To compare the effectiveness and safety of different adjuvant chemotherapy regimens for high-risk triple-negative breast cancer patients predicted by mRNA-lncRNA model

(an open-label, multi-center, randomized controlled study)

Study protocol

Study protocol number: 20150514

Study institute: Fudan University Shanghai Cancer Center

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Contents

1. Study protocol summary (Table 1)

Table1: study protocol summary

Study title	To compare the effectiveness and safety of different adjuvant chemotherapy regimens for high-risk triple-negative breast cancer patients predicted by mRNA-lncRNA model (an open-label, multi-center, randomized controlled study)
Study institute	Fudan University Shanghai Cancer Center
Study objective	To assess the effectiveness and safety of TA(E)C*4-GP*4 regimen and A(E)C*4-T*4 regimen for high-risk patients identified by amRNA-lncRNA signature, and to compare the effectiveness of A(E)C*4-T*4 regimen in high-risk and low-risk patient groups.
Study design	Open-label, multi-center, prospective, randomized controlled study
Study population	Early-stage triple negative breast cancer patients
Number of subjects	503 cases
Treatment protocol	Adjuvant chemotherapy High-risk patients: TA(E)C*4-GP*4 regimen or A(E)C*4-T*4 regimen; Low-risk patients: A(E)C*4-T*4 regimen
Outcome measures	Disease-free survival (DFS), recurrence-free survival (RFS), overall survival (OS)
Evaluation for safety	The safety assessment was conducted based on physical examinations, laboratory tests, and the detection of adverse events and serious adverse events. Adverse events were observed and graded using the NCI-CTCAE version 4.0, following the common toxicity grading standard.
Statistical analysis	Efficacy analysis is conducted in the incorporated intent-to-treat population; The safety analysis included all patients who had undergone at least one protocol therapy dose. All statistical tests were performed using a two-sided approach.

2. Study background

Triple negative breast cancer is a highly heterogeneous subtype of breast cancer. Compared with other breast cancer types (luminal A, luminal B and HER-2 positive), it exhibits aggressive characteristics such as larger size, higher staging, more lymph node metastasis and so on. Due to the heterogeneity of triple-negative breast cancer and the lack of molecular targets, there still lacks well-recognized and effective treatment for TNBC in clinical setting. Previous studies suggest that triple negative breast cancer can be divided into different subtypes, with each subtype characterized

by its own unique gene expression, different subtypes exhibit diverse responses to the same adjuvant chemotherapy regimen. However, there remains a deficiency in effective classification methods in clinical practice to subtype TNBC and tailor individualized treatment strategies.

Long non-coding RNA (lncRNA) is a type of RNA that does not encode proteins and has more than 200 base pairs. In recent years, the role of lncRNA in tumorigenesis and development has been widely confirmed. As for breast cancer, some new regulatory mechanisms of lncRNA have also been proposed. Differentially expressed lncRNAs in tumor tissue may promote tumor formation and malignant phenotype presentation by regulating the expression of target genes. Gupta's team found that lncRNA HOTAIR was specifically overexpressed in metastatic breast cancer specimens, and its expression level was closely related to tumor metastasis and prognosis. Another study showed that the specific low expression of lncRNA GAS5 in breast cancer; Overexpression of GAS5 in breast cancer cell line can inhibit cell proliferation and induce cell apoptosis. The research team of the Cancer Hospital of the Chinese Academy of Medical Sciences has constructed a prognosis prediction model for esophageal squamous cell carcinoma based on differentially expressed lncRNAs in cancer. This model can well predict the outcome of patients. With the deepening of research on lncRNA, more and more evidence suggests that lncRNA can be used as a good prognostic predictor.

In our previous work, our research team used HTA2.0 chip technology to screen out differentially expressed mRNA and lncRNA in cancer tissues by detecting 33 pairs of triple-negative breast cancer and adjacent normal tissues. Based on this, we integrated the transcriptome expression information and follow-up data from an additional 132 patients with triple-negative breast cancer (total of 165 patients), and identified differentially expressed mRNA and lncRNA that were associated with prognosis in triple-negative breast cancer. After strict screening and validation, a total of 5 RNA (3 mRNA and 2 lncRNA) were included in the prognostic prediction model. Survival analysis showed that there was a significant difference in survival between

the high-risk and low-risk patient populations classified by this model (Figure 1). Using a time-dependent ROC curve to test the prognostic prediction performance of the model, it could be found that compared to other common clinical pathological indicators and their integrated comprehensive prediction factors, this model had a larger area under the curve, which indicated better sensitivity and specificity (Figure 2). Multivariate survival analysis further confirmed that this model could be used as an independent predictor for the prognosis of triple-negative patients (Table 1). The team further explored whether the model could predict the sensitivity of triple-negative patients to treatment. The interaction results showed that for survival prediction, there was a significant dependence between the high-risk and low-risk groups of the model and whether to use paclitaxel-based chemotherapy. That is, the relationship between the high-risk and low-risk groups of the model and survival depends on whether paclitaxel-based chemotherapy is used (Table 2). An analysis based on whether taxol-based chemotherapy was used as a stratification factor showed that the survival difference between high-risk and low-risk groups was only present in patients who received taxol-based chemotherapy. This suggests that the low-risk patients predicted by the model have more benefits from paclitaxel chemotherapy. In summary, the prediction model based on the expression information of mRNA and lncRNA can effectively distinguish patients with high and low risk of recurrence, and also has a certain predictive effect on the efficacy of paclitaxel chemotherapy.

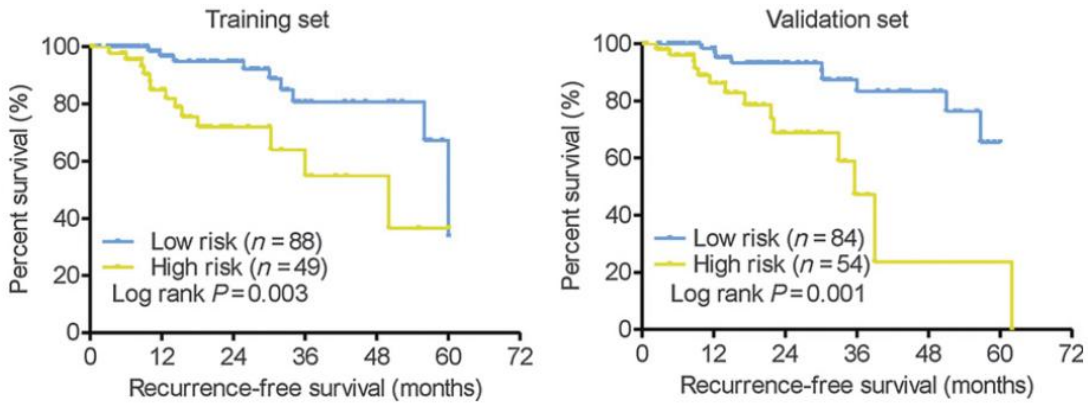

Figure 1: Kaplan-Meier curve diagram for patients with high and low risk recurrence predicted by

the mRNA-lncRNA model. The left figure shows the results of the training set, and the right figure shows the results of the validation set. It can be seen from the figure that regardless of whether it is the training set or the validation set, the prognosis of the high-risk patients predicted by the model is significantly worse than that of the low-risk patients.

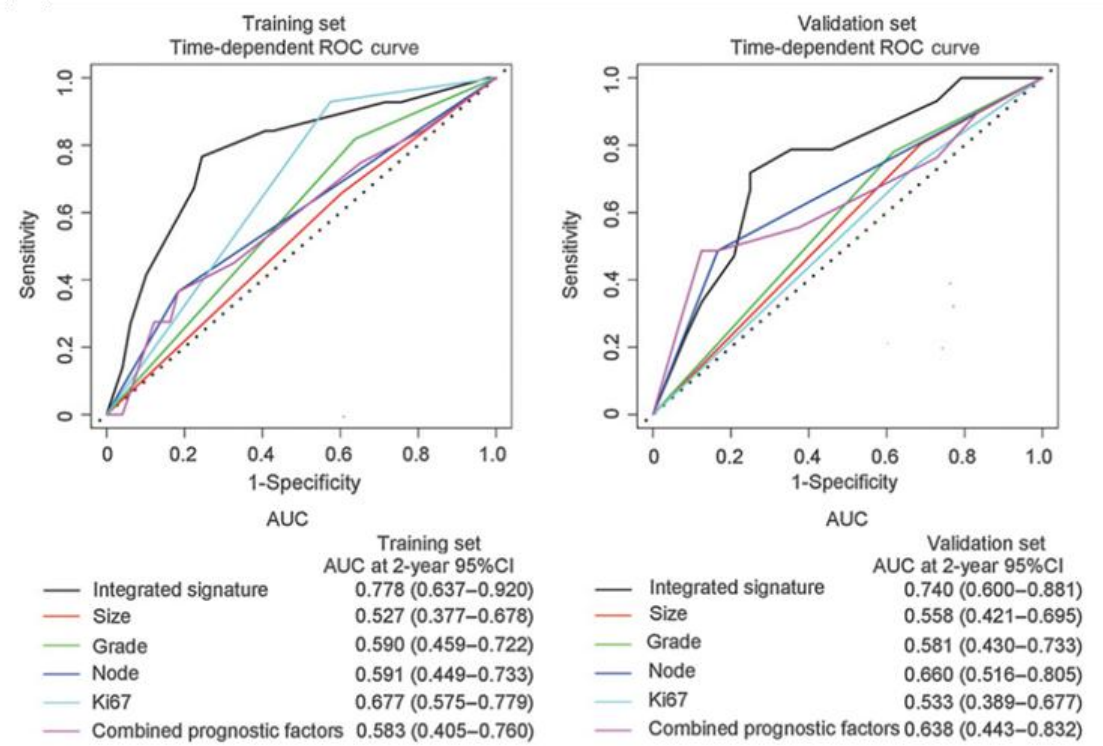

Figure 2: Time-dependent ROC curve diagram. The left figure shows the results of the training set, and the right figure shows the results of the validation set. It can be seen from the figure that regardless of whether it is the training set or the validation set, the area under the curve of the model's prognostic prediction is significantly better than other common prognostic indicators.

Table 1: Results of multivariate Cox proportional hazards regression analysis

	training set		**validation set**	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age (≤50y vs. >50y)	0.20 (0.04-0.98)	0.047	4.40 (0.26-74.48)	0.304
Menopause (No vs. Yes)	0.95 (0.23-3.98)	0.947	0.18 (0.01-2.99)	0.229
Classification (≤II vs. >II)	0.36 (0.10-1.37)	0.136	2.26 (0.56-9.11)	0.252

Size (≤2cm vs. >2cm)	2.32 (0.66-8.11)	0.188	2.64 (0.51-13.77)	0.25
Number of positive lymph nodes (≤3 vs. >3)	2.86 (0.85-9.55)	0.089	4.46 (1.18-16.94)	0.028
Radiotherapy (No vs. Yes)	3.26 (0.91-11.69)	0.070	1.47 (0.39-5.53)	0.57
Chemotherapy (Without taxane vs. With taxane)	1.68 (0.55-5.09)	0.363	0.41 (0.12-1.43)	0.164
Prediction model (Low-risk vs High-risk)	4.63 (1.77-12.07)	0.002	3.89 (1.60-9.47)	0.003

Table 2: Results of interaction analysis

	training set HR (95%CI)	*P*	**validation set** HR (95%CI)	*P*
Chemotherapy (Without taxane vs. With taxane)	1.68 (0.55-5.09)	0.363	0.41 (0.12-1.43)	0.164
Prediction model (Low-risk vs High-risk)	4.63 (1.77-12.07)	0.002	3.89 (1.60-9.47)	0.003
Interaction	7.69 (2.31-25.62)	0.001	4.09 (1.13-14.79)	0.032

Based on previous studies, this study aims to further verify the sensitivity and specificity of the model through prospective clinical trials, and explore more specific and effective adjuvant treatment regimens for triple negative breast cancer patients. The results are expected to provide basis for risk stratification and individualized treatment of triple negative breast cancer patients.

3. Study endpoints

Primary Endpoint: Disease-free survival (DFS) between different treatment arms in the high-risk cohort (arm A vs arm B).

Secondary Endpoints: DFS between high-risk and low-risk cohort receiving same treatment (arm B vs arm C), recurrence-free survival (RFS), overall survival (OS) and Safety

Exploratory Research: Collect blood plasma and tumor specimens from patients participating in the prospective clinical trial for future investigations aimed at refining prognostic/predictive factors in TNBC.

4. Study design

This study is an open-label, multi-center, prospective, randomized controlled study. TNBC patients confirmed by histopathology were included in this study. According to the central laboratory test results of surgical specimens, the high and low risk of patients was determined using the triple negative breast cancer mRNA-lncRNA signature. Patients in the high-risk group were randomly assigned to receive adjuvant chemotherapy with either arm A or arm B regimens. Arm A: TA(E)C*4-GP*4 regimen; Arm B: A(E)C*4-T*4 regimen. Patients in the low-risk group received the same adjuvant chemotherapy as arm B. Arm C: A(E)C*4-T*4 regimen. The specific plans for each arm are detailed later. The specific operation process is shown in Figure 3.

5. Participants enrollment

5.1 Inclusion criteria：

1) Age: 18-70 years old;
2) Expected survival time>12 months.
3) Has baseline ECOG performance status of 0-1.
4) Has undergone breast cancer surgery and subsequently confirmed as invasive unilateral breast cancer through postoperative pathological examination;
5) Clear resection margins after mastectomy or a breast-conserving procedure.
6) positive lymph node or negative lymph node with primary tumor diameter >10 mm.
7) Triple negative breast cancer confirmed by immunohistochemistry: the definition of triple-negative breast cancer refers to the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) in pathological examination. Specifically: ER negative: IHC<1%, PR negative: IHC<1%, HER2 negative: IHC-/+ or IHC++ but FISH/CISH negative.
8) Adequate bone marrow function: absolute neutrophil count ≥ $1.5x10^9$/L, hemoglobin ≥ 100g/L, platelet count ≥ $100x10^9$/L.

9) Adequate liver and kidney function: AST ≤ 60U/L, TBIL ≤ 2.5×ULN, Serum creatinine ≤ 110μmol/L, BUN ≤ 7.1mmol/L.
10) Beginning of chemotherapeutic treatment within 8 weeks of surgery
11) Adequate heart function, with normal ECG and LVEF≥50%.
12) Women of childbearing age are willing to adopt reliable contraceptive measures during the clinical trial, with a negative serum or urine pregnancy test required within 7 days prior to administration.
13) Sign the informed consent form, willingly and able to participate in scheduled visits, treatments, laboratory examinations, and other research procedures.

5.2 Exclusion criteria

1) Has received any systemic therapy for breast cancer (include neoadjuvant and adjuvant chemotherapy, targeted therapy, experimental therapy, etc.).
2) Has evidence of inflammatory breast cancer, bilateral breast cancer, or metastatic breast cancer.
3) Has another malignancy (exceptions include cervical carcinoma in situ or cutaneous basal cell carcinoma that is under control).
4) Has uncontrolled pulmonary disease, severe infection, active digestive ulcer, coagulopathy, severe uncontrolled diabetes, connective tissue disease, or bone marrow dysfunction, etc. Cannot tolerate adjuvant therapy and related treatment.
5) Peripheral neuropathy >1 degree due to any cause.
6) Has a known history of congestive heart failure, uncontrolled or symptomatic angina, arrhythmia, or myocardial infarction, refractory hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 100 mmHg).
7) Coagulation abnormality.
8) Breast cancer during pregnancy and lactation.

9) Has known psychiatric disorder or other reasons that would interfere with cooperation with the requirements of the study.
10) Has a severe hypersensitivity to the components of the study drug.
11) Has undergone major surgery or suffered severe trauma within the first two months prior to the first administration of the study drug.
12) Is currently receiving or recently (within 30 days before enrollment) received another investigational drug or participating in another study.
13) Has a known history of Human Immunodeficiency Virus (HIV).
14) Conditions deemed unsuitable for inclusion by other investigators.

5.3 Criteria of discontinuation

1) Subject requests the discontinuation of study drug.
2) Occurrence of any clinical adverse events, abnormalities in laboratory tests or other medical conditions that may cause the subjects no longer benefit from subsequent treatment.
3) Overall deterioration of health status, unable to continue participating in the trial.
4) Significant deviations from the protocol after enrollment, such as unqualified and non-compliant subjects, etc.;
5) Lost to follow-up;
6) The investigator terminates the study;
7) Other reasons leading to the investigator’s determination that it is impossible to continue the treatment.

5.4 Criteria of withdrawal

1) Requirement to withdraw the consent by subjects

Subjects may voluntarily withdraw from the study at any time. If subjects do not return for scheduled visits as planned, every effort should be made to contact them. In any case, record the subject's results as comprehensively as possible. The investigator should inquire about the reasons for withdrawal, and if possible, request them to undergo the last clinical visit and follow up on unresolved

adverse events. If patient withdraws from the study and retracts the consent, refusing to disclose further information, then further evaluation or collection for additional data are not required. Patients with interruption of follow-up longer than 6 months should be considered as withdrawn from trial.

2) Researchers believe subjects should exit trial due to medical-related consideration.

For the purpose of protecting the subjects, they should be required to exit trial even before completion of scheduled follow-ups; Use of other therapy or medication contraindicated to combined usage that affects the evaluation of safety; Severe adverse event occurs thus unsuitable to accept further clinical visits; Subjects fail to visit on time or lost, featured with poor compliance.

6. Statistical hypothesis and sample size

The sample size, based on the need for sufficient high-risk patients, aimed to test TA(E)C-GP's superiority over A(E)C-T. Based on our previous study results and literature review, for high-risk patients, we predict a 3-year DFS of 90% for the TA(E)C*4-GP*4 regimen and 79% for the A(E)C*4-T*4 regimen. Employing a two-sided significance level of 0.05, a power of 80%, a 3-year enrollment period and 2-year follow-up period, and accounting for a 9% lost to follow-up rate, a total of 335 high-risk TNBC patients with 50 DFS events are required (equally distributed between the control and experimental groups). To compare the efficacy of standard treatment between high and low-risk groups receiving standard treatment, with a 1:1 ratio and a prospective observational trial design, it is planned to include 168 low-risk patients receiving standard treatment. Hence, the overall required sample size for this study is 503.

The primary analysis for high-risk patients will be conducted after collecting 50 DFS events between arm A and arm B or at least 80 months after first patient enrollment.

7. Flow chart

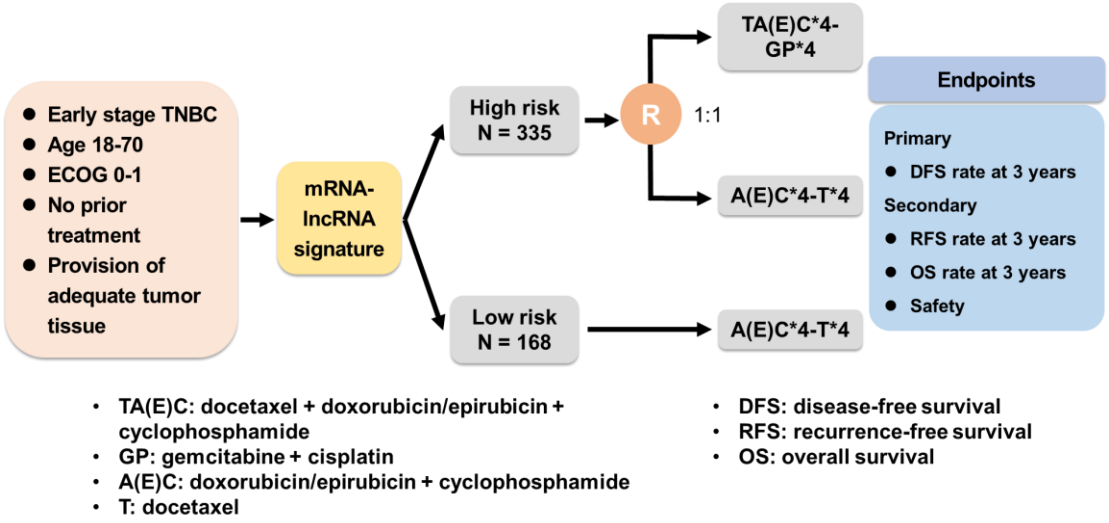

Figure 3: Study schema

8. Investigational drug

This study prospectively validates the utility of the mRNA-lncRNA signature in predicting the prognosis of TNBC patients and their sensitivity to taxane chemotherapy. Additionally, it aims to assess the efficacy and safety of different chemotherapy regimens for high and low-risk patients stratified by this signature. The three regimens designed for the trial adhere to standard treatment protocols, with dosages, routes of administration, frequencies of dosing, courses of treatment, and regulations on concomitant medications all based on drug instructions. The details are as follows：

High-risk patients：

Arm A：TA(E)C*4-GP*4 regimen

Docetaxel 75mg/m^2 iv d1

Doxorubicin 50mg/m^2 or Epirubicin 75 mg/m^2 iv d1

Cyclophosphamide 500 mg/m^2 iv d1

Every 21 days for 4 cycles

Sequential Gemcitabine 1250 mg/m^2 iv d1 and d8

Cisplatin 75 mg/m^2 iv d1

Each cycle of cisplatin use requires hydration for 3 days (d1-3, with at least 1L

of fluids before cisplatin administration) to prevent renal toxicity

Every 21 days for 4 cycles

Arm B：A(E)C*4-T*4 regimen

Doxorubicin 60 mg/m^2 iv or Epirubicin 90 mg/m^2 iv d1

Cyclophosphamide 600 mg/m^2 iv d1

Every 21 days for 4 cycles

Sequential Docetaxel 100 mg/m^2 iv d1

Every 21 days for 4 cycles

Low-risk patients：

Arm C：A(E)C*4-T*4 regimen

Doxorubicin 60 mg/m2 iv or Epirubicin 90 mg/m2 iv d1

Cyclophosphamide 600 mg/m2 iv d1

Every 21 days for 4 cycles

Sequential Docetaxel 100 mg/m2 iv d1

Every 21 days for 4 cycles

9. Supportive treatment and concomitant medications

9.1 Preventive medication

1) Antiemetic therapy

Acute vomiting usually occurs one to two hours after administration and can last for about one week. Standard antiemetic regimen was recommended including 5-HT antagonist, aprepitant and dexamethasone, day 1-3; or use promethazine instead, 25mg im, day 1-3 (for patients who refused to use aprepitant).

2) Intravenous hydration

The renal toxicity of cisplatin mainly involves renal tubular damage. Acute damage is generally observed 10-15 days after administration, with elevated blood urea nitrogen and creatinine levels and decreased creatinine clearance, which is mostly reversible. This study recommends routine hydration treatment during cisplatin use, with specific reference to the cisplatin usage instructions and hydration principles. Generally, it is recommended to hydrate for 3 days, with a daily intake of

at least 2500ml. The amount of intravenous rehydration before and after the day of cisplatin is at least 2L, usually 1L before and after cisplatin (500ml of cisplatin itself is not included, but other fluids are included). Changes in renal tubular function and large amounts of fluid supplementation may cause electrolyte disturbances, and electrolyte disturbances can aggravate renal damage. Therefore, it is recommended to add potassium and magnesium electrolytes at the same time for hydration. According to literature reports, the general amount of potassium chloride is 20-30mmol/L, taking 2L of rehydration per day as an example, which is equivalent to 3-4.5g. It is recommended to supplement magnesium 40-80mmol per cycle of treatment, which is equivalent to add magnesium sulfate with 4.8-9.6g/cycle or adding magnesium chloride supplementation with 2.4-4.8g/cycle, generally after cisplatin. Studies have used magnesium sulfate 10mmol/L (1.2g) daily to add 1000ml of normal saline or magnesium sulfate 2-2.5g (16-20mmol/L) to 1000ml of normal saline. There is also a study showing that adding 8mmol of magnesium before cisplatin can reduce nephrotoxicity. The use of furosemide or mannitol is still controversial.

3) Pre-treatment with dexamethasone prior to using docetaxel

Before using docetaxel, dexamethasone should be used routinely. Please refer to the instructions for using docetaxel and follow clinical practice.

9.2 Supportive treatment

1) Colony stimulating factors (G-CSF)： Primary prophylaxis with pegylated recombinant human granulocyte colony-stimulating factor (PEG-rhG-CSF) is recommended to all patients. PEG-rhG-CSF is subcutaneously injected 24 hours after chemotherapy (6 mg for patients with body weight >45 kg, 3 mg for patients with body weight ≤45 kg). rhG-CSF is administered according to clinical routines and injected subcutaneously (150 μg/kg).

2) Platelet transfusion and/or treatment of thrombocytopenia： For patients with grade 3/4 thrombocytopenia, interleukin-11 (IL-11), TPO, and/or platelet transfusion can be prescribed according to clinical routines.

3) Antibiotics: Some antibiotics (eg. aminoglycoside antibiotics, amphotericin B,

or cephalothin) increase nephrotoxicity when combined with DDP, which should be avoided during treatment. Other drugs can be used based on clinical principal.

9.3 Concomitant medications

In this study, it is necessary to minimize the use of concomitant medications as much as possible. However, if the use of these medications is necessary for the benefit of the patient and d doesn't have an effect on this study, the investigator may use them based on the specific circumstances. All concomitant medications used during the the study must be recorded in the medical record and ICF.

10. Treatment delay and dose adjustment

If the patient cannot meet the following conditions, treatment should be delayed: 1) ANC≥1.5×109/L; 2) PLT≥100×109/L; 3) HGB≥90g/L; 4) Non-hematological toxicity ≤ Grade 1 (excluding hair loss, nausea, etc. that the investigator deems not threatening to patient safety). Patients with grade 3/4 peripheral neurotoxicity should discontinued the treatment, and the treatment will continue until neurotoxicity recovers to ＜grade 2. The treatment for patients with other ≥ grade 2 non-hematological toxicities were interrupted until the toxic effects resolved to a grade lower than 2 (except alopecia, nausea and vomiting). The delay in treatment can be extended up to 14 days to allow recovery from hematologic and non-hematologic toxicities.

The dosage can be adjusted in patients under the following conditions: 1) Grade 4 neutropenia lasting for ≥ 3 days; 2) febrile neutropenia (Grade 3/4 neutropenia accompanied by fever ≥ 38.3 degrees once or ≥ 38.0 degrees for more than one hour); 3) Onset of Grade 4 platelet decline, or bleeding related to platelet reduction; 4) Grade 3 anemia; 5) grade 3/4 nonhematological toxicities; 6) Other toxicities that the investigator deems necessary for dose adjustment. Dose reduction should be based on the most severe toxicity level of the previous treatment course, with the first adjustment of each regimen drug to 75% of the original dose and the second adjustment to 50% of the original dose, and a maximum of two dose reductions are allowed. Which drug should have dose-reduction depends on the toxicity of last cycle

of treatment.

Does reduction of gemcitabine

Pulmonary toxicity: If grade 2 or higher pulmonary infection occurs and is judged to be related to gemcitabine, the use of gemcitabine should be stopped immediately;

Hemolytic uremia: If a patient presents with elevated bilirubin or LDH, increased reticulocytes, severe thrombocytopenia, and/or microvascular disease hemolytic anemia manifestations of renal failure, hemolytic uremia should be considered and gemcitabine should be stopped immediately;

Except for hair loss, nausea/vomiting, if other severe (Grade 3/4) non-hematological toxicities occur and are considered related to gemcitabine, the investigator may consider temporarily suspending or reducing the dose of gemcitabine.

Does reduction of cisplatin

Renal toxicity: Adjust the amount of cisplatin according to the renal function: when the creatinine clearance rate is 45-60 ml/min, reduce the amount of cisplatin by 25%; when the creatinine clearance rate is 30-45 ml/min, reduce the amount of cisplatin by 50%; when the creatinine clearance rate is <30ml/min, suspend the drug until it returns to level 0-1 before starting the next cycle of treatment;

Grade 3/4 neurotoxicity: When the cumulative dose reaches 300-600mg/m2, peripheral neuropathy will occur, mainly manifested as loss of vibration sensation, loss of deep tendon reflex, and significant proprioceptive ataxia. Stop using cisplatin in case of such condition.

Application and treatment of gemcitabine on d8

Dose reduction or treatment delay is permitted for gemcitabine on d8, which is determined by the investigator.

The dosage adjustment of gemcitabine is based on the neutrophil and platelet counts on d8. If the ANC $\geq 1.2*10^9$/L and PLT $\geq 100*10^9$/L on d8, the full dosage of gemcitabine can be given on that day. The dose should be modified according to the table below when patients had a decreased neutrophil and/or platelet counts.

Gemcitabine can also be delayed, but not beyond 7 days (i.e., on the 15th day). If the blood test on the 15th day still shows neutrophil count <1.2*10^9/L and platelet count <100*10^9/L, gemcitabine should not be given on the d8 of this cycle.

Dosing adjustment principles for gemcitabine on d8 of the combined regimen

ANC（*10^9/L）		**Platele tcount (*10^9/L）**	**Percentage of original dose**
≥1.2	and	>75	100
1.0-1.2	or	50-75	75
0.7-1.0	and	≥50	50
<0.7	or	<50	Hold

11. Study Procedures

11.1 Screening

Patients undergo a complete medical history and physical examination at baseline, including blood tests, liver and kidney function tests, ECG, echocardiogram, chest CT, breast ultrasound, MRI, and if necessary, bone scan.

11.2 Clinical Observation and Follow-up

Before start of every cycle of adjuvant chemotherapy, a physical examination is conducted, including evaluation of the breast and regional lymph nodes. In addition, various blood cell counts, liver and kidney function, and ECG were performed before next cycle of treatment. Cardiac ultrasound is performed every two cycles.

After the completion of adjuvant chemotherapy, regular follow-up appointments are scheduled. For the first two years, physical examinations are conducted every three months, including evaluation of the breast and regional lymph nodes, testing of tumor markers (CA15-3, CEA, AFP), and liver and kidney function tests. After the first two years, follow-up appointments are scheduled every six months. Chest CT and mammography are conducted once a year.

12. Evaluation of efficacy and safety

12.1 Efficacy evaluation

The primary endpoint was disease-free survival (DFS), defined as time from random assignment to first relapse (local, regional and distant), contralateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, or carcinoma in situ), or death with any cause. Secondary end points included relapse-free survival (RFS), defined as the time from the date of randomization to local, regional, distant relapse or death with any cause; overall survival (OS), defined as time from randomization until death with any cause.

12.2 Safety evaluation

Safety evaluation includes the observation and recording of all adverse events and serious adverse events, laboratory tests, physical examination, performance status scoring, electrocardiogram, etc. Adverse events are graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 4.0. Investigators have the responsibility to take corresponding treatment measures for adverse events. Investigators should complete the corresponding form in the case report form and determine the relationship between the adverse event or serious adverse event and this study.

13. Study duration

5 years (containing 2-year follow-up)

14. Adverse events

14.1 Definition

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to the treatment, whether or not considered causally related to the treatment.

14.2 Adverse event reporting period

The reporting periods starts from enrollment and lasts till the final follow up. Any adverse event happens during this period should be filled in the case report form.

14.3 Severe adverse event (SAE)

An adverse event is defined as severe adverse event if it agrees with one or more following criteria:

1) Death
2) Life-threatening
3) Hospitalization (initial or prolonged)
4) Disability or Permanent Damage
5) Congenital Anomaly/Brith Defect
6) is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

14.4 Report of severe adverse event

For all severe adverse events the trial should be suspended immediately and corresponding measures to protect subjects should be practiced. Severe adverse events will be recorded in a table and reported to the director of the unit and the sponsor within 24 hours by phone or fax. The investigator shall fill in the severe adverse event report form and fax it to State Food and Drug Administration. Investigator should follow the severe adverse event till resolution. Relevant medical documents should be achieved within original material, including report sheet of laboratory test (e.g. CT examination, electrocardiogram, etc).

14.5 Record and report

For adverse event occurred during the trial, its time of onset, symptoms, severity, duration, treatment and prognosis should be recorded in the case report, so as to evaluate its significance with test compound. The detailed record should be provided, signed and dated by investigators. The adverse events will be graded based on NCI-CTC 4.0. For each symptom, the highest grade experienced since last follow-up should be reported.

Determination of relationship between adverse events and clinical trial:

Attribute to one of the five categories: definitely related, probably related, probably unrelated, unrelated and not appreciable. The first two categories are considered as adverse event and the proportions of adverse events will be calculated.

Definitely related: There is a plausible time relationship between the event and the medication, and the event conforms to the known reactions of the suspected drug; symptoms improve after stopping the drug, and the event recurs after administration is restarted.

Probably related: There is a plausible time relationship between the event and the medication, and the event does not conform to the known reactions of the suspected drug; the clinical state of patients or other treatment may also be responsible for the event.

Probably unrelated: There is no plausible time relationship between the event and the medication, and the event does not conform to the known reactions of the suspected drug; the clinical state of patients or other treatment may also be responsible for the event.

Definitely unrelated: There is no plausible time relationship between the event and the medication, and the event does not conform to the known reactions of the suspected drug. The clinical state of patients or other treatment may also be responsible for the event. The event is eliminated after symptoms improve or other treatment is terminated, and the event recurs after administration is restarted.

Unable to determine: There is no clear time relationship between the event and the medication. The event is similar to the known reactions of the drug. Other drugs used at the same time may also cause corresponding events.

15. Statistical analysis

The statistical analysis are undertaken by professional statisticians who are involved in the entire process, from experimental design and implementation to analysis and summary. A statistical analysis plan will be formulated after the completion of the trial protocol and case report forms. Necessary modifications are made to the plan during the trial as needed. The statistical analysis report should be submitted upon completion of data analysis.

15.1 Data set for statistical analysis

1) Intention-to-treat population (ITT)：All randomized patients were included in ITT.

Efficacy analyses were done on an ITT population in this study.

2）Safety Analysis Set (SS): It refers to subjects who have taken the study drug at least once.

15.2 Selection of statistical method

1) Statistical analysis will be performed using SPSS statistical analysis software and R software.

2）All statistical tests will use two-sided testing. A value of $P < 0.05$ is considered statistically significant.

3）Descriptions of quantitative data in the experimental group

Normally distributed data will be described using the mean ± standard deviation.

Non-normally distributed data will be described using the median (25%-75% percentiles, IQR).

4） Statistical Tests

a. Dropout analysis: number of cases recruited, completed cases, and reasons for dropout.

b. Correlation analysis: Use logistic regression analysis or chi-square test to analyze correlation between study indicators and adverse events.

c. Predictive power analysis: Evaluation of the accuracy of individual or overall prediction of adverse events using the area under the receiver operating characteristic curve.

d. Safety analysis: Safety analysis will be conducted using the safety analysis set. It is necessary to specify the number and percentage of patients with adverse reactions (with the total number of patients in the safety analysis set as the denominator).

16. Data management

a) For all patients who have signed the informed consent form and been screened to enter the study, all items in the case report form must be recorded carefully in detail, and no blank or missing items shall be allowed (blank spaces without records shall be underlined)；

b) All data in the case report form shall be checked with the medical records of subjects to ensure correctness;
c) As the original data, the case report form can only be underlined when any correction is made, and the revised data should be noted aside with the investigator's signature and date;
d) The copy of test report shall be pasted at the specified area of the test report attached to the case report form;
e) Significantly high data or data outside the scope of clinical acceptance should be verified and explained as necessary by the investigator;
f) Please refer to the pathology report form for instructions.

17. Quality control and quality assurance

Unified study protocol, standard operating procedures and quality control procedures should be established by all participating hospitals of this trial. Regular supervision and inspection will be performed during the trial to ensure the implementation of study protocol. The raw data will be reviewed to ensure the consistency with data in the case report form.

18. Ethical principle

The study procedure must strictly conform to the requirement of Good Clinical Practice of SFDA and the Declaration of Helsinki.

18.1 Institutional Ethics Committee (IEC)

This protocol and written informed consent as well as material directly related with subjects shall be submitted to ethics committee of the Fudan University Shanghai Cancer Center. This trial can only initiate after achievement of written approval of the ethics committee. Investigator are required to submit annual report (if applicable) to the ethics committee at least once a year. When the study is suspended and/or completed, the ethics committee should be informed by investigators with written notification; the ethics committee should be informed timely about any change during the study process (e.g. the modification to protocol and/or informed consent form),

and the change can only be realized after approval of ethics committee, unless those made to eliminate the obvious and immediate risk to subjects. The ethics committee should be informed anyway under above-mentioned circumstance. For the participant centers, independent institutional review board of the participating hospitals approved the study protocol separately.

18.2 Informed consent form (ICF)

Prior to enrollment the investigators are responsible for oral and written consent about information including objective, procedure and potential risks of the study to every subject. The subject should be informed about the right to decide whether to participate in the trial and that subject is free to withdraw from trial any time willingly. Subjects or their legal representatives will read and understand the informed consent form and sign it, and keep the copy of signature page.

18.3 Security measures

The medical records (medical history, physical and chemical tests report, etc.) will be integrally stored by hospitals. The doctors (investigators), professional academic committee, ethics committee and health supervision departments will be allowed with access to medical records. The result of this study may be submitted for publishing on medical journals. However, any open report concerning this study will not disclosure individual identity of patients. The privacy of personal medical information of patients should be protected by all permissible means.

19. Data retention and summary

19.1 Data retention

The case report forms will be confirmed with signature by investigators. After completion of trial, all case report forms, detailed materials about classic cases and clinical trial record forms will be sent to sponsor. Original materials relevant to subjects, laboratory results, signed informed consent originals and copy of case report forms will be kept by investigators.

19.2 Summary of trial

The fulfillment of “summary report of clinical trial” will be the responsibility of

study sponsor.

20. References

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Appendix 1: Participating centers

	Hospital
1	Fudan University Shanghai Cancer Center
2	Chongqing Cancer Hospital, Chongqing University
3	Northern Jiangsu People's Hospital
4	Fujian Medical University Union Hospital
5	Shanghai First Maternity and Infant Hospital
6	Obstetrics and Gynecology Hospital of Fudan University
7	The International Peace Maternity & Child Health Hospital of China Welfare Institute