Evaluation of the efficacy and safety of a precise thymalfasin-regulated PRaG regimen for advanced refractory solid tumors: protocol for the open-label, prospective, multicenter study (PRaG5.0 Study)

Informed Consent Form

Version:V1.1

Version date: September 26, 2022

Research center name:

Patient initials:

Patient study number:

Patient contact number:

Patient address:

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Information Disclosure Page

Participant Information Sheet

Dear Patient,

As you are a late-stage solid tumor patient and may meet the inclusion criteria for the " Evaluation of the efficacy and safety of a precise thymalfasin-regulated PRaG regimen for advanced refractory solid tumors: an open-label, prospective, multicenter study (PRaG5.0 Study)". We invite you to participate in this study. Participation is entirely voluntary. If you choose not to participate, all your other rights will remain unchanged and unaffected.

Before making a decision, it is essential that you read and fully comprehend this informed consent document. This document details the study's objectives, methodologies, potential benefits, and associated risks. It also provides an overview of your responsibilities and recommended precautions. This informed consent form is in duplicate. If you decide to participate in this study, you can consult any questions with your research doctor. Upon reaching a clear understanding, both you and your research doctor will sign this document. You will be provided with a copy.

1. Research Objective

The primary objective of this study is to investigate the effectiveness of a precise thymalfasin-regulated regimen that is based on a patient's immune status and combines HFRT with sequential PD-1/PD-L1 inhibitor and GM-CSF therapy for advanced refractory solid tumors. The secondary objective is to assess the safety and toxicity of this treatment.

2. Research Background

Cancer incidence and mortality rates are on the rise, making it a leading cause of death in our country. While treatment modalities for early-stage cancers have advanced, many patients are diagnosed in advanced stages, especially those with extensive metastases. Even after standard treatment, progression often persists. For such cases, there are no longer well-defined standard treatment recommendations, leading to short survival times and poor prognosis. Currently, immunotherapy, particularly with PD-1/PD-L1

inhibitors, is becoming the preferred treatment for advanced-stage patients. Previous PRaG 1.0 studies have shown that combining radiotherapy with immunotherapy can amplify tumor control and better patient survival outcomes.

Thymosin alpha (thymosin peptide α-1, Tα-1) is a widely-recognized immunomodulator, consisting of a peptide composed of 28 amino acids, highly conserved across different species. It has demonstrated effectiveness and safety in treating diseases like lung and liver cancers, melanomas, infections, and chronic hepatitis B. Tα-1 has been proven to be involved in multiple stages of the tumor immune cycle. It can directly act on precursor T cells, promoting their proliferation and maturation, and also affect toll-like receptors 2 and 9 on DC cells, promoting the activation of DC cells, thereby increasing the number of cytotoxic T cells and enhancing the effects of tumor killing. A recent mechanistic study showed that Tα-1 combined with chemotherapy drugs can revert M2 macrophages to M1 in tumor microenvironment, thereby promoting T cell infiltration into tumor microenvironment, and synergistically enhancing the anticancer effects with chemotherapy. Prior clinical trials have indicated that Tα-1 can augment lymphocyte numbers and function. It has demonstrated enhanced clinical efficacy when combined with radiotherapy and chemotherapy. In patients with recurrent metastatic esophageal squamous cell carcinoma after multi-line treatment. Post-treatment, the distant metastasis control group displayed a significant rise in $CD8^{+}$T lymphocyte counts and an elevated lymphocyte to monocyte ratio, signifying an improved clinical prognosis. A recent study involving patients with surgically unresectable stage IIIA-C non-small cell lung cancer treated with Tα-1 and concurrent chemoradiotherapy found that Tα-1 can substantially decreased the onset of grade 2 or higher radiation pneumonia. Additionally, lymphocyte counts in the combination treatment group were notably preserved. These results suggest that combining Tα-1 with chemotherapy, radiotherapy, or immunotherapy can enhance the patient's immune response, optimizing treatment outcomes.

3. Number of Participants and Expected Duration of the Study

This study plans to recruit a total of 60 participants, with an expected duration from 01 aaApril 2023 to 31 December 2025.

4. Research Design

Eligible patients will be treated with Tα-1 (Zadaxin, SciClone Pharmaceuticals) at three different doses based on their absolute T lymphocyte count at baseline. The Tα-1 dose is based on the commonly used dose in previous studies and the dose for severe lymphocytopenia in COVID-19. After 7 days of treatment with Tα-1 for patients with low baseline lymphocytes, patients will receive at least two cycles of the PRaG regimen. The PRaG regimen consists of HFRT delivered on day 1 followed by subcutaneous injection of GM-CSF(Molgramostim, Topleucon, Xiamen Amoytop Biotech) 200μg daily for one week from day 1 to day 7. Administration of PD-1/PD-L1 inhibitor therapy will commence within one week following completion of radiotherapy. The dosage of Tα-1 is adjusted in real-time based on the absolute T lymphocyte count in each PRaG treatment cycle. PRaG and Tα-1 treatments will be repeated every 21 days for at least two cycles until there are no appropriate lesions for irradiation or the tolerance dose of normal tissue is reached. Patients who complete PRaG will proceed with maintenance PD-1/PD-L1 inhibitor and Tα-1 therapy until disease progression or adverse events become intolerable.

5. Research Procedure

You are introduced to this study and after you understand the entire study and your questions have been answered to your satisfaction, you will be asked to sign this informed consent form if you wish to participate in this study.

This study aims to collect and analyze data from medical information from your standard clinical visits. If you consent to participate in this study, each subject will be assigned a unique identifier, and a medical record will be established.

For study eligibility determination (screening), you will be required to complete specific steps, including signed informed consent, demographic data collection,

relevant imaging tests such as CT or MRI within 2 weeks before treatment initiation, and an electrocardiogram one week before treatment. Within 7 days prior to treatment your physician will collect your medical history (including history of previous treatments, surgeries, etc.), measure your vital signs, and conduct a physical examination, score ECOG, PS and quality of life and request you to undergo certain laboratory tests. You will be asked to complete the following labs: complete blood count, urine, and stool tests, liver and kidney function, cardiac enzymes, electrolytes, blood coagulation, tumor markers, thyroid function, glycosylated hemoglobin, and lymphocyte subsets. Check chest X-ray, gastroscopy, cardiac ultrasound will be performed if necessary. For women of childbearing age, a pregnancy test will be conducted to rule out potential pregnancy. For the purpose of identifying novel indicators to evaluate treatment efficacy, approximately 5 mL of your peripheral blood will be drawn for molecular testing before and after the treatment. This is solely for research and you will not receive a report of these findings.

Upon completion of the screening, if you fulfill the criteria, the treatment will be administered as follows:

Based on the patient's baseline count of lymphocyte subsets, a precise dose of Tα-1 regimen will be prescribed as follows.

•For total T lymphocyte counts ≤0.5 times the lower limit of normal (ULN), a loading dose of Tα-1(3.2mg/day from day 1 to day 7) will precede radiotherapy.

•For counts between 0.5 and 1.0 times ULN, a loading dose of thymosin alpha (1.6mg/day from day 1 to day 7) will be administered, followed by radiation therapy, followed by radiotherapy.

•If the count is ≥1.0 times ULN, the loading dose will be omitted, and Tα-1 (1.6mg, thrice weekly) will be given concurrently with radiotherapy.

During subsequent treatment cycles, the Tα-1 administration scheme will be adjusted in real-time based on the above total T lymphocyte levels.

•For counts ≤0.5 times ULN, a loading dose of Tα-1 (3.2mg/day from day 1 to day 7) will be synchronized with radiotherapy during the initial treatment week. For the following two weeks, a maintained dose of Tα-1 (1.6mg, thrice weekly) will be

administered.

•For counts between 0.5 and 1.0 times ULN, a loading dose of Tα-1 (1.6mg/day from day 1 to day 7) will be synchronized with radiotherapy during the initial week. This will be followed by a maintained dose of thymosin alpha (1.6mg, thrice weekly) for the next two weeks.

•For the count is ≥1.0 times ULN, the loading dose will be omitted, and a consistent dose of t Tα-1 (1.6mg, thrice weekly) will be given concurrently with radiotherapy.

•Subsequent treatments will comprise hypofractionated radiotherapy (5 or 8Gy three fractions). On the day of radiotherapy, GM-CSF at 200ug will be administered subcutaneously for seven consecutive days. A PD-1 inhibitor will be introduced within one week after the completion of radiotherapy. During this period, the Tα-1 regimen will be adjusted in real-time based on the total T lymphocyte levels as above.

• After ≥2 cycles of combination therapy of radiotherapy, PD-1/PD-L1 inhibitor, GM-CSF and Tα-1, three combination therapy (PD-1/PD-L1 inhibitor, GM-CSF and Tα-1) will be used for maintenance for at least 6 months. The combination of PD-1 inhibitors and Tα-1 can be continued until disease progression, emergence of intolerable toxic effects, completion of six months of GM-CSF maintenance, or up to one year.

•During the 2-3 treatment cycles, conduct examinations every 21 days including complete blood count, urine, and fecal tests, liver and kidney function tests, myocardial enzymes, electrolytes, blood coagulation function, tumor markers, thyroid function, glycated hemoglobin, and lymphocyte subsets. Chest radiographs, gastroscopy, and cardiac ultrasounds should be performed when necessary.

• Following treatment initiation, disease evaluation using CT/MR/PET-CT will occur bi-monthly. Subsequent evaluations will encompass monitoring of vital signs, physical examinations, blood pressure assessments, complete blood count and urine tests, blood biochemistry, stool tests, blood coagulation, thyroid function, glycated hemoglobin, electrocardiograms, imaging examinations, quality of life scoring, tumor markers, ECOG scoring, recording of adverse events, monitoring of concomitant

medication usage, and adherence to research medication.

6. Potential Risks and Discomforts in Participation

Participation in this study does not involve any additional interventions beyond standard medical treatments. The study drugs may cause side effects, and all possible preventive measures will be undertaken, and participants are encouraged to promptly report any adverse symptoms. Detailed side effects associated with PD-1 inhibitors (nivolumab, pembrolizumab, sintilimab, tislelizumab), PD-L1 inhibitors (durvalumab, envafolimab), GM-CSF(Molgramostim), Tα-1(Zadaxin) are provided below.

Drawing on clinical trial data from tumor participants treated with PD-1/PD-L1 inhibitors, as well as the information from drug manuals, here are the possible side effects listed. But drugs might lead to other side effects not listed here. If you experience any discomfort, please inform the doctor or nurse immediately.

The potentially most common side effects (occurring in >5% of past patients) include fever, abnormal thyroid dysfunction, liver function, fatigue, and rashes.

The possible less common side effects (occurring in >1% of past patients) encompass, abnormal white blood cells and neutrophils counts, abnormal thyroid-stimulating hormone levels, increased uric acid in blood, abnormal liver function, reduced appetite, nausea and vomiting, abdominal pain, cough with phlegm, pneumonia, joint pain or stiffness, hyperglycemia, chills, muscle aches, weakness, stiffness, spasms or paralysis, pain in the arms or legs, headache, dizziness, palpitations, chest discomfort, and chest pain.

Potential, though rare, but serious side effects encompass reduced red blood cells, fatigue, skin reactions (including itching, hives, redness, and dryness), abnormal blood electrolyte (including low levels of blood phosphate, magnesium, and potassium), pneumonia (specifics provided below), enteritis, skin discoloration (localized pigmentation), xerostomia, vomiting, paresthesia in the extremities, dyspnea, altered gustatory perception, flushing, hypertension or hypotension, allergic responses during or between drug infusions, photosensitivity, constipation, dysphagia, gastroesophageal reflux, thrombocytopenia (heightening the risk of hemorrhage), weight decrement,

palpitations, optic disc edema, optic neuritis, hypoxemia, pulmonary injury or failure, pleural effusion, hepatitis, renal injury or failure, hematopoietic abnormalities, oral and gastrointestinal mucosal inflammations, edema in the face, extremities, and appendicular region, elevated inflammatory serum proteins (e.g., lipase), adrenal irregularities, pituitary gland inflammation, visual disturbances (ranging from blurred vision to vision loss), ocular inflammation or hemorrhage, pancreatitis, myocardial or pericardial inflammations, pericardial effusion, hyperglycemia, dehydration, infections (spanning sepsis, pulmonary, and cutaneous infections), constipation, disorientation, dorsalgia, autoimmune conditions, notably Guillain-Barre syndrome (linked with progressive muscular debility or paralysis), thoracic discomfort, meningeal inflammation or loss encircling the central nervous system, drug-induced dermatologic reactions, anomalous hematopoiesis, lymphadenopathy, systemic organ involvement (pertaining to the liver, kidneys, and lungs), a syndrome termed eosinophilic infiltration and drug reaction with systemic symptoms (DRESS), myasthenia gravis (a neuromuscular disorder inducing muscular weakness impacting ocular, facial, respiratory, and swallowing muscles), potential life-threatening or lethal encephalitis, toxic epidermal necrolysis (a severe condition manifested by blistering and epidermal shedding, mimicking extensive burns), rhabdomyolysis (with muscle fiber release into circulation, possibly impairing renal function), and polymyositis (sustained muscular inflammation and debility).

Pneumonia or Pneumonitis: This condition may result in inflammation of the lung. Many individuals with detectable anomalies on X-rays or CT scans remain asymptomatic. However, some present with a range of symptoms from mild to severe, with a few instances culminating in fatality due to pneumonia. Manifestations of pneumonia encompass difficulty breathing, discomfort during respiration, concomitant respiratory and chest pain, cough, breathlessness, elevated respiratory rate, fever, hypoxemia, and fatigue.

Your attending physician and nursing team will vigilantly observe for alterations in your respiratory function and any indicators suggesting the emergence of pneumonia. Routine evaluations, including clinical examinations, non-invasive procedures (pulse

oximetry), hematological assessments, chest radiographs, and/or CT imaging, will be employed to ascertain blood oxygen saturation levels.

Should you encounter any of the following symptoms, it is imperative to notify your physician or nurse promptly:

- New or exacerbated difficulty in breathing;
- Onset or aggravation of chest pain;
- Beginning or intensification of pain or discomfort during breathing;
- New emergence of cough or a marked shift in its nature, such as expectorating excessive sputum, coughing up blood, or any deterioration of these symptoms;
- Alterations in blood oxygen levels;
- Respiratory changes or other pulmonary symptoms accompanied by fever, lethargy, or other related signs.

Should these symptoms manifest, the research physician will request your presence at the clinic for supplementary evaluations, encompassing a clinical examination, oxygen saturation measurement, hematological tests, chest X-rays, and/or a CT scan. Continuous surveillance will monitor any symptom progression in your pulmonary system. Hospital admission may be deemed necessary during this observation phase. Specific interventions may be administered to manage your pneumonia, and a pulmonologist, a specialist in respiratory diseases, might evaluate you.

Occasionally, prolonged administration of anti-inflammatory medications (e.g., glucocorticoids) is essential to address these side effects, which might compromise the body's defences against certain infections, termed opportunistic infections. These infections necessitate treatment with antibiotics or antifungals and can be life-threatening.

You are expected to adhere to your physician's directives, actively participate in your treatment regimen, maintain consistent communication with the research team, and undergo assessments and follow-ups in alignment with the trial protocol.

7. Possible Benefits

The insights garnered from this study aim to enhance our comprehension of the safety and efficacy of radiotherapy in conjunction with immunotherapy. Such insights may pave the way for innovative treatment paradigms for patients diagnosed with advanced multiple metastatic tumors, potentially extending their lifespan and enhancing their prognosis. All enrolled patients will granted Tα-1(Zadaxin, SciClone Pharmaceuticals) and GM-CSF(Molgramostim, Topleucon, Xiamen Amoytop Biotech) at no cost, and free absolute lymphocyte subsets test.

8. Other Optional Therapeutic or Nursing Interventions

If you decide not to participate in this study, your physician will recommend alternative treatment options based on your specific medical condition.

9. Confidentiality and Privacy Authorization

Pertinent Chinese regulations safeguard your health-related data. By endorsing this informed consent form, you grant permission to acquire, utilize, and disseminate your health data to the study physician and associated research personnel. Your initials will be coded and provided to the investigators for research purposes.

Your medical records (study chart/CRF, labs, etc.) will be securely retained at the medical facility attending to you. Laboratory test results will be logged by your physician in your medical record. Authorized personnel, including the investigator, ethics committee, and drug regulatory entities, will have access to your medical records. Any publicized findings from this study will ensure your personal identity remains undisclosed, and we pledge to safeguard your privacy.

10. Voluntary Participation and Option to Withdraw

Your involvement in this research is purely voluntary. You reserve the right to decline participation or withdraw your participation at any stage without jeopardizing your medical care rights or facing discrimination from healthcare providers.

11. Inquiries and Updates

Before endorsing this consent document, all research team members are available to address any queries. If you have further questions, insights, or remarks after signing, feel free to consult the investigator. You will remain informed about the study's developments and progress.

For concerns about your rights and interests, please contact the Ethics Committee of the Second Affiliated Hospital of Soochow University at +86-0512-67783682.

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Informed Consent

Consent Signature Page

I have been informed verbally about this study by the physician responsible for the study or the appropriate researcher, and I have read the written information above.

I have been given ample opportunity to discuss and ask questions about the above study.

I agree to participate in this study and understand that my participation in this study is completely voluntary and that I will cooperate fully with my physician.

I understand that I can withdraw from the study at any time and that my withdrawal will not affect my future medical visits.

By signing this informed consent form, I agree that my personal information data, including my medical information data, will be used as described above.

I understand that I will be given a copy of this informed consent form.

Patient Name:

Patient Signature: _____Date of Signature: _____

Contact phone number: _____

or

Signature of Legal Representative: _____

Date of Signature: _____ (To be used only if subject is incapacitated)

Relationship to the patient: _____

Contact phone number: _____

Signature of Investigator: _____ Date of Signature: _____

Contact phone number: _____