Randomized Controlled Study on the Clinical Efficacy and Safety of RAK Cell Therapy in Advanced Gastric Cancer

Study Protocol
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I. Introduction:

Gastric cancer (GC), a common malignant tumor of the digestive system with high mortality, ranked fifth in incidence and fourth in mortality globally among all cancers according to 2020 global cancer statistics, with over 1 million new cases and approximately 769,000 deaths [1]. China has a high incidence of gastric cancer, with new cases annually exceeding 40% of the world's total. The majority of these cases are diagnosed at an advanced or late stage [2], posing a serious threat to the health and lives of the Chinese population [3].

Comprehensive therapy, primarily based on surgery and combined with chemotherapy, radiotherapy, immunotherapy, and targeted therapy, remains the mainstream treatment strategy for gastric cancer. Due to the high malignancy of gastric cancer, approximately 20% of patients are diagnosed at an advanced stage. Although large clinical trials, such as ToGA [4], Checkmate-649 [5], and ORIENT-16 [6], have established the important role of targeted therapy (represented by Trastuzumab) and immune checkpoint inhibitors (ICIs) in advanced gastric cancer, the median overall survival (OS) for advanced patients receiving comprehensive treatment has exceeded 12 months. However, the median OS for patients with end-stage gastric cancer who develop treatment resistance or progression remains less than 6 months [7]. Furthermore, most gastric cancer patients face the problem of failure of prior therapies and a lack of effective treatment options in the terminal stage of the disease. Therefore, comprehensive treatment for advanced cancer still faces significant challenges.

In light of this, biotherapies represented by lymphocytes (T cells) or natural killer (NK) cells offer new hope for patients who have failed first-line drug therapy and can serve as salvage therapy for advanced gastric cancer. In recent years, CIK cells, RAK cells, CAR-T cells, and TILs cells are among the more promising representatives. RAK cells (RetroNectin activated Killer cells) are immune cells obtained by culturing peripheral blood mononuclear cells (PBMCs) *in vitro* using recombinant human fibronectin (RetroNectin) combined with anti-human CD3 monoclonal antibody and interleukin-2 (IL-2) [8]. The mechanism of action of RAK cells involves the recognition of tumor-derived IL-2 by IL-2 receptors on the RAK cell surface, thereby activating the RAK cells to produce an anti-tumor effect without the need for other auxiliary substances. RAK cell therapy involves collecting the patient's own immune cells, expanding them thousands of times *in vitro* through the aforementioned culture process, enhancing their targeted killing function, and then reinfusing them into the patient to kill pathogens and cancer cells in the blood and tissues, break immune tolerance, and activate and enhance the body's immune capacity. It is characterized by both anti-tumor efficacy and low immunogenicity. RAK cells are derived from cytokines inducing and massively expanding PBMCs *in vitro* to obtain cells with anti-tumor activity. They possess powerful anti-tumor killing capability and the advantage of non-MHC-restricted tumor killing, offering a broad range of applications. Currently, it is an emerging immunotherapy method internationally for various solid tumors and has been used to treat patients with renal cell carcinoma [9, 10], liver cancer [11, 12], etc., achieving certain efficacy. It has also obtained Implied Permission for Clinical

Trials (IND) from China's National Medical Products Administration (NMPA) [13] for use in advanced renal cell carcinoma patients who have failed second-line therapy, and for preventing recurrence in hepatocellular carcinoma patients at high risk of postoperative recurrence.

Results from a recently reported Phase II single-arm clinical trial of RAK cells for postoperative treatment of advanced hepatocellular carcinoma (HCC) showed [14] that none of the 22 patients receiving RAK cell therapy experienced serious adverse events (SAEs). Furthermore, testing for over 120 cytokine types *in vivo* did not indicate the occurrence of biotherapy-related adverse reactions such as cytokine release syndrome in patients receiving RAK cell therapy. Additionally, research on the application of CIK cell therapy, which also belongs to adoptive immunotherapy like RAK cells, in advanced gastric cancer suggested [15] that adverse reactions in gastric cancer patients receiving modified T-cell therapy were mainly Grade 1-2, such as fever, with no Grade 3 or above SAEs reported. These studies suggest that the overall safety profile of RAK cell therapy in patients with solid tumors is manageable [14].

In summary, we plan to utilize the technology for extracting and culturing RAK cells from mononuclear cells (PBMCs) in human umbilical cord blood, and apply adoptive therapy with RetroNectin-activated killer cells (RAK cell therapy) to patients with advanced gastric cancer who have failed prior (second-line or above) therapies. We will compare the tumor efficacy and survival differences between these patients and those receiving third-line therapy for gastric cancer, aiming to provide new evidence and a foundation for the safety and efficacy of this cell therapy in advanced gastric cancer.

II. Research Content

This project employs a prospective, double-blind, randomized controlled trial methodology to comparatively analyze the safety and survival outcomes of human umbilical cord blood RAK cells applied in advanced gastric cancer. Firstly, the maximum tolerated dose (MTD) of RAK cell therapy for patients with advanced gastric cancer will be determined through a dose-escalation trial. Subsequently, the overall survival (OS), progression-free survival (PFS), and incidence of adverse events will be compared between the RAK treatment group and the control group. This aims to explore the efficacy and safety of biotherapy for recurrent or metastatic gastric cancer where frontline therapy has failed, thereby laying the foundation and providing evidence for large-scale, multi-center clinical studies.

Study Design

(1) Study Subjects: Patients with advanced gastric cancer (Stage IV), for whom frontline therapy has failed, hospitalized at the Chinese People's Liberation Army General Hospital.

(2) Randomized Controlled Study:

1. After cases are screened for eligibility based on inclusion/exclusion criteria, dedicated personnel will be notified to assign groups according to a randomization table. Clinicians will then administer the corresponding treatment regimen as indicated:
    - Experimental Group: RAK cell therapy combined with oral TAS-102 (Trifluridine/Tipiracil) treatment.
    - Control Group: Oral TAS-102 treatment plus an infusion simulator treatment.
2. All randomized cases, regardless of whether they complete the corresponding treatment, should not be arbitrarily excluded and must be included in the Intention-To-Treat (ITT) analysis.

3. Blinding: A "double-dummy" method will be used. Based on the randomization results, the investigational drug (RAK cell suspension), TAS-102 (oral drug), and the infusion simulator (normal saline + small amount of fat emulsion) will have their outer packaging marked as either Group A or Group B drugs, making it indistinguishable which is the experimental or control group drug. However, different dosage forms within a group can be distinguished. Medical personnel unaware of the patient's drug group assignment will administer the Group A or Group B drugs to patients according to the corresponding sequence in the randomization table. Unblinding will occur at the end of the study follow-up or when necessary during the treatment process.

(3) Sample Size Calculation:

This study is planned as a prospective, double-blind, randomized controlled clinical trial. Referring to previous studies, the PFS for advanced gastric cancer patients treated with the third-line drug TAS-102 is 2.1 months. It is estimated that the experimental group will increase PFS by approximately 1.5 months to 3.6 months. Using a superiority test with $\alpha=0.05$ and $1-\beta=0.80$ for sample size calculation, the result is 42 subjects for the control group and 42 for the experimental group. Accounting for a 5% dropout rate (3 persons), 45 subjects need to be enrolled per group, resulting in a total study sample size of 90 persons.

(4) Drug Dosage and Administration:

- RAK Cell Therapy Medication: Based on previous publicly reported studies and concentrations used in other tumors, the RAK cell dose will be at the 3×10^9 - 1×10^{10} cell level, once daily, Days 1-3, with each cycle lasting 3 weeks.
- TAS-102 Medication: Based on the results of the RECOURSE [16] and TERRA [17] studies, TAS-102 (Trifluridine/Tipiracil Hydrochloride) will be administered orally at a dose of 35mg/m², twice daily, Days 1-5, with each cycle lasting 3 weeks.
- Administration Method of the Remaining Blinding Simulators in Groups A and B: The infusion simulator for the control group will use normal saline with a small amount of human albumin and fat emulsion to make its dosage form and appearance similar to the RAK cell suspension. It will be infused according to the same dosage and frequency described above.

(5) Primary and Secondary Observation Indicators:

1. Primary Observation Indicators: Overall Survival (OS), Progression-Free Survival (PFS).
2. Secondary Observation Indicators:
   (1) Median Survival Time (MST); Time to Progression (TTP).
   (2) Objective Response Rate (ORR).
   (3) Biotherapy-related adverse reactions.
   (4) Blood immunology indicators: T-cell count, IL-2/IL-6/IL-11, TGF-β, etc.

(6) Inclusion/Exclusion Criteria:

a. Inclusion Criteria:

1. Subjects voluntarily join this study and sign the informed consent form.
2. Age ≥18 years and ≤70 years.
3. Confirmed by gastroscopic pathology or imaging (enhanced CT/PET-CT) as Stage IV gastric cancer or gastroesophageal junction adenocarcinoma (cTanyNanyM1). Metastatic sites include but are not limited to: liver, peritoneum, lungs, pancreas, greater omentum, retroperitoneal lymph nodes, etc.

4. Failure or disease progression after prior frontline anti-tumor therapy (including ineffective first- and second-line chemotherapy, targeted therapy, and immunotherapy for advanced gastric cancer).
5. Have measurable solid tumors (efficacy evaluation standard: RECIST 1.1); tumor assessment via CT scan or MRI must be performed within 28 days before treatment.
6. Physical performance status ECOG 0-3.
7. Expected lifespan ≥1 month.
8. Participants must be able to understand the study procedures and agree to participate in the study by providing written informed consent.

b. Exclusion Criteria:

1. Concurrent other types of malignancy.
2. Severe cardiac, pulmonary, or cerebral system diseases.
3. Expected survival <1 month.
4. Laboratory investigations indicating unsuitability for receiving anti-tumor biotherapy:
   (1) Moderate to severe bone marrow suppression: (HGB <80 g/L; WBC <2.0×10⁹/L; ANC <1.0×10⁹/L; PLT <50×10⁹/L).
   (2) Significantly decreased liver function (Child-Pugh Grade C).
   (3) Severe renal insufficiency (CKD Stage III and above).
   (4) Severe coagulation dysfunction (INR ≥1.5 or APTT >1.5 × ULN).