Neoadjuvant Chemoradiotherapy Combined with Sequential Perioperative Toripalimab in Locally Advanced Esophageal Squamous Cell Cancer

<table>
<thead>
<tr>
<th>Institution</th>
<th>Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University</th>
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<tbody>
<tr>
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<td>Xiumei Ma</td>
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<td>Version Date</td>
<td>April 10, 2020</td>
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Protocol summary

Study title  Neoadjuvant Chemoradiotherapy Combined with Sequential Perioperative Toripalimab in Locally Advanced Esophageal Squamous Cell Cancer

Study phase  Phase II


Institution  Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai

Study objectives  To evaluate the efficacy and safety of preoperative PD-1 inhibitor toripalimab combined with chemoradiotherapy in the treatment for locally advanced esophageal squamous cell carcinoma (ESCC).

Sample size  20

Study Design  Patients with histopathologically confirmed thoracic ESCC and clinical staged as cT1-4aN1-2M0/cT3-4aN0M0 were enrolled. Eligible patients received radiotherapy (23 fractions of 1.8 Gy, 5 fractions a week) with concurrent chemotherapy of paclitaxel/cisplatin (paclitaxel 45mg/m2 and cisplatin 25 mg/m2) on days 1, 8, 15, 22, 29 and two cycles of toripalimab 240 mg every 3 weeks after nCRT for neoadjuvant therapy before surgery, four cycles of toripalimab 240 mg every 3 weeks for adjuvant therapy after surgery.

Inclusion criteria  1. Age 18-75 years, male or female;
2. Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition)
3. Without any previous treatment;
4. ECOG performance score is 0-1;
5. The indexes of hematology, biochemistry and organ function meet the following requirements: white blood cell count (WBC) \( \geq 3.0 \times 10^9/L \); neutrophil count (ANC) \( \geq 1.5 \times 10^9/L \); platelets \( \geq 85 \times 10^9/L \); hemoglobin \( \geq 9g/dL \); total bilirubin \( \leq 14.4\mu mol/L \); ALT \( \leq 75U/L \); serum creatinine \( \leq 104\mu mol/L \) and creatinine clearance rate \( >60 \text{ mL/min} \);
6. Women of childbearing age must undergo a pregnancy test within 7 days before enrolling in the treatment, and those who are negative can be enrolled. Patients of childbearing age and their sexual partners agree to use reliable methods of contraception before entering the study, during the study, and at least 180 days after the end of the study;
7. Ability to understand the study and sign informed consent.
| Exclusion criteria | 1. Patients with active infection within 2 weeks before the first use of the study drug or need to be treated with oral or intravenous antibiotics;  
2. A history of interstitial lung disease or non-infectious pneumonia;  
3. Patients whose clinician judges surgery as the first choice for the best treatment;  
4. A history of autoimmune diseases or abnormal immune system;  
5. Patients who cannot tolerate chemoradiotherapy or surgery due to severe cardiac, lung dysfunction.  
6. Patients diagnosed with any other malignant tumor within 5 years before enrollment, except for malignant tumors with low risk of recurrence and risk of death, such as fully treated basal cell or squamous cell skin and carcinoma in situ of the cervix;  
7. Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of Toripalimab, and the chemotherapeutic drugs paclitaxel or cisplatin;  
8. A history of immunodeficiency, including a positive HIV test result or other acquired or congenital immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation;  
9. Women during pregnancy or lactation;  
10. Other situations not suitable for enrollment. |
| --- | --- |
| Endpoints | **Primary endpoint:**  
Major pathological response rate (MPR)  
**Secondary endpoints:**  
Disease-free survival; Overall survival rate; Safety; Surgical complication rate |
| Statistical analysis | Statistical analyses were conducted using SPSS 25.0 software. DFS and OS will be analyzed using the Kaplan-Meier method and log-rank test. The safety analysis was based on descriptive statistics. |
1. Study Background

Esophageal cancer is a common and highly lethal malignant tumor in China. In the past, the main treatment approach was comprehensive therapy based on surgery, but its effectiveness has been unsatisfactory. Specifically, locally advanced esophageal cancer has a poor prognosis, with a 5-year survival rate of only 20.6%-34% for stage II-III patients who receive surgery as the sole treatment. The CROSS study conducted in the Netherlands in 2012 identified the role of preoperative neoadjuvant chemoradiotherapy in the treatment of locally advanced esophageal cancer: the median postoperative survival of patients who received neoadjuvant chemoradiotherapy alone increased from 24 months to 49 months. Preoperative neoadjuvant therapy can improve survival in patients with locally advanced esophageal cancer and achieve a pathological complete response rate of about 30%. Patients who achieve a pathological complete response experience significantly prolonged survival, with a 5-year survival rate ranging from 40% to 60%. The NEOCRTEC5010 study also demonstrated that the overall survival after neoadjuvant chemoradiotherapy for esophageal squamous cell carcinoma patients reached 100 months. However, there is still a need to enhance the effectiveness of preoperative neoadjuvant therapy, for more than half of these patients can not reach major pathological response leading high recurrence rate, and also about 10% of patients experience disease progression and miss the chance for surgery.

PD-1 monoclonal antibodies have demonstrated therapeutic advantages in clinical studies of advanced or metastatic esophageal cancer, either as monotherapy or in combination with other drugs. However, there is still a lack of reliable clinical evidence on whether PD-1 can improve the efficacy of neoadjuvant therapy for esophageal cancer. Preliminary results from a small sample trial presented at the 2019 ASCO conference suggest that PD-1 monoclonal antibodies can improve the pathological response rate in neoadjuvant therapy for esophageal cancer. Toripalimab, a PD-1 monoclonal antibody, has been approved for treating melanoma and has brought significant survival benefits to melanoma patients. It has also shown effectiveness in applications for lung cancer, esophageal cancer, and other tumors. The JS001-CT5 study showed that toripalimab, used in patients with metastatic esophageal squamous cell carcinoma, has definite efficacy and controllable safety, indicating its potential application in the neoadjuvant treatment for locally advanced esophageal cancer.

2. Study Objectives

To investigate the feasibility and safety of perioperative toripalimab combined with neoadjuvant chemoradiotherapy (paclitaxel and cisplatin) in the treatment of locally advanced esophageal squamous cell carcinoma.

2.1 Primary study endpoints

Major Pathological Response Rate (MPR): No more than 10% of tumor cells were found in neoadjuvant surgical specimens.

2.2 Secondary study endpoints

Disease-free survival; overall survival rate; safety and surgical complication rate.

3. Study Design
This project is a prospective single-arm, exploratory study with a small sample size of 20 patients to initially observe the efficacy and safety.

4. Study Population

4.1 Inclusion criteria

1) Age 18-75 years, male or female;

2) Histologically confirmed T1b-4aN2-3M0 ESCC (AJCC/UICC TNM staging, 8th Edition)

3) Without any previous treatment;

4) ECOG performance score is 0-1;

5) The indexes of hematology, biochemistry and organ function meet the following requirements: white blood cell count (WBC) $\geq 3.0 \times 10^9$/L; neutrophil count (ANC) $\geq 1.5 \times 10^9$/L; platelets $\geq 85 \times 10^9$/L; hemoglobin $\geq 9$g/dL; total bilirubin $\leq 14.4$μmol/L; ALT $\leq 75$U/L; serum creatinine $\leq 104$μmol/L and creatinine clearance rate $>60$ mL/min;

6) Women of childbearing age must undergo a pregnancy test within 7 days before enrolling in the treatment, and those who are negative can be enrolled. Patients of childbearing age and their sexual partners agree to use reliable methods of contraception before entering the study, during the study, and at least 180 days after the end of the study;

7) Ability to understand the study and sign informed consent.

4.2 Exclusion criteria

1) Patients with active infection within 2 weeks before the first use of the study drug or need to be treated with oral or intravenous antibiotics;

2) A history of interstitial lung disease or non-infectious pneumonia;

3) Patients whose clinician judges surgery as the first choice for the best treatment;

4) A history of autoimmune diseases or abnormal immune system;

5) Patients who cannot tolerate chemoradiotherapy or surgery due to severe cardiac, lung dysfunction.

6) Patients diagnosed with any other malignant tumor within 5 years before enrollment, except for malignant tumors with low risk of recurrence and risk of death, such as fully treated basal cell or squamous cell skin and carcinoma in situ of the cervix;

7) Known or suspected allergy or hypersensitivity to monoclonal antibodies, any ingredients of Toripalimab, and the chemotherapeutic drugs paclitaxel or cisplatin;

8) A history of immunodeficiency, including a positive HIV test result or other acquired or congenital
immunodeficiency diseases, a history of organ transplantation or allogeneic bone marrow transplantation;

9) Women during pregnancy or lactation;

10) Other situations not suitable for enrollment.

4.3 Removal criteria
Subjects who are enrolled but violate the protocol should be removed, including: (1) mis-diagnosis; (2) not meeting the inclusion criteria but meeting the exclusion criteria; (3) having incomplete data for evaluation of efficacy and safety. The reason of rejection should be explained and recorded. However, patients who have received treatment and have safety record should be included in the safety analysis.

4.4 Drop-out/Withdrawal criteria
Subjects could withdraw from the study at any time and for any reason. The investigator could withdraw a subject from the study including the following reasons: serious adverse events, trial protocol violation, poor compliance, lack of efficacy, trial discontinuation is determined to be necessary for subjects by the investigator.

4.5 Discontinuation criteria
Serious adverse events, or other events that affect subject safety; Radiographic disease progression or metastasis; Patients receive other anti-tumor treatments that affect the results of the study; The subject withdrew his informed consent; Other conditions that the investigator considers necessary to terminate the study drug treatment.

5. Treatment Plan
1.1 Pre-treatment staging
All patients underwent medical history taking, physical examination, routine laboratory tests, echocardiography, cardiac ultrasound and pulmonary function test. Pre-treatment tumor staging was performed through contrast-enhanced chest CT, esophagogastroduodenoscopy diagnostic biopsy with endoscopic ultrasound (EUS), esophageal barium imaging, magnetic resonance imaging (MRI) of the esophagus, and positron emission tomography-computed tomography (PET-CT).

1.2 Neoadjuvant treatment and outcome measurements
Neoadjuvant radiotherapy was given by means of intensity-modulated radiotherapy (IMRT). A total dose of 41.4 Gy radiation was given by 23 fractions with 1.8 Gy per fraction and five fractions per week. The chemotherapy regimen included paclitaxel (45 mg/m2) and cisplatin (25 mg/m2) were administrated weekly on days 1, 8, 15, 22 and 29. Two cycles of toripalimab 240 mg were given intravenously every 3 weeks after completion of chemoradiotherapy. The first cycle of toripalimab was administrated within 1-3 days after completion of radiotherapy. 3 weeks after the administration of the second cycle of immunotherapy, disease reevaluation was performed, including physical examination, routine laboratory test, echocardiography,
pulmonary function test, contrast-enhanced chest CT, MRI, and PET-CT, to restage and exclude cases not suitable for surgery.

5.3 Surgery and adjuvant treatment

Surgery was scheduled for 4-6 weeks after the second treatment cycle of toripalimab. A minimal invasive McKeown esophagectomy was performed, including two-field lymphadenectomy or three-field lymphadenectomy in individual patients by a single experienced surgeon. Adjuvant treatment should be started within 12 weeks after surgery. Toripalimab 240 mg were given every 3 weeks in 4 cycles for adjuvant treatment.

6. Dose adjustment

6.1 Dose adjustment of chemotherapy drugs

Neutropenia is the dose-limiting toxicity in paclitaxel monotherapy. Other possible adverse events include hypersensitivity, skin reactions, gastrointestinal toxicity (nausea, vomiting, stomatitis, diarrhea), alopecia, muscle weakness, mild injection site reactions (phlebitis), peripheral neurotoxicity, and fluid retention/edema. Cisplatin monotherapy is associated with major adverse events such as ototoxicity, peripheral neuropathy, renal failure, and vomiting. When subjects experience multiple toxicities and there are differing dose adjustment principles, the minimum dose is chosen. If the drug dose is reduced due to drug-related toxicity, it cannot be increased back to the original dose and should continue at the reduced dose.

6.1.1 Dose adjustment grade of chemotherapeutic agents:

<table>
<thead>
<tr>
<th></th>
<th>Original dose (100%)</th>
<th>-1 Dose level (90%)</th>
</tr>
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<tbody>
<tr>
<td>cisplatin</td>
<td>25mg/m2</td>
<td>22.5mg/m2</td>
</tr>
<tr>
<td>paclitaxel</td>
<td>45mg/m2</td>
<td>40.5mg/m2</td>
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6.1.2 Adjustment for bone marrow toxicity:

<table>
<thead>
<tr>
<th>ANC ≥ 1,000/mm³ and platelets ≥ 100,000/mm³</th>
<th>full doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC &lt; 1,000/mm³ Or platelets &lt; 100,000/mm³</td>
<td>Postpone chemotherapy for one week, if ANC ≥ 1,000/mm³ and platelets ≥ 100,000/mm³ in the next week examination, the full dose chemotherapy with both drugs should be continued. If only partial recovery of bone marrow toxicity, both drugs should be administered at a dose level of -1.</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>Postpone both chemotherapy and radiotherapy until ANC ≥ 1,000/mm³ and platelet ≥ 100,000/mm³, then both drugs will be given at -1 dose level, the suspension time should not exceed 2 weeks.</td>
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6.1.3 Adjustment for nephrotoxicity:

<table>
<thead>
<tr>
<th>CCr ≥ 50 ml/min</th>
<th>full doses</th>
</tr>
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<tbody>
<tr>
<td>CCr &lt; 50 ml/min</td>
<td>Paclitaxel was used as scheduled, and cisplatin was postponed. If CCr ≥ 50ml/min next week, resume the previous dose of cisplatin. If the CCr</td>
</tr>
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remains <50 ml/min in the next week, continue to postpone cisplatin, but not beyond 2 weeks, otherwise withdraw from the trial.

### 6.1.4 Adjustment for neurotoxicity:

| Level 3 or 4 | Paclitaxel and cisplatin were postponed. |
| Level 2      | Reduce paclitaxel and cisplatin doses to dose levels of-1. |
| Level 1      | Full-dose administration of paclitaxel and cisplatin. |

### 6.1.5 Adjustment of the mucositis

| Level 3 or 4 | Paclitaxel was postponed but not beyond 2 weeks, otherwise withdrawn from the trial. When returning to grade 2 or lower, reduce to -1 dose level. No reduction in cisplatin dose is required. |

### 6.2 Dose adjustment of toripalimab

<table>
<thead>
<tr>
<th>Toxocities</th>
<th>Severity</th>
<th>Adjustment Scheme</th>
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<tbody>
<tr>
<td>Diarrhea/colitis</td>
<td>Grade 2-3</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>Termination</td>
</tr>
<tr>
<td>Hepatic dysfunction</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3 or 4</td>
<td>Termination</td>
</tr>
<tr>
<td>Renal failure or nephritis</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3/4</td>
<td>Termination</td>
</tr>
<tr>
<td>Endocrine disorders</td>
<td>Grade 2-3 hyperthyroidism</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 2-3 hypophysitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 2 adrenal insufficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 3 hyperglycemia or diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypothyroidism</td>
<td>Termination</td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperthyroidism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hypophysitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 adrenal insufficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4 hyperglycemia or diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Grade 2</td>
<td>Postpone until recovery to Grade 0-1</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4 or recurrent Grade 2</td>
<td>Suspension</td>
</tr>
</tbody>
</table>
6.3 Dose adjustment of radiotherapy

The toxicity during radiotherapy will be assessed using (CTCAE v4.0). Radiotherapy should be continued if grade 3 toxicity is unrelated to radiotherapy. Radiotherapy should be discontinued if grade 4 toxicity is observed. If grade 3 radiation-related toxicity is observed, actively treat the symptoms. If the grade 3 reaction reduces or disappears, continue with the radiotherapy. If any of the following toxicities are present, subjects should be excluded from the study: esophageal perforation and heavy hemorrhage, non-healing esophageal tracheal leakage, myocardial infarction, heart failure, severe arrhythmias, and radiation pneumonia with dyspnea. If radiotherapy is temporarily interrupted or cumulatively interrupted for more than 2 weeks due to various reasons, the patient will be considered a major deviation, but follow-up will continue.

7. Outcomes evaluation
7.1 Efficacy evaluation

The primary endpoint is major pathological response (MPR) rate. The pathological examination should report the tumor extension, lymph node status, resection margins, and tumor regression grade (TRG). The TRGs of the primary tumor were described using a previously reported method as follows: grade 1, no evidence of vital residual tumor cells; grade 2, 10% or fewer vital residual tumor cells; grade 3, 11 to 50%; and grade 4, more than 50%. Pathological complete response (pCR) was defined as the absence of viable tumor cells in the resected cancer specimen. Major pathological response (MPR) was defined as less than 10% vital residual tumor cells (TRG1 and TRG2). MRI evaluation will be performed during nCRT in the third week to evaluate the early response. 3 weeks after the administration of the second cycle of immunotherapy and before surgery, disease reevaluation will be performed, including physical examination, routine laboratory test, echocardiography, pulmonary function test, contrast-enhanced chest CT, MRI, and PET-CT, to restage and exclude cases not suitable for surgery.

Secondary endpoints include DFS and OS. DFS is calculated from the date of surgery until the date of death from any cause or the date of first documented disease progression whichever came first, and OS is calculated...
from the date of randomization until the date of death from any cause or the date of last follow-up, whichever came first. During the adjuvant treatment radiological evaluation will be performed before the first and fourth cycle of immunotherapy, and during follow-up evaluation will be performed every 3 month in the first two years and then every 6 month until 5 years.

7.2 Safety evaluation

The toxicity of chemotherapy and radiotherapy will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v 4.0). The post-operative complications will be evaluated according to Clavien-Dindo classification. All AEs occurring during the trial should be recorded in the CRF. Changes in patients' subjective symptoms (nausea, vomiting, poor appetite, alopecia, etc.), PS scores, blood routine, hepatic and renal function should be recorded every week during nCRT and before every cycle of toripalimab and thyroid function, adrenal cortical function, myocardial enzymes, BNP, etc. should be recorded before every cycle of toripalimab. All postoperative complications occurring within 30 days after surgery were recorded according to Clavien-Dindo Classification and ECG definition, respectively.

7.3 Exploratory analysis

PD-L1 expression was assessed on pretreatment formalin-fixed paraffin-embedded (FFPE) sections using an immunohistochemistry (IHC) assay (PD-L1 IHC 22C3). PD-L1 expression was evaluated by tumor proportion score (TPS) and PD-L1 expression positive was defined as TPS ≥1%. Pretreatment (baseline) biopsy and surgical resected specimen were used to assess tumor-infiltrating CD8+ lymphocytes by IHC. Flow cytometry was used to measure peripheral lymphocyte subsets consisting of T cells (CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells), regulatory T cells (CD3+CD4+CD25+CD127- Treg cells), natural killer cells (CD16+CD56+ NK cells), B cells (CD19+ B cells), and activation antigens (CD3+HLA-DR+/CD3+(%), CD8+CD38+/CD8+(%), CD8+HLA-DR+/CD8+(%), CD4+HLA-DR+/CD4+(%), CD4+CD38+/CD4+(%) before and after nCRT, after one cycle of toripalimab, before surgery and during adjuvant treatment. The cytokines including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, TNF-α, IFN-α, and IFN-γ were also measured by flow cytometry at the same times.

8. Data management

8.1 Case report form (CRF)

To ensure accuracy of information and facilitate efficient analysis, it is imperative that the data be promptly recorded in the CRF. Under normal circumstances, the CRF should not be modified. However, if there are genuine errors that need correction, the investigator must sign at the location of the alteration to document the change. After the study, duplicate copies of the completed CRF will be distributed to both the sponsor and investigator as per standard protocol. Once the data has been thoroughly reviewed by the Clinical Research Associate (CRA), it will be entered into a secure database for further analysis. At this stage, any further amendments to the CRF will no longer be possible, as the contents will have been finalized. According to GCP, documentation should be properly preserved by the investigators for more than 5 years.

8.2 Severe adverse event (SAEs) handling and reporting methods
Accurately complete the adverse event record, recording the occurrence time, severity, duration, measures taken, and outcome of the adverse events. Explain the criteria for judging the severity of adverse events and the 5-level classification criteria for determining the relationship between adverse events and investigational drugs (definitely related, probably related, possibly unrelated, unrelated, and cannot be determined). Any serious adverse events occurring during the clinical trial period must be reported to the principal investigator of the research institution, the ethics committee of the drug clinical research base, and the sponsor within 24 hours.

9. Ethics requirements
Before conducting this experiment, the protocol, proposed informed consent form, and other materials provided to patients must be submitted for review by the Institutional Ethics Committee (IEC). A written approval with the committee's signature and date indicating their endorsement is required prior to the start of the experiment. Any amendments to the research plan, informed consent form, or materials provided to the patients must be approved by the committee.

The clinical trial can only proceed after obtaining the informed consent of the subjects and having them sign the informed consent form. During the study, the rights of the subjects should be ensured, and confidentiality principles should be followed regarding subject information.

10. Statistical analysis
SPSS version 22.0 will be used for statistical analysis. All statistical tests are two-tailed, and a P-value less than or equal to 0.05 is considered statistically significant for the tested differences (excluding those specifically stated otherwise). Descriptive statistics for quantitative variables will include mean, standard deviation, median, minimum value, maximum value, lower quartile (Q1), upper quartile (Q3). Categorical variables will be described with counts and percentages for each category. For comparisons between two groups, appropriate methods will be used based on the type of variable. Group comparisons for quantitative data will use either independent samples t-test (assuming homogeneity of variance and normal distribution) or Wilcoxon rank-sum test. Chi-square test or exact probability method (if chi-square test is not applicable) will be used for categorical data, and Wilcoxon rank-sum test or CMH test will be used for ordinal data. Tumor pathological remission rate, drug-related adverse events, perioperative data, and incidence of complications will be reported as descriptive statistics. The Kaplan-Meier method will be used for analysis of DFS and OS.