**To explore the efficacy and safety of neoadjuvant chemotherapy combined with immunotherapy followed by sequential endoscopic resection in patients with cT1bN0M0 esophageal cancer**

### 1. Objective and content of the clinical trial

 **1.1 Objective**

The objective of this study is to evaluate the efficacy and safety of neoadjuvant chemotherapy (NAC) combined with immunotherapy followed by sequential endoscopic resection (ER) in patients with cT1bN0M0 esophageal cancer (EC).

 **1.2 Main contents**

This is a single-center, prospective single-arm study whose objective is to evaluate the efficacy and safety of NAC combined with immunotherapy followed by sequential ER in patients with cT1bN0M0 EC.

### 2. Background

Esophageal cancer (EC) is the ninth most common cancer and the sixth most common cause of cancer death worldwide. The incidence of EC varies substantially among regions, with the highest incidence occurring in East Asia, Central Asia, and eastern and southern Africa. Endoscopic submucosal dissection (ESD) is currently the preferred treatment for early EC tumors and precancerous lesions; a number of studies in China and abroad have confirmed the safety and efficacy of ESD in the treatment of early EC. Multiple guidelines recommend superficial EC (that is, with invasion depths limited to M1-M2) as the absolute indication for endoscopic treatment. However, the treatment for preoperatively evaluated superficial EC with no obvious lymph node metastasis and an invasion depth of T1b is controversial, with options other than surgery often considered. In clinical practice, among patients with head and neck tumors, such as oropharyngeal carcinoma, who have a long history of smoking and/or alcohol consumption, approximately 30% have early EC. The overall background mucosa of these early EC patients is poor, their primary lesions are often large and irregular, and the remaining esophagus contains multiple lesions that may or may not be positively stained with iodine. Following head and neck chemotherapy combined with immunotherapy, gastroscopy shows that for a substantial percentage of these patients, the size of the main esophageal lesion is significantly reduced, and the background mucosa of the whole esophagus is significantly improved. According to relevant reports, additional chemotherapy and immunotherapy for patients with early EC following noncurative resection can further improve the background mucosa, thereby reducing the incidence of metachronous carcinoma. To evaluate the efficacy and safety of neoadjuvant chemotherapy (NAC) combined with immunotherapy followed by sequential endoscopic resection (ER), a single-center prospective study is being conducted to investigate the effective treatment rate and adverse reactions in patients with cT1bN0M0 EC.

### 3. Trial inclusion and exclusion criteria

 **Inclusion criteria:**

 1) Age ≥18 years, ≤75 years;

 2) EC case-related specimens confirmed to be esophageal squamous cell carcinoma by biopsy according to at least one of the following

 a) The presence of at least one iodine-unstained area (USL) measuring ≥ 3 cm in at least one direction and reaching or exceeding 3/4 of the full circumference of the esophagus;

b) Lesion infiltration to the submucosa, as determined by combined gastroscopy and endoscopic ultrasound;

 3) Complete imaging staging, including gastroscopy, endoscopic ultrasound, chest computed tomography (CT), brain magnetic resonance imaging (MRI), and bone scan (if the result is positive, bone metastasis must be confirmed by MRI or a CT scan at the corresponding location); endoscopic ultrasound (EUS) and chest CT must not reveal lymph nodes in the mediastinum with a short axis greater than 10 mm, and B-type ultrasound can be used to rule out lymph node metastasis in the abdominal organs and the neck;

 4) No history of other malignant tumors;

 5) Good physical condition and the ability to tolerate gastroscopy and biopsy;

 6) Willingness to provide written informed consent to participate in this clinical study and understand their responsibilities during the study process.

 **Exclusion criteria:**

 1) Esophageal adenocarcinoma and precancerous lesions;

 2) Current antiplatelet, anticoagulant, or thrombolytic therapy (such as warfarin, heparin, clopidogrel, aspirin, streptokinase, and urokinase);

 3) Myocardial infarction or other severe heart diseases in the past 6 months;

 4) Serious cerebrovascular diseases, such as cerebral hemorrhage and cerebral infarction, in the past 6 months;

 5) Previous surgery or chemotherapy and immunotherapy other than radiofrequency therapy, cryotherapy, photodynamic therapy, or ER due to esophageal tumors;

 6) Unmanaged mental illness and/or known drug or alcohol dependence that has not been cured that limits their ability to understand or follow the instructions related to informed consent, posttreatment instructions or follow-up guidelines;

 7) Incomplete clinical data (ambiguous diagnosis, incomplete medical history, incomplete medication records, etc.);

 8) Regional lymph node metastasis confirmed by imaging examination and needle biopsy;

 9) Signs of eosinophilic esophagitis found on endoscopic examination and/or histological examination;

 10) Grade C or D active reflux esophagitis;

 11) Allergy to platinum or paclitaxel;

 12) Pregnancy/lactation;

 13) Inability to provide signed informed consent.

### 4. Discontinuation of the trial/trial treatments and procedures

 1) If the sponsor finds that the safety of the subjects may be affected or the implementation of the trial may change the ethics committee’s approval for the continuation of the trial, the sponsor should immediately notify all involved institutions and investigators and take corresponding measures;

 2) Deviation from the ethics committee approved trial protocol that may affect the rights, safety and health of the subjects or the scientific nature and integrity of the trial will be assessed for acceptability regarding continuation of the trial;

 3) The subject initiates the request to stop the trial.

### 5. Time of inclusion

 After the subjects confirm that they understand the clinical trial protocol, pass the preliminary screening, and provide informed consent, the investigators will review and approve the clinical trial.

#### 5.1 Expected overall duration of the clinical trial and the reasons for this duration

The trial is expected to last for 2 years, mainly limited by the speed of subject enrollment. Currently, 10 subjects are initially scheduled to be enrolled within three months; therefore, a suitable duration is needed to ensure the enrollment of a sufficient number of subjects.

#### 5.2 Expected duration for each participated subject

 The entire trial process is divided into a treatment period and an observation period. After determining whether they meet the inclusion and exclusion criteria and following their provision of signed informed consent, the subjects will receive 3 cycles of neoadjuvant concurrent chemotherapy and 2 cycles of immunotherapy; within 2 weeks of the end of neoadjuvant therapy, the endoscopic assessment (narrow band imaging (NBI)+magnifying endoscopy (ME)+iodine staining) will be performed again. If the main lesions demonstrate a significant reduction in size with respect to the pretreatment level (definition: the lesion occupies less than 3/4 of the esophageal lumen and demonstrates a reduced surface elevation and improved submucosal infiltration according to EUS, while biopsy reveals an improved pathological type with respect to the pretreatment level), the subject will be included in the sensitive to chemotherapy combined with immunotherapy group (Group A); if lesion regression is not evident, the subject will be enrolled in the stable/insensitive to chemotherapy combined with immunotherapy group (Group B). Within 5 weeks after the end of neoadjuvant therapy, all patients in Group A will undergo ER, that is, complete resection (even circumferential resection), according to the clinically visible extent of the lesion prior to chemotherapy combined with immunotherapy. Group B patients who strongly request endoscopic treatment will be automatically enrolled in group C, and those who wish to undergo surgical treatment will be automatically enrolled in group D; for these patients, the postoperative pathology should be accurately assessed, and appropriate remedial treatment options, such as radiotherapy or surgery, can be applied.

####  5.3 Number of subjects required for the clinical trial

 This study is a single-center nonrandomized controlled clinical trial. A total of 40 patients are planned to be enrolled, including 20 patients in Group B.

### 6. Trial procedure

All patients will undergo accurate assessment via endoscopy (white light + NBI + ME + iodine staining + EUS). The location, morphology, size, range of involvement, pathological results, possible depth of invasion, and esophageal background mucosal classification of the lesions will be recorded in detail before treatment. For patients with cT1bN0M0 EC who meet the inclusion and exclusion criteria after the first comprehensive evaluation, including endoscopy, multidisciplinary consultation including the thoracic surgery, internal medicine, and radiotherapy departments will be conducted to further determine whether the patient can be included in the trial. All subjects who meet the inclusion requirements should sign informed consent forms to receive NAC combined with immunotherapy for treating their esophageal lesions. After the effect is evaluated, endoscopic submucosal dissection will be performed.

 **6.1 Course of treatment**

 All patients will undergo surgery under general anesthesia and catheterization, followed by white-light endoscopy and narrow-band imaging (NBI) endoscopy. Iodine staining with 20-30 ml of 1.25% Lugol solution will be performed to determine and evaluate the lesion boundary. The location, length, and circumference of the lesion will be recorded in detail. A DualKnife will be used to electrosurgically mark 1-2 mm around the identified lesion site.

A transparent cap will be installed at the front end of the EUS probe. Then, physiological saline containing 0.04 mg/ml methylene blue and 0.002 mg/ml adrenaline will be submucosally injected with a needle to form a liquid cushion separating the lesion from the muscle layer. Then, mucosal dissection will be performed around the marked points using the DualKnife in electrodesiccation mode, followed by submucosal dissection along the pre-incision site also with the DualKnife, with supplementary injection into the submucosa as needed. If bleeding or blood vessel exposure occurs during the dissection, the DualKnife or hot biopsy forceps will be used for hemostasis via electrocoagulation. Finally, the entire lesion will be completely removed.

After the end of treatment, the residual marker points around the artificial ulcer and any resection wound bleeding and perforation will be monitored. Any bleeding and exposed blood vessels will be treated with hot biopsy forceps. If there is a depression or perforation, the depression should be clamped with metal clamps. Following the end of this treatment, the wound will continue to be monitored for 5-10 minutes to ensure that there is no active bleeding.

 **6.2 Pathological evaluation**

 6.2.1 Reconstructed pathological images: The reconstructed endoscopic pathological images of the resected lesions in Group A and Group C will obtained and analyzed to determine whether the iodine-stained area of the lesion has been reduced after chemotherapy combined with immunotherapy to a size corresponding to pathological intramucosal carcinoma at the histopathological level (pT1a), i.e., clinical complete response (cCR).

 6.2.2 Follow-up biopsy: Each follow-up will involve gastroscopy (white light + NBI + ME + iodine staining) + CT examination of the neck, chest, and upper abdomen. Biopsy will be performed on all USLs found in the treatment area (TA) after iodine staining during endoscopy, and specific information of these USLs will be recorded in detail. In addition, for the nonresection areas in Groups A and C (i.e., those who have undergone chemotherapy combined with immunotherapy), biopsies will be taken every 2 cm for pathological examination. If any new USL is found outside the TA during the follow-up period, a biopsy specimen should be collected, and the biopsy fragments should be placed in separate containers and labeled according to the location. USLs outside the TA will be treated according to standard practices under hospital standards.

 **6.3 Postoperative observation and follow-up**

 6.3.1 Primary outcomes

 1) Effective treatment rate (eTR) for cT1bN0M0 EC patients in Groups A and C after NAC combined with immunotherapy;

 2) Sustainability of complete remission in nonresection areas in Group A and C patients showing cCR over long-term follow-up;

 3) Three- and 5-year overall survival (OS) rates of cT1bN0M0 EC patients who have undergone NAC combined with immunotherapy followed by sequential ER;

 6.3.2 Secondary outcomes

 1) Toxic and side effect percentages of preoperative NAC combined with immunotherapy;

 2) Comparison of the postoperative complication rates in Groups A and C and evaluation of the effects of NAC combined with immunotherapy followed by sequential ER on quality of life (QoL) of cT1bN0M0 EC patients in different groups.

 3) Five-year OS rate, disease-free survival (DFS), local control rate, distant metastasis rate and QoL of patients in Group C;

 4) Identification of patients (clinical factors + genetic profile characteristics) who would benefit from NAC and immunotherapy (chemotherapy- and immunotherapy-sensitive groups) to guide individualized treatment.

 5) Identification of high-risk recurrence factors (clinical factors + genetic factors) of cT1bN0M0 EC patients treated with NAC combined with immunotherapy followed by sequential ER and establishment of a recurrence prediction model.

### 7. Follow-up evaluation

Principle: Following the completion of ER, the patients will be followed up once every 3 months for the first 2 years, once every 6 months for the next 3 to 5 years, and once a year thereafter; thus, all patients participating in the trial will be followed up for at least 5 years after enrollment. The follow-up data will include: 1) survival, 2) local recurrence, 3) regional lymph node metastasis, 4) distant metastasis, 5) whether the patient receives other treatments (endoscopic surgery, radiofrequency ablation (RFA)/coagulation, resurgery, adjuvant therapy, etc.), 6) QoL scale assessment (Appendix 3 and Appendix 4), and 7) acute and chronic adverse reactions after radiotherapy (Appendix 5 and Appendix 6).

 **7.1 Primary endpoint**

 1) The eTR of cT1bN0M0 EC patients treated with NAC and immunotherapy combined with ER, defined as the proportion of ER specimens with pathologically confirmed remission after NAC and immunotherapy;

 **7.2 Secondary endpoints**

 1) Adverse reactions in cT1bN0M0 EC patients after NAC and immunotherapy combination with ER;

 2) Three-year OS rate of cT1bN0M0 EC patients after NAC and immunotherapy combination with ER;

OS: Time from the date of NAC and immunotherapy to the date of death or last follow-up;

 3) Three-year DFS rate, locoregional-free survival (LRFS), distant metastasis-free survival (DMFS), local control rate, distant metastasis rate, and QoL of cT1bN0M0 patients receiving NAC and immunotherapy combined with ER;

DFS: Time from the date of NAC and immunotherapy to the date of tumor recurrence and metastasis, death or follow-up;

LRFS: Time from the date of NAC and immunotherapy to the date of local or regional lymph node metastasis, death or last follow-up;

DMFS: Time from the date of NAC and immunotherapy to the date of distant metastasis, death or last follow-up;

 Recurrence events include local recurrence, regional lymph node recurrence and distant metastasis;

 Local control rate refers to the proportion of patients who are free from local recurrence and regional lymph node recurrence;

 Distant metastasis rate: the proportion of patients with distant metastasis;

### 9. Statistical analysis

 Descriptive analyses will be used to summarize the study results. For continuous variables, the number, mean, standard deviation, median, minimum and maximum values of effectively observed events will be provided. For categorical variables, frequencies and percentages will be used. Unless otherwise specified, the statistical evaluations will be conducted with two-sided tests at an α level of 0.05, including Student’s t test or the Wilcoxon rank-sum test for continuous variables and the chi-square test or Fisher’s exact test for categorical variables. Other statistical methods could be used as appropriate. The statistical analyses will be performed using SPSS or other validated statistical software.

 Before enrollment, the purpose, procedures and possible risks of the study, as well as the subjects’ rights, will be comprehensively described by the physicians to the included subjects, who will be asked to sign the informed consent form, included in the study file as an appendix, prior to study participation.

 **(2) Approval of the trial protocol**

 Before the start of the trial, the protocol should be approved by the Ethics Committee of the participating institutions.

 **(3) Informed consent process and informed consent form**

Please see the appendix for details on the informed consent process and a copy of the informed consent form.