Informed Consent Form

Project Name: Patient Derived Organoids to Observe the Clinical Consistency of Personalized Neoadjuvant Therapy for Resectable Esophageal Squamous Cell Carcinoma

Project

Research Institution: Zhongshan Hospital affiliated to Fudan University

Principal Investigator: Lijie Tan

Sponsor: Zhongshan Hospital affiliated to Fudan University

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You have been invited to participate in a clinical study on the predictive efficacy of neoadjuvant personalized therapy for resectable esophageal squamous cell carcinoma using human tumor organoid models. Please read this informed consent carefully and make a decision on whether to participate in this study. Participating in this study is entirely your own choice, but you will only have the opportunity to enter the study after signing this informed consent form. When your study doctor or researcher discusses the informed consent form with you, you can ask them to explain any parts you do not understand. We encourage you to discuss with your family and friends before making a decision to participate in this study. You have the right to refuse to participate in this study and can withdraw from the study at any time without penalty or loss of your rights. If you are participating in another study, please inform your study doctor or researcher. The background, objectives, study process, and other important information of this study are as follows:

Research background

Esophageal cancer is one of the common malignant digestive tract tumors in China. For most resectable esophageal cancers, neoadjuvant therapy can reduce the tumor stage, increase the success rate of surgery, prolong patient survival, and improve the quality of life without increasing mortality. In recent years, immunotherapy for esophageal tumors has developed rapidly. However, the response of different patients to neoadjuvant immunotherapy varies significantly. To further improve the benefit rate of neoadjuvant immunotherapy, we urgently need to explore more effective efficacy prediction models.

Organoids are a three-dimensional cell-derived tissue culture that can self-organize into isolated "mini-organs." Patient tumor-derived organoids can better preserve the original tumor characteristics and interpatient variability, with structural and genetic integrity, and can be cultured long-term as well as form biobanks. In the future of precision medicine, patient tumor organoids will have broad application prospects, such as personalized prediction of drug efficacy, investigation of drug resistance mechanisms, and potential translational research.

To better simulate the interaction between immune cells and tumor cells in vivo, the technique frequently used is immune co-culture, which involves culturing tumor organoids together with the patient's autologous immune cells. The method of separately proliferating organoids and peripheral blood immune cells and then co-culturing them can maintain the activity of immune cells for a longer period and continuously amplify and add activated autologous immune cells. Multiple studies have confirmed the feasibility of patient tumor organoid models, and some research has validated that peripheral blood immune cell co-culture models can well predict the efficacy of neoadjuvant immunotherapy regimens in diseases such as gastric cancer, colorectal cancer, and skin

cancer. This study aims to provide precise guidance for efficacy prediction and personalized medication of neoadjuvant treatment for esophageal cancer.

Research Objective

The main purpose of this study is to establish a tumor organoid model for patients with esophageal squamous cell carcinoma. By comparing the drug sensitivity results of different chemotherapy-immunotherapy combined regimens and their consistency with clinical efficacy, this study will evaluate the feasibility of using an esophageal squamous cell carcinoma organoid immune co-culture model to guide the selection of clinical chemotherapy-immunotherapy combined regimens. Additionally, this model will be used to explore potential drug mechanisms and drug resistance mechanisms.

III. Research Process

How many people will participate in this study?

Approximately 30 people will participate in this study conducted at our hospital.

Research Steps

If you agree to participate in this study, please sign this informed consent form.

This study is divided into 4 phases, including the screening period, organoid modeling period, and follow-up period.

Screening period:

After signing the informed consent form, you will need to undergo a screening period examination. The examination items include medical history, collection of demographic data, height and weight measurement, medical history of past illnesses, physical examination, vital signs, 12-lead electrocardiogram (12-ECG), infectious disease screening, and routine laboratory tests, etc.

After the completion of the screening period, the organoid modeling period will begin, and the patient will receive treatment according to the clinical plan formulated by the responsible physician.

Additional biological samples need to be collected before the start of treatment: one endoscopic tumor biopsy sample; blood samples of 10mL may be required depending on the situation.

Pathological specimens of surgical tissues are retained after treatment.

Organoid modeling period:

Organoid modeling is performed using gastrointestinal tumor biopsy samples. Analysis of pathological and immunohistochemical consistency with patient surgical tissues is conducted. If successful modeling occurs, a drug sensitivity experiment is performed.

1) Organoid culture methods

① Collect surgical tissue, digest, rinse, and resuspend in organoid culture medium;

② Add matrix gel, mix until homogeneous, then inoculate into culture dishes, place in a 37℃ constant temperature incubator for fixation;

③ After fixation, add organoid culture medium, place in a 37℃ constant temperature incubator.;

④ Change the culture medium every 2-3 days until ESCC organoids are cultured.

⑤ Identification of tumor organoid models

⑥ Collect appropriate organoid samples and perform consistency identification with the source tissue;

⑦ Screen appropriate organoids for paraffin embedding, sectioning, HE staining, and immunohistochemical staining, and compare with the pathology and immunohistochemistry of the source tissue.

2) Culture of Tumor-Invading Lymphocytes (TIL)

① Collect tumor tissue, take about 0.1g, clean the tissue, and cut it into small pieces of about $1mm^3$.

② Small tissue pieces are transferred to different wells of the 24-well plate and immune cell induction medium is added;

③ After observing immune cell expansion, filter or sort, enrich immune cells, and continue culture;

④ Expand to a sufficient number of immune cells, then freeze for the next experiment.

3) Peripheral blood mononuclear cell (PBMC) culture

① Collect blood samples, use EasySepTM Direct Human PBMC Isolation Kit to isolate PBMC;

② Perform organoid culture first;

③ Use TrypLE to digest the cultured organoids into small clusters, mix the organoids with PBMC at a certain ratio for co-culture.

4) Drug sensitivity test

According to the experimental design, immune cell co-culture and the addition of immune combination chemotherapy drugs were performed.

Sensitivity of the co-culture model to immune combination chemotherapy drugs was detected through microscopy and immunohistochemical staining.

Follow-up period (2 years):

Safety follow-up (inpatient or telephone follow-up): Adverse reactions related to the treatment drug within 90 days after the last dose, followed up every 30 days, including survival status, adverse reactions, concomitant medications, and concomitant treatment information.

Survival follow-up (phone follow-up): The first follow-up 30 days after surgery, and then every 3 months thereafter, to collect survival information and data on antitumor treatment and disease progression time after the study ends, until the participant dies, is lost to follow-up, or the study is terminated.

How long will this study last?

This study is planned to last approximately 2 years (including the follow-up period), and your participation in this study will not affect your treatment plan.

You can choose to withdraw from the study at any time without losing any benefits you should receive. However, if you decide to withdraw from the study during the course of the study, we encourage you to discuss it with your doctor first. If you experience a serious adverse event, or your study doctor determines that continuing in the study is not in your best interest, he/she will decide to withdraw you from the study. The sponsor or regulatory agency may also require the termination of the study during the study period. But your withdrawal will not affect your normal medical treatment and rights.

Biological samples collected during the study

During the research process, your esophageal tumor biological samples and the data generated from sample testing will be collected, stored, and used for the purposes of this trial as informed. Intellectual property rights or potential commercial value generated from the research results will not be shared with you. The biological samples collected by the study will be tested at the Organoid Laboratory of the Pathology Department, Zhongshan Hospital affiliated to Fudan University (No. 180 Fenglin Road, Xuhui District, Shanghai). The endoscopic biopsy samples collected in this study will be used entirely for organoid modeling. After successful modeling, drug sensitivity testing and exploratory research on potential clinical efficacy-related mechanisms will be conducted. Postoperative pathological specimens will be used to determine the clinical efficacy of neoadjuvant therapy and exploratory research on potential clinical efficacy-related mechanisms. The use and storage of all samples will be conducted within Zhongshan Hospital affiliated to Fudan University. All samples will be destroyed on site after the study concludes. The information collected in this study will be stored at Zhongshan Hospital affiliated to Fudan University and will be kept for 5 years after the trial ends.

IV. Risks and Benefits

1. What are the risks of participating in this study?

The potential risks associated with participating in this study are as follows. You should discuss these risks with your research physician, or if you prefer, with your regular physician:

Routine treatment risks: drug-related adverse reactions, surgical complications, etc.

Additional risks of participating in the study: This study will not cause physical trauma. However, there may be risks related to information security. We will do our utmost to protect the information you provide from being disclosed and will make every effort within the legal framework to protect your personal privacy.

If you experience any discomfort during the study, or if your condition changes in any new way, or if any unexpected situation occurs, whether or not it is related to the study, you should promptly notify your research physician.

What are the benefits of participating in the research?

This study will provide you with a tumor organoid drug sensitivity test report, which may help in selecting your medication treatment plan. However, you need to be aware that the results carry the risk of false positives or false negatives and should be interpreted with caution.

Potential benefits: We hope that the information obtained from your participation in this study in the future will benefit you or other patients with the same condition.

5. Alternative treatment options

This study does not interfere with your clinical treatment plan; it only collects your information or data and collects biological samples for testing, with no alternative treatment options.

VI. Confidentiality of Personal Information

During this study, for the purpose of the study, the research team may need to access your medical history, collect necessary medical records and test results from the past, and other personal information. After you sign this informed consent form, it means you allow the research team to contact providers who can offer you other medical assistance to obtain necessary medical information about you during the time they provide medical services to you. Only members of the research team can access your medical information and identify your identity. Without violating the principle of confidentiality and relevant regulations, the sponsor's authorized monitors, auditors, ethics committees, and regulatory authorities may review your original medical records to verify clinical trial data.

During the study, we will collect your personal information and research data. To ensure privacy, we will encode data that can directly identify you, such as your name and contact information, so that no one can determine your identity. If the results of this study are published in medical journals or presented at scientific conferences, information that can identify your identity will not be disclosed.

Your permission to use and share personal information can be revoked at any time. If necessary, you can contact your responsible physician. If you do so, you will no longer be able to continue participating in the study. After that, researchers will no longer collect new health data that can identify your personal information. However, the already collected health data may still be used and shared with other researchers, as described in this informed consent form. To ensure the scientific validity and credibility of this study, you may not be able to view some records related to the study before it ends. When the study ends, you can request from the research physician to view the health data collected during the study process. After viewing, you can raise any issues regarding incorrect personal information.

Personal information collected or generated during this study and data generated by the research process will be encoded and stored at Zhongshan Hospital affiliated to Fudan University, and will be destroyed 5 years after the study ends. The encoded personal information and research data will be provided to the collaborator "Chuangxin International Biotechnology (Guangzhou) Co., Ltd." in a way that cannot identify your personal information to help interpret the research results.

Except for this study, this information will not be used again in the future.

VII. Feedback on Research Results

The overall results of the study (excluding your personal information) will be described on https://www.chictr.org.cn after the study ends, and you can retrieve them by searching with the keywords of the study title.

VIII. About research costs and related compensation

1. Costs of drugs/equipment used in the study and related examinations

Preoperative routine physical examinations, neoadjuvant therapy medications, and surgeries are all part of standard medical care. The study does not incur additional costs, and no free drugs, equipment, or examination fees are provided. Additional biological samples are collected as part of routine examinations, and no separate sample collection process is conducted.

Compensation for participating in the research

Blood samples may be collected from some participants in this study, and compensation for nutritional expenses of 50-100 yuan will be provided based on the amount of blood collected.

Compensation/Reimbursement in case of injury

If you suffer an injury related to this research, you may receive free treatment provided by Zhongshan Hospital affiliated with Fudan University, or compensation/reimbursement will be made in accordance with relevant Chinese laws.

IX. The Rights of Participants and Related Precautions

1. Your Rights

Throughout the entire process of participating in the research, you are voluntary. If you decide not to participate in this study, it will not affect other treatments you should receive. You have the right to withdraw from the trial at any stage without discrimination or unfair treatment, and your corresponding medical treatment and rights will not be affected. If there is an update that may affect your rights and safety, you will need to sign an updated informed consent form again to obtain new information.

2. Precautions

As a participant, you need to provide truthful information about your medical history and current physical condition; inform the research physician of any discomfort discovered during this research period; do not take restricted medications, foods, etc., as informed by the physician; inform the research physician if you have recently participated in or are currently participating in other research; cooperate with a series of medical activities including treatment and follow-up.

X. Contact Information for Relevant Information

If any important new information arises during the research process that may affect your willingness to continue participating in the study, your physician will promptly notify you. If you have any questions about your research data, or if you wish to know the findings of this study after it concludes, you can raise any questions about this research at any time and receive corresponding answers.

The Ethics Committee has reviewed and approved this study. If you have any questions related to your rights/interests, or if you wish to report difficulties, dissatisfaction, or concerns encountered during the participation in this study, or if you wish to provide suggestions or opinions related to this study, please contact the Medical Ethics Committee of Zhongshan Hospital affiliated to Fudan University, Tel: 021-31587871, Email: ec@zs-hospital.sh.cn.

Informed Consent Signature Page

Informed Consent Statement:

I have been informed of the purpose, background, procedures, risks, and benefits of this research. I have been given sufficient time and opportunity to ask questions, and I am satisfied with the answers. I consent to participate in this study.

I have also been informed that I should contact [whoever] if I have questions, wish to report difficulties, concerns, suggestions for the research, or wish to obtain further information or help with the research.

I know that I can choose not to participate in this study, or withdraw from this study at any time during the study without any reason.

I have been informed that if my condition worsens, or if I experience a serious adverse event, or if my research physician determines that continuing to participate in the study is not in my best interest, he/she will decide to withdraw me from the study. No consent from me is required, and the funding source or regulatory agency may also terminate the study during the study period. If this occurs, the research physician will notify me promptly, and the research physician will also discuss other options with me.

I will receive a copy of this informed consent form, which includes my signature and the researcher's signature.

I understand that participation in this study requires the use of my personal information and biological samples, and I agree to the use and processing of my personal information and biological samples for the purposes stated in this informed consent form.

☐ I agree ; ☐ I disagree ; ☐ I can’t decide

Subject Signature: Date:

Guardian Signature: Relationship with Subject ( ) Date:

(Applicable when the subject is unable to act/has limited capacity, then a guardian's signature and date are required)

Witness for justice signature: Date:

(Applicable when the subject cannot read this informed consent form, then a witness for justice is required to witness that the researcher has informed the subject of all the contents of the informed consent form, the subject has expressed willingness to participate, the witness for justice needs to sign and sign the date)

Researcher signature: Date:

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