	Follow-up Assessments (every 90 ± 14 day) via telephone
Day: **Procedure:**	**-21 to -1**					
Informed consent[2]	x					
Inclusion/exclusion criteria review	x					
Demographics	x					
Medical, surgical, cancer, and medication history	x					
Archival tissue	x					
Physical examination	x					

Vital signs	x					
ECOG	x					
EORTC QLQ-OV28	x					
Optional serial tumor biopsy[4]	x					
Blood sample for exploratory biomarkers	x					
CBC	x					
Serum chemistry	x					
Coagulation	x					
Serum CA-125	x					
TSH, T3 or FT3, and FT4	x					
Urinalysis	x					

Cytokine	x					
Blood manganese concentration	x					
ECG	x					
Tumor assessment (RECIST)[5]	x					
Concomitant medications	x					
Adverse event monitoring	x					
nab-paclitaxel	x					
cisplatin	x					
anti-PD-1 antibody	x					
Manganese chloride	x					

Concomitant medications	x					
Survival state	x					

Abbreviations: AE = adverse event; CBC = complete blood count; CT = computed tomography; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; MRI = magnetic resonance imaging; RECIST = Response Evaluation Criteria in Solid Tumors;

Note:

1. Screening period:Screening period, from day -28 to day -1, after the subject's voluntarily signed written informed consent, screening shall be conducted to confirm whether the subject meets the test eligibility (baseline period, day -7 to day -1, if all the evaluation time of the screening period is within 7 days before the first dose, all the evaluation content of the baseline period need not be repeated, if more than 7 days,Some assessments should be re-evaluated at baseline, including entry review, physical examination, vital signs, ECOG score, blood routine, urine routine, blood biochemistry, coagulation function, 12-lead electrocardiogram;Blood pregnancy test within 72 hours before first dosing and weight measurement within 24 hours).

2. Informed consent must be obtained before entering the screening period of clinical trials.

3. EOT visit should be completed within 7 days of the last dose of study drug.

4. In patients who consent to serial biopsies, fresh tumor sample is to be obtained at screening, 1 to 3 days before or on C3D1 prior, and when possible, at the time of disease progression. See the Study Manual for details on sample collection and processing. The serial biopsies at different time points should be on the same lesion preferably. A core biopsy is recommended; if an excisional or incisional biopsy is to be performed, it must be conducted on a non-target lesion. If a patient has had a biopsy within 12 weeks prior to entering screening, that biopsy may be accepted as the screening biopsy.

5. Tumor assessment per RECIST via CT or MRI (chest, abdomen, and pelvis [brain, only if clinically indicated]) required at screening, every 6 weeks (42 ±7 days) until progression; at the time of progression, a final follow up

set of images is required if not done within the last 4 weeks. The same modality (CT or MRI) should be used throughout the study for a given patient. If the chest or brain CT/MRI is clear at screening, repeat imaging of these areas is not required in the absence of clinical indication requiring follow-up. Positron emission tomography/CT may be used according to RECIST v1.1 guidelines. Bone scans should be conducted per standard of care. Timing of images will not be delayed for treatment interruptions, and tumor assessment should occur according to this schedule regardless of whether study treatment is interrupted. If a patient discontinues treatment for a reason other than progression or death, withdrawal of consent, or loss to follow-up, scans and CA-125 testing should continue at the specified intervals.

4) Collect medical history, treatment history, allergy history and personal history: previous medical history, present medical history, allergy history, drug abuse history, smoking history, alcohol history, surgical history, menstrual history, recent and current drug use history, etc. The medical history will include all active conditions and any conditions diagnosed within the last 10 years that the investigator deems clinically significant. Collect and study tumor history and related treatment history, including disease stage, pathological diagnosis, diagnosis date and previous treatment history, treatment for initial diagnosis, chemotherapy, radiotherapy, surgical treatment, molecular targeted drugs, checkpoint inhibitor therapy, participation in other clinical studies and other anti-tumor therapy. Previous detection results of molecular markers, etc. (Copies of outpatient medical records, discharge summaries, pathology reports, etc., must be signed by the investigator).

5) Physical examination, including measurement of height and weight, is required. If the screening period exceeds 7 days from the initial dosing, a second examination should be conducted at baseline with weight measured within 24 hours prior to the first dosing.

6) Vital signs: If the screening period exceeds 7 days prior to the initial dosing, a second baseline examination is mandatory.

7) ECOG score: If the screening period is more than 7 days after the first dosing, a second score will be performed at baseline.

8) Optional tumor biopsy for biomarker testing

For patients who are available for biopsy and agree for sequential biopsies, baseline tumor biopsies are retained. Refer to the study manual for detailed information on sample collection and processing.

9) Collect Samples (whole blood) for blood manganese and cytokine analysis

10) Tumor assessment (CT/MRI) for determination of measurable disease (RECIST

v1.1)

For chest examination, the preferred imaging modality for screening is CT examination. For abdominal and pelvic (brain, if clinically necessary) imaging, MRI is the preferred method; however, CT can be used if MRI is not feasible. If no abnormalities are detected in the chest and/or brain MRI/CT during screening, repeat examinations should only be performed when there are clinical indications that necessitate follow-up. Positron emission tomography (PET)/CT can be utilized in accordance with RECIST v1.1 guidelines.

11) Bone scan should be performed according to clinical indications (when bone metastasis is suspected).

12) Laboratory examination

-Blood routine

- Urine routine

- Bowel routine

- Serum biochemistry: liver and kidney function, electrolytes

- Blood clotting

- Thyroid function (TSH, T3 or FT3, and FT4)

- Tumor markers (CA125 must be checked)

- Blood manganese levels

- Interleukin (IL-1/6/8/10, CRP, TNF-a)

13) 12-lead electrocardiogram: If the screening period is more than 7 days from the first dosing, a second examination is required at baseline

14) Virological examination: Including hepatitis B surface antigen (HBsAg), hepatitis

B surface antibody (anti-HBS), Hepatitis Be antigen (HBeAg), Hepatitis Be antibody (anti-HBE), and Hepatitis B core antibody (anti-HBC) (Hepatitis B DNA was selected if necessary according to the five results of hepatitis B and related medical history), hepatitis C antibody (hepatitis C RNA was selected if necessary according to the results of hepatitis C antibody and related medical history) and HIV and treponema pallidum antibody detection;

15) Quantitative evaluation of EORTC QLQ-OV28: within 7 days before first dosing

16) Record adverse events (if any) resulting from the study procedure

17) Record combined drug administration/non-drug treatment

8.2 Administration period

Treatment cycle: Manganese chloride or placebo was nebulized inhaled at 0.4mg/kg/d once daily in a 3-week cycle. The immunochemotherapy consisted of nab-paclitaxel (200 mg/m^2, administered intravenously on day 1 in a 3-week cycle), cisplatin (60 mg/m^2, administered intravenously on day 1-2 in a 3-week cycle) and anti-PD-1 antibody (200 mg, administered intravenously on day 3 in a 3-week cycle). Sustain dosing until disease progression, use of antitumor therapy other than the protocol, toxicity intolerance, withdrawal of informed consent, occurrence of other reasons for discontinuation of study therapy or subject death/loss of follow-up, or treatment duration reaching 24 months, whichever occurs first.

Enrollment: Subjects were not permitted to enter the treatment group until their eligibility for enrollment was confirmed and treatment assignment was randomized. The enrollment date was defined as the completion of randomization and acquisition of randomization tables.

The following assessments should be completed before each cycle of dosing

1) Informed consent for treatment, sign informed consent and date

2) Second review of the inclusion/exclusion criteria.

3) Vital signs: Complete before each cycle of dosing

4) Weight: Weight was measured within 24 hours prior to each dosing cycle

5) ECOG scoring

6) Laboratory examination

-Blood routine

- Urine routine

- Bowel routine

- Serum biochemistry: liver and kidney function, electrolytes

- Blood clotting

- Thyroid function (TSH, T3 or FT3, and FT4)

- Tumor markers (CA125 must be checked)

- Blood manganese levels

- Interleukin (IL-1/6/8/10, CRP, TNF-a)

7) 12-lead electrocardiogram

8) Quantitative evaluation of EORTC QLQ-OV28

9) Record adverse events (if any) resulting from the study procedure

10) Peripheral blood of C1D1 and C3D1 was collected for single cell sequencing analysis

11) The tumor tissue will be punctured and fresh tumor specimens will be obtained during the screening period or C1D1. Subsequently, on C3D1, another puncture of the tumor tissue will be performed to collect additional fresh tumor specimens.

Imaging and tumor assessment: The tumor should undergo imaging evaluation every 6 weeks following the initial administration of the drug. MRI/CT scans should be scheduled on specified calendar days throughout the study for the prescribed imaging tests, ensuring that any delay in treatment dosing does not impede timely evaluation

PK blood collection and detection of peripheral immune cell changes: PK and detection of immune cell samples were collected according to the sampling schedule (Table 2). The procedure is detailed in the laboratory manual

8.3 End of Treatment/Early Withdrawal/Unplanned Visits

End-of-treatment visits are required for end-of-treatment or early withdrawal, and end-of-treatment visits are required within 7 days of confirmation of treatment completion. For safety reasons, the investigator may increase the number of visits to the subject, i.e., unplanned visits, as needed during the trial if the subject has an adverse event or abnormal laboratory test, or if the subject is unwell in any way. The investigator should document each unplanned visit for the subject in the unplanned visit section of the original medical record and eCRF, etc.

End of treatment, early withdrawal, or unplanned visits may include, but are not limited to:

1) Vital signs
2) Physical examination
3) ECOG score
4) Laboratory examination

-Blood routine

- Urine routine

- Bowel routine

- Serum biochemistry: liver and kidney function, electrolytes

- Blood clotting

- Thyroid function (TSH, T3 or FT3, and FT4)

- Tumor markers (CA125 must be checked)

- Blood manganese levels

- Interleukin (IL-1/6/8/10, CRP, TNF-a)

5) 12-lead electrocardiogram

6) Quantitative evaluation of EORTC QLQ-OV28

7) Imaging and tumor assessment:

   - If not performed within the last 4 weeks, a final set of imaging images will be required at the time of disease progression.

   - If a patient discontinues treatment for reasons other than progression or death, withdrawal of consent, or loss to follow-up, imaging and CA- 125 testing should be performed every 9 weeks (63 ± 7 days) up to 1 year, and continuously every 12 weeks (84 ± 7 days) thereafter.

8) Record adverse events

9) Record combined drug administration/non-drug treatment

Note: Unscheduled visits are at the discretion of the investigator based on the subject's condition.

8.4 Security follow-up

Post-discontinuation safety follow-up was performed 30 (±7) days after the last dose or before initiation of new antitumor therapy, whichever occurred first, with the following examinations:

1) Vital Signs;

2) Physical Examination;

3) ECOG Rating;

4) Laboratory tests:

- blood routine
- urine routine
- Stool routine
- Serum biochemistry: liver and kidney function, electrolytes
- Blood clotting
- Thyroid function (TSH, T3 or FT3, and FT4)
- Tumor markers (CA125 must be checked)
- Blood manganese levels
- Interleukin (IL-1/6/8/10, CRP, TNF-α)

5) 12-lead electrocardiogram

6) Quantitative evaluation of EORTC QLQ-OV28

7) Imaging and tumor assessment:

8) Record adverse events

9) record combined drug administration/non-drug treatment

If the safety follow-up is less than 7 days from the end-of-treatment visit, the end-of-treatment visit may be substituted for the safety follow-up and does not need to be repeated, but the items required for the safety follow-up need to be supplemented.

8.5 Survival follow-up

Progression-free survival (PFS) and overall survival (OS) will continue to be followed up after safety follow-up.

PFS follow-up: PFS follow-up is required for subjects who discontinue treatment without disease progression. After safety follow-up, subjects will undergo imaging assessments to evaluate PFS every 6 weeks (every 12 weeks after 48 weeks of initial dosing) until the disease progression, receives new antitumor therapy, loses the visit, or dies. The time of each follow-up visit, the results of the imaging evaluation, and other antitumor therapy information need to be documented.

OS follow-up: OS follow-up is required for all subjects treated. After safety follow-up, subjects may be followed up by telephone contact for survival every 12 weeks (±14 days) to evaluate the OS until the subject dies, is lost to follow-up, or is no longer collected by the sponsor (whichever occurs earliest). The time of each follow-up visit and follow-up information needs to be recorded.

AE monitoring: SAEs and ECIs need to be captured within 90 days of cessation of study treatment (or at least 30 days post-treatment if the patient is starting an alternative anti-cancer therapy)

If a patient starts an alternative anticancer treatment, information on subsequent anticancer treatments will need to be obtained through post-treatment.

8.6 Unplanned assessments

For any patient diagnosed with MDS/AML during the study period, perform a bone marrow aspirate, biopsy and sample collection (whole blood) for cytogenetic analysis.

9. Statistical Analysis

9.1 Statistical Hypotheses

Null hypothesis: test group ORR ≤ control group ORR.

Alternative hypothesis: test group ORR > control group ORR.

The null hypothesis will be tested at a one-sided 0.025 level.

9.2 Sample Size Estimation

Primary endpoint is ORR per Investigator assessment. The study is designed to test the null hypothesis that the objective response is the same between the manganese plus immunochemotherapy arm and the immunochemotherapy alone arm vs. the alternative hypothesis that manganese promotes the objective response. The reference ORR was calculated based on the prior clinical trials with ICB plus chemotherapy in platinum resistant ovarian cancer, including the JAVELIN 200 study with a 13.3% ORR in ICB plus chemotherapy group. Approximately 45 patients will be randomized 2:1 (approximately 30 and 15 patients in the Mn^{2+} and placebo group, respectively) to provide a 80% power at the 5% level of statistical significance.

9.3 Analysis Populations

The statistical analysis populations in this study will include:

- Full Analysis Set (FAS): Determined by the intention-to-treat (ITT) principle, consisting of all participants randomized into the study.

- Per-Protocol Set (PPS): A subset of the FAS, including all participants randomized, completed treatment according to the protocol, and with no significant protocol deviations. Analyses based on the PPS will supplement those based on the FAS. The PPS must be determined by the project medical team and the statistical team before the final analysis database is locked. All protocol deviations, including those judged to significantly affect study outcomes, will be listed in the report.

- Safety Set (SS): All randomized subjects who received at least one dose of the study drug and underwent at least one post-baseline safety assessment.

- Pharmacokinetic Analysis Set (PKAS): Includes all subjects who received the study drug, with no significant protocol deviations affecting PK evaluation, and who have evaluable PK data.

9.4 Statistical Methods

9.4.1 General Methods

Descriptive statistics will be summarized or tabulated for all variables obtained at each observation time point, unless the program or Statistical Analysis Plan (SAP) determines that statistical descriptions are not needed at a particular time point. In general, continuous variables (e.g., age) will be statistically described using the number of observations, mean, standard deviation, median, minimum, and maximum values; categorical variables will be statistically described using frequency counts of each category and their percentages.

The final analysis of the study will be based on data collected throughout the study period and as described in the following analyses. Specific statistical analysis descriptions will be detailed in the statistical analysis plan.

9.4.2 Efficacy Analysis

All the ITT subjects who have undergone randomization regardless of whether assigned treatment received will be accepted as the efficacy-evaluable population.

For continuous variables such as the number of patients, mean, standard deviation, median, minimum, and maximum values in clinical study protocols, statistical analysis will be performed using two-sided 95% confidence intervals (CIs) based on the Clopper-Pearson method. Time-to-event analyses will utilize the Kaplan-Meier (KM) method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model. Comparisons between two groups of Laboratories tests were performed by paired *t* test.

9.4.2.1 Primary Efficacy Parameter

The primary study endpoint is the Objective Response Rate (ORR), defined as the proportion of subjects achieving Complete Response (CR) or Partial Response (PR) as assessed by RECIST V1.1 criteria, along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified， Cochran-Mantel-Haenszel test.

9.4.2.2 Secondary Efficacy Parameter(s)

The Disease Control Rate (DCR) will be assessed as a secondary study endpoint, defined as the proportion of subjects achieving CR, PR, or Stable Disease (SD) as assessed by RECIST V1.1 criteria, along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified Cochran-Mantel-Haenszel test.

Progression-free survival, overall survival and duration of response were estimated using the Kaplan-Meier method, with 95% CIs were calculated with

calculated with the Brookmeyer-Crowley method, and were compared between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

9.4.2.3 CA125

Proportion of subjects with optimal clinical benefit as assessed by Gynecological Cancer Inter Group CA-125 Response rate criteria,along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified Cochran-Mantel-Haenszel test。

9.4.2.4 Biomarker Analysis

For each patient in the study, peripheral blood specimens will be prospectively collected; as well as for some patients for whom tumor tissue puncture is available, tumor tissue samples will be retained, evaluated and archived to support exploratory biomarker analysis.

The comparison of Mn2+ concentrations in peripheral blood, serum cytokines and chemokines between the experimental and control groups will be conducted using paired *t*-tests.

The same method will be used to analyze the correlation of PD-L1 expression (retrospective analysis), BRCA gene mutations, HRD scores, immune cell infiltration, and other exploratory biomarkers with clinical response.

9.4.2.5 EORTC QLQ-OV28 Analysis

Changes in EORTC QLQ-OV28 (European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Ovarian Cancer Module 28) questionnaire scores will be summarized through descriptive statistics, summarizing the outcome changes from baseline to the last safety follow-up for each treatment group in the FAS. Completion rates for each questionnaire at planned time points will be

summarized. Assessments will be conducted among patients both at baseline and post-baseline.

9.4.3 Safety Analysis

Unless specified otherwise, the following key safety parameters will be assessed in accordance with the study plan and overall:

1) The incidence of TEAEs during the first cycle compared to the second and subsequent cycles;

2) The incidence of TEAE in patients during treatment or within 30 days of the last dose of study drug;

3) The incidence of SAEs and ECIs in patients during treatment or within 90 days after the last dose of study medication;

4) The incidence of any new malignant tumor;

5) Changes in clinical laboratory parameters (hematology, biochemistry, thyroid function, coagulation, urinalysis), vital signs, ECOG scores, electrocardiogram parameters, physical examinations, and concomitant medication use.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system, categorized by System Organ Class (SOC) and Preferred Term (PT), and graded according to CTCAE v5.0.

Adverse event analysis is defined as the analysis of any treatment-related events occurring during the study, including 1) the entire treatment period from the first dose of study medication to 30 days after the last dose; 2) any SAE or ECI occurring within 90 days after cessation of study treatment (or at least 30 days if the patient begins alternative cancer treatment); 3) any event present at baseline that worsens in intensity during treatment or by the end of the study and is considered drug-related.

The number and percentage of patients experiencing treatment-related TEAEs

(definite, probable, or possible relationship) and those experiencing SAEs will be summarized by treatment group and overall. In these tables, each patient will be counted once for the incidence rate in descriptive analysis (i.e., the most relevant or most severe). Furthermore, the severity of TEAEs will also be summarized by SOC and PT. No formal hypothesis testing will be analyzed for AE incidence rates.

All adverse events occurring in the study will be listed in the patient data listings. Detailed records of patient deaths, SAEs and adverse events leading to withdrawal will also be recorded.

9.4.4 Pharmacokinetic Analysis

Patients who have received at least one cycle of the study drug and have measurable drug concentrations will be included in the PK analysis. The number of cases, mean, standard deviation, median, minimum, maximum, coefficient of variation, geometric mean, and the coefficient of variation of the geometric mean will also be reported.

9.4.5 Baseline Descriptive Statistics

Descriptive statistical analysis methods will be used to summarize the demographic and baseline characteristics of the subjects, including age, gender, ethnicity, height, weight, Body Mass Index (BMI), and prior treatments. This analysis will be based on the full analysis set.

9.4.6 Interim Analysis

To minimize the risk of exposing patients to ineffective treatment, an assessment of the benefit comparison between the trial and control groups will be conducted during the study, a formal interim analysis of Overall Survival (OS) is planned when a pre-specified number of ORR events has been reached for the final ORR analysis.

10. Quality Management

10.1 Quality Control

10.1.1 Qualifications of Research Units and Researchers

Research units must have the qualifications for conducting clinical drug trials, and the facilities and conditions of the department hosting the project should meet the requirements for safely and effectively conducting clinical trials. Researchers must possess the professional expertise, qualifications, and ability to undertake this clinical trial and must have undergone training in Good Clinical Practice (GCP) regulations and this specific protocol. Before the trial begins, the principal investigator at the center should organize protocol training for the researchers, and only those who have passed the training can participate in the trial. The researchers involved in this trial should be relatively fixed, and any replacement researchers should join after training and the Clinical Trial Participant Authorization Form should be updated in a timely manner.

Researchers are required to prepare and keep all observation results for each study participant. These observations must be recorded fully and accurately, including other data related to the clinical study. Researchers will also continuously perform all appropriate safety assessments.

10.2 Ensuring Compliance

1) Participant Compliance: The informed consent process and the signing of the consent form are crucial for ensuring participant compliance. Researchers must thoroughly explain the informed consent, ensuring participants fully understand the trial's details, processes, and their rights and obligations, such as adhering to medication schedules, follow-up appointments, and completing necessary clinical observations and tests.

2) Researcher Compliance: Researchers must strictly follow the trial protocol and

relevant regulations. Their compliance is manifested in three main aspects: selecting qualified participants, controlling influencing factors, and observing and evaluating efficacy indicators, the specific contents are as follows：

a) strictly adhere to the trial protocol and regulations without significant deviations;

b) Selection of eligible subjects to participate in the trial and signing of the informed consent form;

c) Subjects were enrolled in strict adherence to the order specified in the protocol, and experimental observations were made and recorded;

d) The administration of reasonable treatment to the subject, and that all effects of its contributing factors (including test drugs, co-administration) and other measures can be measured, evaluated and judged by objective, credible, scientific and practicable criteria;

e) Adverse events, especially serious adverse events, are handled, recorded and reported in a timely manner;

f) Strict adherence to Standard Operating Procedures (SOPs) for pilot studies.

g) Data management and statistical processing are in strict compliance with standard operating procedures.

10.3 Data Collection and Management Responsibilities

Research data will be entered into the eCRF by researchers or authorized personnel at the research center. Before initiating the center or entering data, researchers and authorized personnel will be properly trained and appropriate security measures taken.

The eCRF should be completed as soon as possible during or after the visit period and updated regularly to reflect the latest status of participants. To minimize assessment discrepancies, it's vital to ensure the same individual completes all baseline and

subsequent efficacy and safety evaluations for each participant. Researchers must review the data to ensure accuracy and correctness of all information entered into the eCRF. If certain assessments weren't conducted or some information is unavailable, not applicable, or unknown, it should be recorded in the eCRF. Researchers are responsible for electronically signing verified data.

CRA will review the eCRF for completeness and consistency, comparing it with original documents to ensure key data consistency. All data entries, corrections, and modifications are the responsibility of the researcher or designated personnel, with CRA having no data entry authority. Data submitted to the data server in the eCRF and any changes made will be logged in the audit trail, recording the reason for changes, the operator's name, and the time and date of modification. Roles and permissions for research center personnel responsible for data entry will be predetermined.

Unless specified otherwise, the eCRF will only serve as a data collection form, not as original documentation. Original documents, which prove the existence of the participant, eligibility criteria, and all records of participation in the study, including laboratory records, ECG results, memos, pharmacy dispensing records, participant folders, etc., are maintained by the researcher or hospital.

Researchers are responsible for maintaining all original documents, regardless of the duration of a participant's involvement in the study, and must submit a complete eCRF for each participant.

AE and concomitant diseases/history will be coded using MedDRA, and concomitant/previous medication using WHO-Drug.

10.4 Archiving Study Documents

Essential clinical documents must be preserved to demonstrate the study's validity and the integrity of the collected data. The master file should be established at the beginning of the study, maintained throughout, and retained according to appropriate

regulations. Documents in clinical trials (protocols and amendments, completed eCRFs, signed ICFs, etc.) must be stored and managed following GCP requirements. Research centers should keep these documents for 5 years after the study ends. The sponsor should retain clinical trial materials for 5 years after the drug is approved for marketing.

Research documents should be stored securely to allow future access or data tracing, considering safety and environmental risks.

Without written permission from the sponsor and researcher, no study documents may be destroyed. Only after notifying the sponsor and obtaining written consent can study documents be transferred to a third party that meets document retention requirements or moved to a compliant third party.

10.5 Clinical Trial Standards

The "Good Clinical Practice (GCP)" and the "Declaration of Helsinki" serve as the legal backbone ensuring the scientific integrity and ethical principles of this clinical study and serve as the legal and regulatory guidelines for this clinical research. Any person involved in the trial, especially those appointed by the sponsor for monitoring and auditing, has the right to correct actions deviating from GCP and the Declaration of Helsinki. This is especially true when participant rights and protections are at stake, authorizing the termination of the clinical trial if necessary.

10.6 Informed Consent

Before the clinical trial begins, researchers must fully inform participants about the trial's nature, objectives, potential benefits and risks, alternative treatments, and the participant's rights and obligations. This ensures participants understand and agree to participate, evidenced by signing the "Informed Consent Form". This form is a crucial document for ensuring participant safety, rights, and health throughout the trial. Informed consent is one of the trial documents that ensures that the safety, rights and

health of subjects are safeguarded and protected during the trial, any clinical trial is conducted in a way that does not jeopardize the health and rights of the subjects.

The informed consent form must be signed and dated; a copy will be given to the patient and the investigator will retain a copy as part of the clinical study record. Written consent must be obtained before any research activities begin, and all consent procedures and timings must be documented.

If protocol changes are needed, then the ICF may need to be revised to reflect the changes in the protocol and re-signed by all subsequent enrollees in the clinical study, as well as by all current enrollees in the clinical study.

10.7 Ethics Committee and Trial Document Review

Like the informed consent form, the ethics committee is vital for protecting participant rights. It reviews clinical trial documents and oversees compliance with the protocol, participant rights protection, especially in handling, tracking, and following up on serious adverse events, ensuring the trial is conducted under scientific and ethical principles. Prior to the start of the trial, this protocol should be discussed and revised by the study leader and submitted to the ethics committee of the research center for written approval, and the investigator should use a copy of the ethical approval as the basis for initiating and implementing the trial.

10.8 Establishing Comprehensive Center SMP and SOP

Research entities should establish comprehensive regulations and standard management procedures (SMP) to enhance GCP and regulatory training for researchers, ensuring smooth trial conduct. Standard operating procedures (SOPs) for experimental studies should be established and improved, and implemented and perfected in actual experimental studies, so that the entire experimental study is proceduralized, institutionalized, standardized and formatted.

11. Supporting Documents and Operational Considerations

11.1 Ethics

11.1.1 Approval of Trial Documents by Ethics Committee Before Research Begins

Prior to the start of the trial, this protocol needs to be discussed and revised by the study leader of the research center and submitted to the ethics committee of the research center for written approval, and the center should use the ethical approval document as the basis for initiating and implementing this trial, and the documents submitted to the ethics committee include:

1) A copy of the drug clinical trial approval;
2) The revised clinical trial protocol was discussed by the center's research leaders;
3) informed consent forms;
4) case report forms;
5) investigator's brochure;
6) A copy of the quality control report of the test drug;
7) other required documents by the ethics committee.

11.1.2 Obtaining Informed Consent from Participants Before Research Begins

Before participant screening, researchers must explain the clinical study in detail, ensuring participants understand the trial and their rights and obligations. Informed consent must be voluntarily signed by the participant or their legal guardian.

11.1.3 Effective Handling and Follow-up of Any Adverse Events (AEs) During the Study

Any adverse event that occurs during the trial will allow the investigator to decide

whether to terminate treatment based on the condition. Any adverse events occurring, the investigator will immediately administer the correct treatment or rescue therapy to protect the safety of the subject.

All AEs are to be promptly addressed to ensure participant safety and followed up until resolved or stabilized. Follow-up visits may take the form of inpatient, outpatient, home visit, telephone, or correspondence, depending on the severity of the adverse event, the proximity of the subject's residence to the hospital, and the medical expertise of the center, respectively.

11.1.4 Study Outcomes Favor Participants Over Risks

This protocol follows the Declaration of Helsinki Ethical Guidelines for Human Medical Research and complies with the ethical guidelines for human medical research to benefit all subjects as much as possible.

Although there is no guarantee that the results of this trial will be as expected, subjects participating in this trial may experience improvement in their condition.

11.2 Publication

Information on the publication of study results is included in the clinical trial protocol.

11.3 Research Committee

A research committee will be established to review and assess study data continuously, safeguarding the interests and safety of participants. Detailed information on membership, main responsibilities and corresponding procedures are recorded in the Research Committee Statutes.

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Appendix 1. ECOG performance status scoring criteria

Level	Performance status
0	Fully active, capable of all pre-disease performance without restriction
1	Ambulatory and capable of light or sedentary work (light house work or office work) but restricted in physically strenuous activity
2	Ambulatory and capable of all self-care but incapable of any work activities; up and about more than 50% of waking hours
3	Capable of limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely bedridden, incapable of any self-care
5	Dead

Appendix 2. 2014 FIGO staging for ovarian cancer, fallopian tube cancer, and peritoneal cancer

I		Tumor confined to the ovaries or fallopian tube(s)
	IA	Tumor limited to 1 ovary (capsule intact) or fallopian tube, no tumor on the ovarian or fallopian tube surface, and no malignant cells in the ascites or peritoneal washings
	IB	Tumor limited to both ovaries (capsule intact) or fallopian tubes, no tumor on the ovarian or fallopian tube surface, and no malignant cells in the ascites or peritoneal washings
	IC	Tumor limited to one or both ovaries or fallopian tubes, with any of the following: IC1 Surgical spill intraoperatively; IC2 Capsule ruptured before surgery or tumor on the ovarian or fallopian tube surface; or IC3 Malignant cells in the ascites or peritoneal washings
II		Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below the pelvic brim) or peritoneal cancer (Tp)
	IIA	Extension and/or implants on the uterus and/or fallopian tubes and/or ovaries
	IIB	Extension to other pelvic intraperitoneal tissues
III		Tumor involves 1 or both ovaries or fallopian tubes or primary peritoneal cancer with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and (or) metastasis to the retroperitoneal lymph nodes Metastasis to retroperitoneal lymph nodes with or without microscopic peritoneal involvement beyond the pelvis
	IIIA	IIIA1 Positive retroperitoneal lymph node only (cytologically or histologically confirmed) IIIA1(i) Metastasis ≤ 10 mm in greatest dimension (note: this is the tumor dimension and not the lymph node dimension) IIIA1(ii) Metastasis >10 mm in the greatest dimension IIIA2 Microscopic extrapelvic (above the pelvic brim), peritoneal involvement with or without positive retroperitoneal lymph nodes
	IIIB	Macroscopic peritoneal metastasis beyond the pelvic brim ≤ 2 cm in greatest dimension, with or without positive retroperitoneal lymph nodes
	IIIC	Macroscopic peritoneal metastasis beyond the pelvic brim > 2 cm in greatest dimension, with or without positive retroperitoneal lymph nodes (Note 1)
IV		Distant metastasis excluding peritoneal metastasis
	IVA	Pleural effusion with positive cytology
	IVB	Metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of abdominal cavity) (Note 2)

Note 1: Includes extension of the tumor to the capsule of the liver and spleen without parenchymal involvement of either organ.

Note 2: Parenchymal metastases are stage IVB.

Appendix 3. Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria

1. The measurability of the tumor at the baseline level

1.1 Definition:

At the baseline level, tumors/lymph nodes are classified as measurable and unmeasurable based on the following definitions.

1.1.1 Measurable lesions

Tumors: For measurable lesions, there must be at least 1 diameter that can be accurately measured (recorded as the longest diameter). The longest diameter should be **≥ 10 mm on CT (recommended CT slice thickness is ≤ 5 mm),** ≥10 mm on clinical examination using conventional instruments (tumors that cannot be accurately measured by conventional calliper devices will be recorded as unmeasurable), and ≥ 20 mm on chest X-ray. **Malignant lymph nodes are pathologically enlarged and measurable, and the short-axis diameter (SAD) of a single lymph node should be**

≥ 15 mm on CT (recommended CT slice thickness is ≤ 5 mm). At baseline and follow-up, only the SAD will be measured and followed up.

1.1.2 Unmeasurable lesions

All other lesions include small lesions (longest diameter <10 mm or pathological lymph node SAD ≥ 10 mm to < 15 mm) and unmeasurable lesions. Unmeasurable lesions include meningeal disease, ascites, pleural or pericardial effusion, inflammatory breast cancer, cutaneous/pulmonary lymphangitis carcinomatosis, abdominal mass that cannot be diagnosed and followed up by imaging, and cystic lesions.

1.1.3 Special concerns for lesion measurement

Bone lesions, cystic lesions, and lesions that have received local treatment will be noted:

- Bone lesions:

- Bone scans, positron emission tomography (PET) scans or plain films are not suitable for measuring bone lesions but will be used to confirm the presence or disappearance of bone

lesions.If osteolytic lesions or mixed osteolytic/osteogenic lesions have a definite soft tissue composition that meets the above definition of measurability and are evaluable using tomographic imaging techniques such as CT or MRI, then these

lesions will be considered measurable.

- Osteogenic lesions are unmeasurable lesions.

➢ Cystic lesions:

- Lesions that meet the definition criteria for simple cysts on radiography will not be considered malignant just because they are simple cysts by definition. They are neither measurable lesions nor unmeasurable lesions.
- If a cystic metastatic lesion meets the definition criteria for measurability, it can be regarded as measurable. However, if cystic and noncystic lesions simultaneously present in the same patient, noncystic lesions will be preferentially

selected as target lesions.

Locally treated lesions:

- Lesions located at sites that have received radiotherapy or other local treatments will generally be regarded as unmeasurable lesions unless there is clear progression of the lesion. The study protocol will elaborate the conditions under

which these lesions are measurable.

1.2 Measurement method description

1.2.1 Measurement of lesions

During clinical evaluations, all tumor measurements will be recorded in the record system. All baseline evaluations of tumor size will be completed before and as close as possible to the start of treatment and **must be completed within 28 days (4 weeks) before the start of treatment.**

1.2.2 Evaluation methods

The same techniques and methods will be used for baseline evaluations and subsequent lesion measurements. Except for lesions that cannot be evaluated by imaging but can only be evaluated by clinical examination, all lesions will be evaluated by imaging.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and have a diameter ≥ 10 mm at the time of measurement (such as skin nodules). For patients with skin lesions,

it is recommended to archive colour photographs containing scales to measure lesion sizes. When the lesions are evaluable using both imaging and clinical examinations, imaging will be selected whenever possible because imaging is more objective and can be reviewed repeatedly after the end of the study.

Chest X-ray: When tumor progression is set as a primary endpoint, chest CT is preferred because CT is more sensitive than X-ray, especially for new lesions. Chest X- ray is only applicable when the measured lesion has a clear boundary and the lungs are well ventilated.

CT and MRI: CT is currently the best reproducible method for efficacy evaluations. The measurability in this guideline is defined based on a CT slice thickness ≤ 5 mm. If the CT slice thickness is >5 mm, the minimum measurable lesion will be 2 times the slice thickness. MRI is also acceptable in some cases (e.g., whole-body scan).

Ultrasound: Ultrasound will not be used to measure lesion size. Due to its operational dependence, ultrasound is not repeatable and technical and operational homogeneity cannot be guaranteed among different measurements. New lesions detected by ultrasound during the treatment period will be confirmed by CT or MRI. If CT radiation exposure is a concern, MRI will be used instead.

Endoscopy and laparoscopy: These techniques are not recommended for the objective evaluation of tumors. However, they will be used to obtain biopsy specimens for CR confirmation or for confirmation of recurrence after CR or in surgical resection. Tumor markers: Tumor markers alone will not be used to evaluate objective tumor response. However, if the baseline level of a tumor marker exceeds the ULN, it will not be used to evaluate CR unless its level returns to normal. The variation in tumor markers in different diseases needs to be taken into account when formulating the measurement criteria into the protocol. Specific criteria for CA-125 (recurrent ovarian cancer) and prostate-specific antigen (recurrent prostate cancer) responses have been published. In addition, the Gynecologic Cancer Intergroup (GCIG) has developed CA-125 progression criteria, which will soon be added to the objective tumor evaluation criteria of the first-line treatment regimens for ovarian cancer.

Cytological/histological techniques: Under conditions specified in the protocol, these techniques can be used to identify PR and CR (such as the presence of residual benign tumor tissue in germ cell tumors). When exudation may be a potential side effect of a certain therapy (such as treatment with taxane compounds or angiogenesis inhibitors) and a measurable tumor meets the criteria for response or SD, the occurrence or exacerbation of tumor-related exudation during the treatment will be diagnosed by cytological techniques to distinguish between response (or SD) and disease progression.

2. Tumor response evaluation

2.1 Evaluation of all tumors and measurable lesions

To evaluate objective response or possible future progression, it will be necessary to perform a baseline evaluation of the total tumor burden as a reference for the subsequent measurements. In the clinical protocols with objective response as the primary endpoint, only patients with measurable lesions at baseline are eligible. Measurable lesions are defined as the presence of at least one measurable lesion. For trials with disease progression (disease progression time or degree of progression on a fixed date) as the primary endpoint, the inclusion criteria for the protocols will clarify whether only patients with measurable lesions are eligible or patients with no measurable lesions are also eligible.

2.2 Baseline records of target lesions and nontarget lesions

When there is more than 1 measurable lesion during a baseline evaluation, all measurable lesions will be recorded and measured, but the **total number of measurable lesions will not exceed 5 (no more than 2 in each organ)**. For patients with 1 or 2 organs involved, up to 2 or 4 target lesions will be selected for baseline measurements. The target lesions will be selected based on the size (the longest diameter) and will represent all the involved organs, and the measurement will be highly reproducible. If the measurement of the largest lesion is not reproducible, the largest one among reproducible lesions will be selected.

Special attention will be paid to lymph nodes because they are normal tissues and can be detected by imaging even without tumor metastasis. Pathological lymph nodes defined as measurable nodules or even target lesions must meet the following criteria. The CT measurement of the SAD must be ≥ 15 mm. Only the SAD will be measured at baseline. Radiologists usually use the SAD of a nodule to determine whether the nodule has undergone metastasis. The size of a nodule is generally represented by the two-dimensional data from imaging (the axial plane on CT and the axial, sagittal or coronal plane on MRI). The minimum value is the SAD. For example, a 20 mm × 30 mm abdominal nodule has an SAD of 20 mm and can be regarded as malignant and measurable. In this example, 20 mm is the measured value of the nodule. Nodules with a diameter ≥ 10 mm but < 15 mm will not be considered **target lesions. Nodules < 10 mm will not be categorized as pathological nodules and will not be recorded** and further observed.

The sum of the diameters of all target lesions (including the longest diameter of nonnodular lesions and the SAD of nodular lesions) will be reported as the baseline sum of the diameters. If the diameters of

lymph nodes are included, as mentioned above, only the SAD is included. The baseline sum of the diameters will be used as the reference value for the baseline level of the disease.

All other lesions, including pathological lymph nodes, will be considered nontarget lesions and will not need be measured but will be recorded during the baseline evaluation. For example, the lesion will be classified as “existing”, “missing” or, in rare cases, “clear progression”. Extensive target lesions will be recorded with target organs (e.g., numerous enlarged pelvic lymph nodes or large-scale liver metastasis).

2.3 Criteria for response

2.3.1 Evaluation of target lesions

CR: All target lesions have disappeared, and the SADs of all pathological lymph nodes (including target nodules and nontarget nodules) have reduced to <10 mm.

PR: The sum of the diameters of all target lesions is at least 30% higher than the diameter sum at baseline.

PD: The sum of the diameters of all target lesions is at least 20% higher than the reference diameter sum, which is the minimum value of the sum of the diameters of all target lesions measured during the entire study period (if the diameter sum at baseline is the smallest, the baseline value is used as the reference). In addition, the absolute value of the diameter sum must have increased by at least 5 mm (**the appearance of 1 or more new lesions is also considered PD**).

SD: SD is a status between PR and PD, where the reduction in the target lesion does not reach PR and the increase in the target lesion does not reach PD. The minimum value of the diameter sum will be used as a reference.

2.3.2 Precautions for target lesion evaluations

Lymph nodes: Even if the size of the lymph nodes identified as target lesions decreases to less than 10 mm, the actual SAD corresponding to the baseline will be recorded for each measurement (consistent with the anatomical plane of the baseline measurement). This means that if a lymph node is the target lesion, even if CR is achieved, it cannot be concluded that the lesion has disappeared completely because the SAD of a normal lymph node is defined as <10 mm. In the CRF or other recording forms, it is necessary to specifically record the target lesion (lymph node) at a specific location: for CR, the SADs of all lymph nodes must be <10 mm; for PR, SD and PD, the measured value of the SAD of the target lymph node will be included in the sum of the diameters of the target lesions.

Target lesions that are too small to be measured: In clinical studies, all lesions (nodules or non-nodules) recorded at baseline should be measured again and recorded, even if the lesions are very small (e.g., 2 mm). However, sometimes a lesion may be too small, resulting in very fuzzy CT images. In this case,

the radiologists cannot accurately define the exact size of the lesion and may report it as “too small to be measured”. However, it is very important to record a value on the CRF in this condition. In this trial, even if the radiologist believes that the lesion may have disappeared, he/she will still record 0 mm on the CRF. If the lesion does exist but is too fuzzy to be accurately measured, the default size will be 5 mm (note: this condition is unlikely to occur in lymph nodes because they generally have a measurable size under normal

conditions or are often surrounded by adipose tissue as in the retroperitoneal cavity, whereas if the lymph nodes cannot be measured accurately, the default size will also be 5 mm). The default size of 5 mm is derived from the slice thickness of the CT scan (however, this value does not change with the slice thickness of the CT scan). Because the probability of the repeated occurrence of the same measurement value is small, the use of this default value will reduce the risk of an erroneous evaluation. However, it should be reiterated that if the radiologist can obtain the exact lesion size, even if the diameter of the lesion is less than 5 mm, the actual size will be recorded.

Dissociated or combined lesions: When a nonnodular lesion dissociates into fragments, the sum of the diameters of the fragments will be calculated by adding the longest diameters of all fragments. Similarly, the planes between combined lesions can be used to distinguish each lesion, and then the longest diameter of each lesion can be calculated. However, if the combined lesions fuse into an inseparable lesion, the longest diameter should be the longest diameter of the entire fused lesion.

2.3.3 Evaluation of nontarget lesions

This section defines the response criteria for nontarget tumors. Although some nontarget lesions are measurable, they will not be measured but will be qualitatively evaluated at the time points planned in the protocol.

CR: All nontarget lesions have disappeared, and tumor markers have returned to normal levels; all lymph nodes are of nonpathological size (SAD $\leq$ 10 mm).

PR/non-PD: The presence of one or more nontarget lesions and/or the persistent presence of abnormally high tumor marker levels.

PD: Clear progression of existing nontarget lesions. **Note: The appearance of one or more new lesions is also considered PD**.

2.3.4 Special considerations for rating the progression of nontarget lesions

The following is a supplementary explanation of the definition of the progression of nontarget lesions: when patients have measurable nontarget lesions, to achieve ‘unequivocal progression’ on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently

to merit discontinuation of therapy. However, a general size increase in 1 or more nontarget lesions often cannot meet the criteria for progression. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare..

The situation that all nontarget lesions are unmeasurable may occur in some phase III trials, in which the inclusion criteria do not stipulate the necessity of measurable lesions. The overall evaluation is based on the above criteria, but measurable lesion data are absent in this situation. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease. For example, such progression can be described as an increase in tumor burden equivalent to an additional 73% increase in volume (equivalent to a 20% increase in the diameters of measurable lesions), the change in peritoneal exudation from “minimal” to “massive”, the change in lymphatic lesions from “local” to “extensively expanded”, or “sufficient to change the treatment method” in the protocol. Examples include pleural exudate increasing from a trace amount to a large amount, lymphatic involvement spreading distally from the primary site, or a description of "necessity for treatment changes" in the protocol. In this clinical study, if there is clear progression, the patient will be considered to have disease progression at that time point. It is best to have objective criteria that can be applied to the evaluation of unmeasurable lesions. Note that the supplementary criteria must be reliable.

2.3.5 New lesions

The appearance of new malignant lesions indicates disease progression; therefore, the evaluation of new lesions is critical. Currently, there is no specific standard for the imaging detection of lesions. However, the finding of a new lesion should be unequivocal. For example, progression cannot be attributed to different imaging techniques, changes in imaging morphology, or lesions other than tumors (e.g., some so-called new bone lesions are merely cured original lesions or the recurrence of original lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, a necrotized liver lesion may be classified as a new cystic lesion on the CT report, although it is not.

Lesions that are detected during the follow-up but were not found in the baseline examination will be considered new lesions and suggestive of disease progression. For example, if a patient with visceral lesions in the baseline examination is found to have metastases during a CT or MRI cranial examination, intracranial metastases will be considered the basis of disease progression even if the patient did not undergo a cranial examination during the baseline examination. If a new lesion is equivocal, for example

because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

In addition to fluorodeoxyglucose (FDG)-PET, additional tests are generally required for supplementary confirmation. It is rational to combine FDG-PET and complementary CT results to evaluate progression (especially for new suspected diseases). New lesions can be confirmed by FDG-PET using the following procedures.

Negative baseline FDG-PET results together with the positive FDG-PET results in a subsequent follow-up indicate disease progression.

In cases with no baseline FDG-PET and positive FDG-PET results in a subsequent follow-up, if the new lesion indicated by the positive FDG-PET is confirmed by CT, then disease progression is confirmed; if the new lesion indicated by the positive results of the follow-up FDG-PET is not confirmed by CT, another CT should be performed to confirm the lesion (if confirmed, the disease progression time is counted from the initial discovery of the lesion); if the lesion is confirmed to be an existing lesion recorded in a past CT examination and no progression of the lesion has been found by imaging, then there is no disease progression.

2.4 Evaluation of the best overall efficacy

The best overall efficacy will be evaluated based on the best response from the start of treatment to the end of treatment, and all requisites will be considered for confirmation. Sometimes, the response occurs after the end of treatment. Therefore, the protocol will clarify whether an efficacy evaluation after the end of the treatment is included in the best overall efficacy evaluation. The protocol will clarify how any new treatment before progression affects the best response. The best response mainly depends on target lesion and nontarget lesion results, as well as the presence of new lesions. In addition, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement. Specifically, in nonrandomized trials, response is the primary goal, and efficacy, i.e., PR or CR, must be confirmed to determine the best overall efficacy.

2.4.1 Time point response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 summarises the overall response of a patient population with measurable disease at baseline at each time point. The evaluation of patients with no measurable lesions (no target lesions) is detailed in Table 2.

2.4.2 Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

2.4.3 Best overall response

At all time points, once all the patient data are available, the best overall response will be determined.

Evaluation of the best overall response when confirmation of CR or PR is not required: The best treatment response is the best response at all time points (e.g., if the efficacy in a patient is rated SD for the first cycle, PR for the second cycle, and PD for the last cycle, then the best overall response for the patient is PR). When the best overall response is rated SD, the requirement for the minimum interval from baseline to SD specified in the protocol must be met. If the minimum interval requirement is not met, even if the best overall response is rated SD, the evaluation result will not recognized, and the best overall response of the patient will be determined by a subsequent evaluation. For example, if a patient response is rated SD for the first cycle and PD for the second cycle but does not meet the minimum interval requirement, the best overall response is PD. Similarly, if a patient response is rated SD for the first cycle but the patient becomes lost to follow-up after the first cycle, the best overall response is considered NE.

Evaluation of the best overall response when confirmation of CR or PR is required: If each subject meets the criteria for PR or CR and the efficacy is confirmed at a subsequent time point (usually 4 weeks later) specifically mentioned in the protocol, the best overall response can be declared CR or PR. In this situation, the best overall response is detailed in Table 3.

2.4.4 Special tips for efficacy evaluations

When nodular lesions are included in the total target lesion evaluation and the size of the nodule decreases to the "normal" size (<10 mm), a lesion size scan report will be generated. To avoid the over-evaluation of efficacy based on an increase in nodule size, the measurement results will be recorded even if the nodule is normal. As mentioned earlier, this means that even for subjects with a CR, the nodule size will not be recorded as 0 on the CRF. If the efficacy needs to be confirmed during treatment, repeated "unmeasurable" time points will complicate the evaluation of the best efficacy. The analysis plan must state that these missing data/evaluations will be clearly explained when determining efficacy. For example, in most trials, PR-NE-PR can be regarded as confirmation of efficacy.

When treatment must be discontinued due to the deterioration in the general health status of a participant that is not supported by objective evidence, the efficacy will be reported as symptomatic progression. Even after the discontinuation of treatment, objective progression will be evaluated whenever possible. Symptomatic deterioration is a reason for the discontinuation of treatment but is not an objective response evaluation. The objective response of such subjects will be evaluated based on the conditions of target and nontarget lesions, as detailed in Tables 1 to 3.

Conditions defined as early progression, early death and NE are special cases and will be clearly described for each regimen (depending on the treatment interval and treatment cycle).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. If abnormal imaging results for local lesions are considered to represent lesion fibrosis or scar formation, FDG-PET can be used as an evaluation standard, similar to biopsy, to confirm CR. In this situation, the application of FDG-PET will be prospectively described in the protocol, and the specialized medical literature reporting this situation will be cited as support. However, limitations of FDG-PET and biopsy (including their resolution and sensitivity) will lead to false positive results in the evaluation of CR.

Table 1 – Time point response: patients with target (+/– non-target) disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is *truly* met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

2.5 Frequency of tumor re-evaluations

The frequency of tumor re-evaluations during treatment will be determined by the treatment regimens and will occur in accordance with the type of and schedule for treatment. However, in the phase II trial in which the benefit of treatment was not clear, it is reasonable to conduct follow-up every 6 to 8 weeks (at the end of a cycle), and the length of the time interval will be adjusted under special schemes or

circumstances. The protocol will specifically indicate which tissue sites need to be evaluated at baseline (usually those tissue sites that are most likely to be closely related to the metastatic lesions of the tumor type under study) and the frequency of re-evaluation. Under normal circumstances, target lesions and nontarget lesions will be rated at each evaluation. In some optional cases, the evaluation frequency of some nontarget lesions will be lower; for example, bone scans should be repeated only when the treatment efficacy for the target disease is confirmed to be CR or when bone lesion progression is suspected.

After the end of treatment, the re-evaluation of tumors depends on whether the response rate or the occurrence of a certain event (progression/death) is used as the clinical trial endpoint. If the clinical trial endpoint is the occurrence of a certain event (e.g., TTP/DFS 1/PFS), routine re-evaluations, as specified in the protocol, are required. In randomized comparative trials, the scheduled evaluations should be listed in the study schedule (e.g., 6-8 weeks of treatment or 3-4 months after the treatment) and should not be affected by other factors, such as treatment delays, dosing intervals, and any other events that may cause an imbalance in the treatment arm regarding the selection of disease evaluation time.

2.6 Efficacy evaluation/response confirmation

2.6.1 Confirmation

For nonrandomized clinical trials with efficacy as the primary endpoint, PR and CR must be confirmed to ensure that the efficacy is not the result of an erroneous evaluation. Confirmation also allows a reasonable interpretation of the results in the presence of historical data, and efficacy in the historical data of these trials should also be confirmed. However, in all other cases, such as randomized trials (Phase II or III) or studies with SD or PD as the primary endpoint, efficacy confirmation is no longer necessary because it is of no value for the interpretation of the experimental results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded. In the case of SD, at least 1 measurement must meet the SD criteria specified in the protocol within the minimum time interval after the start of treatment (generally no less than 6-8 weeks).

2.6.2 Total response period

The total response period is the interval from the time when the CR or PR (whichever is measured first) criteria are first met to the time of the first actual recording of disease recurrence or progression (the minimum measured value recorded during treatment is used as the reference for disease progression). The total CR period is the interval from the time when the CR criteria are first met to the time of the

first actual recording of disease recurrence or progression.

2.6.3 SD period

The SD period is the interval from the start of treatment to disease progression (in a randomized trial, from the time of randomization), and the smallest sum during treatment is used as the reference (if the baseline sum is the smallest, it is used as the reference for PD calculations). The clinical relevance of SD varies in different studies and different diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease. Note: The response period, SD period, and PFS are affected by the follow-up frequency after the baseline evaluation. Defining the standard follow-up frequency is outside the scope of this guideline. The frequency of follow-up should consider many factors, such as disease type and stage, treatment cycle and standard specifications. However, if intertrial comparisons are required, the limitations of the accuracy of these measurement endpoints should be considered.

Appendix 4. CA-125 criteria for response evaluation in ovarian cancer

The Response Evaluation Criteria in Solid Tumors (RECIST) are currently the internationally accepted method- ology for assessing tumor response in all cancer trials. The RECIST criteria, a simplification of the former WHO criteria, are based on measurable target lesions and do not make use of serum tumor markers levels in evaluation of tumor response.

In ovarian cancer, often, there are no reliably measurable target lesions due to predominantly diffuse peritoneal disease. Hence, serum CA-125 levels are extensively used to assess response and define progression in ovarian cancer. To standardize the use of CA-125 levels in ovarian cancer trials, the Gynecologic Cancer Intergroup (GCIG) has recently published definitions of CA-125 response and CA-125 progression.

The GCIG CA-125 response is defined as at least 50% reduction in serum CA-125 levels from pre-treatment levels provided the response is maintained for 28 days. This definition is a simplification of the Rustin's CA-125 criteria, which specifies a 50% response as well as a 75% response [4]. Briefly, a 50% CA-125 response is defined by Rustin as a 50% decrease of serum CA-125 levels after two samples and confirmed by a final sample. A 75% CA-125 response is defined as a serial decrease of N75%, over three CA-125 samples. In each case, the final sample had to be at least 28 days after the previous sample.

Recently, a Danish group has found the GCIG-CA-125 criteria to be a better prognostic tool than the RECIST criteria in second-line treatment of ovarian cancer. This retrospective study is likely to give impetus to the widespread adoption of CA-125 criteria in the clinical as well as trial setting.

However, we would urge caution in adopting the GCIG CA-125 criteria. We propose that that the adoption of Rustin's 75% response criteria might be more appropriate instead of the proposed 50% response criteria based on our experience in using WHO criteria and Rustin's CA-125 criteria in a phase II trial. We evaluated the efficacy of Oxaliplatin and 5-Fluorouracil/Leucovorin combination in twenty-seven ovarian cancer patients relapsing within 2 years of prior platinum chemotherapy. Using the CA-125 levels measured at baseline, tumor response was prospectively assessed by Rustin's CA-125 criteria. All the patients with an objective response by WHO criteria demonstrated a 75% CA-125 response while none of the

patients with progressive disease by WHO criteria showed a CA-125 response. Only two patients in our trial had a 50% CA-125 response and both these patients had stable disease only by WHO criteria (Table 1). This prospective data set suggests that the stringent 75% CA-125 criteria are more likely to mirror the RECIST response criteria and in turn more likely to be useful in measuring tumor response in patients with non-evaluable disease. On the other hand, the 50% response criteria are more likely to overestimate the tumor response rate.

Table 1
Correlation between WHO response and CA-125 response

	Rustin's CA-125 response (best tumor response)			
	75% response	50% response	No response	Not evaluable[a]
WHO criteria (best tumor response)				
Complete response	100%	–	–	–
Partial response	100%	–	–	–
Stable disease	43%	29%	29%	–
Progressive disease	–	–	86%	14%
Not evaluable[b]	43%	–	43%	14%

[a] Two patients were not evaluable by CA-125 criteria because only one sample of CA-125 was available.

[b] Seven patients were non-evaluable by WHO criteria because of the absence of reliable, bi-dimensionally measurable target lesions.

An independent retrospective study from a Danish group confirms our prospective observation that the 50% CA-125 criteria overestimate tumor response. Moreover, from a practical point of view, the 50% response rate is also more likely to be influenced by medical procedures such as ascites and pleural effusion drainage, which are quite commonly used in management of relapsed ovarian cancer patients. The 75% response rate is less likely to be affected by medical procedures.

The test data set provided by GCIG on its web site also supports our suggestion since all, except one, responder in that data set would still be classified as a responder under the 75% response definition. Hence, the adoption of the stringent 75% CA-125 response instead of the 50% response criteria has the potential to avoid overestimation of tumor response rate without a significant risk of underestimation. Needless to add, that this small prospective data set from a phase II trial needs to be validated by a large dataset.

Appendix 5. NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.0

Please go to the following website to access the NCI CTCAE version 5.0:

https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0/

Appendix 6. NCCN Guidelines of Management of Immunotherapy-Related Toxicities

Please go to the following website to access the NCCN Guidelines of Management of Immunotherapy-Related Toxicities:

https://www.nccn.org/guidelines/guidelines-detail?category=3&id=1486

Protocol

A Single-Blind, Placebo-Controlled, Randomized, Phase 2 study of Manganese, Sintilimab plus Nab-paclitaxel and Platinum *vs.* Placebo, Sintilimab plus Nab-paclitaxel and Platinum in Platinum-Resistant/Refractory Ovarian Cancer

Clinical Research Organization： Chinese PLA General Hospital

Medical Monitor： Weidong Han

Development Phase: 2

Date of Original Protocol: 10 June 2019

Date of Protocol Amendment 1: 20 October 2019

Date of Protocol Amendment 2: 21 May 2020

Date of Protocol Amendment 3: 15 December 2020

Version of Protocol: 4.0

INVESTIGATOR SIGNATURE PAGE

Declaration of the Principal Investigator

Title: A Single-Blind, Placebo-Controlled, Randomized, Phase 2 study of Manganese, Sintilimab plus Nab-paclitaxel and Platinum vs. Placebo, Sintilimab plus Nab-paclitaxel and Platinum in Platinum-Resistant/Refractory Ovarian Cancer

I have read this study protocol, including all appendices. By signing this protocol, I agree to conduct the clinical study, following approval by an Independent Ethics Committee (IEC)/Institutional Review Board (IRB), in accordance with the study protocol, the current International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), and applicable regulatory requirements. I will ensure that all personnel involved in the study under my direction will be informed about the contents of this study protocol and will receive all necessary instructions for performing the study according to the study protocol.

Principal Investigator

_____ _____

Name: Date

Title:

Institution:

SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor/Company: Chinese PLA General Hospital</td></tr>
<tr><td colspan="2">Title of Study:</td></tr>
<tr><td colspan="2">Name of Investigational Product: Manganese Chloride, nab-paclitaxel, Platinum chemotherapy, Sintilimab</td></tr>
<tr><td>Study Period (years):

Estimated date first patient enrolled: December 2020

Estimated date last patient completed: December 2023</td><td>Phase of Development:

2</td></tr>
<tr><td colspan="2">Objectives:

Primary objectives:

The primary objective of the study is investigator-assessed ORR per RECIST v1.1, defined as the percentage of patients with at least 1 efficacy assessment who achieved a complete or partial response (CR/PR).

The primary safety endpoint is the incidence of treatment-related AEs per CTCAE v5.0 in patients who received at least one dose of study treatment.

Secondary objectives:

To evaluate additional measures of clinical benefit as assessed by the Investigators, the secondary objectives of the study are as follows:

1) Progression-free survival (PFS) by RECIST v1.1

2) Overall survival (OS)

3) Disease control rate (DCR) by RECIST v1.1

4) Duration of response (DOR) by RECIST v1.1

5) Gynecological Cancer InterGroup CA-125 response rate

6) Quality of life score by FACT-O

Exploratory objectives：

1) Concentration of blood Mn^{2+} was detected by the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) recommended by ATSDR</td></tr>
</table>

2) serum cytokines and chemokines were analyzed by LEGEND^{plex} Bead-based Immunoassays (BioLegend) according to the manufacturer's instructions.

3) To correlate Concentration of blood $Mn^{2+}$with other immune-related biomarkers and with efficacy outcomes.

4) To identify the biomarker-based patient population that would derive benefit from the combination treatment based on the cytokine and cellular profile of blood.

Methodology:

Overall

This phase 2, randomized, single-blind, placebo-controlled study evaluating the efficacy and safety of combination treatment with manganese chloride or placebo plus immunochemotherapy in patients with platinum-resistant/refractory OC. The study is detailed as follows:

1) recurrent epithelial high-grade serous ovarian, fallopian tube, or primary peritoneal cancer who are currently platinum-resistant (defined as progression within 6 months following last dose of most recent platinum) or platinum-refractory (defined as lack of response or progression within 1 month since the last dose)；

2) Participants were randomly assigned in a 2:1 ratio to the test and control groups, and stratified by platinum-free interval；

—Test group:

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle)

Manganese chloride was nebulized inhaled at 0.4mg/kg twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle.

—Control group:

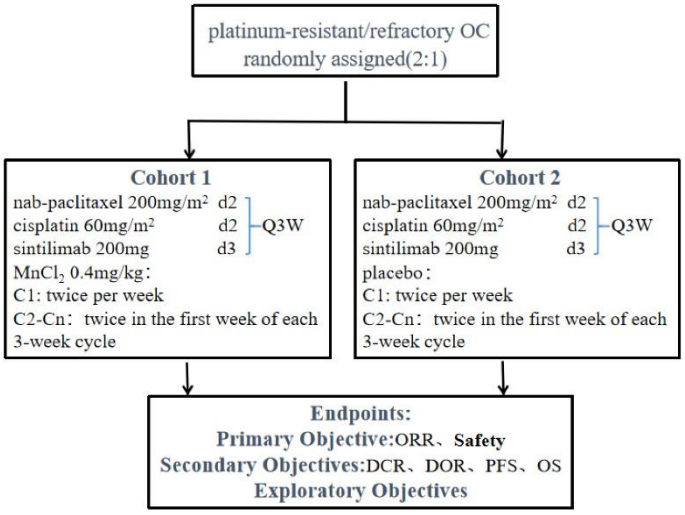
nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle)

Placebo was nebulized inhaled twice per week in the first 3-week cycle, and thereafter twice in the first week of each 3-week cycle.

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.



General Study Conduct

In this study, the screening period did not exceed 21 days, and qualified subjects who completed the screening examination and evaluation were randomly divided into blind groups and entered the study treatment period (every 21 days was a treatment cycle), and they were treated and visited in accordance with the protocol.

Following informed consent, all patients will undergo screening procedures within 21 days prior to the first dose of study treatment to determine eligibility for study entry.

Screening procedures include medical, surgical, cancer, and medication history; complete physical examination, including vital signs, height, and weight; Eastern Cooperative Oncology Group (ECOG) performance status; clinical laboratory tests (complete blood count [CBC], coagulation, chemistry, thyroid-stimulating hormone [TSH], triiodothyronine [T3] or free T3 [FT3], free thyroxine [FT4], serum CA-125), blood Mn^{2+} parameters and electrocardiogram (ECG). see Study Manual for details on sample collection and preparation. Radiographic evaluations (magnetic resonance imaging [MRI, preferred method]) or computed tomography [CT] of abdomen, and pelvis must be conducted at screening to determine extent of disease and confirm presence of measurable disease. The chest must be examined by computed tomography [CT]. Head scan will be conducted if clinically indicated; bone scans will be conducted per standard of care. Scans performed prior to the signing of the informed consent form (ICF) as part of routine clinical management are acceptable

for use as initial tumor imaging if they are of diagnostic quality and are performed within 21 days prior to first dose date.

Safety assessments conducted throughout the treatment period include symptom-directed physical examination, vital signs, ECGs, ECOG performance status, and clinical laboratory assessments (CBC, coagulation [Phase 1 only], chemistry, TSH, T3 or FT3, FT4, urinalysis, CA-125, blood Mn^{2+} parameters). Radiographic evaluations all patients to assess extent of disease will be conducted every 6 weeks (42 days ± 7 days) after Cycle 1/Day 1 while on study treatment independent of cycle delays and/or dose interruptions and/or at any time when progression of disease is suspected. All radiographic images/scans will be sent to a central imaging vendor upon acquisition and archived for future evaluation if needed. Per RECIST v1.1, patients who achieved complete response (CR) or partial response (PR) should have the response confirmed; tumor imaging for confirmation of response may be performed at the earliest 3 weeks after the first indication of response, or at the next scheduled scan, whichever is clinically indicated.

Safety and tolerability will be evaluated throughout the study by recording adverse events, monitoring vital signs and physical examination, laboratory evaluation of safety, and 12-lead electrocardiogram (ECG).

All AE and SAEs experienced by a patient, regardless of suspected causation, will be monitored until the AE or SAE is resolved, until abnormal laboratory values are returned to baseline or normalized, until there is a satisfactory explanation for observed changes, until the patient loses follow-up, or until the patient dies.

Number of Patients (Planned): 80

Criteria for Inclusion:

1. Patients with histologically or cytologically confirmed epithelial ovarian cancer, primary fallopian tube cancer, or peritoneal cancer.
2. Patients who have received at least one chemotherapy regimen based on platinum compounds.
3. Patients who experienced disease progression during treatment or within 6 months after completion of the above treatment regimen.
4. Patients with at least one measurable lesion.
5. Patients who have received immunotherapy (anti-PD-1 antibodies, anti-PD-L1 antibodies), or bevacizumab, are not restricted from enrollment.
6. Expected survival of more than 6 months.
7. Age over 18 years.

8. Patient weight > 40 kg.

9. ECOG performance status ≤ 2.

10. Peripheral blood white blood cell count > 3.5×10^9 /L.

11. Bone marrow reserve and liver and kidney function (the following laboratory tests should be performed before initial treatment to prove):

- Absolute neutrophil count (ANC) ≥ 1,000/mm^3;

- Hemoglobin > 80g/dL;

- Platelet count > 80,000/mm^3;

- ALT/AST < 3×ULN;

- Serum creatinine < 3×ULN;

- Total bilirubin level < 3×ULN.

12. No significant hereditary diseases. Normal liver and kidney biochemical indicators.

13. At least 4 weeks since the end of previous ovarian cancer treatment, and substantial recovery from adverse reactions of previous treatment (graded according to CTCAE5.0, excluding alopecia).

14. No treatment with paclitaxel (albumin-bound) plus platinum in the past 3 months.

15. Voluntary participation and signing of informed consent, compliance with the trial treatment plan and visit schedule, and cooperation in observing adverse events and efficacy.

Criteria for Exclusion:

1. Absolute neutrophil count (ANC) < 1×10^9/L or platelets < 80×10^9/L or hemoglobin < 80g/dL (according to the normal values of the clinical trial center).

2. Serum total bilirubin levels higher than 3 times the upper limit of the reference range.

3. ALT, AST, or ALP levels higher than 3 times the upper limit of the reference range when there are no liver metastases; ALT, AST, or ALP levels higher than 5 times the upper limit of the reference range when there are liver metastases.

4. Patients receiving known strong inhibitors (e.g., ketoconazole) or inducers (e.g., rifampicin or St. John's Wort) of CYP3A4, or strong inhibitors (e.g., gemfibrozil) or inducers of CYP2C8; patients receiving treatment with potent P-gp inhibitors or inducers, or patients who cannot stop treatment with these agents for 2 weeks

before the start of the study.

5. Toxic reactions to previous antitumor therapy that have not recovered to Grade 0 or 1 (excluding alopecia).

6. Organ failure:

- Heart: Grade III or IV.

- Liver: Grade C according to Child-Pugh B liver function classification.

- Kidney: Renal failure and uremia.

- Lung: Severe respiratory failure symptoms.

- Brain: Consciousness disorders.

7. T-cell tumors such as T-cell lymphoma or T-cell leukemia.

8. Major surgery within 4 weeks before enrollment, or planned major surgery during the study (excluding diagnostic surgery).

9. HIV antibody positive, or other acquired, congenital immunodeficiency diseases, or patients with a history of organ transplantation.

10. History of Parkinson's disease or epilepsy, or occurrence of diseases that can induce seizures within 12 months before the study (including a history of transient ischemic attack, stroke, traumatic brain injury with consciousness disorders).

11. CTCAE grade 2 infections that do not respond to treatment or active clinically serious infections with CTCAE > grade 2.

12. Patients with active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection requiring treatment.

13. Patients with a history of allergic reactions to study drug components or suspected allergies.

14. Chronic diseases requiring immunotherapy or hormone therapy.

15. Patients who have received any investigational drug treatment within 30 days before the first dose of the trial drug.

16. Subjects with symptoms of brain or leptomeningeal metastasis.

17. Subjects with a history of malignant tumors (non-study tumor) within 3 years before the first dose of the trial drug.

18. History of the following diseases: interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases including diabetes, hypertension, pulmonary fibrosis, acute lung disease, abdominal infection, etc.

19. Subjects with clinical symptoms of intestinal obstruction persisting before the first dose of the experimental drug; subjects with incomplete intestinal obstruction who have returned to normal within 1 month before the first dose of the experimental drug can be included in the study after discussion by the researchers.

Investigational Product:

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle)

Manganese chloride was nebulized inhaled at 0.4mg/kg twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle.

Statistical Methods:

Sample Size Estimation

Primary endpoint is ORR per Investigator assessment. The study is designed to test the null hypothesis that the objective response is the same between the manganese plus immunochemotherapy arm and the immunochemotherapy alone arm *vs.* the alternative hypothesis that manganese promotes the objective response. The reference ORR was calculated based on the prior clinical trials with ICB plus chemotherapy in platinum resistant ovarian cancer, including the JAVELIN 200 study with a 13.3% ORR in ICB plus chemotherapy group. Approximately 78 patients will be randomized 2:1 (approximately 52 and 26 patients in the Mn^{2+} and placebo group, respectively) to provide a 90% power at the 5% level of statistical significance.

General Methods

For continuous variables such as the number of patients, mean, standard deviation, median, minimum, and maximum values in clinical study protocols, statistical analysis will be performed using two-sided 95% confidence intervals (CIs) based on the Clopper-Pearson method. Time-to-event analyses will utilize the Kaplan-Meier (KM) method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model. Comparisons between two groups of laboratories tests were performed by paired *t* test.

Efficacy

ORR and DCR will be summarized using descriptive statistics including number, percentage and 2-sided 95% CI.

Duration of response, PFS, and OS will be summarized using the Kaplan-Meier method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

Interim Analysis:

To minimize the risk of exposing patients to ineffective treatment, an assessment of the benefit comparison between the trial and control groups will be conducted during the study, a formal interim analysis of Overall Survival (OS) is planned when a pre-specified number of ORR events has been reached for the final ORR analysis.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	The absolute neutrophil count
APTT	Activated partial thromboplatin time
AST	Aspartate transaminase
AUC	Area under curve
BIL	Bilirubin
BLD	Urine latent blood
BMI	Body mass index
BOR	Best overall response
CK	Creatine kinase
CL	Clearance
Cmax	Peak concentration
CMH	Cochran mantel haenszel
CNS	Central nervous system

CR	Complete response
Cr	Serum creatinine
CRA	Clinical research associte
CRF	Case report form
eCRF	Electronic case report form
CRO	Contract research organization
CT	Computed tomography
CL	Clearance
DBIL	Direct bilirubin
DCR	Disease control rate
DOR	Duration of response
ECG	Electrocardiogram
ECOG	Eastern cooperative oncology group
EOS	Eosinophil
EPO	Erythropoietin
FACT-O	Functional Assessment of Cancer Therapy-Ovarian Cancer
FIB	Fibrinogen
GCP	Good clinical practice
G-CSF	Granulocyte colony stimulating factor
GLP	Good laboratory practice of drug

GLU	Urine glucose
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCT	Hematocrit
HCV	Hepatitis C virus
HGB	Hemoglobin
HIV	Human immunodeficiency virus
HR	Hazard ratio
5-HT3	5-Hydroxytryptamine
IB	Investigator's brochure
IDMC	Independent data monitoring committee
IEC	Independent ethics committee
IHC	Immunohistochemistry
INR	International normalized ratio
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
ITT	Intention-to-treat
IV	Intravenous injection

KET	Ketone
LD	Lactic dehydrogenase
LEU	Leucocytes in urine
LYMPH	Lymphocyte percentage and counting
MedDRA	Medical dictionary for regulatory activities
MON	Monocytes percentage and counting
mPFS	Median progression free survival
MRI	Magnetic resonance imaging
NCI-CTCAE	National cancer institute common terminology criteria for adverse events
NEUT	Neutrophils percentage and counting
NIT	Nitrite
NMPA	National medical products administration
QA	Quality assurance
QC	Quality control
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PD-L1	Programmed cell death-ligand 1

PFS	Progress-free survival
PK	Pharmacokinetics
PLT	Platelet count
PPS	Per protocol set
PR	Partial response
PRO	Protein in urine
PT	Prothrombin time
Q3W	3 weeks using a
RBC	Red blood cell count
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Standard analysis software
SD	Stable disease
SG	Specific gravity
SMP	Standard management procedure
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TBIL	Total bilirubin

TEAE	Treatment emergent adverse events
TESAE	Treatment of serious adverse events
TNF	Tumor necrosis factor
TP	Total protein
TT	Thrombin time
TTP	Time-to-progress
WBC	White blood cell count
WHO-Drug	World health organization drug dictionaries

1. BACKGROUND

Ovarian cancer is a common malignant tumor of the female reproductive system, with the incidence rate occupying the 3rd place and the mortality rate ranking the first among gynecological malignant tumors. Every year,52,000 women are diagnosed with ovarian cancer in China, and about 22,000 people die as a result of the disease. 75% of the patients diagnosed for the first time are already in the advanced stage (Stage III/IV), and after receiving ideal tumor cytoreduction and standardized chemotherapy, 85% of them still suffer from relapses[1-3], and the emergence of Platinum-resistant response, and at present there is no effective standard treatment plan for platinum-resistant relapsed/refractory ovarian cancer worldwide.

Studies have shown that ovarian cancer expresses tumor-specific antigens, which can induce spontaneous anti-tumor immune responses from immunotherapy[4-5], providing some theoretical support for the application of PD-1/L1 inhibitors and other drugs that modulate acquired immune responses in ovarian cancer. However, clinical studies have confirmed that monotherapy with immune checkpoint inhibitors in recurrent/refractory ovarian cancer yields low objective response rates (ORR) ranging from 8.0% to 15.0% [6-9] , with a median PFS of 2 months; the ORR of immune checkpoint inhibitors combining with chemotherapy or anti-angiogenic drugs is also only 15%-20%, and the study of JAVELIN-200 has shown that the median PFS and OS of doxorubicin liposomal combined with avelumab are 3.7 months and 15.7 months[10] , which did not significantly improve the clinical efficacy, in response to the study, Lancet Oncol published a review "Immunotherapy in ovarian cancer: we are not there yet" in 2021[11] , suggesting that the JAVELIN-200 study is a failure, for the immunotherapy in ovarian cancer. The failure of the JAVELIN-200 study brought to a close the key question of immunotherapy in ovarian cancer and opened up new hypotheses and hopes. Therefore, further exploration of combination therapy strategies to improve clinical efficacy, prognosis and survival in relapsed/refractory ovarian cancer is necessary.

The reason for the poor efficacy of immune-detection point inhibitors in ovarian cancer may be related to the "cold tumor" property, the low frequency of pro-inflammatory immune cells and immunosuppressive signals in the tumor microenvironment of ovarian cancer[12] . Recent studies have shown that the STING (Stimulator of Interferon Genes) signaling pathway plays a key role in stimulating anti-

tumor immunity and converting "cold tumors" into "hot tumors"[13-16]. STING signaling pathway is one of the major natural immune pathways triggered by the body during antiviral processes. The cGAS-STING signaling pathway senses intracellular viral DNA, activates the transcription factors IRF3/7 and NF-κB, and produces cytokines such as type I interferon (IFN), which induces an immune response. Several studies have confirmed that the cGAS-STING pathway also has an anti-tumor effect, and the mechanisms include: 1) inducing tumor cell senescence; 2) promoting tumor cell autophagy; 3) promoting tumor cell apoptosis; 4) enhancing acquired anti-tumor response. DNA fragments of tumor cells in the tumor microenvironment, which are recognized and absorbed into the intracellular compartment by DC cells, activate the cGAS-STING pathway, induce type I interferon, promote antigen presentation and the activation of $CD8^+$ T cells, and activate acquired immunity; at the same time, locally increased mature DC cells can enhance the tumor-specific T cell response, thus playing an important role in tumor immunosurveillance and antitumor process[17-23] .

At present, many research institutions and pharmaceutical companies around the world have invested heavily in the research and development of STING agonists, including CDNs (synthetic cyclic dinucleotides), non-CDNs, and bacterial vector drugs, of which CDNs are the first-generation STING agonists, such as ADU-S100, MK-1454 and so on. Nearly 14 clinical studies of STING agonists combined with Immune checkpoint inhibitors for the treatment of solid tumors and lymphomas have been conducted internationally, and nearly 20 preclinical drug trials are under development. Among them, the clinical study data of ADU-S100 and MK-1454 combination therapy were reported in ASCO 2019 and ESMO 2018[24-25], respectively, and the four clinical studies containing the two drugs did not have significant breakthroughs in clinical efficacy, and the results of other STING agonist-based drugs have still not been published, and it has been reported that many of the clinical studies have been called off. Analyzing of treatment failure may be due to unstable drug metabolism and poor cell permeability; New STING agonists with non-CDN structures, such as liposomes, polymers, and nanoparticles, are currently being developed. These face challenges related to complex preparation methods and high production costs [26-27].

Studies have shown that metal ions also play an important role in immune regulation, such as Ca^{2+} is involved in the activation of T cells; K^+ can maintain T cell stemness and enhance T cell function; K^+, Ca^{2+} and Na^+ are involved in the occurrence of inflammation[28-31]. Therefore, the immunomodulatory function of metal ions can be utilized for the treatment of diseases, and "metal immunotherapy" has been proposed in the journal Nature 2021.

Prof. Jiang Zhengfan's team at Peking University found that manganese ions (Mn^{2+}) play an important role in activating the cGAS-STING pathway: Virus infection of cells resulted in the release of Mn^{2+} from manganese-storing organelles such as mitochondria and Golgi apparatus into the cytoplasm and extracellular space. The significantly increased concentration of Mn^{2+} in the cytoplasm has a dual activation effect on the cGAS-STING pathway: Mn^{2+} enhances the sensitivity of cGAS to DNA and promotes its synthesis of the second messenger, cGAMP; Mn^{2+} improves the binding capacity of STING and cGAMP, and Mn^{2+} released into the cytoplasm puts the cGAS-STING pathway in a state of hyper-activation, which greatly enhances its ability to respond to cytoplasmic DNA and even activating it at DNA levels that were initially non-responsive. And when mice were deficient in Mn^{2+}, the anti-DNA viral ability was significantly inhibited[32] . This study is the first to show that the trace element Mn^{2+} can play a dual role as an alarm and agonist in the body's natural immunity against infections. Mn^{2+} -STING agonist, unlike many agonists that have been developed so far, is an essential trace element that is naturally occurring inside the cell; it is more easily absorbed by the body as a trace element supplement.

To further confirm the anti-tumor effects of Mn^{2+}-STING agonists, our team collaborated with Professor Zhengfan Jiang's team to conduct both basic research and Phase I clinical studies. The basic study showed that the growth of tumor cells and metastatic lesions in manganese-deficient mice was significantly accelerated and increased, confirming that under physiological conditions, Mn^{2+} as an essential trace element plays an indispensable role in tumor immunosurveillance. Exogenous administration of Mn^{2+} can effectively activate the intracellular cGAS-STING pathway in mice, significantly promote the ability of antigen-presenting cells, such as DCs and macrophages, to present tumor-specific antigens, and enhance the infiltration and specific killing function of acquired immune cells in tumor tissues. The combination of Mn^{2+} and anti-PD-1 antibody (manganese immunotherapy) has been shown to significantly enhance the anti-tumor effect of anti-PD-1 antibody in several animal models. In the phase I clinical study of manganese immunotherapy for relapsed refractory solid tumors, the objective remission rate was 45.5%, the disease control rate reached 81.8%, and no serious adverse events were observed, which preliminarily confirms the efficacy and safety of Mn^{2+}-based therapy[33] .

Our team conducted a phase I clinical study in patients with advanced metastatic solid tumor, and the preliminary results showed that the objective remission rate of the manganese immunotherapy group was as high as 45.5%, the disease control rate was 90.9%, and the type I interferon in peripheral blood was significantly increased.

Manganese immunotherapy might significantly improve the clinical efficacy of relapsed and refractory ovarian cancer, which is considered to be related to the unique tumor microenvironment of ovarian cancer. However, the specific mechanism needs to be further analyzed. Moreover, further expansion of clinical samples is needed to confirm the efficacy and safety in relapsed refractory ovarian cancer.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is investigator-assessed ORR per RECIST v1.1, defined as the percentage of patients with at least 1 efficacy assessment who achieved a complete or partial response (CR/PR).

The primary safety endpoint is the incidence of treatment-related AEs per CTCAE v5.0 in patients who received at least one dose of study treatment.

2.2. Secondary Objectives

To evaluate additional measures of clinical benefit as assessed by the Investigators, the secondary objectives of the study are as follows:

1) Progression-free survival (PFS) by RECIST v1.1

2) Overall survival (OS) by RECIST v1.1

3) Disease control rate (DCR) by RECIST v1.1

4) Duration of response (DOR) by RECIST v1.1

5) Gynecological Cancer InterGroup CA-125 response rate

6) Quality of life score by FACT-O

2.3. Exploratory Objectives

The exploratory objectives of the study are as follows:

1) Concentration of blood Mn^{2+} was detected by the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) recommended by ATSDR

2) serum cytokines and chemokines were analyzed by LEGEND^{plex} Bead-based Immunoassays (BioLegend) according to the manufacturer's instructions

3) To correlate concentration of blood Mn^{2+}, serum cytokines and chemokines with efficacy outcomes

4) PBMCs cellular profile was characterized by scRNA-seq

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This phase 2, randomized, single-blind, placebo-controlled study evaluating the efficacy and safety of combination treatment with manganese chloride or placebo plus immunochemotherapy in patients with platinum-resistant/refractory OC. The study is detailed as follows:

1) recurrent epithelial high-grade serous ovarian, fallopian tube, or primary peritoneal cancer who are currently platinum-resistant (defined as progression within 6 months following last dose of most recent platinum) or platinum-refractory (defined as lack of response or progression within 1 month since the last dose);

2) Participants were randomly assigned in a 2:1 ratio to the test and control groups, and stratified by platinum-free interval;

—Test group：

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle)

Manganese chloride was nebulized inhaled at 0.4mg/kg twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle.

—Control group：

nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

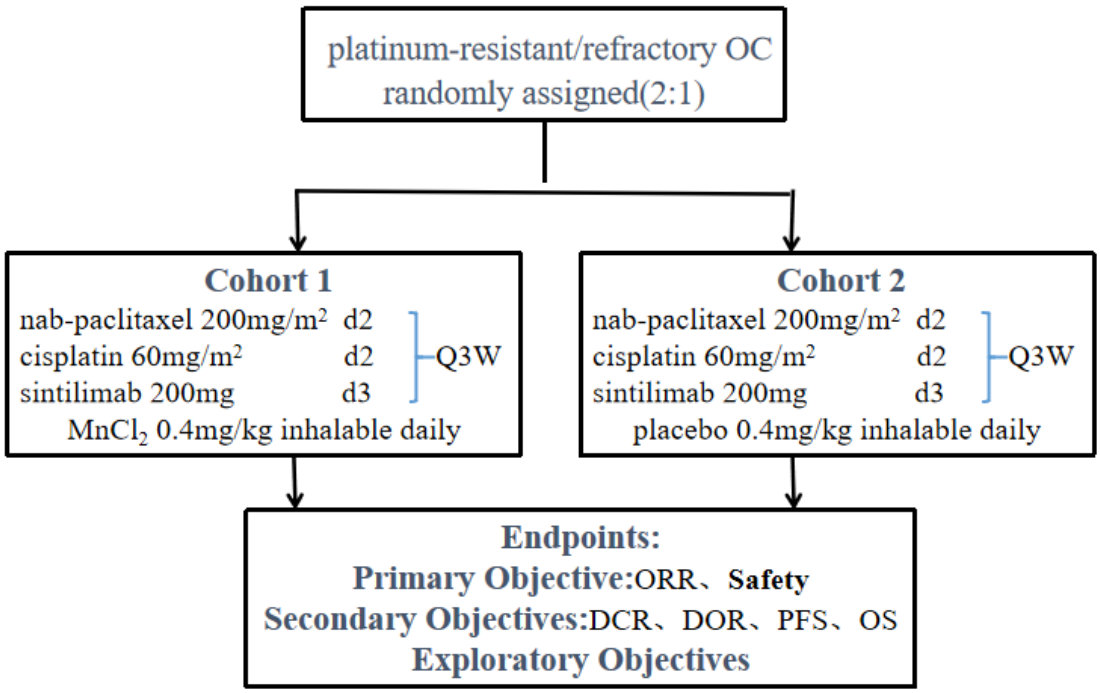
cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle)

sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle)

Placebo was nebulized inhaled twice per week in the first 3-week cycle, and thereafter twice in the first week of each 3-week cycle.

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.

Figure 1. Study Schema



Cohort 1：Test group (Mn^{2+} plus immunochemotherapy); Cohort 2：Control group (); n=3,4,5…

The study will be conducted in conformance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of International Council for Harmonisation.

3.2 General Study Conduct

In this study, the screening period did not exceed 21 days, and qualified subjects who completed the screening examination and evaluation were randomly divided into blind groups and entered the study treatment period (every 21 days was a treatment cycle), and they were treated and visited in accordance with the protocol.

Following informed consent, all patients will undergo screening procedures within 21 days prior to the first dose of study treatment to determine eligibility for study entry.

Screening procedures include medical, surgical, cancer, and medication history; complete physical examination, including vital signs, height, and weight; Eastern

Cooperative Oncology Group (ECOG) performance status; clinical laboratory tests (complete blood count [CBC], coagulation, chemistry, thyroid-stimulating hormone [TSH], triiodothyronine [T3] or free T3 [FT3], free thyroxine [FT4], serum CA-125), blood Mn^{2+} parameters and electrocardiogram (ECG). see Study Manual for details on sample collection and preparation) and will be sent to a centralized laboratory for biomarker testing. Radiographic evaluations (magnetic resonance imaging [MRI, preferred method]) or computed tomography [CT] of abdomen, and pelvis must be conducted at screening to determine extent of disease and confirm presence of measurable disease. The chest must be examined by computed tomography [CT]. Head scan will be conducted if clinically indicated; bone scans will be conducted per standard of care. Scans performed prior to the signing of the informed consent form (ICF) as part of routine clinical management are acceptable for use as initial tumor imaging if they are of diagnostic quality and are performed within 21 days prior to first dose date.

Safety assessments were conducted throughout the treatment period include symptom-directed physical examination, vital signs, ECGs, ECOG performance status, and clinical laboratory assessments (CBC, coagulation, chemistry, TSH, T3 or FT3, FT4, urinalysis, CA-125, blood Mn^{2+} parameters). Radiographic evaluations all patients to assess extent of disease will be conducted every 6 weeks (42 days ±7 days) after Cycle 1/Day 1 while on study treatment independent of cycle delays and/or dose interruptions and/or at any time when progression of disease is suspected. All radiographic images/scans will be sent to a central imaging vendor upon acquisition and archived for future evaluation if needed. Per RECIST v1.1, patients who achieved complete response (CR) or partial response (PR) should have the response confirmed; tumor imaging for confirmation of response may be performed at the earliest 3 weeks after the first indication of response, or at the next scheduled scan, whichever is clinically indicated.

Safety and tolerability will be evaluated throughout the study by recording adverse events, monitoring vital signs and physical examination, laboratory evaluation of safety, and 12-lead electrocardiogram (ECG).

All AEs and SAEs experienced by a patient, regardless of suspected causation, will be monitored until the AE or SAE is resolved, until abnormal laboratory values are returned to baseline or normalized, until there is a satisfactory explanation for observed changes, until the patient loses follow-up, or until the patient dies.

3.3 Randomization and Blind Method

This is a prospective, randomized, single-blind clinical investigation. Participants will be blinded to their treatment assignment and the study site personnel will be trained not to disclose the treatment assignment to the subject. Participants will be given a treatment drug or placebo with similar physical properties to reduce the possibility of unblinding. Participants blinding must be maintained until the completion of the trial.

The physician performing the treatment will not be blinded to the assigned treatment. Thus, the efficacy evaluations were performed by blinded radiologists and a different physician in order to reduce bias and maintain participants blinding. If clinical follow-up with a study physician is deemed necessary at the protocol required follow-up time points, the different physician (or designee) must conduct the follow-up clinical visits. Site personnel will be adequately trained such that the physician (or designee) conducting the clinical follow-up is adequately blinded to the treatment received by the participants. For unscheduled visits, subjects may see the physician who performed the treatment. The treating physician should prevent unblinding of the subject when they conduct any non-protocol related visits.

4. STUDY POPULATION

4.1 Inclusion Criteria:

1) Patients with histologically or cytologically confirmed epithelial ovarian cancer, primary fallopian tube cancer, or peritoneal cancer.

2) Patients who have received at least one chemotherapy regimen based on platinum compounds.

3) Patients who experienced disease progression during treatment or within 6 months after completion of the above treatment regimen.

4) Patients with at least one measurable lesion.

5) Patients who have received immunotherapy (anti-PD1 antibodies, anti-PD-L1 antibodies), or bevacizumab, are not restricted from enrollment.

6) Expected survival of more than 6 months.

7) Age over 18 years.

8) Patient weight > 40 kg.

9) ECOG performance status ≤ 2.

10) Peripheral blood white blood cell count > 3.5×10^9/L.

11) Bone marrow reserve and liver and kidney function (the following laboratory tests should be performed before initial treatment to prove):

- Absolute neutrophil count (ANC) ≥ 1,000/mm^3.
- Hemoglobin > 80 g/dL.
- Platelet count > 80,000/mm^3.
- ALT/AST < 3×ULN

- Serum creatinine < 3×ULN.

- Total bilirubin level < 3×ULN.

12) No significant hereditary diseases.

13) Normal liver and kidney biochemical indicators.

14) At least 4 weeks since the end of previous ovarian cancer treatment, and substantial recovery from adverse reactions of previous treatment (graded according to CTCAE5.0, excluding alopecia).

15) No treatment with paclitaxel (albumin-bound) plus platinum in the past 3 months.

16) Voluntary participation and signing of informed consent, compliance with the trial treatment plan and visit schedule, and cooperation in observing adverse events and efficacy.

4.2 Exclusion Criteria:

Subjects meeting any of the following conditions cannot be selected as subjects:

1) Absolute neutrophil count (ANC) < 1×10^9/L or platelets < 80×10^9/L or hemoglobin < 80 g/dL (according to the normal values of the clinical trial center).

2) Serum total bilirubin levels higher than 3 times the upper limit of the reference range.

3) ALT, AST, or ALP levels higher than 3 times the upper limit of the reference range when there are no liver metastases; ALT, AST, or ALP levels higher than 5 times the upper limit of the reference range when there are liver metastases.

4) Patients receiving known strong inhibitors (e.g., ketoconazole) or inducers (e.g., rifampicin or St. John's Wort) of CYP3A4, or strong inhibitors (e.g., gemfibrozil) or inducers of CYP2C8; patients receiving treatment with potent P-gp inhibitors or inducers, or patients who cannot stop treatment with these agents for 2 weeks before the start of the study.

5) Toxic reactions to previous antitumor therapy that have not recovered to Grade 0 or 1 (excluding alopecia).

6) Organ failure:

- Heart: Grade III or IV.

- Liver: Grade C according to Child-Pugh B liver function classification.

- Kidney: Renal failure and uremia.

- Lung: Severe respiratory failure symptoms.

- Brain: Consciousness disorders.

7) T-cell tumors such as T-cell lymphoma or T-cell leukemia.

8) Major surgery within 4 weeks before enrollment, or planned major surgery during the study (excluding diagnostic surgery).

9) HIV antibody positive, or other acquired, congenital immunodeficiency diseases, or patients with a history of organ transplantation.

10) History of Parkinson's disease or epilepsy, or occurrence of diseases that can induce seizures within 12 months before the study (including a history of transient ischemic attack, stroke, traumatic brain injury with consciousness disorders).

11) CTCAE grade 2 infections that do not respond to treatment or active clinically serious infections with CTCAE > grade 2.

12) Patients with active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection requiring treatment.

13) Patients with a history of allergic reactions to study drug components or suspected allergies.

14) Chronic diseases requiring immunotherapy or hormone therapy.

15) Patients who have received any investigational drug treatment within 30 days

before the first dose of the trial drug.

16) Subjects with symptoms of brain or leptomeningeal metastasis.

17) Subjects with a history of (non-study tumor) malignant tumors within 3 years before the first dose of the trial drug.

18) History of the following diseases: interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases including diabetes, hypertension, pulmonary fibrosis, acute lung disease, abdominal infection, etc.

19) Subjects with clinical symptoms of intestinal obstruction persisting before the first dose of the experimental drug; subjects with incomplete intestinal obstruction who have returned to normal within 1 month before the first dose of the experimental drug can be included in the study after discussion by the researchers.

4.3 Discontinuation from Treatment

Termination of study treatment does not mean withdrawal from the study. Subjects who discontinue study treatment must continue to complete the remaining follow up as required by the protocol. The investigator should consider arranging for the subject to terminate study treatment if any of the following conditions occur:

1) The second confirmed complete response.

2) The subject is unwilling to continue:

- The subject discontinues the drug on their own and withdraws from the trial.
- Loss to follow-up due to work, changes in living environment, or accidental accidents.
- The subject withdraws informed consent.

2) Intolerable adverse events:

- Due to significant abnormal laboratory indicators or adverse events related to

safety (such as severe allergic reactions), the investigator believes it is not appropriate to continue the use of the investigational drug based on ethical considerations.

- Requires rescue due to severe adverse events.

- Other serious illnesses occurred during the trial period, and the investigator judged it inappropriate to continue treatment.

3) Disease progression or the need for other treatments:

- Imaging studies define disease progression.

- It is judged by the investigator that new antitumor treatment or other treatments not allowed by the protocol are needed.

- If the subject experiences tumor progression during the trial, study treatment should be terminated. However, if the investigator believes that continuing study treatment may have clinical benefits and the subject does not receive other tumor treatment measures, the subject can continue study treatment after discussion by the investigator.

4) Other reasons that the investigator believes to be impossible to continue study treatment:

- Significant protocol deviations discovered after enrollment.

- If the delay in treatment exceeds 21 days (the time of one treatment cycle) from the next planned treatment time, the investigator should consider whether to withdraw from the clinical trial based on the actual benefits to the subject.

4.4 Study Withdrawal

Subjects can freely withdraw from the study at any time, and the investigator or sponsor may arrange for the subject to withdraw from the study due to safety concerns, inability to comply with the trial protocol, or other reasons. Reasons for subjects'

withdrawal may include:

- The subject withdraws informed consent and refuses to participate in the study further.
- Other necessary reasons for withdrawal from the study as determined by the investigator.
- At any time, patients are found not to meet the enrollment criteria.
- Loss to follow-up.
- Subject death.
- The sponsor terminates the study.

The reasons for subjects' withdrawal must be recorded in the case report form and the subject's medical record.

5. STUDY MEDICATION

5.1 Investigational Drugs

5.1.1 Paclitaxel (Albumin-bound)

a. Mechanism:

1) In the process of cell division, microtubule proteins that make up the spindle apparatus (comprising α-tubulin and β-tubulin) can bind to specific sites on microtubule proteins in cancer cells, preventing their depolymerization. This leads to the accumulation of microtubule proteins within the cell. And microtubule proteins and microtubule dimers can interconvert, during cell division, spindle microtubules cannot be rapidly synthesized by microtubule proteins, causing the cell division to arrest in the G2 and M phases. This prevents replication and ultimately leads to the death of cancer cells, thereby acting to kill cancer cells.

2) Albumin-bound paclitaxel (nab-paclitaxel): Utilizing unique nanotechnology to bind hydrophobic paclitaxel with albumin, it takes advantage of the natural transport mechanism of albumin (gp60-albumin-SPARC) to distribute paclitaxel more in tumor tissues, achieving higher concentrations within tumor cells.

3) Immunogenicity: Enhancing the release of tumor antigens and improving the anti-tumor response of immune checkpoint inhibition. In addition, albumin-bound paclitaxel can activate Toll-like receptors and enhance dendritic cell activity.

5.1.2 Cisplatin

Cisplatin is a heavy metal complex formed by combining divalent platinum with two chlorine atoms and two ammonia molecules. Similar to bifunctional alkylating agents, it can inhibit the DNA replication process. DDP cells are the most sensitive, and at high concentrations, it can also inhibit RNA and protein synthesis. This substance has an effect on anaerobic cells and can diffuse through charged cell membranes after entering the human body. Currently, it is believed that the primary site of action for DDP is in the purine and pyrimidine bases of DNA.

Cisplatin belongs to the category of cell cycle non-specific drugs. It possesses cytotoxicity and can inhibit the DNA replication process in cancer cells while damaging structures on their cell membranes. As a result, it exhibits a strong broad-spectrum anticancer effect.

5.1.3 Sintilimab

Sintilimab is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Sintilimab injection is a sterile, non-pyrogenic lyophilized powder for IV infusion supplied in single-use Type I glass vial containing 100 mg of Sintilimab. The product is preservative-free, white to off-white powder and free from visible foreign matter.

5.1.4 Manganese Chloride

Manganese chloride with the molecular formula $MnCl_2$ and a molecular mass of 125.84, is also known as chloromanganese, dichloromanganese, tetrahydrate manganese chloride, and so on. Hydrated manganese chloride appears as rose-colored monoclinic crystals, while anhydrous manganese chloride appears as pink crystals. In clinical settings, $MnCl_2$ is often used for intravenous nutrition to supplement the body's required manganese metal element.

5.2 Administration

5.2.1 Manganese Administration

Participants were randomly assigned to the test group and the control group in a 2:1 ratio.

The administration method of $MnCl_2$ in the experimental group was as follows:

1) Manganese chloride was nebulized inhaled at 0.4mg/kg twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle.

2) nebulized inhaled

- Body Position: Prior to treatment, cough up phlegm to prevent hindrance to the penetration of aerosol droplets. During nebulized inhalation, it is recommended to sit, semi-sit, or lie on one's side, while avoiding the supine position as much as possible. If a supine position is necessary, the head of the bed should be raised by 30°. During treatment, patients should take slow and deep inhalations, pause briefly at the end of inhalation to allow for deeper penetration of the aerosol droplets.

- For patients undergoing long-term nebulized inhalation therapy, the amount of aerosol used must be moderate. Over-humidification can lead to increased phlegm production. In critically ill patients with altered mental status or reduced cough reflex, the inability to expectorate phlegm promptly can worsen their condition or even lead to death. If the nebulization is insufficient, it can be challenging to achieve the treatment goals.

- During nebulized inhalation, water vapor can effectively moisten the airways. However, the expelled aerosol has some pressure, which displaces the surrounding air from entering the respiratory tract and reduces oxygen intake. Therefore, for patients with asthma, breathing difficulties, severe hypoxia, and those with pneumonia complicated by heart failure, it is necessary to improve the above symptoms, increase oxygen intake before proceeding with nebulized inhalation. Inhalation time should be short, around 5 minutes per session, to prevent worsening of hypoxia. Pay close attention to changes in the patient's condition during nebulized inhalation. If symptoms such as coughing and shortness of breath occur, stop nebulized inhalation immediately, increase oxygen intake, perform back percussion, drink water, and consider the next nebulized inhalation treatment only when the symptoms have eased. Additionally, check the temperature, dosage, and body position during nebulization, and make necessary adjustments.
- To prevent cross-infections in the respiratory tract during nebulized inhalation therapy, the following measures should be taken: the nebulizer must be thoroughly disinfected before use and replaced daily; when not in use, there should be no liquid residue in the entire system to prevent bacterial growth; sterile solution should be used during nebulization therapy.

The placebo administration method for the control group was as follows:

1) In the first cycle, a placebo was nebulized and inhaled twice per week during the initial 3-week cycle.
2) In the second cycle and each subsequent cycle, a placebo was nebulized and inhaled twice in the first week of each 3-week cycle.
3) The nebulization inhalation method was the same as that of the test group.

5.2.2 Anti-PD-1 antibody plus chemotherapy treatment

Enrolled patients will receive intravenous chemotherapy plus anit-PD-1 Antibody. The immunochemotherapy consisted of nab-paclitaxel (200 mg/m^2, administered

intravenously on day 2 in a 3-week cycle), cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle) and sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle).

If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.

5.3. Dose Modification

According to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE version 5.0), if any grade 3 or 4 adverse events occur during treatment and the investigator determines that they are related to the research protocol, the treatment must be interrupted. If the toxicity returns to normal or grade 1（or lower） within 21 days, the patient can resume treatment with the investigational drug. However, if grade 3 or 4 adverse events recur despite preventive measures, the dose level may be reduced as needed. If the adverse events occur again, treatment should be interrupted again, and after resolution, the dose must be further reduced. Dose reduction should not exceed two times. If a patient has already experienced a maximum of two dose reductions and the toxicity has not returned to normal or grade 1 adverse events within 21 days, treatment must be permanently discontinued.

To maintain the dose intensity and cumulative dose of this study, a reasonable dose adjustment plan will be implemented to minimize the occurrence of dose reductions and treatment delays. Any subject with a treatment delay must be assessed weekly until adequate hematological and non-hematological parameters are met. Then, the treatment plan will proceed in the normal sequence.

All treatment interruptions and dose reductions (including any missed doses) and the reasons for reduction/interruption should be documented in the electronic Case Report Form (eCRF).

5.3.1. Dose Reductions or Delays for cisplatin and nab-paclitaxel

5.3.1.1 Guidelines for Hematologic Toxicity

Initial therapy modifications will consist of cycle delay and/or dose reduction as directed. Treatment decisions will be based on the ANC rather than the total white cell count.

Before each treatment cycle, blood routine tests will be conducted. Medication can be administered if the absolute neutrophil count (ANC) is ≥ 1000/μL and the platelet count is ≥ 75,000/μL. Subjects with an ANC between 1000-1500/μL or a platelet count between 75,000-100,000/μL will be able to continue treatment, with dose modifications as shown in Table 1. If the specified values are not reached, treatment may be postponed for up to three weeks until these values are achieved. For subjects who do not recover their counts within 3 weeks as shown in Table 1, the investigator should be notified.

Table 1. Dose Modifications for Dose Limiting Hematologic Toxicity or Reduced ANC (1000–1500/μL) or Reduced Platelets (75,000–100,000/μL)

ANC	PLT	Regimen –1 Level	Regimen –2 Level	Regimen –3 Level
Yes	No	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue
Yes	Yes	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue
No	Yes	nab-paclitaxel 170 mg/m^2 and add G-CSF	nab-paclitaxel 140 mg/m^2 and add G-CSF	Discontinue

*Considering that hematological toxicity is primarily associated with nab-paclitaxel, dose adjustments should primarily focus on adjusting the dose of nab-paclitaxel.

Guidelines for the Use of Hematopoietic Cytokines

The use of hematopoietic growth factors is restricted as follows:

a) Generally, subjects should not receive prophylactic filgrastim (G-CSF), PEG-filgrastim (Neulasta), or sargramostim (GM-CSF) unless they experience treatment delays, dose omissions, or complications of recurrent neutropenia. Specifically, hematopoietic growth factors should not be used to avoid modifications of the initial treatment dose as specified in the protocol. However, subjects may also receive growth factor treatment for complications of neutropenia based on institutional treatment guidelines.

b) If dose adjustments are required per dosing modification guidelines, it is recommended to initiate daily subcutaneous administration within 24-72 hours after the last chemotherapy.

c) Subjects will not receive prophylactic platelet stimulants.

d) Subjects may receive erythropoiesis-stimulating agents, iron supplements, and/or blood transfusions to correct treatment-related anemia. The treating physician should be aware of the potential risks of erythropoiesis-stimulating agents, including the potential risk of shortening tumor progression or disease-free survival. These agents are used solely to avoid red blood cell transfusions and should not be used in patients with uncontrolled hypertension, as they may increase the risk of thrombotic events in cancer patients.

e) Subjects should not receive amifostine or other protective agents.

5.3.1.2 Guidelines for Non-Hematologic Toxicity

The management of grade 3 or 4 non-hematological toxicities related to treatment (excluding alopecia, fatigue, allergic reactions, nausea, vomiting, constipation, diarrhea, hypokalemia, hypomagnesemia, hypocalcemia, hyponatremia, and hypophosphatemia) should follow the specific dose level adjustments outlined in this section. The table 2

below is applicable to dose level modifications related to non-hematological toxicities:

Table 2. Modifications for Non-Hematologic Toxicity

Drug	Regimen –3 Level (Continued treatment-related CTCAE Grade 3 or 4 AE or SAE ≥ 28 days)	Regimen –2 Level (2nd dose reduction for treatment-related CTCAE Grade 3 or 4 AE or SAE where prophylaxis is not considered feasible)	Regimen –1 Level (1st dose reduction for treatment-related CTCAE Grade 3 or 4 AE or SAE where prophylaxis is not considered feasible)	Regimen Starting Dose
nab-paclitaxel	Discontinue	140mg/m^2	170 mg/m^2	200 mg/m^2
cisplatin	Discontinue	40 mg/m^2	50 mg/m^2	60 mg/m^2

Abbreviations: AE = adverse event; CTCAE = Common Terminology for Adverse Events; SAE = serious adverse event.

Peripheral Neuropathy

Peripheral neuropathy Grade 2 (or greater) requires reduction of one dose level in nab-paclitaxel (both dosing schedules) and delay in all subsequent therapy for a maximum of three weeks until recovered to Grade 1. If peripheral neuropathy fails to recover to Grade 1 by a maximum delay of three weeks from time therapy is due, the medical monitor should be contacted. If neuropathy of grade 2 (or higher) recurs after two dose reductions of nab-paclitaxel, the medical monitoring device should be contacted. If grade 3 or higher neuropathy occurs, the use of nab-paclitaxel should be discontinued.

Renal Toxicity

Renal toxicity is primarily caused by cisplatin. If creatinine levels increase to grade 3 or higher during the treatment process, it is necessary to reduce the dose level of cisplatin and delay treatment for up to 3 weeks until it recovers to grade 1 or lower. If grade 3 (or higher) elevation in creatinine cannot be restored within three weeks or does

not recover despite dose adjustments, treatment with cisplatin should be discontinued, and subsequent treatment cycles should not include cisplatin but should be replaced with carboplatin.

Hepatic Toxicity

If there is a Grade 3 (or higher) increase in SGOT (AST), SGPT (ALT), alkaline phosphatase, or bilirubin, it is necessary to reduce the dose of nab-paclitaxel by one level and delay treatment for up to 3 weeks until it recovers to Grade 1 or lower. If the Grade 3 (or higher) increase does not recover within three weeks or does not recover despite dose adjustment, treatment should be discontinued, and the investigator should be contacted.

Hypersensitivity Reaction

In general, the occurrence of allergic reactions to cisplatin or albumin-bound paclitaxel is not considered a dose-limiting toxicity. With measures such as adjusting infusion rates and strict drug management to prevent allergic reactions, full doses can still be administered. However, if despite these safety measures, repeated attempts at infusion still result in severe hypersensitivity reactions, the drug should be discontinued for the participant. Severe allergic reactions to albumin-bound paclitaxel do not need to be rechallenged, and after an allergic reaction to cisplatin, it can be replaced with carboplatin for treatment.

Other Toxicity

For alopecia, nausea, constipation, diarrhea, hypokalemia, hypocalcemia, hypomagnesemia, hyponatremia, or hypophosphatemia, dose adjustments are not required. It is recommended to manage and correct nausea, constipation, diarrhea, and electrolyte abnormalities using standard medical measures.

Nausea, Vomiting, or Fatigue

For ≥ 3-grade nausea, vomiting, or fatigue mainly caused by cisplatin, prophylactic antiemetic therapy should be administered before dosing until symptoms are relieved to ≤1-grade. If active prophylactic antiemetic therapy is given, and the patient still cannot be relieved, experiencing severe electrolyte imbalances, shock, or other life-threatening events, cisplatin should not be used in subsequent treatment cycles, and it should be replaced with carboplatin.

Other non-hematologic toxicity dose adjustments are as follows:

a) For any 3-grade non-hematologic adverse events (except manageable nausea/vomiting, constipation, diarrhea, hypokalemia, hypomagnesemia, hypophosphatemia, or hyponatremia) considered related to the study treatment, treatment should be initiated, and the dose of the drug most likely to cause toxicity should be reduced by one dose level until symptoms are relieved to ≤1-grade or return to normal. If >3-grade adverse events persist for 3 weeks or recur after recovery, the subject may discontinue treatment after discussing with the investigator.

b) Any 4-grade non-hematologic adverse events related to treatment (except manageable nausea/vomiting, constipation, diarrhea, hypokalemia, hypocalcemia, hypomagnesemia, hypophosphatemia, or hyponatremia) should be discussed with the investigator, and the subject may stop treatment or reduce the dose.

5.3.2 Dose Reductions or Delays for sintilimab

Adverse events (both non-serious and serious) related to exposure to sintilimab may be indicative of an immune-related condition. These AEs can occur shortly after the first dose or several months after the last dose of treatment. According to Table 3, if drug-related toxicities and serious or life-threatening adverse events occur, the use of sintilimab must be discontinued. Please also refer to Clinical Event of Interest (ECIs).

Table 3 provides detailed information on dose interruptions and discontinuations related to sintilimab toxicity. Reducing the dose of sintilimab is not allowed.

Table 3 Sintilimab Dose Modifications for Non-hematologic Toxicities

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hypothyroidism		Continued while thyroid replacement therapy is instituted	Continued while thyroid replacement therapy is instituted
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue.
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with sintilimab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Infusion reaction	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue.
AST, ALT, or increased bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue	Permanently discontinue.
Diarrhea/colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or

			less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue.
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4 or Recurrent 2	Permanently discontinue	Permanently discontinue.
Renal failure or nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All other drug related toxicity	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Abbreviations: AE = adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase; T1DM=type 1 diabetes mellitus

The reasons for interrupting or discontinuing the administration of sintilimab should be documented in the eCRF (electronic case report form).

5.3.3 Dose Reductions or Delays for Manganese chloride or placebo

Exposure to Manganese chloride primarily causes manganese ion poisoning. Since

manganese poisoning is rare in everyday life and there is limited treatment experience, it is recommended to carefully observe patients and reduce their exposure risk. If a case of manganese ion poisoning occurs, usage should be stopped, and reducing the dose for further use is not allowed.

5.4. Packaging, Labeling, and Storage

5.4.1 Basic Information on Drugs

1) Storage and Preservation of Manganese Chloride:

   Drug Specification: 100mg (10ml) per bottle.

   Drug Storage: Keep in dark place

   The appearance of the placebo is the same as that of manganese chloride, and drug storage follows the same guidelines as for manganese chloride.

2) Sintilimab injection is packaged in small boxes, with each box containing 1 vial, and each vial has a capacity of 10ml: 100mg, stored in a light-protected manner at 2-8°C.

3) Nab-paclitaxel (albumin-bound) is available in vials with a capacity of 100mg each.

4) Cisplatin is available in vials with a capacity of 10mg each.

The label text of the study treatments will comply with Good Manufacturing Practice and national legislation to meet the requirements of the participating countries. The study treatment will be open-label and non-patient-specific.

All study treatment supplies must be stored in accordance with the Pharmacy Manual instructions and package labeling. Until dispensed or administered to the patients, the study treatment will be stored in a securely locked area, accessible to authorized personnel only.

5.4.2 Drug Packaging and Labels

Each package box is affixed with a drug label. The drug label contains information such as protocol number, drug name, drug code, specification, production batch number, expiration date, storage conditions, usage instructions, precautions, and sponsor information, etc.

The vials are also affixed with drug labels containing the same information as the package box labels. The drug labels include the protocol number, drug name, drug code, specification, production batch number, expiration date, storage conditions, and sponsor information, etc.

All packaging and drug labels for investigational drugs are clearly marked with 'For Clinical Trial Use Only.

5.5. Drug Accountability

5.5.1 Drug Dispensing

The Investigator or designee is responsible for maintaining accurate dispensing records of the study treatments throughout the clinical study.

Details of maintaining drug accountability, including information on the accountability log, will be provided in the Pharmacy Manual.

All dispensation and accountability records will be available for Sponsor review. The study monitor will assume the responsibility to reconcile the study treatment accountability log. The pharmacist will dispense study treatment for each patient according to the protocol and Pharmacy Manual, if applicable.

5.5.2 Drug Dispensing, Retrieval, and Disposal

The management, dispensing, and retrieval of investigational drugs are the responsibility of designated personnel at each research center. Researchers must ensure that all investigational drugs are used exclusively for participants in this study, and their

dosage and usage should follow the research protocol. Any remaining or expired investigational drugs must be returned to the sponsor or disposed of in accordance with medical waste standards.

If there are quality issues such as turbidity or precipitation with the investigational drug, another vial of the drug may be used, and the sponsor should be promptly contacted for replacement.

Investigational drugs should be stored as per label requirements, and the dispensing and retrieval of each unit of investigational drug should be promptly recorded on a dedicated record sheet.

Monitors are responsible for overseeing the supply, use, storage, and handling of remaining drugs in the investigational drug process.

5.6 Past and Concurrent Drug Therapy

Throughout the entire research process, medications can be taken for the treatment of pre-existing conditions present before the subject's enrollment or for new conditions that may arise during the study. In addition to excluding medications prohibited by the criteria and protocol, researchers may decide to administer other medications that are necessary for the safety and health of the participants. Researchers can provide any necessary concomitant medication or treatment to provide comprehensive therapeutic support.

Apart from the investigational treatment, any medications taken by patients during the study, including herbal and other non-traditional medicines, as well as blood products or vaccines, are considered concurrent medications. All concurrent medications must be documented in the eCRF (electronic Case Report Form). Each concurrent medication must include the following information in the eCRF: generic name, route of administration, start date, end date, dosage, and indication. Any changes in the dosage or regimen of concurrent medications must also be recorded in the eCRF.

During screening, patients will be asked about any medications they have taken in the past 30 days. During subsequent study visits, patients will be asked about the concurrent medications they are currently taking or have taken.

5.6.1 Permitted Concomitant Medication or Treatment

The following medications are allowed at the discretion during the study period:

1) Long-term medications required for underlying conditions such as hypertension and diabetes.

2) Medications determined by the investigator to be in compliance with the protocol requirements (such as concomitant medications for treating various adverse events).

— The short or long-acting granulocyte colony stimulating factor (G-CSF) can be given if the patients suffer severe neutropenia (above grade 3) and stopped 24 hours before treatment.

— Hydration and alkalization as routine treatment during chemotherapy will be allowed.

— Appropriate auxiliary drug, such as liver-protective, heart-protective and stomach-protective, are permitted to perform concomitant with investigational drugs

— Prophylactic Antiemetics: Prophylactic antiemetic drugs may be administered based on the common gastrointestinal adverse reactions observed in previous studies (such as nausea and vomiting). Depending on the actual condition of the subject, prophylactic antiemetic drugs may be given before the study treatment. Recommended prophylactic antiemetic drugs include, but are not limited to, 5-HT3 receptor antagonists, NK-1 receptor antagonists, gastric mucosal protectants, etc. Prophylactic antiemetic treatment should be administered according to the standard treatment protocols of medical institutions, published guidelines, and the respective drug labels.

3) Supportive Treatment for Alleviating Tumor-Related Symptoms, such as

bisphosphonate therapy for bone metastases.

4) Researchers, prioritizing the well-being and safety of the subjects, may provide symptomatic supportive treatment for drug toxicity reactions or to manage tumor symptoms.

5.6.2 Prohibited Concomitant Medication or Treatment

During the trial, the use of any other antitumor treatments not specified in the protocol is prohibited. This includes cytotoxic drugs, radiotherapy, biologic therapy, immunosuppressive agents, or any other investigational drugs with anticancer properties. Traditional Chinese medicines with anticancer effects are also not allowed to be used in this study. Hormone replacement therapy (e.g., oral contraceptives) or local corticosteroid therapy (e.g., topical or inhaled) not intended for antitumor treatment is prohibited. The use of any other clinical research drugs besides the investigational drug specified in this protocol is also not allowed. If a subject requires such medications, they should be excluded from the trial.

5.7 Treatment Compliance

The dosage and administration method of the investigational drug for each subject will be recorded on the corresponding eCRF (electronic Case Report Form) for each cycle. Reasons for delayed or missed administrations should be documented. Compliance with treatment will be assessed using this information. Subjects will self-administer nebulized inhalation of manganese chloride/placebo and keep records on a log card. They will be required to return any unused empty packages for inventory during the next scheduled visit.

6. ENDPOINTS AND METHODS OF ASSESSMENT

6.1 Tumor Response Evaluation

The efficacy endpoints of this study's combined treatment regimen will be

evaluated based on the RECIST v1.1 criteria.Tumor marker data (CA-125) will not be used to define objective response or disease progression but may serve as reference data for clinical decision-making. Clinical criteria, such as the GCIG criteria detailed in Appendix F, will also be considered for patients with ovarian cancer presenting clinical events (e.g., bowel obstruction) without radiological evidence of disease progression.

Efficacy assessments will primarily be conducted through imaging data, with imaging examinations completed at the times specified in Table 6 and archived for future evaluation.

Tumor imaging for pelvic, abdominal, and skull lesions should be performed using magnetic resonance imaging (preferred) or CT, and chest imaging should be performed using CT. The same imaging technique should be used throughout the trial.

If the chest is normal at the time of screening, repeat imaging of the area is not necessary in the absence of clinical indications that require follow-up.Bone scans should be conducted based on clinical necessity.

6.1.1 Timing of Imaging Evaluations

Initial imaging at screening must be performed within 15 days prior to the first dose study treatment date.

Scans performed prior to ICF sign-off as part of routine clinical management may be used as initial tumor imaging if they are of diagnostic quality and performed within 15 days prior to the date of the first dose.

The first study imaging assessment should occur 6 weeks after the initial study treatment; for Progressive Disease (PD), a confirmation is required 3 weeks later. Subsequent imaging should be conducted every 6 weeks, or more frequently if clinically indicated and suspected of disease progression. After 1 year of imaging assessments, patients will undergo imaging every 12 weeks. Imaging should not be delayed by delays in cycle initiation or longer intervals between cycles of combination therapy.

According to RECIST v1.1, Complete Response (CR) or Partial Response (PR) should be confirmed through repeated tumor imaging assessments. Imaging to confirm responses can be performed 3 weeks after the first response or at the next scheduled examination (i.e., 6 weeks later), as clinically necessary.

Imaging will continue until any of the following occurs:

- Initiation of a new anti-tumor treatment,

- Withdrawal of consent by the patient,

- Death,

- End of the study.

Patients who discontinue study treatment for reasons other than disease progression will continue to have imaging assessments at the same frequency as their original follow-up.

6.1.2 Response Assessment by RECIST

RECIST v1.1 will be used by investigators as the primary measure for assessing tumor response, disease progression, and as the basis for all protocol guidelines related to disease status.

Detailed information on RECIST v1.1, including the assessment of target and non-target lesions and definitions of response, can be found in Appendix 3.

6.2 Primary Endpoint

6.2.1 Objective Response Rate (ORR)

Within the entire intention-to-treat population, the proportion of subjects achieving a Complete Response (CR) or Partial Response (PR) as assessed by RECIST from enrollment until the end of treatment for any reason.

Proportion of subjects achieving complete response (CR) or partial response (PR)

as assessed by the investigator according to RECIST from the start of enrollment to the end of treatment for any reason in all INTENTION-TO-TREAT populations.

Data obtained prior to progression, or last assessed in the absence of progression, will be included in the objective response assessment. CR assessments should be confirmed through CT/MRI evaluations. Any CR or PR occurring after further anti-cancer treatment will not be included in the ORR analysis.

6.2.2 Treatment-Emergent Adverse Events (TEAEs) and Severity

This includes the incidence of treatment emergent serious adverse events (TESAEs), abnormalities in clinical laboratory markers, and other examination results.

6.3 Secondary Endpoints

6.3.1 Progression-Free Survival (PFS)

PFS is defined from enrollment to the time of objective tumor progression or death from any cause, whichever comes first. For subjects who die without reported progression, progression is assumed to have occurred on the date of death. Patients who did not have disease progression but withdrew from the study due to toxicity or other reasons, PFS was calculated as if they had been last identified.

6.3.2 Overall Survival (OS)

Overall survival will be assessed as a secondary endpoint and is defined as the time from date of first dose of study treatment to the date of death by any cause. New malignancy information will also be collected as part of this assessment.

6.3.3 Disease Control Rate (DCR)

Disease control rate will be assessed as a secondary endpoint and is defined as the proportion of patients achieving CR, PR, or stable disease (SD) as assessed by the Investigator per RECIST v1.1.

6.3.4 Duration of Response (DOR)

For participants confirmed to have achieved a complete response (CR) or partial response (PR), the DOR is defined as the time from the first documented objective response to the first occurrence of tumor progression or death due to any cause, whichever comes first.

6.3.5 CA-125 response rate

A response according to GCIG CA125 Criteria has occurred if there is at least a 50% reduction in CA125 levels from the pre-treatment (baseline) sample. The response must be confirmed and maintained for at least 28 days.

6.4. Pharmacokinetics

PK samples will be carried out according to samples schedule specified in the program and the checks will be carried out in the central laboratory. Sampling methods, sample storage, transportation and analysis are detailed in the Laboratory Manual provided by the Sponsor's designated central laboratory.

6.5. Biomarkers

Tumor tissue and peripheral blood samples will be collected according to the protocol requirements, and the changes of blood manganese concentration, immune cells and tumor microenvironment before and after treatment will be analyzed.Sampling methods, sample storage, transportation and analysis are detailed in the Laboratory Manual provided by the Sponsor's designated central laboratory.

6.6 Health status assessment

The health status of the subjects will be assessed using the FACT-G (Version 4) questionnaire. Five main areas are covered (mobility, self-care, daily activities,

pain/discomfort, and anxiety/depression) as well as an overall visual analog scale of health status.

7. ADVERSE EVENTS

7.1 Definition of Adverse Events

Investigators and any designated compliant personnel are responsible for investigating, documenting, evaluating, and tracking safety events, including Adverse Events (AEs), Serious Adverse Events (SAEs), and medication overdoses.

The AE is defined as any unfavorable medical occurrence in a clinical trial subject after administration of the trial medication, which can manifest as symptoms, signs, illness, or laboratory abnormalities, regardless of causality with the trial medication.

The AE could be the exacerbation of preexisting symptoms, signs, laboratory abnormalities, newly diagnosed diseases, and clinically significant laboratory abnormalities that were temporally related to drug use, regardless of whether they were causally related to the drug. Events caused by research procedures, collected after informed consent, must be recorded as AEs.

7.1.1 Serious Adverse Events

A Serious Adverse Event (SAE) refers to an AE that meet any of the following criteria at any stage of the clinical study:

1) Results in death;
2) Life threatening (where "life-threatening" means immediate risk of death at the time of the event, not hypothetical future severity);
3) Leads to hospitalization or prolongation of hospitalization;

Generally, hospitalization implies that the subject, due to the severity of their condition, cannot be observed and/or treated in an outpatient setting or doctor's office

and requires overnight stay at a hospital or emergency room for observation and/or treatment. Complications during hospitalization are considered AEs. An AE that complicates or prolongs hospital stay or meets other serious criteria is deemed "serious." When it is uncertain if hospitalization occurred or was necessary, the AE should be considered "serious."

4) Results in persistent or significant disability/incapacity (significantly impairs normal life functions);

5) Other significant medical events that could result in the conditions listed above if not treated. Medical and scientific judgment must be applied to decide whether other situations require rapid reporting, such as important medical events that may not immediately result in life-threatening situations, death, or hospitalization but could jeopardize the subject or require medical intervention to prevent one of the listed outcomes, and are generally considered serious.

Note: The following hospitalizations do not count as SAEs:

1) Stays in the emergency room or other hospital departments for less than 24 hours (unless deemed a significant medical event or life-threatening by the researcher based on facts);

2) Hospitalizations scheduled as part of the clinical study protocol;

3) Treatments or surgeries scheduled before study enrollment (should be recorded in the trial's original documentation and subject CRF);

4) Elective or planned treatments for pre-existing conditions not related to the study disease and not worsened;

5) Hospitalizations for selective treatments of pre-existing conditions that have not worsened compared to baseline;

6) Hospitalizations for medical management, insurance, or social reasons (e.g., check-up hospitalizations, rehabilitation/nursing home stays, or if the subject has no

caregiver).

7.1.2 Suspected and Unexpected Serious Adverse Reactions (SUSARs)

SUSARs refer to serious adverse reactions that are both unexpected and suspicious, exceeding the severity and nature anticipated in the Brochure, the marketed product's label, or the Summary of Product Characteristics.

7.2 Collection and Recording of Adverse Events

7.2.1 Collection of Adverse Events During Follow-up Period

The collection and reporting period for AEs begins after the participant signs the informed consent form (if not caused by the study procedure, it's recorded as medical history with specific dates and times) and continues until the end of the safety follow-up visit. AEs deemed unrelated to study treatment by the investigator will no longer be collected after this point. However, all AEs must be followed up until any of the following occurs:

1) Death or loss to follow-up;

2) The participant withdraws consent for further data collection;

3) Study termination.

7.2.2 Recording of Adverse Events

Researchers should record AEs or SAEs using medical terminology/concepts, avoiding colloquialisms and abbreviations. All AEs, including SAEs, must be recorded on an adverse event form. Researchers must comprehensively record every adverse event, including diagnosis (or symptoms, signs including laboratory abnormalities if no diagnosis), onset and end dates and times (if applicable), CTCAE severity grade and changes, whether it is a serious adverse event, actions taken regarding the investigational product, treatments given for the AE, and the outcome of the event, the relationship between the adverse event and the investigational product, and the

causality assessment with the study procedure.

For serious adverse events, researchers must also provide the date on which the AE met SAE criteria, the date on which the researcher became aware of the SAE, the criteria for SAE severity, admission and discharge dates (if applicable), possible causes of death (if applicable), the date of death (if applicable), and whether an autopsy was conducted (if applicable), along with the rationale for the relevance judgment and a description of the SAE.

Notes on AE Recording:

1) If a diagnosis is made, the diagnosis rather than individual symptoms and signs is recorded. If symptoms and signs cannot be determined to be caused by the diagnosis at reporting, they are recorded as separate AEs/SAEs. If symptoms and signs are determined to be caused by the diagnosis, only the diagnosis is reported, including the symptoms and signs. AEs are removed from the symptoms and signs record, and SAEs are updated with follow-up reports.

2) AEs resulting from other events or clinical sequelae should trace back to the primary event, unless the secondary event is more severe or constitutes an SAE. Significantly clinical secondary events occurring at different times from the primary event should be recorded separately.

3) Recurrent AEs are those that had resolved but reoccurred between two assessment points and should be recorded upon each occurrence.

4) Symptoms/signs present during the trial screening period are recorded and reported as adverse events only if they worsen in severity, frequency, or nature after trial entry, excluding condition worsening under study.

5) Symptoms and signs resulting from disease progression are not reported as AEs or SAEs unless the progression or symptoms change more severely than expected, or the researcher considers them related to the trial drug or procedure. Deaths should be reported as SAEs only if they result from an AE or if the cause of death is

undetermined, reported as "death of unknown cause."

7.2.3 Assessment of Adverse Event Severity

CTCAE terms and grades: Researchers will use descriptions and grading scales from the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 5.0 for AE reporting.

For events without assigned CTCAE grades, severity levels ranging from mild to life-threatening, corresponding to Grades 1 through 5, are defined as follows:

- Grade 1, Mild: asymptomatic or mild symptoms; only clinical or diagnostic observations required; no intervention indicated.
- Grade 2, Moderate: minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).
- Grade 3, Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4, Life-threatening consequences; urgent intervention indicated.
- Grade 5, Death related to AE.

7.2.4 Assessment of Causality Between Adverse Events and the Investigational Product.

Researchers should assess the reasonable possibility between the adverse event and the investigational product. "Reasonable possibility" implies that there are facts, evidence, and/or arguments suggesting a causal relationship rather than the inability to exclude causality.

Researchers will use clinical judgment and refer to the product information of marketed products to determine causality. The determination of causality will take full account of other causes, such as underlying conditions, concomitant treatments, and

other risk factors, as well as other events that are causally related to the study drug.

For each case of AE/SAE, Researchers had to document in the clinical record that they had reviewed the AE/SAE and made a causal assessment. In some cases, Researchers may not be able to provide sufficient additional information about SAEs that have occurred in the initial report, and Researchers are required to make a causal assessment of each event based on the available information. Researchers may update their view of the causal assessment as a result of subsequent follow-up information, but the SAE follow-up report needs to be supplemented with this updated causal assessment.

If researchers does not provide a causal assessment of an AE, including an SAE, the AE is considered to have a possible association with the trial drug that cannot be ruled out until the Researchers provides clarification.

The determination of whether an adverse event (AE) is attributed to the trial drug or other causes (e.g., natural history of the underlying disease, concomitant treatments) depends on the understanding of several aspects:

1) The known pharmacology of the trial drug.

2) The clinical and/or pathophysiological rationale.

3) Reactions observed in similar products in the past or reported in literature as related to the product.

4) The reasonableness of the temporal relationship between the event and drug administration or discontinuation.

AEs are categorized into five levels of association with the trial drug: definite, probable, possible, unlikely, and unrelated, with "definite, probable, possible" being considered related to the trial drug.

Criteria for determination include:

a. Definite – The AE *is clearly related* to the study treatment.

Evidence of drug use, reasonable temporal sequence, more plausible than other causes, positive dechallenge, positive rechallenge if feasible.

b. Probable – The AE *is likely related* to the study treatment.

Evidence of drug use, reasonable temporal sequence, more plausible than other causes, positive dechallenge.

c. Possible – The AE *may be related* to the study treatment.

Evidence of drug use, reasonable temporal association, can be explained by other causes.

d. Unlikely – The AE *is doubtfully related* to the study treatment.

Evidence of drug use, other causes may explain the AE better, negative or unclear dechallenge.

e. Unrelated – The AE *is clearly NOT related* to the study treatment.

No drug use, no temporal association with drug use, or another definite cause for the AE.

7.3 Adverse Reactions to Treatment Drugs

7.3.1 Chemotherapy Drug Adverse Reactions:

1) Hematological system: anemia, bone marrow suppression, thrombocytopenia, neutropenia and neutropenia with fever were reported in the clinical trials of this product. Neutropenia was dose-related and returned to normal. In a randomized controlled clinical trial involving patients with metastatic breast cancer, paclitaxel for injection (albumin-bound) was administered at a dose of 260mg/m^2 every 3 weeks; The dose of paclitaxel injection group was 175mg/m^2, once every 3 weeks. The incidence of neutropenia less than 500/mm^2 (grade 4) was 9% in European and American patients. The incidence of grade 4 neutropenia was 7% in Chinese patients.

2) Allergic reactions: Allergic reactions have been reported in clinical trials of this product. In randomized controlled clinical trials conducted in European and American patients, grade 1 or 2 allergic reactions occurred on the day of administration of this drug, manifested as dyspnea (1% of the patients), skin flushing, hypotension, chest pain, and arrhythmia (<1% of the patients). In a randomized controlled clinical trial conducted in China, 3% of patients had grade 1 or 2 skin reactions on the day of administration, which were manifested as skin itching and rash. There are no data on the use of this product in patients with a history of allergy to paclitaxel or human serum albumin.

3) Cardiovascular system: arrhythmia, bradycardia, cardiac arrest, congestive heart failure, edema, left ventricular dysfunction, supraventricular tachycardia, tachycardia, flushing, hypotension, hypertension and atrioventricular block have been reported in clinical trials. In randomized controlled clinical trials of patients with metastatic breast cancer in Europe and the United States, 5% of patients had a decrease in blood pressure during the 30-minute dosing period, and <1% of patients had bradycardia. In Chinese patients, blood pressure decreased in 7% patients, and bradycardia occurred in less than 1% patients. These changes in vital signs are usually asymptomatic and require neither special management nor treatment discontinuation.

4) Respiratory system: cough, dyspnea, interstitial pneumonia, pleural effusion, pulmonary embolism, pulmonary thromboembolism, and radiation pneumonitis have been reported in clinical trials. In the randomized controlled clinical trials of metastatic breast cancer patients in Europe and the United States, 12% reported dyspnea, 7% reported cough, and less than 1% had pneumothorax. In a randomized controlled clinical trial of patients with metastatic breast cancer in China, cough or dyspnea occurred in 2% of patients after treatment.

5) Nervous system: Sensory neuropathy and sensory peripheral neuropathy have been reported in clinical trials. The frequency of sensory neuropathy was positively

correlated with the cumulative dose administered. Discontinuation of treatment due to sensory neurotoxicity accounted for 3% (7/229) of all patients. Of the 24 patients (10%) with grade 3 peripheral sensory neurotoxicity, 14 had symptomatic improvement after 22 days（the median）, with 10 patients continuing treatment after dose reduction and 2 withdrawing from treatment. Of the 10 patients with no documented improvement, 4 discontinued treatments because of sensory neuropathy.

6) Gastrointestinal reactions: severe nausea and vomiting were the main limiting toxicities. Acute vomiting usually occurs 1 to 2 hours after administration and can last for about a week. Therefore, it is necessary to use this product with potent antiemetic agents to control acute vomiting, such as 5-hydroxytryptamine 3 (5-HT3), receptor antagonist antiemetic agent ondansetron, etc.

7) Nephrotoxicity: Cumulative and dose-related renal dysfunction are the main limiting toxicities of cisplatin. Generally, a dose of more than 90mg/m2 per day is a risk factor for nephrotoxicity,mainly renal tubular injury. Acute renal damage usually occurs 10-15 days after treatment. The increase of blood urea nitrogen (BUN) and creatinine (Cr) and the decrease of creatinine clearance rate are reversible in most cases, repeated high-dose therapy can cause persistent mild to moderate kidney damage at present, there is no effective means to prevent nephrotoxicity caused by this product except hydration.

7.3.2 Immune-mediated AE

Anti-PD-1 antibody can result in some immune-mediated adverse events probably due to T cell activation and proliferation. The immune-mediated AE is defined as an adverse event of unknown etiology associated with drug exposure and consistent with an immune phenomenon, and after ruling out neoplastic, infectious, metabolic, toxin or other etiologic causes.

Once the immune-mediated adverse reaction is noted, appropriate work-up should be performed, and steroid therapy may be considered if clinically necessary.

However, the patient must be informed that the clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from anti-PD-1 antibody. The skin rash/toxicity should be treatment as ESMO Clinical Practice Guidelines, 2017.

7.3.3 Manganese-related AE

Manganese is suggested as a bridge between the innate and adaptive immune reaction. The immune-mediated AE may be also observed after the manganese administration. Once the immune-mediated adverse reaction is noted, appropriate work-up should be performed, and the usage of Manganese Chloride should be discontinued. The overdose of manganese has been reported to cause a neurologic disorder, just like the Parkinsonian Syndrome. The blood concentration of Manganese must be monitored on time. Once the Manganese is overdosed and/or the neurologic AE appears, manganese chloride must be discontinued.

7.4 Management of Treatment-related Toxicity

1) Unacceptable toxicity requires study withdrawal and monitoring until resolution.
2) Administration will be interrupted and supportive care will be administered according to guidelines for ≥Grade 3 toxicities that believed to be drug-related
3) If the toxicity subsides or recovers to ≤ grade 2 within 21 days, treatment can be resumed with the test drug at the same dose.

7.5 Reporting of Serious Adverse Events (SAEs)

All initial and follow-up SAEs during the study, regardless of causality to the study drug, must be reported to the sponsor or designated person within 24 hours of discovery. SAEs post safe follow-up period won't be reported unless deemed drug-related.

Any non-serious AE escalating to SAE criteria should be reported as a new SAE. If an SAE occurs, the investigator should complete the SAE report form as much detail

as possible. Detailed SAE reports should include symptoms, severity, occurrence and treatment timing, actions taken, time of follow-up, outcomes, Source of the report, basic information about the subject, name of the test drug, name of the SAE, and drug causality, etc.

7.6 The report of deaths

All deaths occurring during the study or follow-up period must be reported. If death is directly due to disease progression, it should be informed to the study monitor at the next monitoring visit and recorded in the Case Report Form (CRF), but not reported as an SAE within the study; if death is not due to disease progression or the study disease, it should be reported as an SAE to the study monitor within 24 hours. The report should specify the primary cause of death and any related reasons.

7.7 Identification and Assessment of Risk Factors

By summarizing safety data from clinical trials, analyze the presentation and pattern of adverse reactions/events, such as time-dependency, demographic factors (age, gender, race, etc.), correlation with dose, drug concentration and administration method, and underlying diseases (e.g., renal impairment), to identify factors that might affect the frequency of adverse reactions/events. Clarify drug-related adverse reactions and adverse events that cannot be excluded from drug correlation to assess the causes of drug safety risks (high-risk factors and populations at high risk).

In safety evaluation, propose risk control/minimization measures based on all safety data from clinical trials, providing a basis for the drug's risk/benefit assessment. These measures usually include describing conditions for safe and effective use of the drug in the proposed labeling, with attention to clinically relevant information to minimize potential risks during post-marketing clinical use.

8.Research Processes

Before study, subjects must read and sign the latest version of the informed consent form approved by the Ethics Committee. The study steps required by the program shall be meticulously planned to ensure timely completion within the specified time frame, to the best of their ability. The investigator shall document the cause and promptly notify the research team sponsor of any unforeseen circumstances. Additional tests may be added if the investigator deems it necessary.

8.1 Screening (Day -21 to Day -1)

The screening evaluation should be completed within 28 days prior to study commencement, unless otherwise specified. The examination and evaluation conducted prior to the signing of the informed consent shall not be repeated within the specified time window, in order to safeguard the rights and interests of the subjects. 1. The screening period (-28 to -1 days) including the baseline period of -7 to -1 days, requires completion of the following study procedures to ensure patient eligibility for inclusion in the study.

1) Informed consent, signed informed consent and dated;

   The signing of a single research ICF will precede any research program.

2) Review the inclusion/exclusion criteria: If the screening period exceeds 7 days from the initial dosing, a second assessment must be conducted at baseline

3) Demographic information should be collected, including the subject's full name, gender, age (date of birth), ethnicity, and other relevant details.

Table 4 Schedule of Events

Cycle/Visit:	Screening[1]	Cycle 1	Subsequent Cycles	EOT[3]	Safety Follow-up	Follow-up Assessments (every 90 ± 14 day) via telephone
Day: **Procedure:**	**-21 to -1**					
Informed consent[2]	x					
Inclusion/exclusion criteria review	x					
Demographics	x					
Medical, surgical, cancer, and medication history	x					
Archival tissue	x					
Physical examination	x					

Vital signs	x					
ECOG	x					
FACT-O	x					
Optional serial tumor biopsy[4]	x					
Blood sample for exploratory biomarkers	x					
CBC	x					
Serum chemistry	x					
Coagulation	x					
Serum CA-125	x					
TSH, T3 or FT3, and FT4	x					
Urinalysis	x					

Cytokine	x					
Blood manganese concentration	x					
ECG	x					
Tumor assessment (RECIST)[5]	x					
Concomitant medications	x					
Adverse event monitoring	x					
nab-paclitaxel	x					
cisplatin	x					
sintilimab	x					
Manganese chloride	x					

Concomitant medications	x					
Survival state	x					

Abbreviations: AE = adverse event; CBC = complete blood count; CT = computed tomography; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; MRI = magnetic resonance imaging; RECIST = Response Evaluation Criteria in Solid Tumors;

Note:

1. Screening period:Screening period, from day -28 to day -1, after the subject's voluntarily signed written informed consent, screening shall be conducted to confirm whether the subject meets the test eligibility (baseline period, day -7 to day -1, if all the evaluation time of the screening period is within 7 days before the first dose, all the evaluation content of the baseline period need not be repeated, if more than 7 days,Some assessments should be re-evaluated at baseline, including entry review, physical examination, vital signs, ECOG score, blood routine, urine routine, blood biochemistry, coagulation function, 12-lead electrocardiogram;Blood pregnancy test within 72 hours before first dosing and weight measurement within 24 hours).

2. Informed consent must be obtained before entering the screening period of clinical trials.

3. EOT visit should be completed within 7 days of the last dose of study drug.

4. In patients who consent to serial biopsies, fresh tumor sample is to be obtained at screening, 1 to 3 days before or on C3D1 prior, and when possible, at the time of disease progression. See the Study Manual for details on sample collection and processing. The serial biopsies at different time points should be on the same lesion preferably. A core biopsy is recommended; if an excisional or incisional biopsy is to be performed, it must be conducted on a non-target lesion. If a patient has had a biopsy within 12 weeks prior to entering screening, that biopsy may be accepted as the screening biopsy.

5. Tumor assessment per RECIST via CT or MRI (chest, abdomen, and pelvis [brain, only if clinically indicated]) required at screening, every 6 weeks (42 ±7 days) until progression; at the time of progression, a final follow up

set of images is required if not done within the last 4 weeks. The same modality (CT or MRI) should be used throughout the study for a given patient. If the chest or brain CT/MRI is clear at screening, repeat imaging of these areas is not required in the absence of clinical indication requiring follow-up. Positron emission tomography/CT may be used according to RECIST v1.1 guidelines. Bone scans should be conducted per standard of care. Timing of images will not be delayed for treatment interruptions, and tumor assessment should occur according to this schedule regardless of whether study treatment is interrupted. If a patient discontinues treatment for a reason other than progression or death, withdrawal of consent, or loss to follow-up, scans and CA-125 testing should continue at the specified intervals.

4) Collect medical history, treatment history, allergy history and personal history: previous medical history, present medical history, allergy history, drug abuse history, smoking history, alcohol history, surgical history, menstrual history, recent and current drug use history, etc. The medical history will include all active conditions and any conditions diagnosed within the last 10 years that the investigator deems clinically significant. Collect and study tumor history and related treatment history, including disease stage, pathological diagnosis, diagnosis date and previous treatment history, treatment for initial diagnosis, chemotherapy, radiotherapy, surgical treatment, molecular targeted drugs, checkpoint inhibitor therapy, participation in other clinical studies and other anti-tumor therapy. Previous detection results of molecular markers, etc. (Copies of outpatient medical records, discharge summaries, pathology reports, etc., must be signed by the investigator).

5) Physical examination, including measurement of height and weight, is required. If the screening period exceeds 7 days from the initial dosing, a second examination should be conducted at baseline with weight measured within 24 hours prior to the first dosing.

6) Vital signs: If the screening period exceeds 7 days prior to the initial dosing, a second baseline examination is mandatory.

7) ECOG score: If the screening period is more than 7 days after the first dosing, a second score will be performed at baseline.

8) Optional tumor biopsy for biomarker testing

For patients who are available for biopsy and agree for sequential biopsies, baseline tumor biopsies are retained. Refer to the study manual for detailed information on sample collection and processing.

9) Collect Samples (whole blood) for blood manganese and cytokine analysis

10) Tumor assessment (CT/MRI) for determination of measurable disease (RECIST

v1.1)

For chest examination, the preferred imaging modality for screening is CT examination. For abdominal and pelvic (brain, if clinically necessary) imaging, MRI is the preferred method; however, CT can be used if MRI is not feasible. If no abnormalities are detected in the chest and/or brain MRI/CT during screening, repeat examinations should only be performed when there are clinical indications that necessitate follow-up. Positron emission tomography (PET)/CT can be utilized in accordance with RECIST v1.1 guidelines.

11) Bone scan should be performed according to clinical indications (when bone metastasis is suspected).

12) Laboratory examination

-Blood routine

- Urine routine

- Bowel routine

- Serum biochemistry: liver and kidney function, electrolytes

- Blood clotting

- Thyroid function (TSH, T3 or FT3, and FT4)

- Tumor markers (CA125 must be checked)

- Blood manganese levels

- Interleukin (IL-1/6/8/10, CRP, TNF-a)

13) 12-lead electrocardiogram: If the screening period is more than 7 days from the first dosing, a second examination is required at baseline

14) Virological examination: Including hepatitis B surface antigen (HBsAg), hepatitis

B surface antibody (anti-HBS), Hepatitis Be antigen (HBeAg), Hepatitis Be antibody (anti-HBE), and Hepatitis B core antibody (anti-HBC) (Hepatitis B DNA was selected if necessary according to the five results of hepatitis B and related medical history), hepatitis C antibody (hepatitis C RNA was selected if necessary according to the results of hepatitis C antibody and related medical history) and HIV and treponema pallidum antibody detection;

15) Quantitative evaluation of FACT-O: within 7 days before first dosing

16) Record adverse events (if any) resulting from the study procedure

17) Record combined drug administration/non-drug treatment

8.2 Administration period

Treatment cycle: Manganese chloride was nebulized inhaled at 0.4mg/kg twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle. The immunochemotherapy consisted of nab-paclitaxel (200 mg/m^2, administered intravenously on day 1 in a 3-week cycle), cisplatin (60 mg/m^2, administered intravenously on day 1-2 in a 3-week cycle) and sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle). Sustain dosing until disease progression, use of antitumor therapy other than the protocol, toxicity intolerance, withdrawal of informed consent, occurrence of other reasons for discontinuation of study therapy or subject death/loss of follow-up, or treatment duration reaching 24 months, whichever occurs first.

Enrollment: Subjects were not permitted to enter the treatment group until their eligibility for enrollment was confirmed and treatment assignment was randomized. The enrollment date was defined as the completion of randomization and acquisition of randomization tables.

The following assessments should be completed before each cycle of dosing

1) Informed consent for treatment, sign informed consent and date

2) Second review of the inclusion/exclusion criteria.

3) Vital signs: Complete before each cycle of dosing

4) Weight: Weight was measured within 24 hours prior to each dosing cycle

5) ECOG scoring

6) Laboratory examination

   -Blood routine

   - Urine routine

   - Bowel routine

   - Serum biochemistry: liver and kidney function, electrolytes

   - Blood clotting

   - Thyroid function (TSH, T3 or FT3, and FT4)

   - Tumor markers (CA125 must be checked)

   - Blood manganese levels

   - Interleukin (IL-1/6/8/10, CRP, TNF-a)

7) 12-lead electrocardiogram

8) Quantitative evaluation of FACT-O

9) Record adverse events (if any) resulting from the study procedure

10) Peripheral blood of C1D1 and C3D1 was collected for single cell sequencing analysis

11) The tumor tissue will be punctured and fresh tumor specimens will be obtained during the screening period or C1D1. Subsequently, on C3D1, another puncture of

the tumor tissue will be performed to collect additional fresh tumor specimens.

Imaging and tumor assessment: The tumor should undergo imaging evaluation every 6 weeks following the initial administration of the drug. MRI/CT scans should be scheduled on specified calendar days throughout the study for the prescribed imaging tests, ensuring that any delay in treatment dosing does not impede timely evaluation

PK blood collection and detection of peripheral immune cell changes: PK and detection of immune cell samples were collected according to the sampling schedule (Table 2). The procedure is detailed in the laboratory manual

8.3 End of Treatment/Early Withdrawal/Unplanned Visits

End-of-treatment visits are required for end-of-treatment or early withdrawal, and end-of-treatment visits are required within 7 days of confirmation of treatment completion. For safety reasons, the investigator may increase the number of visits to the subject, i.e., unplanned visits, as needed during the trial if the subject has an adverse event or abnormal laboratory test, or if the subject is unwell in any way. The investigator should document each unplanned visit for the subject in the unplanned visit section of the original medical record and eCRF, etc.

End of treatment, early withdrawal, or unplanned visits may include, but are not limited to:

1) Vital signs
2) Physical examination
3) ECOG score
4) Laboratory examination

-Blood routine

- Urine routine

- Bowel routine

- Serum biochemistry: liver and kidney function, electrolytes

- Blood clotting

- Thyroid function (TSH, T3 or FT3, and FT4)

- Tumor markers (CA125 must be checked)

- Blood manganese levels

- Interleukin (IL-1/6/8/10, CRP, TNF-a)

5) 12-lead electrocardiogram

6) Quantitative evaluation of FACT-O

7) Imaging and tumor assessment:

   - If not performed within the last 4 weeks, a final set of imaging images will be required at the time of disease progression.

   - If a patient discontinues treatment for reasons other than progression or death, withdrawal of consent, or loss to follow-up, imaging and CA- 125 testing should be performed every 9 weeks (63 ± 7 days) up to 1 year, and continuously every 12 weeks (84 ± 7 days) thereafter.

8) Record adverse events

9) Record combined drug administration/non-drug treatment

Note: Unscheduled visits are at the discretion of the investigator based on the subject's condition.

8.4 Security follow-up

Post-discontinuation safety follow-up was performed 30 (±7) days after the last dose or before initiation of new antitumor therapy, whichever occurred first, with the following examinations:

1) Vital Signs;

2) Physical Examination;

3) ECOG Rating;

4) Laboratory tests:

- blood routine
- urine routine
- Stool routine
- Serum biochemistry: liver and kidney function, electrolytes
- Blood clotting
- Thyroid function (TSH, T3 or FT3, and FT4)
- Tumor markers (CA125 must be checked)
- Blood manganese levels
- Interleukin (IL-1/6/8/10, CRP, TNF-α)

5) 12-lead electrocardiogram

6) Quantitative evaluation of FACT-O

7) Imaging and tumor assessment:

8) Record adverse events

9) record combined drug administration/non-drug treatment

If the safety follow-up is less than 7 days from the end-of-treatment visit, the end-of-treatment visit may be substituted for the safety follow-up and does not need to be repeated, but the items required for the safety follow-up need to be supplemented.

8.5 Survival follow-up

Progression-free survival (PFS) and overall survival (OS) will continue to be followed up after safety follow-up.

PFS follow-up: PFS follow-up is required for subjects who discontinue treatment without disease progression. After safety follow-up, subjects will undergo imaging assessments to evaluate PFS every 6 weeks (every 12 weeks after 48 weeks of initial dosing) until the disease progression, receives new antitumor therapy, loses the visit, or dies. The time of each follow-up visit, the results of the imaging evaluation, and other antitumor therapy information need to be documented.

OS follow-up: OS follow-up is required for all subjects treated. After safety follow-up, subjects may be followed up by telephone contact for survival every 12 weeks (±14 days) to evaluate the OS until the subject dies, is lost to follow-up, or is no longer collected by the sponsor (whichever occurs earliest). The time of each follow-up visit and follow-up information needs to be recorded.

AE monitoring: SAEs and ECIs need to be captured within 90 days of cessation of study treatment (or at least 30 days post-treatment if the patient is starting an alternative anti-cancer therapy)

If a patient starts an alternative anticancer treatment, information on subsequent anticancer treatments will need to be obtained through post-treatment.

8.6 Unplanned assessments

For any patient diagnosed with MDS/AML during the study period, perform a bone marrow aspirate, biopsy and sample collection (whole blood) for cytogenetic analysis.

9. Statistical Analysis

9.1 Statistical Hypotheses

Null hypothesis: test group ORR ≤ control group ORR.

Alternative hypothesis: test group ORR > control group ORR.

The null hypothesis will be tested at a one-sided 0.025 level.

9.2 Sample Size Estimation

Primary endpoint is ORR per Investigator assessment. The study is designed to test the null hypothesis that the objective response is the same between the manganese plus immunochemotherapy arm and the immunochemotherapy alone arm vs. the alternative hypothesis that manganese promotes the objective response. The reference ORR was calculated based on the prior clinical trials with ICB plus chemotherapy in platinum resistant ovarian cancer, including the JAVELIN 200 study with a 13.3% ORR in ICB plus chemotherapy group. Approximately 78 patients will be randomized 2:1 (approximately 52 and 26 patients in the Mn^{2+} and placebo group, respectively) to provide a 90% power at the 5% level of statistical significance.

9.3 Analysis Populations

The statistical analysis populations in this study will include:

- Full Analysis Set (FAS): Determined by the intention-to-treat (ITT) principle, consisting of all participants randomized into the study.

- Per-Protocol Set (PPS): A subset of the FAS, including all participants randomized, completed treatment according to the protocol, and with no significant protocol deviations. Analyses based on the PPS will supplement those based on the FAS. The PPS must be determined by the project medical team and the statistical team before the final analysis database is locked. All protocol deviations, including those judged to significantly affect study outcomes, will be listed in the report.

- Safety Set (SS): All randomized subjects who received at least one dose of the study drug and underwent at least one post-baseline safety assessment.

- Pharmacokinetic Analysis Set (PKAS): Includes all subjects who received the study drug, with no significant protocol deviations affecting PK evaluation, and who have evaluable PK data.

9.4 Statistical Methods

9.4.1 General Methods

Descriptive statistics will be summarized or tabulated for all variables obtained at each observation time point, unless the program or Statistical Analysis Plan (SAP) determines that statistical descriptions are not needed at a particular time point. In general, continuous variables (e.g., age) will be statistically described using the number of observations, mean, standard deviation, median, minimum, and maximum values; categorical variables will be statistically described using frequency counts of each category and their percentages.

The final analysis of the study will be based on data collected throughout the study period and as described in the following analyses. Specific statistical analysis descriptions will be detailed in the statistical analysis plan.

9.4.2 Efficacy Analysis

All the ITT subjects who have undergone randomization regardless of whether assigned treatment received will be accepted as the efficacy-evaluable population.

For continuous variables such as the number of patients, mean, standard deviation, median, minimum, and maximum values in clinical study protocols, statistical analysis will be performed using two-sided 95% confidence intervals (CIs) based on the Clopper-Pearson method. Time-to-event analyses will utilize the Kaplan-Meier (KM) method, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method, and were compared between the trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model. Comparisons between two groups of Laboratories tests were performed by paired *t* test.

9.4.2.1 Primary Efficacy Parameter

The primary study endpoint is the Objective Response Rate (ORR), defined as the proportion of subjects achieving Complete Response (CR) or Partial Response (PR) as assessed by RECIST V1.1 criteria, along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified， Cochran-Mantel-Haenszel test.

9.4.2.2 Secondary Efficacy Parameter(s)

The Disease Control Rate (DCR) will be assessed as a secondary study endpoint, defined as the proportion of subjects achieving CR, PR, or Stable Disease (SD) as assessed by RECIST V1.1 criteria, along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified Cochran-Mantel-Haenszel test.

Progression-free survival, overall survival and duration of response were estimated using the Kaplan-Meier method, with 95% CIs were calculated with

calculated with the Brookmeyer-Crowley method, and were compared between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

9.4.2.3 CA125

Proportion of subjects with optimal clinical benefit as assessed by Gynecological Cancer Inter Group CA-125 Response rate criteria,along with two-sided 95% CIs using the Clopper-Pearson method. Comparison between the trial groups was performed using stratified Cochran-Mantel-Haenszel test。

9.4.2.4 Biomarker Analysis

For each patient in the study, peripheral blood specimens will be prospectively collected; as well as for some patients for whom tumor tissue puncture is available, tumor tissue samples will be retained, evaluated and archived to support exploratory biomarker analysis.

The comparison of Mn2+ concentrations in peripheral blood, serum cytokines and chemokines between the experimental and control groups will be conducted using paired t-tests.

The same method will be used to analyze the correlation of PD-L1 expression (retrospective analysis), BRCA gene mutations, HRD scores, immune cell infiltration, and other exploratory biomarkers with clinical response.

9.4.2.5 FACT-O Analysis

Changes in FACT-O (Functional Assessment of Cancer Therapy-Ovarian Cancer) questionnaire scores will be summarized through descriptive statistics, summarizing the outcome changes from baseline to the last safety follow-up for each treatment group in the FAS. Completion rates for each questionnaire at planned time points will be summarized. Assessments will be conducted among patients both at baseline and post-

baseline.

9.4.3 Safety Analysis

Unless specified otherwise, the following key safety parameters will be assessed in accordance with the study plan and overall:

1) The incidence of TEAEs during the first cycle compared to the second and subsequent cycles;

2) The incidence of TEAE in patients during treatment or within 30 days of the last dose of study drug;

3) The incidence of SAEs and ECIs in patients during treatment or within 90 days after the last dose of study medication;

4) The incidence of any new malignant tumor;

5) Changes in clinical laboratory parameters (hematology, biochemistry, thyroid function, coagulation, urinalysis), vital signs, ECOG scores, electrocardiogram parameters, physical examinations, and concomitant medication use.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system, categorized by System Organ Class (SOC) and Preferred Term (PT), and graded according to CTCAE v5.0.

Adverse event analysis is defined as the analysis of any treatment-related events occurring during the study, including 1) the entire treatment period from the first dose of study medication to 30 days after the last dose; 2) any SAE or ECI occurring within 90 days after cessation of study treatment (or at least 30 days if the patient begins alternative cancer treatment); 3) any event present at baseline that worsens in intensity during treatment or by the end of the study and is considered drug-related.

The number and percentage of patients experiencing treatment-related TEAEs (definite, probable, or possible relationship) and those experiencing SAEs will be

summarized by treatment group and overall. In these tables, each patient will be counted once for the incidence rate in descriptive analysis (i.e., the most relevant or most severe). Furthermore, the severity of TEAEs will also be summarized by SOC and PT. No formal hypothesis testing will be analyzed for AE incidence rates.

All adverse events occurring in the study will be listed in the patient data listings. Detailed records of patient deaths, SAEs and adverse events leading to withdrawal will also be recorded.

9.4.4 Pharmacokinetic Analysis

Patients who have received at least one cycle of the study drug and have measurable drug concentrations will be included in the PK analysis. The number of cases, mean, standard deviation, median, minimum, maximum, coefficient of variation, geometric mean, and the coefficient of variation of the geometric mean will also be reported.

9.4.5 Baseline Descriptive Statistics

Descriptive statistical analysis methods will be used to summarize the demographic and baseline characteristics of the subjects, including age, gender, ethnicity, height, weight, Body Mass Index (BMI), and prior treatments. This analysis will be based on the full analysis set.

9.4.6 Interim Analysis

To minimize the risk of exposing patients to ineffective treatment, an assessment of the benefit comparison between the trial and control groups will be conducted during the study, a formal interim analysis of Overall Survival (OS) is planned when a pre-specified number of ORR events has been reached for the final ORR analysis.

10. Quality Management

10.1 Quality Control

10.1.1 Qualifications of Research Units and Researchers

Research units must have the qualifications for conducting clinical drug trials, and the facilities and conditions of the department hosting the project should meet the requirements for safely and effectively conducting clinical trials. Researchers must possess the professional expertise, qualifications, and ability to undertake this clinical trial and must have undergone training in Good Clinical Practice (GCP) regulations and this specific protocol. Before the trial begins, the principal investigator at the center should organize protocol training for the researchers, and only those who have passed the training can participate in the trial. The researchers involved in this trial should be relatively fixed, and any replacement researchers should join after training and the Clinical Trial Participant Authorization Form should be updated in a timely manner.

Researchers are required to prepare and keep all observation results for each study participant. These observations must be recorded fully and accurately, including other data related to the clinical study. Researchers will also continuously perform all appropriate safety assessments.

10.2 Ensuring Compliance

1) Participant Compliance: The informed consent process and the signing of the consent form are crucial for ensuring participant compliance. Researchers must thoroughly explain the informed consent, ensuring participants fully understand the trial's details, processes, and their rights and obligations, such as adhering to medication schedules, follow-up appointments, and completing necessary clinical observations and tests.

2) Researcher Compliance: Researchers must strictly follow the trial protocol and

relevant regulations. Their compliance is manifested in three main aspects: selecting qualified participants, controlling influencing factors, and observing and evaluating efficacy indicators, the specific contents are as follows：

a) strictly adhere to the trial protocol and regulations without significant deviations;

b) Selection of eligible subjects to participate in the trial and signing of the informed consent form;

c) Subjects were enrolled in strict adherence to the order specified in the protocol, and experimental observations were made and recorded;

d) The administration of reasonable treatment to the subject, and that all effects of its contributing factors (including test drugs, co-administration) and other measures can be measured, evaluated and judged by objective, credible, scientific and practicable criteria;

e) Adverse events, especially serious adverse events, are handled, recorded and reported in a timely manner;

f) Strict adherence to Standard Operating Procedures (SOPs) for pilot studies.

g) Data management and statistical processing are in strict compliance with standard operating procedures.

10.3 Data Collection and Management Responsibilities

Research data will be entered into the eCRF by researchers or authorized personnel at the research center. Before initiating the center or entering data, researchers and authorized personnel will be properly trained and appropriate security measures taken.

The eCRF should be completed as soon as possible during or after the visit period and updated regularly to reflect the latest status of participants. To minimize assessment discrepancies, it's vital to ensure the same individual completes all baseline and

subsequent efficacy and safety evaluations for each participant. Researchers must review the data to ensure accuracy and correctness of all information entered into the eCRF. If certain assessments weren't conducted or some information is unavailable, not applicable, or unknown, it should be recorded in the eCRF. Researchers are responsible for electronically signing verified data.

CRA will review the eCRF for completeness and consistency, comparing it with original documents to ensure key data consistency. All data entries, corrections, and modifications are the responsibility of the researcher or designated personnel, with CRA having no data entry authority. Data submitted to the data server in the eCRF and any changes made will be logged in the audit trail, recording the reason for changes, the operator's name, and the time and date of modification. Roles and permissions for research center personnel responsible for data entry will be predetermined.

Unless specified otherwise, the eCRF will only serve as a data collection form, not as original documentation. Original documents, which prove the existence of the participant, eligibility criteria, and all records of participation in the study, including laboratory records, ECG results, memos, pharmacy dispensing records, participant folders, etc., are maintained by the researcher or hospital.

Researchers are responsible for maintaining all original documents, regardless of the duration of a participant's involvement in the study, and must submit a complete eCRF for each participant.

AE and concomitant diseases/history will be coded using MedDRA, and concomitant/previous medication using WHO-Drug.

10.4 Archiving Study Documents

Essential clinical documents must be preserved to demonstrate the study's validity and the integrity of the collected data. The master file should be established at the beginning of the study, maintained throughout, and retained according to appropriate

regulations. Documents in clinical trials (protocols and amendments, completed eCRFs, signed ICFs, etc.) must be stored and managed following GCP requirements. Research centers should keep these documents for 5 years after the study ends. The sponsor should retain clinical trial materials for 5 years after the drug is approved for marketing.

Research documents should be stored securely to allow future access or data tracing, considering safety and environmental risks.

Without written permission from the sponsor and researcher, no study documents may be destroyed. Only after notifying the sponsor and obtaining written consent can study documents be transferred to a third party that meets document retention requirements or moved to a compliant third party.

10.5 Clinical Trial Standards

The "Good Clinical Practice (GCP)" and the "Declaration of Helsinki" serve as the legal backbone ensuring the scientific integrity and ethical principles of this clinical study and serve as the legal and regulatory guidelines for this clinical research. Any person involved in the trial, especially those appointed by the sponsor for monitoring and auditing, has the right to correct actions deviating from GCP and the Declaration of Helsinki. This is especially true when participant rights and protections are at stake, authorizing the termination of the clinical trial if necessary.

10.6 Informed Consent

Before the clinical trial begins, researchers must fully inform participants about the trial's nature, objectives, potential benefits and risks, alternative treatments, and the participant's rights and obligations. This ensures participants understand and agree to participate, evidenced by signing the "Informed Consent Form". This form is a crucial document for ensuring participant safety, rights, and health throughout the trial. Informed consent is one of the trial documents that ensures that the safety, rights and

health of subjects are safeguarded and protected during the trial, any clinical trial is conducted in a way that does not jeopardize the health and rights of the subjects.

The informed consent form must be signed and dated; a copy will be given to the patient and the investigator will retain a copy as part of the clinical study record. Written consent must be obtained before any research activities begin, and all consent procedures and timings must be documented.

If protocol changes are needed, then the ICF may need to be revised to reflect the changes in the protocol and re-signed by all subsequent enrollees in the clinical study, as well as by all current enrollees in the clinical study.

10.7 Ethics Committee and Trial Document Review

Like the informed consent form, the ethics committee is vital for protecting participant rights. It reviews clinical trial documents and oversees compliance with the protocol, participant rights protection, especially in handling, tracking, and following up on serious adverse events, ensuring the trial is conducted under scientific and ethical principles. Prior to the start of the trial, this protocol should be discussed and revised by the study leader and submitted to the ethics committee of the research center for written approval, and the investigator should use a copy of the ethical approval as the basis for initiating and implementing the trial.

10.8 Establishing Comprehensive Center SMP and SOP

Research entities should establish comprehensive regulations and standard management procedures (SMP) to enhance GCP and regulatory training for researchers, ensuring smooth trial conduct. Standard operating procedures (SOPs) for experimental studies should be established and improved, and implemented and perfected in actual experimental studies, so that the entire experimental study is proceduralized, institutionalized, standardized and formatted.

11. Supporting Documents and Operational Considerations

11.1 Ethics

11.1.1 Approval of Trial Documents by Ethics Committee Before Research Begins

Prior to the start of the trial, this protocol needs to be discussed and revised by the study leader of the research center and submitted to the ethics committee of the research center for written approval, and the center should use the ethical approval document as the basis for initiating and implementing this trial, and the documents submitted to the ethics committee include:

1) A copy of the drug clinical trial approval;
2) The revised clinical trial protocol was discussed by the center's research leaders;
3) informed consent forms;
4) case report forms;
5) investigator's brochure;
6) A copy of the quality control report of the test drug;
7) other required documents by the ethics committee.

11.1.2 Obtaining Informed Consent from Participants Before Research Begins

Before participant screening, researchers must explain the clinical study in detail, ensuring participants understand the trial and their rights and obligations. Informed consent must be voluntarily signed by the participant or their legal guardian.

11.1.3 Effective Handling and Follow-up of Any Adverse Events (AEs) During the Study

Any adverse event that occurs during the trial will allow the investigator to decide

whether to terminate treatment based on the condition. Any adverse events occurring, the investigator will immediately administer the correct treatment or rescue therapy to protect the safety of the subject.

All AEs are to be promptly addressed to ensure participant safety and followed up until resolved or stabilized. Follow-up visits may take the form of inpatient, outpatient, home visit, telephone, or correspondence, depending on the severity of the adverse event, the proximity of the subject's residence to the hospital, and the medical expertise of the center, respectively.

11.1.4 Study Outcomes Favor Participants Over Risks

This protocol follows the Declaration of Helsinki Ethical Guidelines for Human Medical Research and complies with the ethical guidelines for human medical research to benefit all subjects as much as possible.

Although there is no guarantee that the results of this trial will be as expected, subjects participating in this trial may experience improvement in their condition.

11.2 Publication

Information on the publication of study results is included in the clinical trial protocol.

11.3 Research Committee

A research committee will be established to review and assess study data continuously, safeguarding the interests and safety of participants. Detailed information on membership, main responsibilities and corresponding procedures are recorded in the Research Committee Statutes.

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Appendix 1. ECOG performance status scoring criteria

Level	Performance status
0	Fully active, capable of all pre-disease performance without restriction
1	Ambulatory and capable of light or sedentary work (light house work or office work) but restricted in physically strenuous activity
2	Ambulatory and capable of all self-care but incapable of any work activities; up and about more than 50% of waking hours
3	Capable of limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely bedridden, incapable of any self-care
5	Dead

Appendix 2. 2014 FIGO staging for ovarian cancer, fallopian tube cancer, and peritoneal cancer

I		Tumor confined to the ovaries or fallopian tube(s)
	IA	Tumor limited to 1 ovary (capsule intact) or fallopian tube, no tumor on the ovarian or fallopian tube surface, and no malignant cells in the ascites or peritoneal washings
	IB	Tumor limited to both ovaries (capsule intact) or fallopian tubes, no tumor on the ovarian or fallopian tube surface, and no malignant cells in the ascites or peritoneal washings
	IC	Tumor limited to one or both ovaries or fallopian tubes, with any of the following: IC1 Surgical spill intraoperatively; IC2 Capsule ruptured before surgery or tumor on the ovarian or fallopian tube surface; or IC3 Malignant cells in the ascites or peritoneal washings
II		Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below the pelvic brim) or peritoneal cancer (Tp)
	IIA	Extension and/or implants on the uterus and/or fallopian tubes and/or ovaries
	IIB	Extension to other pelvic intraperitoneal tissues
III		Tumor involves 1 or both ovaries or fallopian tubes or primary peritoneal cancer with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and (or) metastasis to the retroperitoneal lymph nodes Metastasis to retroperitoneal lymph nodes with or without microscopic peritoneal involvement beyond the pelvis
	IIIA	IIIA1 Positive retroperitoneal lymph node only (cytologically or histologically confirmed) IIIA1(i) Metastasis ≤ 10 mm in greatest dimension (note: this is the tumor dimension and not the lymph node dimension) IIIA1(ii) Metastasis >10 mm in the greatest dimension IIIA2 Microscopic extrapelvic (above the pelvic brim), peritoneal involvement with or without positive retroperitoneal lymph nodes
	IIIB	Macroscopic peritoneal metastasis beyond the pelvic brim ≤ 2 cm in greatest dimension, with or without positive retroperitoneal lymph nodes
	IIIC	Macroscopic peritoneal metastasis beyond the pelvic brim > 2 cm in greatest dimension, with or without positive retroperitoneal lymph nodes (Note 1)
IV		Distant metastasis excluding peritoneal metastasis
	IVA	Pleural effusion with positive cytology
	IVB	Metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of abdominal cavity) (Note 2)

Note 1: Includes extension of the tumor to the capsule of the liver and spleen without parenchymal involvement of either organ.

Note 2: Parenchymal metastases are stage IVB.

Appendix 3. Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria

1. The measurability of the tumor at the baseline level

1.1 Definition:

At the baseline level, tumors/lymph nodes are classified as measurable and unmeasurable based on the following definitions.

1.1.1 Measurable lesions

Tumors: For measurable lesions, there must be at least 1 diameter that can be accurately measured (recorded as the longest diameter). The longest diameter should be **≥ 10 mm on CT (recommended CT slice thickness is ≤ 5 mm),** ≥10 mm on clinical examination using conventional instruments (tumors that cannot be accurately measured by conventional calliper devices will be recorded as unmeasurable), and ≥ 20 mm on chest X-ray. **Malignant lymph nodes are pathologically enlarged and measurable, and the short-axis diameter (SAD) of a single lymph node should be**

≥ 15 mm on CT (recommended CT slice thickness is ≤ 5 mm). At baseline and follow-up, only the SAD will be measured and followed up.

1.1.2 Unmeasurable lesions

All other lesions include small lesions (longest diameter <10 mm or pathological lymph node SAD ≥ 10 mm to < 15 mm) and unmeasurable lesions. Unmeasurable lesions include meningeal disease, ascites, pleural or pericardial effusion, inflammatory breast cancer, cutaneous/pulmonary lymphangitis carcinomatosis, abdominal mass that cannot be diagnosed and followed up by imaging, and cystic lesions.

1.1.3 Special concerns for lesion measurement

Bone lesions, cystic lesions, and lesions that have received local treatment will be noted:

- Bone lesions:

- Bone scans, positron emission tomography (PET) scans or plain films are not suitable for measuring bone lesions but will be used to confirm the presence or disappearance of bone

lesions.If osteolytic lesions or mixed osteolytic/osteogenic lesions have a definite soft tissue composition that meets the above definition of measurability and are evaluable using tomographic imaging techniques such as CT or MRI, then these

lesions will be considered measurable.

- Osteogenic lesions are unmeasurable lesions.

➢ Cystic lesions:

- Lesions that meet the definition criteria for simple cysts on radiography will not be considered malignant just because they are simple cysts by definition. They are neither measurable lesions nor unmeasurable lesions.
- If a cystic metastatic lesion meets the definition criteria for measurability, it can be regarded as measurable. However, if cystic and noncystic lesions simultaneously present in the same patient, noncystic lesions will be preferentially

selected as target lesions.

Locally treated lesions:

- Lesions located at sites that have received radiotherapy or other local treatments will generally be regarded as unmeasurable lesions unless there is clear progression of the lesion. The study protocol will elaborate the conditions under

which these lesions are measurable.

1.2 Measurement method description

1.2.1 Measurement of lesions

During clinical evaluations, all tumor measurements will be recorded in the record system. All baseline evaluations of tumor size will be completed before and as close as possible to the start of treatment and **must be completed within 28 days (4 weeks) before the start of treatment.**

1.2.2 Evaluation methods

The same techniques and methods will be used for baseline evaluations and subsequent lesion measurements. Except for lesions that cannot be evaluated by imaging but can only be evaluated by clinical examination, all lesions will be evaluated by imaging.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and have a diameter ≥ 10 mm at the time of measurement (such as skin nodules). For patients with skin lesions,

it is recommended to archive colour photographs containing scales to measure lesion sizes. When the lesions are evaluable using both imaging and clinical examinations, imaging will be selected whenever possible because imaging is more objective and can be reviewed repeatedly after the end of the study.

Chest X-ray: When tumor progression is set as a primary endpoint, chest CT is preferred because CT is more sensitive than X-ray, especially for new lesions. Chest X- ray is only applicable when the measured lesion has a clear boundary and the lungs are well ventilated.

CT and MRI: CT is currently the best reproducible method for efficacy evaluations. The measurability in this guideline is defined based on a CT slice thickness ≤ 5 mm. If the CT slice thickness is >5 mm, the minimum measurable lesion will be 2 times the slice thickness. MRI is also acceptable in some cases (e.g., whole-body scan).

Ultrasound: Ultrasound will not be used to measure lesion size. Due to its operational dependence, ultrasound is not repeatable and technical and operational homogeneity cannot be guaranteed among different measurements. New lesions detected by ultrasound during the treatment period will be confirmed by CT or MRI. If CT radiation exposure is a concern, MRI will be used instead.

Endoscopy and laparoscopy: These techniques are not recommended for the objective evaluation of tumors. However, they will be used to obtain biopsy specimens for CR confirmation or for confirmation of recurrence after CR or in surgical resection. Tumor markers: Tumor markers alone will not be used to evaluate objective tumor response. However, if the baseline level of a tumor marker exceeds the ULN, it will not be used to evaluate CR unless its level returns to normal. The variation in tumor markers in different diseases needs to be taken into account when formulating the measurement criteria into the protocol. Specific criteria for CA-125 (recurrent ovarian cancer) and prostate-specific antigen (recurrent prostate cancer) responses have been published. In addition, the Gynecologic Cancer Intergroup (GCIG) has developed CA-125 progression criteria, which will soon be added to the objective tumor evaluation criteria of the first-line treatment regimens for ovarian cancer.

Cytological/histological techniques: Under conditions specified in the protocol, these techniques can be used to identify PR and CR (such as the presence of residual benign tumor tissue in germ cell tumors). When exudation may be a potential side effect of a certain therapy (such as treatment with taxane compounds or angiogenesis inhibitors) and a measurable tumor meets the criteria for response or SD, the occurrence or exacerbation of tumor-related exudation during the treatment will be diagnosed by cytological techniques to distinguish between response (or SD) and disease progression.

2. Tumor response evaluation

2.1 Evaluation of all tumors and measurable lesions

To evaluate objective response or possible future progression, it will be necessary to perform a baseline evaluation of the total tumor burden as a reference for the subsequent measurements. In the clinical protocols with objective response as the primary endpoint, only patients with measurable lesions at baseline are eligible. Measurable lesions are defined as the presence of at least one measurable lesion. For trials with disease progression (disease progression time or degree of progression on a fixed date) as the primary endpoint, the inclusion criteria for the protocols will clarify whether only patients with measurable lesions are eligible or patients with no measurable lesions are also eligible.

2.2 Baseline records of target lesions and nontarget lesions

When there is more than 1 measurable lesion during a baseline evaluation, all measurable lesions will be recorded and measured, but the **total number of measurable lesions will not exceed 5 (no more than 2 in each organ)**. For patients with 1 or 2 organs involved, up to 2 or 4 target lesions will be selected for baseline measurements. The target lesions will be selected based on the size (the longest diameter) and will represent all the involved organs, and the measurement will be highly reproducible. If the measurement of the largest lesion is not reproducible, the largest one among reproducible lesions will be selected.

Special attention will be paid to lymph nodes because they are normal tissues and can be detected by imaging even without tumor metastasis. Pathological lymph nodes defined as measurable nodules or even target lesions must meet the following criteria. The CT measurement of the SAD must be ≥ 15 mm. Only the SAD will be measured at baseline. Radiologists usually use the SAD of a nodule to determine whether the nodule has undergone metastasis. The size of a nodule is generally represented by the two-dimensional data from imaging (the axial plane on CT and the axial, sagittal or coronal plane on MRI). The minimum value is the SAD. For example, a 20 mm × 30 mm abdominal nodule has an SAD of 20 mm and can be regarded as malignant and measurable. In this example, 20 mm is the measured value of the nodule. Nodules with a diameter ≥ 10 mm but < 15 mm will not be considered **target lesions. Nodules < 10 mm will not be categorized as pathological nodules and will not be recorded** and further observed.

The sum of the diameters of all target lesions (including the longest diameter of nonnodular lesions and the SAD of nodular lesions) will be reported as the baseline sum of the diameters. If the diameters of

lymph nodes are included, as mentioned above, only the SAD is included. The baseline sum of the diameters will be used as the reference value for the baseline level of the disease.

All other lesions, including pathological lymph nodes, will be considered nontarget lesions and will not need be measured but will be recorded during the baseline evaluation. For example, the lesion will be classified as “existing”, “missing” or, in rare cases, “clear progression”. Extensive target lesions will be recorded with target organs (e.g., numerous enlarged pelvic lymph nodes or large-scale liver metastasis).

2.3 Criteria for response

2.3.1 Evaluation of target lesions

CR: All target lesions have disappeared, and the SADs of all pathological lymph nodes (including target nodules and nontarget nodules) have reduced to <10 mm.

PR: The sum of the diameters of all target lesions is at least 30% higher than the diameter sum at baseline.

PD: The sum of the diameters of all target lesions is at least 20% higher than the reference diameter sum, which is the minimum value of the sum of the diameters of all target lesions measured during the entire study period (if the diameter sum at baseline is the smallest, the baseline value is used as the reference). In addition, the absolute value of the diameter sum must have increased by at least 5 mm (**the appearance of 1 or more new lesions is also considered PD**).

SD: SD is a status between PR and PD, where the reduction in the target lesion does not reach PR and the increase in the target lesion does not reach PD. The minimum value of the diameter sum will be used as a reference.

2.3.2 Precautions for target lesion evaluations

Lymph nodes: Even if the size of the lymph nodes identified as target lesions decreases to less than 10 mm, the actual SAD corresponding to the baseline will be recorded for each measurement (consistent with the anatomical plane of the baseline measurement). This means that if a lymph node is the target lesion, even if CR is achieved, it cannot be concluded that the lesion has disappeared completely because the SAD of a normal lymph node is defined as <10 mm. In the CRF or other recording forms, it is necessary to specifically record the target lesion (lymph node) at a specific location: for CR, the SADs of all lymph nodes must be <10 mm; for PR, SD and PD, the measured value of the SAD of the target lymph node will be included in the sum of the diameters of the target lesions.

Target lesions that are too small to be measured: In clinical studies, all lesions (nodules or non-nodules) recorded at baseline should be measured again and recorded, even if the lesions are very small (e.g., 2 mm). However, sometimes a lesion may be too small, resulting in very fuzzy CT images. In this case,

the radiologists cannot accurately define the exact size of the lesion and may report it as “too small to be measured”. However, it is very important to record a value on the CRF in this condition. In this trial, even if the radiologist believes that the lesion may have disappeared, he/she will still record 0 mm on the CRF. If the lesion does exist but is too fuzzy to be accurately measured, the default size will be 5 mm (note: this condition is unlikely to occur in lymph nodes because they generally have a measurable size under normal

conditions or are often surrounded by adipose tissue as in the retroperitoneal cavity, whereas if the lymph nodes cannot be measured accurately, the default size will also be 5 mm). The default size of 5 mm is derived from the slice thickness of the CT scan (however, this value does not change with the slice thickness of the CT scan). Because the probability of the repeated occurrence of the same measurement value is small, the use of this default value will reduce the risk of an erroneous evaluation. However, it should be reiterated that if the radiologist can obtain the exact lesion size, even if the diameter of the lesion is less than 5 mm, the actual size will be recorded.

Dissociated or combined lesions: When a nonnodular lesion dissociates into fragments, the sum of the diameters of the fragments will be calculated by adding the longest diameters of all fragments. Similarly, the planes between combined lesions can be used to distinguish each lesion, and then the longest diameter of each lesion can be calculated. However, if the combined lesions fuse into an inseparable lesion, the longest diameter should be the longest diameter of the entire fused lesion.

2.3.3 Evaluation of nontarget lesions

This section defines the response criteria for nontarget tumors. Although some nontarget lesions are measurable, they will not be measured but will be qualitatively evaluated at the time points planned in the protocol.

CR: All nontarget lesions have disappeared, and tumor markers have returned to normal levels; all lymph nodes are of nonpathological size (SAD $\leq$ 10 mm).

PR/non-PD: The presence of one or more nontarget lesions and/or the persistent presence of abnormally high tumor marker levels.

PD: Clear progression of existing nontarget lesions. **Note: The appearance of one or more new lesions is also considered PD**.

2.3.4 Special considerations for rating the progression of nontarget lesions

The following is a supplementary explanation of the definition of the progression of nontarget lesions: when patients have measurable nontarget lesions, to achieve ‘unequivocal progression’ on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently

to merit discontinuation of therapy. However, a general size increase in 1 or more nontarget lesions often cannot meet the criteria for progression. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare..

The situation that all nontarget lesions are unmeasurable may occur in some phase III trials, in which the inclusion criteria do not stipulate the necessity of measurable lesions. The overall evaluation is based on the above criteria, but measurable lesion data are absent in this situation. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease. For example, such progression can be described as an increase in tumor burden equivalent to an additional 73% increase in volume (equivalent to a 20% increase in the diameters of measurable lesions), the change in peritoneal exudation from “minimal” to “massive”, the change in lymphatic lesions from “local” to “extensively expanded”, or “sufficient to change the treatment method” in the protocol. Examples include pleural exudate increasing from a trace amount to a large amount, lymphatic involvement spreading distally from the primary site, or a description of "necessity for treatment changes" in the protocol. In this clinical study, if there is clear progression, the patient will be considered to have disease progression at that time point. It is best to have objective criteria that can be applied to the evaluation of unmeasurable lesions. Note that the supplementary criteria must be reliable.

2.3.5 New lesions

The appearance of new malignant lesions indicates disease progression; therefore, the evaluation of new lesions is critical. Currently, there is no specific standard for the imaging detection of lesions. However, the finding of a new lesion should be unequivocal. For example, progression cannot be attributed to different imaging techniques, changes in imaging morphology, or lesions other than tumors (e.g., some so-called new bone lesions are merely cured original lesions or the recurrence of original lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, a necrotized liver lesion may be classified as a new cystic lesion on the CT report, although it is not.

Lesions that are detected during the follow-up but were not found in the baseline examination will be considered new lesions and suggestive of disease progression. For example, if a patient with visceral lesions in the baseline examination is found to have metastases during a CT or MRI cranial examination, intracranial metastases will be considered the basis of disease progression even if the patient did not undergo a cranial examination during the baseline examination. If a new lesion is equivocal, for example

because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

In addition to fluorodeoxyglucose (FDG)-PET, additional tests are generally required for supplementary confirmation. It is rational to combine FDG-PET and complementary CT results to evaluate progression (especially for new suspected diseases). New lesions can be confirmed by FDG-PET using the following procedures.

Negative baseline FDG-PET results together with the positive FDG-PET results in a subsequent follow-up indicate disease progression.

In cases with no baseline FDG-PET and positive FDG-PET results in a subsequent follow-up, if the new lesion indicated by the positive FDG-PET is confirmed by CT, then disease progression is confirmed; if the new lesion indicated by the positive results of the follow-up FDG-PET is not confirmed by CT, another CT should be performed to confirm the lesion (if confirmed, the disease progression time is counted from the initial discovery of the lesion); if the lesion is confirmed to be an existing lesion recorded in a past CT examination and no progression of the lesion has been found by imaging, then there is no disease progression.

2.4 Evaluation of the best overall efficacy

The best overall efficacy will be evaluated based on the best response from the start of treatment to the end of treatment, and all requisites will be considered for confirmation. Sometimes, the response occurs after the end of treatment. Therefore, the protocol will clarify whether an efficacy evaluation after the end of the treatment is included in the best overall efficacy evaluation. The protocol will clarify how any new treatment before progression affects the best response. The best response mainly depends on target lesion and nontarget lesion results, as well as the presence of new lesions. In addition, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement. Specifically, in nonrandomized trials, response is the primary goal, and efficacy, i.e., PR or CR, must be confirmed to determine the best overall efficacy.

2.4.1 Time point response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 summarises the overall response of a patient population with measurable disease at baseline at each time point. The evaluation of patients with no measurable lesions (no target lesions) is detailed in Table 2.

2.4.2 Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

2.4.3 Best overall response

At all time points, once all the patient data are available, the best overall response will be determined.

Evaluation of the best overall response when confirmation of CR or PR is not required: The best treatment response is the best response at all time points (e.g., if the efficacy in a patient is rated SD for the first cycle, PR for the second cycle, and PD for the last cycle, then the best overall response for the patient is PR). When the best overall response is rated SD, the requirement for the minimum interval from baseline to SD specified in the protocol must be met. If the minimum interval requirement is not met, even if the best overall response is rated SD, the evaluation result will not recognized, and the best overall response of the patient will be determined by a subsequent evaluation. For example, if a patient response is rated SD for the first cycle and PD for the second cycle but does not meet the minimum interval requirement, the best overall response is PD. Similarly, if a patient response is rated SD for the first cycle but the patient becomes lost to follow-up after the first cycle, the best overall response is considered NE.

Evaluation of the best overall response when confirmation of CR or PR is required: If each subject meets the criteria for PR or CR and the efficacy is confirmed at a subsequent time point (usually 4 weeks later) specifically mentioned in the protocol, the best overall response can be declared CR or PR. In this situation, the best overall response is detailed in Table 3.

2.4.4 Special tips for efficacy evaluations

When nodular lesions are included in the total target lesion evaluation and the size of the nodule decreases to the "normal" size (<10 mm), a lesion size scan report will be generated. To avoid the over-evaluation of efficacy based on an increase in nodule size, the measurement results will be recorded even if the nodule is normal. As mentioned earlier, this means that even for subjects with a CR, the nodule size will not be recorded as 0 on the CRF. If the efficacy needs to be confirmed during treatment, repeated "unmeasurable" time points will complicate the evaluation of the best efficacy. The analysis plan must state that these missing data/evaluations will be clearly explained when determining efficacy. For example, in most trials, PR-NE-PR can be regarded as confirmation of efficacy.

When treatment must be discontinued due to the deterioration in the general health status of a participant that is not supported by objective evidence, the efficacy will be reported as symptomatic progression. Even after the discontinuation of treatment, objective progression will be evaluated whenever possible. Symptomatic deterioration is a reason for the discontinuation of treatment but is not an objective response evaluation. The objective response of such subjects will be evaluated based on the conditions of target and nontarget lesions, as detailed in Tables 1 to 3.

Conditions defined as early progression, early death and NE are special cases and will be clearly described for each regimen (depending on the treatment interval and treatment cycle).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. If abnormal imaging results for local lesions are considered to represent lesion fibrosis or scar formation, FDG-PET can be used as an evaluation standard, similar to biopsy, to confirm CR. In this situation, the application of FDG-PET will be prospectively described in the protocol, and the specialized medical literature reporting this situation will be cited as support. However, limitations of FDG-PET and biopsy (including their resolution and sensitivity) will lead to false positive results in the evaluation of CR.

Table 1 – Time point response: patients with target (+/– non-target) disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is *truly* met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

2.5 Frequency of tumor re-evaluations

The frequency of tumor re-evaluations during treatment will be determined by the treatment regimens and will occur in accordance with the type of and schedule for treatment. However, in the phase II trial in which the benefit of treatment was not clear, it is reasonable to conduct follow-up every 6 to 8 weeks (at the end of a cycle), and the length of the time interval will be adjusted under special schemes or

circumstances. The protocol will specifically indicate which tissue sites need to be evaluated at baseline (usually those tissue sites that are most likely to be closely related to the metastatic lesions of the tumor type under study) and the frequency of re-evaluation. Under normal circumstances, target lesions and nontarget lesions will be rated at each evaluation. In some optional cases, the evaluation frequency of some nontarget lesions will be lower; for example, bone scans should be repeated only when the treatment efficacy for the target disease is confirmed to be CR or when bone lesion progression is suspected.

After the end of treatment, the re-evaluation of tumors depends on whether the response rate or the occurrence of a certain event (progression/death) is used as the clinical trial endpoint. If the clinical trial endpoint is the occurrence of a certain event (e.g., TTP/DFS 1/PFS), routine re-evaluations, as specified in the protocol, are required. In randomized comparative trials, the scheduled evaluations should be listed in the study schedule (e.g., 6-8 weeks of treatment or 3-4 months after the treatment) and should not be affected by other factors, such as treatment delays, dosing intervals, and any other events that may cause an imbalance in the treatment arm regarding the selection of disease evaluation time.

2.6 Efficacy evaluation/response confirmation

2.6.1 Confirmation

For nonrandomized clinical trials with efficacy as the primary endpoint, PR and CR must be confirmed to ensure that the efficacy is not the result of an erroneous evaluation. Confirmation also allows a reasonable interpretation of the results in the presence of historical data, and efficacy in the historical data of these trials should also be confirmed. However, in all other cases, such as randomized trials (Phase II or III) or studies with SD or PD as the primary endpoint, efficacy confirmation is no longer necessary because it is of no value for the interpretation of the experimental results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded. In the case of SD, at least 1 measurement must meet the SD criteria specified in the protocol within the minimum time interval after the start of treatment (generally no less than 6-8 weeks).

2.6.2 Total response period

The total response period is the interval from the time when the CR or PR (whichever is measured first) criteria are first met to the time of the first actual recording of disease recurrence or progression (the minimum measured value recorded during treatment is used as the reference for disease progression). The total CR period is the interval from the time when the CR criteria are first met to the time of the

first actual recording of disease recurrence or progression.

2.6.3 SD period

The SD period is the interval from the start of treatment to disease progression (in a randomized trial, from the time of randomization), and the smallest sum during treatment is used as the reference (if the baseline sum is the smallest, it is used as the reference for PD calculations). The clinical relevance of SD varies in different studies and different diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease. Note: The response period, SD period, and PFS are affected by the follow-up frequency after the baseline evaluation. Defining the standard follow-up frequency is outside the scope of this guideline. The frequency of follow-up should consider many factors, such as disease type and stage, treatment cycle and standard specifications. However, if intertrial comparisons are required, the limitations of the accuracy of these measurement endpoints should be considered.

Appendix 4. CA-125 criteria for response evaluation in ovarian cancer

The Response Evaluation Criteria in Solid Tumors (RECIST) are currently the internationally accepted method- ology for assessing tumor response in all cancer trials. The RECIST criteria, a simplification of the former WHO criteria, are based on measurable target lesions and do not make use of serum tumor markers levels in evaluation of tumor response.

In ovarian cancer, often, there are no reliably measurable target lesions due to predominantly diffuse peritoneal disease. Hence, serum CA-125 levels are extensively used to assess response and define progression in ovarian cancer. To standardize the use of CA-125 levels in ovarian cancer trials, the Gynecologic Cancer Intergroup (GCIG) has recently published definitions of CA-125 response and CA-125 progression.

The GCIG CA-125 response is defined as at least 50% reduction in serum CA-125 levels from pre-treatment levels provided the response is maintained for 28 days. This definition is a simplification of the Rustin's CA-125 criteria, which specifies a 50% response as well as a 75% response [4]. Briefly, a 50% CA-125 response is defined by Rustin as a 50% decrease of serum CA-125 levels after two samples and confirmed by a final sample. A 75% CA-125 response is defined as a serial decrease of N75%, over three CA-125 samples. In each case, the final sample had to be at least 28 days after the previous sample.

Recently, a Danish group has found the GCIG-CA-125 criteria to be a better prognostic tool than the RECIST criteria in second-line treatment of ovarian cancer. This retrospective study is likely to give impetus to the widespread adoption of CA-125 criteria in the clinical as well as trial setting.

However, we would urge caution in adopting the GCIG CA-125 criteria. We propose that that the adoption of Rustin's 75% response criteria might be more appropriate instead of the proposed 50% response criteria based on our experience in using WHO criteria and Rustin's CA-125 criteria in a phase II trial. We evaluated the efficacy of Oxaliplatin and 5-Fluorouracil/Leucovorin combination in twenty-seven ovarian cancer patients relapsing within 2 years of prior platinum chemotherapy. Using the CA-125 levels measured at baseline, tumor response was prospectively assessed by Rustin's CA-125 criteria. All the patients with an objective response by WHO criteria demonstrated a 75% CA-125 response while none of the

patients with progressive disease by WHO criteria showed a CA-125 response. Only two patients in our trial had a 50% CA-125 response and both these patients had stable disease only by WHO criteria (Table 1). This prospective data set suggests that the stringent 75% CA-125 criteria are more likely to mirror the RECIST response criteria and in turn more likely to be useful in measuring tumor response in patients with non-evaluable disease. On the other hand, the 50% response criteria are more likely to overestimate the tumor response rate.

Table 1
Correlation between WHO response and CA-125 response

	Rustin's CA-125 response (best tumor response)			
	75% response	50% response	No response	Not evaluable[a]
WHO criteria (best tumor response)				
Complete response	100%	–	–	–
Partial response	100%	–	–	–
Stable disease	43%	29%	29%	–
Progressive disease	–	–	86%	14%
Not evaluable[b]	43%	–	43%	14%

[a] Two patients were not evaluable by CA-125 criteria because only one sample of CA-125 was available.

[b] Seven patients were non-evaluable by WHO criteria because of the absence of reliable, bi-dimensionally measurable target lesions.

An independent retrospective study from a Danish group confirms our prospective observation that the 50% CA-125 criteria overestimate tumor response. Moreover, from a practical point of view, the 50% response rate is also more likely to be influenced by medical procedures such as ascites and pleural effusion drainage, which are quite commonly used in management of relapsed ovarian cancer patients. The 75% response rate is less likely to be affected by medical procedures.

The test data set provided by GCIG on its web site also supports our suggestion since all, except one, responder in that data set would still be classified as a responder under the 75% response definition. Hence, the adoption of the stringent 75% CA-125 response instead of the 50% response criteria has the potential to avoid overestimation of tumor response rate without a significant risk of underestimation. Needless to add, that this small prospective data set from a phase II trial needs to be validated by a large dataset.

Appendix 5. NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.0

Please go to the following website to access the NCI CTCAE version 5.0:

https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0/

Appendix 6. NCCN Guidelines of Management of Immunotherapy-Related Toxicities

Please go to the following website to access the NCCN Guidelines of Management of Immunotherapy-Related Toxicities:

https://www.nccn.org/guidelines/guidelines-detail?category=3&id=1486

Protocol Version Number (Approval Date) **Summary of Key Changes**
v1.0 (10 June 2019): Initial version
v2.0 (20 October 2019) ***Note: No patients enrolled because of COVID-19*** Protocol was amended to: 1) Identify sintilimab as the anti-PD-1 antibody used in this trial 2) Excluded subjects with previous or concurrent other malignancies from this trial 3) Reduce administration frequency of manganese chloride from once daily to pulse-dosage regimen (administered for 3 consecutive cycles and stopped for 2 cycles, then followed with the schedule of 2 weeks of administration then 2 weeks of rest) 4) Increase the anticipated enrollment to 80 patients to provide a stronger power to 90%
V3.0 (21 May 2020) ***Note: Protocol not activated*** Protocol was amended to: 1) Include the scRNA-seq analysis of PMBCs to the laboratory assessment 2) Remove the conditions for “up to a maximum of 12 cycles of treatment” from the treatment discontinuation criteria
V4.0 (15 December 2020) ***Note: Actual performed protocol*** Protocol was amended to: 1) Remodel the anticipated completed date to December 2023 2) Adjust the administration frequency of manganese chloride to “twice per week in the first 3-week cycle, and thereafter 0.4mg/kg twice in the first week of each 3-week cycle” 3) Update FACT-O score as the patient report outcome assessment

Statistical Analysis Plan

A Single-Blind, Placebo-Controlled, Randomized, Phase 2 study of Manganese, Sintilimab plus Nab-paclitaxel and Platinum vs. Placebo, Sintilimab plus Nab-paclitaxel and Platinum in Platinum-Resistant/Refractory Ovarian Cancer

Trial Protocol Version: v4.0 Date: Dec 15, 2020

目录

1. Introduction

1.1 Preamble

This statistical analysis plan (SAP) describes the statistical methods that will be used for analysis and reporting of the phase 2, single-blind, placebo-controlled, randomized study to assess the safety and efficacy of manganese (Mn^{2+}) primed sintilimab, nab-paclitaxel plus platinum versus placebo combined the immunochemotherapy in subjects with platinum-resistant/refractory ovarian, fallopian tube, or peritoneal cancers. All analyses conform to the specifications laid out in the statistics chapter of the trial protocol. The SAP must be approved prior to database lock for corresponding analysis. This SAP is to support publication. The methods used for the final analysis are specified below.

The protocol version V4.0 dated Dec 20 2020 serves as reference.

1.2 Protocol Objectives

1.2.1 Primary objectives

- To compare the objective response rate (ORR) of patients randomized to the Mn^{2+} group (Mn^{2+} primed sintilimab, nab-paclitaxel plus platinum) versus placebo group (placebo combined sintilimab, nab-paclitaxel plus platinum), as assessed by the investigator.
- To compare the incidence of treatment-related adverse events (TRAEs) of patients randomized to the Mn^{2+} versus placebo group using CTCAE v5.0.

1.2.2 Key Secondary Objectives

- To compare the progression-free survival (PFS) of patients randomized to the Mn^{2+} versus placebo group, as assessed by the investigator.

- To compare the overall survival (OS) of patients randomized to the Mn^{2+} versus placebo group, as assessed by the investigator.
- To compare the disease control rate (DCR) of patients randomized to the Mn^{2+} versus placebo group, as assessed by the investigator.

1.2.3 Other Secondary Objectives

- To compare the duration of response (DOR) in patients randomized to the Mn^{2+} versus placebo group.
- To compare the cancer antigen (CA-125) response rate (RR) per Gynecologic Cancer Intergroup (GCIG) CA-125 criteria in patients randomized to the Mn^{2+} versus placebo group.
- To compare the patient reported outcome (PRO) using the Functional Assessment of Cancer Therapy-Ovarian Cancer (FACT-O) assessment from patients randomized to the Mn^{2+} versus placebo group.

1.2.4 Exploratory Objectives

- To evaluate concentrations of blood Mn^{2+} using the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) recommended by ATSDR.
- To assess serum cytokines and chemokines using LEGENDplex Bead-based Immunoassays.
- To characterize cellular profile of PBMCs using single-cell RNA sequencing
- To correlate concentration of blood Mn^{2+} with other immune-related biomarkers and with efficacy outcomes.
- To identify the biomarker-based patient population that would derive benefit from the combination treatment based on the tumor tissue molecular profile and circulating biomarkers.

1.3 Study Endpoints

1.3.1 Primary Endpoints

- ORR: defined as the percentage of patients with at least 1 efficacy assessment who achieved the best response of complete or partial response (CR/PR) as assessed by the Investigator per RECIST v1.1.
- TRAEs: the safety and tolerability of the treatment of the Mn^{2+} and placebo groups.

1.3.2 Key Secondary Endpoints

- PFS: defined as the time from the date of randomization until investigator-assessed progressive disease [PD] or death per RECIST v1.1, whichever occurred first.
- OS: defined as the time from date of randomization until the date of death. Patients alive at the time of analysis will be censored at the last date known to be alive.
- DCR: defined as defined as the percentage of patients with at least 1 efficacy assessment who achieved the best response of CR, PR or stable disease [SD] as assessed by the Investigator per RECIST v1.1.

1.3.3 Other Secondary Endpoints

- DOR: defined as the time from initial response until investigator-assessed PD or death occurred for all participants who had a confirmed objective response.
- CA-125 response determined using the GCIG criteria defined in Appendix 4 of the Protocol. CA-125 response per GCIG criteria will be determined programmatically.
- PRO from FACT-O questionnaire.

1.3.4 Exploratory Endpoints

- Blood Mn^{2+} concentration was assessed by ICP-AES.
- Serum cytokines and chemokines were analyzed by bead-based immunoassays.
- Correlate between the concentration of blood Mn^{2+}, serum cytokines/chemokines and the efficacy outcomes
- PBMCs cellular profile was characterized by scRNA-seq.

2. Study Methods

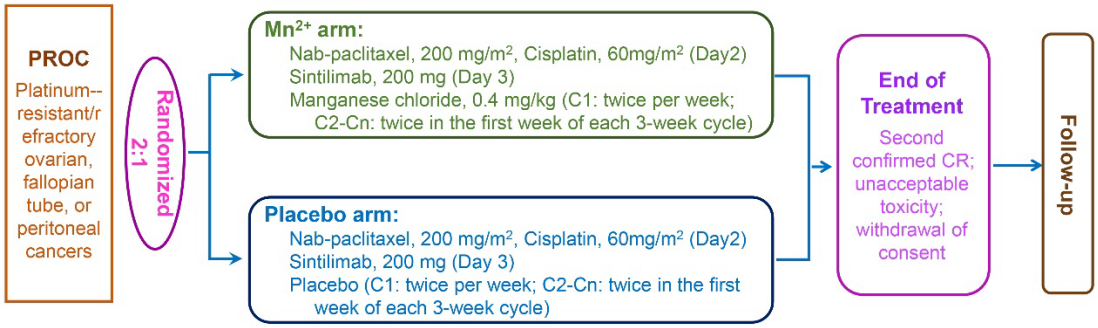
2.1 Study Overview

This is a prospective randomized placebo-controlled single-blind phase 2 trial. The study is designed to compare the safety and efficacy of Mn^{2+} primed sintilimab, nab-paclitaxel plus platinum versus placebo combined sintilimab, nab-paclitaxel plus platinum in patients with platinum-resistant/refractory ovarian, fallopian tube, or peritoneal cancers.

Enrolled patients were allocated to treatment using minimization with a random element and stratified by platinum-free interval (PFI). The participants were assigned in a 2:1 ratio to receive manganese chloride or placebo plus immunochemotherapy. Patients were blinded to the treatment allocation. Manganese chloride (0.4mg/kg) or placebo was nebulized inhaled twice per week in the first 3-week cycle, and thereafter twice in the first week of each 3-week cycle. The immunochemotherapy consisted of nab-paclitaxel (200 mg/m^2, administered intravenously on day 2 in a 3-week cycle), cisplatin (60 mg/m^2, administered intravenously on day 2 in a 3-week cycle) and sintilimab (200 mg, administered intravenously on day 3 in a 3-week cycle). If patients with known hypersensitivity to cisplatin, carboplatin (area under the curve [AUC] 4 mg/mL per min) was used as alternative.

2.2 Study Schema

Figure 1. Study flowchart



2.3 Study Population

All patients diagnosed with platinum-resistant/refractory ovarian, fallopian tube, or peritoneal cancer were eligible for this study. Enrolled patients provided the written informed consent, and were allocated to treatment using minimization with a random element and stratified by platinum-free interval (PFI). The participants were assigned in a 2:1 ratio to receive manganese chloride or placebo plus immunochemotherapy.

The intention-to-treat (ITT) population were consisted of all randomized patients without withdrawal of informed consent. For the efficacy analysis, all randomized patients will be analyzed (according to the intention-to-treat-principle) within the group they were randomized into, not regarding their actual further treatment.

The safety population included all participants who underwent randomization and received at least one dose of the assigned treatment.

2.4 Sample Size

The prior clinical trials with ICB plus chemotherapy in platinum resistant ovarian

cancer, including the JAVELIN 200 study, achieved a 13.3% ORR in ICB plus chemotherapy group. Referring to the results of these previous reports and our phase I study, we assume a proportion of objective response in the Placebo Arm of 14% and 50% in the Mn^{2+} Arm. An alpha level of 5%, a statistical power of 90% will be set. The ratio of patients in both arms will be 2:1 (Mn^{2+}: Placebo). A sample size of 78 patients (52 in the Mn^{2+} Arm and 26 in the Placebo Arm) is estimated to show the difference between both groups.

2.5 Blinding

Participants were blinded to the treatment allocation. Patients will be given a treatment drug or placebo with similar physical properties to reduce the possibility of unblinding. Participants blinding must be maintained until the completion of the trial.

The physician performing the treatment will not be blinded to the assigned treatment. Thus, if clinical follow-up with a study physician is deemed necessary at the protocol required follow-up time points, a different physician (or designee) must conduct the follow-up clinical visits in order to reduce bias and maintain participants blinding.

2.6 Timing of outcome assessments

Responses were assessed by the same radiologist per RECIST 1.1, using magnetic resonance imaging (MRI) or computed tomography (CT) at baseline, every 2 cycles during treatment, and 12 weeks after discontinuing treatment until PD, death, initiation of subsequent treatment, loss to follow-up, withdrawal of consent, or study closure. Post-progression follow-up was performed every 3 months for OS. Patient-reported outcome questionnaire FACT-O (Functional Assessment of Cancer Therapy-Ovarian Cancer) was administered at baseline, prior to each cycle of treatment, end of treatment, and follow-up.

3. Statistical Principles

3.1 Confidence Intervals and P Values

The level of significance is fixed at 0.05 and the confidence intervals will be 95%.

3.2 Missing Data

Attempts will be made to acquire the primary and secondary endpoints as completely and accurately as possible for all patients who at least started the trial intervention. Missing event dates will result in censoring at the date last known event-free.

Descriptive analyses will be based on complete case.

3.3 Analysis Population

3.3.1 Screened

The Screened population includes all patients entered into the eligibility evaluation who have signed an informed consent.

3.3.2 ITT population

The ITT population is defined as all patients randomized to the study, regardless of whether or not patients received study treatment.

3.3.3 Per Protocol Population

A subset of the ITT population, including all participants randomized, completed treatment according to the protocol, and with no significant protocol deviations.

3.3.4 CA-125-Evaluable Population

The CA-125-Evaluable population is defined as all patients randomized to the study who have received at least 1 dose of study drug, and who have at least 1 post-baseline CA-125 evaluation.

3.3.5 Safety population

All randomized patients who received at least 1 dose of assigned treatment will be included in the Safety population.

4. Trial Population

4.1 Eligibility

There will be **no exceptions** to eligibility requirements at the time of registration. Questions about eligibility criteria should be addressed prior to registration or randomization.

The eligibility criteria for this study have been carefully considered. Eligibility criteria are standards used to ensure that patients who enter this study are medically appropriate candidates for this therapy. For the safety of the patients, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions be made to these criteria for admission to the study.

Patients must fulfill all of the following criteria to be eligible for admission to the study.

4.2 Patient Characteristics

Patient characteristics will be summarized and listed for the ITT population by the treatment to which they were randomized (planned treatment).

4.2.1 Demography

Data collected about the following patient characteristics at the screening visit will be summarized as follows:

- Age (collected at the time of patient registration)
- Sex (Childbearing potential [female only, yes/no])

4.2.2 Baseline data

- Physical Examination
- Vital signs (height, weight, body surface area, temperature)
- Medical history events

4.2.3 Disease Characteristics

- ECOG performance status (0/1/2)
- Primary diagnosis (epithelial ovarian cancer, fallopian tube cancer, primary peritoneal cancer)
- Histology
- Disease stage at initial diagnosis
- Primary platinum-free interval (<6Months vs ≥6 to <12Months)

  Defined as time from last dose of first line platinum therapy to the date of disease progression and/or relapse following first line therapy.

- Platinum-free interval (<1Months vs ≥1 to <6Months)

  Defined as time from last dose of latest line platinum therapy to the date of disease progression and/or relapse following that line of therapy.

4.2.4 Treatment history and Gene Mutations

- Prior systemic therapy
- Prior exposure to bevacizumab
- Prior exposure to nab-paclitaxel
- Prior exposure to anti-PD-1 treatment
- Prior to PARP inhibitors
- Any BRCA mutations
- Homologous recombination deficiency (HRD)
- Microsatellite stable status
- Tumor mutational burden

5. Efficacy analyses

5.1 Primary efficacy outcome analysis

The protocol specifies the following primary efficacy endpoint:

- **ORR** as assessed by the investigator

The ORR will be calculated as the number of patients with a best overall response of CR or PR divided by the number of patients in the ITT population. Patients without at least 1 post-baseline RECIST assessment will be treated as non-responders.

Objective response was estimated for each treatment group, along with two-sided 95% CIs using the Clopper-Pearson method. ORR will be analyzed using a Cochran-Mantel-Haenszel (CMH) test stratified by the randomization stratification factors to test for differences between the Mn^{2+} and placebo groups. *P*-value and 95% CIs for ORR will be provided. Risk ratio and its 95% CI will also be reported.

5.2 Secondary Efficacy Analyses

The protocol describes the following key secondary efficacy endpoints:

- **PFS as assessed by the investigator**
- **OS**
- **DCR per RECISR v1.1**

5.2.1 PFS

PFS is defined as the time from the date of randomization until the date of PD or death from any cause, whichever occurs first. PFS is defined based on radiological assessments and determined by the Investigator.

The distribution of PFS will be summarized using the Kaplan-Meier method. Median times will be estimated for each treatment from the 50th percentile of the corresponding Kaplan-Meier estimates, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method. The comparisons between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

5.2.2 OS

OS is defined as the time from the date of randomization until the date of death from any cause. Patients who are alive or lost to follow-up at the analysis are censored at the last known date at which they were known to be alive. When an analysis cutoff date is implemented, only deaths occurring on or before the cutoff date are counted as OS events. Patients whose date of death or the last date at which the patient was known to be alive will be censored at the analysis cutoff date.

The distribution of OS will be summarized using the Kaplan-Meier method. Median times will be estimated for each treatment from the 50th percentile of the corresponding Kaplan-Meier estimates, with 95% CIs were calculated with calculated

with the Brookmeyer-Crowley method. The comparisons between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

5.2.3 DCR

The DCR will be calculated as the number of patients with a best overall response of CR, PR or SD divided by the number of patients in the ITT population. Patients without at least 1 post-baseline RECIST assessment will be treated as non-responders.

Disease control was estimated for each treatment group, along with two-sided 95% CIs using the Clopper-Pearson method. *P*-value and 95% CIs for DCR will be provided. Risk ratio and its 95% CI will also be reported.

5.3 Other Efficacy Analyses

5.3.1 DOR

DOR is defined as the time from the date of the first response (CR or PR), whichever occurs first, to the date of PD or death from any cause, whichever occurs first. DOR is only defined for patients who have an objective response.

Patients with an overall response of CR or PR must have a repeat tumor assessment performed no less than 4 weeks after the criteria for response are first met. The first date at which a CR or PR response was noted will be used to calculate DOR, not the date of the confirmatory tumor assessment.

The distribution of DOR will be summarized using the Kaplan-Meier method. Median times will be estimated for each treatment from the 50th percentile of the corresponding Kaplan-Meier estimates, with 95% CIs were calculated with calculated with the Brookmeyer-Crowley method. The comparisons between the 2 trial groups using the stratified log-rank test. Hazard ratios and corresponding CIs were analyzed with the stratified Cox proportional-hazards model.

5.3.2 GCIG CA-125 criteria clinical response rate

A CA-125 response is defined as a ⩾50% reduction in CA-125 levels from baseline. The response must be confirmed and maintained for at least 28 days. The CA-125 response will be conducted using the CA-125-Evaluable population. The date of response corresponds to the date when the CA-125 level is first reduced by 50%. The summary table for CA-125 will include the number (percentage) of patients in the CA-125-Evaluable population, and that sample size will then be used as the denominator for CA-125 response rate.

5.3.3 Patient report outcome

Change in patient-reported outcome from baseline to the last safety follow-up visit for the FACT-O subscales was assessed in patients with a baseline and postbaseline score for the specific subscale.

5.4 Exploratory efficacy analyses

The protocol describes the following exploratory efficacy analyses:

- Evaluate alternation of blood Mn^{2+} concentration and the association with response to the study treatment
- Evaluate alternation of serum cytokines and chemokines and the association with response to the study treatment
- Characterize treatment-induced alternation of PBMCs cellular profile

5.5 Efficacy analysis on subgroups of patients

ORR, PFS, and OS will be analyzed with the following groups of patients:

- Age (<57years; ⩾57years)
- Histologic grade (Epithelial)

- FIGO stage (III; IV)
- ECOG (0; 1)
- Previous treatment lines (<1; ≥4)
- Primary PFI (<6months; ≥6months)
- Most recent PFI (<1months; 1-6months)
- Prior Bevacizumab treatment (Yes; No)
- Prior Nab-Paclitaxel treatment (Yes; No)
- Prior anti-PD-1 treatment (Yes; No)
- Prior PARPi treatment (Yes; No)
- BRCA mutated (Positive; Negative)
- HRD (Positive; Negative)

The summaries for time to event variables include the number and percentage of events, median and its 95% CI, 2-sided *P*-value, and hazard ratio (Mn^{2+} group to placebo group) and its 95% CI.

The forest plot of time to event variables will display the number of subjects, median of each subgroup, hazard ratio ((Mn^{2+} group to placebo group) and its 95% CI.

The summaries for ORR include the ORR and its 95% CI, and risk ratio and its 95% CI. The forest plot of ORR will display the number of subjects, difference of the two group, risk ratio and its 95% CI. All subgroup analyses will be unstratified.

Due to potential small number of patients will be eligible for DOR analysis, no subgroup analyses will be conducted for DOR.

6. Safety analyses

6.1 Exposure

The following information will be provided.

Treatment doses and administration of manganese chloride, sintilimab, nab-paclitaxel, and cisplatin (or carboplatin) will be calculated and summarized. Number of cycles administered per patient of the study treatment will be summarized, also the number and percentage of patients with each number of cycles received will be shown.

Number and percentage of patients with delays, reductions, interruptions and withdrawals will be tabulated. The total number of cycles affected, and reasons given by each of these actions will be analyzed.

6.2 Adverse events (AEs)

6.2.1 AEs recording

AEs will be documented on the adverse event form and monitored continuously throughout the study from the time of informed consent until the end of the safety follow-up visit or until the event has resolved, stabilized, or returned to baseline. AEs deemed unrelated to study treatment by the investigator will no longer be collected after this point. However, all AEs must be followed up until death or loss to follow-up, withdraws consent for further data collection, and study termination, whichever occurs first.

AEs are documented and graded using Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Researchers should record AEs or SAEs using medical terminology/concepts, avoiding colloquialisms and abbreviations. Researchers must comprehensively record every adverse event, including diagnosis (or symptoms, signs including laboratory abnormalities if no diagnosis), onset and end dates and times (if applicable), CTCAE severity grade and changes, whether it is a serious adverse event,

actions taken regarding the investigational product, treatments given for the AE, and the outcome of the event, the relationship between the adverse event and the investigational product, and the causality assessment with the study procedure.

6.2.2 Treatment-related AEs

AEs are defined as any unfavorable medical occurrence in a clinical trial subject after administration of the trial medication, which can manifest as symptoms, signs, illness, or laboratory abnormalities, regardless of causality with the trial medication. Medical history conditions that exist before the initiation of study drug but worsen in severity during the study will also be recorded as an AE and will be included as treatment-related in the summary tables and listings.

AEs that are definitely, probably or possibly related to the study drug will be considered as related to the study drug. AEs with missing or unknown relationship to the study drug are considered as related to the study drug. AEs with the closest relatedness to the study drug will be used for summaries.

6.2.3 Serious AEs

Serious AEs are defined as an AE that meet any of the following criteria at any stage of the clinical study:

- results in death (fatal)
- life threatening (where "life-threatening" means immediate risk of death at the time of the event, not hypothetical future severity)
- leads to hospitalization or prolongation of hospitalization
- results in persistent or significant disability/incapacity
- other significant medical events that could result in the conditions listed above if not treated

6.2.4 Immune-mediated AEs

The immune-mediated AEs are defined as adverse event of unknown etiology associated with drug exposure and consistent with an immune phenomenon, and after ruling out neoplastic, infectious, metabolic, toxin or other etiologic causes.

6.2.5 Manganese-related AEs

The overdose of manganese has been reported to cause a neurologic disorder, just like the Parkinsonian Syndrome. The blood concentration of Mn^{2+} must be monitored on time. Once the manganese is overdosed and/or the neurologic AE appears, manganese chloride must be discontinued.

6.3 Clinical laboratory results

Laboratory test results (including hematology, coagulation, serum chemistry, and urinalysis) and abnormal laboratory values will be presented in data listings.

CTCAE Version 5.0 laboratory grades will also be presented. CTCAE grades will be derived based on laboratory results and will not factor in clinical evaluations. Shift tables summarizing the changes from baseline in severity of laboratory grades will be provided for laboratory parameters graded according to CTCAE Version 5.0.

6.4 Vital Signs

Vital signs (including temperature, pulse rate, systolic blood pressure, diastolic blood pressure, and respiratory rate) will be collected throughout the study.

Vital signs results will be classified into 4 or 5 categories (low, borderline low, normal, borderline high, or high). Shift tables based on this classification will be summarized from baseline to the last post-baseline visit value.

7. Patient report outcome (PRO)

Analyses for PRO, including quality of life and healthcare resource utilization, will be covered by a separate, independent analysis plan.

8. Blood sample analyses

The exploratory analyses of the blood sample, including Mn^{2+} concentration, serum cytokines and chemokines, and PBMC scRNA-seq for this study will be covered by a separate, independent analysis plan.

9. Statistical software

All analysis and reporting will be undertaken using StataMP 17 software (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.)