Safety and Efficacy of Docetaxel, Darolutamide, and Homoharringtonine Combined with Androgen Deprivation Therapy in Neoadjuvant Treatment of High-Risk Prostate Cancer: A Multicenter Prospective, Single-Arm Clinical Study

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I. Basis for Project Establishment

1. Current Research Status in China and Abroad

Prostate cancer (PCa) has become a major threat to male urogenital health. Prostate cancer is a highly heterogeneous malignant tumor. This significant heterogeneity is manifested, on the one hand, by the diversity of clinical courses; patients with indolent disease can survive for years without progression, while aggressive disease can metastasize rapidly and become incurable. On the other hand, it is manifested by spatial and clonal genomic diversity. The high heterogeneity of prostate cancer, some of which can rapidly develop bone and lymph node metastases, is a major cause of death in prostate cancer patients.

The global cancer statistics report published by the WHO International Agency for Research on Cancer shows that in 2020, there were 1,414,259 new cases of prostate cancer worldwide, accounting for 7.3% of all malignant tumors, with an incidence rate second only to breast and

lung cancer. In recent years, the incidence of prostate cancer in China has been increasing. Although the widespread availability of prostate-specific antigen (PSA) screening has improved the early diagnosis rate of prostate cancer, many patients are already at an advanced stage at initial diagnosis. Consequently, the overall survival of prostate cancer in China is poor, with a 5-year survival rate of only about 70%, far lower than the over 90% in developed countries. Therefore, improving tumor control in patients with advanced prostate cancer is a critical issue that urgently needs to be addressed clinically. A growing number of studies are focusing on the significance of radical prostatectomy (RP) in the treatment of high-risk prostate cancer. In these cases, the incidence of perioperative complications appears manageable, and patients may benefit from radical surgery. However, since radical prostatectomy alone has proven insufficient, the combination of neoadjuvant systemic therapy and radical prostatectomy has been applied in the multimodal treatment of prostate cancer. Androgen deprivation therapy (ADT) is the foundational treatment for high-risk prostate cancer, but the 3-year biochemical recurrence-free survival (bRFS) rate after ADT combined with radical prostatectomy (RP) is only 50%-60%. The STAMPEDE study showed that ADT combined with docetaxel (75 mg/m², once every 3 weeks, for 6 cycles) increased the 10-year overall survival (OS) rate of HRPC patients by 10%, establishing the central role of docetaxel in combined therapy for HRPC. Subsequent phase II studies (e.g., reported in European Urology in 2019) further confirmed that ADT + docetaxel preoperative induction therapy achieved pathological downstaging (pT3 → pT2) in 30%-40% of patients, with a negative margin (R0) rate increased to 65%-70%, approximately 20 percentage points higher than ADT alone.

Second-generation androgen receptor inhibitors (ARi) such as darolutamide and apalutamide, due to their high selectivity and low blood-brain barrier penetration, have shown great potential in the treatment of high-risk prostate cancer. The ARAMIS study (NEJM, 2019) confirmed that darolutamide combined with ADT significantly prolonged metastasis-free survival in non-metastatic castration-resistant prostate cancer (nmCRPC), but preoperative induction studies in localized HRPC are still in the exploratory stage. A phase II study published in the Journal of Clinical Oncology in 2022 showed that after 6 cycles of induction therapy with the triple combination of ADT + darolutamide + docetaxel, the proportion of HRPC patients with PSA reduced to below the detection limit (<0.01 ng/mL) reached 58%, and the pathological complete response (pCR) rate was 12%, significantly higher than the ADT + docetaxel doublet regimen (32% and 3%, respectively), with no significant increase in the incidence of grade 3 or higher adverse events (AEs) (mainly neutropenia, incidence 35%). Our research group combined spatial transcriptomics (ST) and single-cell RNA sequencing (scRNA-seq) to analyze clinical samples of prostate cancer from our center. We identified a subtype of small cell-like prostate cancer cells with significantly enriched protein synthesis pathways. Through in vivo and in vitro experimental validation, we identified the protein translation inhibitor homoharringtonine (HHT) as having the function of inhibiting prostate cancer progression. Furthermore, through spatial proteomics analysis, we also identified the conserved pattern of the translation initiation factor eIF1A in prostate cancer progression, further suggesting the potential of HHT as a targeted therapeutic agent for prostate cancer. Additionally, our preliminary work through a prospective single-arm clinical trial has preliminarily validated its efficacy and safety in patients with

castration-resistant prostate cancer. Therefore, we plan to initiate an investigator-initiated trial to determine whether neoadjuvant docetaxel, darolutamide, and homoharringtonine combined with androgen deprivation therapy can be effective in high-risk prostate cancer patients before radical prostatectomy, increasing the pathological complete response (pCR) or minimal residual disease (MRD) rate, and improving overall survival.

2. Entry Point and Significance of This Study
Prostate cancer has a high incidence rate and is a major disease threatening the health of elderly men in China. Although neoadjuvant androgen deprivation therapy combined with radical prostatectomy reduces the rate of positive surgical margins, it has not significantly improved survival. However, comprehensive neoadjuvant therapy based on ADT has shown certain potential for clinical application.

Previous study findings suggest that the efficacy limitations of the neoadjuvant treatment regimen combining docetaxel with androgen deprivation therapy are associated with protein synthesis. Homoharringtonine (HHT) is currently the only small-molecule translation elongation inhibitor approved by the U.S. Food and Drug Administration (FDA). Based on this, we plan to conduct a prospective interventional study aimed at validating whether the intensified quadruple regimen formed by adding darolutamide and homoharringtonine (HHT) to the standard regimen can further significantly enhance efficacy.

II. Research Content
1. Research Objectives
(1) Primary Research Objectives
To evaluate, after radical prostatectomy, the pathological complete response (pCR) or minimal residual disease (MRD) rate, PSA response, and postoperative biochemical progression-free survival (bPFS) in high-risk prostate cancer patients treated with the quadruple regimen of docetaxel, darolutamide, homoharringtonine, and androgen deprivation therapy combined with surgery.

To compare the differences in primary efficacy endpoints between the prospective cohort and historical controls.

(2) Secondary Research Objectives
To evaluate the pathological response (including positive surgical margins, tumor size, extraprostatic extension, seminal vesicle invasion, and lymph node involvement, etc.) after radical prostatectomy, changes in TNM staging before and after neoadjuvant therapy, and other progression-free survival in patients of the prospective cohort.

To evaluate the safety of the quadruple regimen of docetaxel, darolutamide, homoharringtonine, and androgen deprivation therapy combined with surgery for prostate cancer (based on CTCAE version 5.0 criteria), and to assess patients' quality of life (using the EORTC QLQ-C30 questionnaire).

(3) Exploratory Research Objectives
Through observation of clinical outcomes and testing of biological samples, to explore the following scientific questions:

To explore the biological mechanisms underlying the efficacy of neoadjuvant docetaxel, darolutamide, and homoharringtonine combined with androgen deprivation therapy using bulk, single-cell/spatial transcriptomics, single-cell ATAC sequencing, intratumoral microbiota detection, proteomics, and tissue microarray construction before and after treatment;

For patients with poor efficacy, to explore the biological mechanisms of resistance to neoadjuvant docetaxel, darolutamide, and homoharringtonine combined with androgen deprivation therapy, and to analyze optimized or combined treatment strategies;

To collect patient blood and urine samples for testing, including whole-genome sequencing, metabolomics, and proteomics, to identify biomarkers of response to neoadjuvant docetaxel, darolutamide, and homoharringtonine combined with androgen deprivation therapy, enabling precise selection of target patients.

2. Research Content
(1) Selection of Study Sites:
The primary center for this study will be the Department of Urology, Zhongda Hospital, Southeast University. Sub-centers will be the Department of Urology of the First Affiliated Hospital of Bengbu Medical University, the Department of Urology of Wuxi Xishan People's Hospital, the Department of Urology of Nantong First People's Hospital, the Department of Urology of Nantong Tumor Hospital, the Department of Urology of Northern Jiangsu People's Hospital, and the Department of Urology of Xuzhou Central Hospital.

(2) Selection of Study Subjects:
1) Source of Study Subjects
Prostate cancer patients attending the Department of Urology, Zhongda Hospital, Southeast University, the First Affiliated Hospital of Bengbu Medical University, Wuxi Xishan People's Hospital, Nantong First People's Hospital, Nantong Tumor Hospital, Northern Jiangsu People's Hospital, and Xuzhou Central Hospital who meet the inclusion criteria.

2) Inclusion Criteria
① Age ≥ 18 years and ≤ 85 years; ② Histologically or cytologically confirmed prostate cancer; ③ High-risk prostate cancer: meeting at least one of the following criteria: clinical stage T3-T4, or Gleason score 8-10, or PSA > 20 ng/mL, or presence of distant metastasis (clinical stage M1); ④ ECOG (Eastern Cooperative Oncology Group) performance status score of 0-1; ⑤ All patients voluntarily sign informed consent and are able to comply with treatment and follow-up.

3) Exclusion Criteria

① Any prior or ongoing treatment for prostate cancer, including radiotherapy, chemotherapy, ADT, etc.; ② Prior prostatectomy; ③ Any other serious underlying medical, psychiatric, or psychological conditions that, in the investigator's judgment, may affect treatment; ④ Allergy to any of the study drugs; ⑤ Refusal to undergo radical prostatectomy; ⑥ Deemed unsuitable for participation in this clinical trial by the investigator's judgment.

(3) Sample Size Estimation:
Taking $\alpha = 0.05$ (one-sided), $\beta = 0.20$. Referring to previous literature, the pCR/MRD rate for neoadjuvant therapy in prostate cancer is approximately 9.1-46%. The historical control pCR/MRD rate (p_0) is taken as 27.5%; the expected pCR/MRD rate (p_1) for this trial is 40%. Using a z-test with pooled variance, it was calculated that approximately 84 patients would need to be enrolled to provide about 80% statistical power. Considering a 10% dropout rate, the final planned sample size is 94 cases. The enrollment ratio for the primary center (Department of Urology, Zhongda Hospital, Southeast University) and the six sub-centers is 1:1:1:1:1:1:1.

(4) Subject Selection and Allocation Method:
This study is a prospective, single-arm clinical trial. Eligible patients will be consecutively enrolled into the study after assessment by investigators at each center.

Allocation method is as follows:
The total planned sample size is 94 cases.
To ensure balanced contribution of cases from each center, the principle of proportional allocation by center is adopted. There are a total of 7 participating centers (the primary center and 6 sub-centers), with an enrollment ratio set at 1:1:1:1:1:1:1.
Based on this calculation, each center is planned to enroll approximately $94 / 7 \approx 13.4$ cases. To ensure completion of the total sample size, it is planned that the primary center (Department of Urology, Zhongda Hospital, Southeast University) enrolls 14 cases, and each of the six sub-centers enrolls 13 cases, totaling $14 + (13 \times 6) = 92$ cases. The remaining 2 cases will be flexibly allocated based on the enrollment progress of each center.

Intervention Regimen:
Enrolled patients will uniformly receive the following neoadjuvant treatment regimen: Docetaxel 75 mg/m², intravenous infusion, once every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles + Darolutamide 600 mg, orally, twice daily, starting from day 1 of treatment and continued until 1 week before surgery + Homoharringtonine 1 mg intravenous infusion in 250 mL 5% glucose injection, once daily for two consecutive days, repeated every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles + Continuous androgen deprivation therapy. All patients will undergo radical prostatectomy within 3 weeks (± 7 days) after the completion of treatment.

(5) Informed Consent of Study Subjects:
Prospective Intervention Cohort: This study will strictly follow the principles of the Declaration of Helsinki. All enrolled patients must be fully informed of the study purpose,

procedures, potential risks, and benefits before participating in any study-related procedures and must voluntarily sign a written informed consent form.

(6) Evaluation Indicators:
1) Primary Evaluation Indicators
① pCR or MRD Rate:
pCR (Pathological Complete Response) is defined as the absence of morphologically identifiable cancer in the prostatectomy specimen.
MRD (Minimal Residual Disease) is defined as a maximum cross-sectional diameter of residual tumor ⩽ 5 mm, and using RCB (Residual Cancer Burden) ⩽ 0.25 cm^3 (tumor volume ⩽ 0.5 cm^3 × tumor cellularity ⩽ 50%). Tumor volume is calculated by three-dimensional volume estimation based on the largest cross-sectional dimension of the tumor and the number of cross-sections, with adjustment for tumor cellularity.

② PSA Response Rate: Defined as the comparison of PSA levels before neoadjuvant therapy and at the end of the neoadjuvant therapy cycle (before surgery).

③ Biochemical Progression-Free Survival (bPFS) after Radical Prostatectomy.

2) Secondary Evaluation Indicators
① Pathological response after radical prostatectomy (including positive surgical margins, tumor size, extraprostatic extension, seminal vesicle invasion, and lymph node involvement) in the prospective cohort; ② Changes in TNM staging on imaging after neoadjuvant therapy and before surgery; ③ Other progression-free survival (progression includes radiographic progression, castration resistance, need for further therapeutic intervention, etc.); ④ Safety indicator: CTCAE version 5.0 adverse event grading; ⑤ Quality of life score: EORTC QLQ-C30 questionnaire.

3) Exploratory Evaluation Indicators
① Mechanisms of treatment efficacy: transcriptional, proteomic, and microbiome characteristics before and after treatment, as well as in patients who achieve the primary endpoint; ② Mechanisms of treatment resistance: transcriptional, proteomic, and microbiome characteristics before and after treatment, as well as in patients who do not achieve the primary endpoint; ③ Effective biomarkers: changes in liquid biopsy indicators in patients who achieve and those who do not achieve the primary endpoint.

(7) Intervention Methods:
Docetaxel:
75 mg/m^2, intravenous infusion, once every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles. To prevent allergic reactions and fluid retention, oral dexamethasone 8 mg is administered twice daily on the day before, the day of, and the day after docetaxel infusion.

Darolutamide:

600 mg, orally, twice daily, starting from day 1 of treatment and continued until 1 week before surgery. To be taken with food.

Homoharringtonine:
1 mg homoharringtonine intravenous infusion + 250 mL 5% glucose injection, once daily for two consecutive days, repeated every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles.

Androgen Deprivation Therapy:
Luteinizing hormone-releasing hormone (LHRH) analogs such as goserelin. Specific dosage: goserelin 3.6 mg administered as a subcutaneous injection into the anterior abdominal wall once every 28 days. (Specific drug dosages should follow the instructions in the package insert and the guidelines for the diagnosis and treatment of prostate cancer.)

3. Research Methods
(1) Study Design
Previous study findings suggest that the efficacy limitations of the neoadjuvant treatment regimen combining docetaxel with androgen deprivation therapy are associated with protein synthesis. Homoharringtonine (HHT) is currently the only small-molecule translation elongation inhibitor approved by the U.S. Food and Drug Administration (FDA). Based on this, we plan to conduct a prospective interventional study aimed at validating whether the intensified quadruple regimen formed by adding darolutamide and homoharringtonine (HHT) to the standard regimen can further significantly enhance the depth of pathological response and improve patient outcomes through multi-mechanism synergy.

(2) Enrollment and Treatment Methods
This study will be a prospective, single-arm, multicenter interventional study.
Enrollment: Newly diagnosed high-risk prostate cancer patients meeting the inclusion/exclusion criteria for the prospective cohort will be consecutively enrolled.
Treatment Methods: Enrolled patients will uniformly receive the following combined neoadjuvant treatment regimen: Docetaxel: 75 mg/m², intravenous infusion, once every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles. Premedication with oral dexamethasone according to the standard regimen is required before administration to prevent allergies and fluid retention; Darolutamide: 600 mg, orally, twice daily, starting from day 1 of treatment and continued until 1 week before surgery. It is recommended to be taken with food; Homoharringtonine: 1 mg in 250 mL 5% glucose injection, intravenous infusion, once daily for two consecutive days, repeated every 3 weeks (administered in weeks 1, 4, and 7), for a total of 3 cycles; Continuous androgen deprivation therapy: e.g., goserelin 3.6 mg subcutaneous injection into the anterior abdominal wall once every 28 days, continued until surgery.

Surgery and Follow-up:
After starting neoadjuvant therapy, all patients in the prospective cohort will be followed up at the hospital during each treatment cycle, with repeat blood tests including PSA, and

collection of patient blood and urine samples. After all treatment cycles are completed, imaging examinations will be repeated to assess efficacy, and patients will undergo radical prostatectomy within 3 weeks (± 7 days) after the end of treatment. Postoperative follow-up will be conducted according to the specified protocol.

(3) Efficacy Evaluation:
Efficacy is evaluated through regular patient follow-ups including blood PSA levels and imaging-assisted examinations. The pCR or MRD rate after radical prostatectomy serves as a key indicator for measuring efficacy.

(4) Statistical Analysis
Descriptive statistics for categorical indicators will be presented as counts and percentages. Intergroup comparisons for continuous indicators will be performed using independent t-tests or Mann-Whitney U tests; intragroup comparisons will use paired t-tests or Wilcoxon signed-rank tests. Categorical indicators will be analyzed using the chi-square test or Fisher's exact test. All statistical tests will be one-sided, and a P-value $\leqslant 0.05$ will be considered statistically significant.

(5) Risk Assessment and Management Plan
1) Risk Assessment
In this interventional clinical study, during the administration of docetaxel, darolutamide, homoharringtonine combined with androgen deprivation therapy, adverse reactions may occur. Common adverse reactions include myelosuppression, sinus tachycardia, anorexia, nausea, vomiting, etc. All adverse reactions are graded according to CTCAE version 5.0. Therefore, if adverse reactions occur during treatment, they should be managed promptly to avoid treatment delays or misdiagnosis and prevent irreversible consequences.

2) Management Plan
Throughout the interventional clinical study process, researchers must strictly adhere to scientific research ethics. The information collection process must strictly protect patient privacy and not harm any patient's interests. During patient treatment, liver and kidney function, electrolytes, and other indicators will be regularly monitored, and telephone follow-ups will be conducted regularly to ensure timely diagnosis of the patient's condition and avoid delays in treatment. During treatment, if grade 1-2 adverse reactions occur, the medication regimen remains unchanged with active symptomatic management. If a grade 3 adverse reaction occurs, the docetaxel dose for the next cycle is reduced to 60 mg/m². If it recurs, it is reduced to 45 mg/m². If it recurs again, docetaxel is discontinued. For darolutamide, if restarted, the dose is reduced to 400 mg twice daily. If the reaction recurs, it is discontinued. For homoharringtonine, the dose is reduced to 0.5 mg. If a grade 4 adverse reaction occurs, the medication must be discontinued.

(6) Follow-up
The follow-up period includes safety follow-up and survival follow-up.
Safety Follow-up: When a subject discontinues study treatment, safety follow-up visits will

be conducted before and after drug administration (± 3 days) during each treatment cycle, before and after surgery (± 7 days), and within one month after surgery (± 7 days). Blood routine tests, liver and kidney function, and other indicators will be rechecked, and patient blood and urine samples will be collected.
Survival Follow-up: After completing two safety follow-up visits, survival follow-ups will be conducted every three months (± 7 days) during the first year, along with safety assessments. From the second year onwards, survival and safety follow-ups will be conducted every six months (± 7 days) (including rechecking blood routine, liver and kidney function, and collecting patient blood and urine samples). Follow-up continues until subject death, withdrawal from the study, or completion of 5 years of study follow-up (whichever occurs first).

4. Research Technical Route
[Image description: The diagram outlines the study workflow: 1. Enrollment of high-risk prostate cancer patients (meeting inclusion/exclusion criteria) from 7 centers; 2. Administration of the neoadjuvant quadruple therapy (Docetaxel + Darolutamide + HHT + ADT) for 3 cycles; 3. Assessment during treatment (PSA, imaging, blood/urine sample collection); 4. Radical prostatectomy within 3 weeks post-treatment; 5. Postoperative sample analysis (pathology, transcriptomics, proteomics, etc.); 6. Follow-up for safety and survival endpoints; 7. Data analysis and outcome evaluation.]

5. Feasibility Analysis
(1) Theoretical Feasibility: The proposed work is based on the results of preliminary research and synthesized with the latest international research progress. This study addresses the issue of currently lacking effective treatment options for patients with localized high-risk/very high-risk, locally lymph node metastatic, or metastatic prostate cancer. It aims to utilize homoharringtonine, the only small-molecule translation elongation inhibitor approved by the U.S. FDA, combined with androgen deprivation therapy to treat prostate cancer cells. The rationale for the project is solid, experimental conditions are mature, and the approach is theoretically feasible. The key experimental techniques required for the project have been mastered and are routinely performed in the laboratory. Additionally, the research group has established a prostate cancer biospecimen bank in the preliminary work, ensuring the availability of population samples needed for this study.

(2) Technical Feasibility: This study is a single-arm clinical trial. The regimen involves a combination of docetaxel, darolutamide, homoharringtonine, and androgen deprivation therapy. Homoharringtonine is a drug currently used for conditions such as myelodysplastic syndromes, chronic myeloid leukemia, and polycythemia vera, and its safety in the general population has been preliminarily validated. This study represents a new application for an old drug, aiming to provide clinical evidence for the use of HHT in patients with advanced prostate cancer.

7. Research Plan and Expected Progress
Timeline Main Work Content

January 2026 – December 2026 Complete clinical case enrollment, implement corresponding treatments and evaluations, collect data.
December 2026 – December 2027 Organize, process, and analyze clinical data; translate analysis results into publications, etc.

8. Expected Research Outcomes

(1) Demonstrate that compared to historical controls, this quadruple neoadjuvant therapy regimen more effectively reduces patient PSA levels, achieves a higher rate of imaging downstaging, and significantly prolongs biochemical progression-free survival (bPFS).
(2) Elucidate that this quadruple regimen is superior to traditional doublet regimens in suppressing pathological responses in post-radical prostatectomy specimens (e.g., reducing positive surgical margin rates, decreasing seminal vesicle invasion, and lymph node involvement).
(3) Obtain safety data for this quadruple regimen in neoadjuvant therapy for prostate cancer, clarifying that its adverse reaction profile is manageable and patient quality of life is acceptable.
(4) Through exploratory research, preliminarily reveal the mechanisms of action and resistance of this combined regimen, and identify potential predictive biomarkers of efficacy, providing a basis for subsequent precision treatment.

III. Working Foundation and Conditions

Accumulated Research Work and Achievements Related to This Project:

1. Combined Application of Spatial Transcriptome Sequencing and Single-Cell RNA Sequencing

To comprehensively analyze the spatial heterogeneity of prostate cancer samples, we performed 10x Visium spatial transcriptome sequencing analysis on prostate biopsy tissues from 8 patients (including normal tissue N, prostatic intraepithelial neoplasia tissue PIN, and prostate cancer tissue). Histopathological results showed that the 8 samples morphologically represented different prostate cancer subtypes. Further analysis of the molecular expression characteristics of different samples revealed that atypical glandular tumors exhibited histological features of small cell carcinoma. Subsequently, we referred to these tumors as small cell-like prostate cancer (SCLPC).

Based on dimensionality reduction and clustering, spots from all samples were grouped into 8 major clusters. According to marker gene expression, clusters C1-C3 were defined as stroma, and clusters C4-C8 as epithelial regions. Combined with inferCNV analysis, clusters C4 and C5 were identified as tumor regions. Subpopulations C4/C5 showed high histological similarity to neuroendocrine prostate cancer (NEPC). Using GSEA, IHC, and differential expression analysis between SCLPC and Adeno subtypes, we found that C4/C5 did not exhibit distinct neuroendocrine features and showed significantly weaker AR activity. Furthermore, through GSEA, TCGA cohort analysis, and scoring methods, we found that upregulated genes in C4/C5 were enriched in functional categories related to stemness (e.g., MYC and WNT signaling), cancer aggressiveness (e.g., metastasis and squamous cell carcinoma), proliferation (e.g., cell cycle and DNA repair), RNA metabolism (e.g., mRNA

processing and splicing), and translation (e.g., ribosome pathway). In conclusion, we defined an aggressive primary prostate cancer subtype characterized by non-neuroendocrine features, stem cell features, low AR activity, and associated with poor clinical prognosis.

Simultaneously, we performed single-nucleus RNA sequencing (snRNA-seq) on frozen surgical tissue from two representative samples (SCLPC subtype and Adeno subtype), obtaining 12,913 cells grouped into 15 major clusters. Pseudo-time analysis of epithelial cells showed that two cancerous epithelial groups (G1/7) were clearly separated from normal epithelium (G0). CellphoneDB cell communication analysis revealed that epithelial groups with stem/progenitor characteristics (G0/12/10) showed significantly increased interactions with other cell types in the microenvironment compared to mature G3/4 luminal cells. Additionally, the G7 and G1 populations in the data corresponded to SCLPC and Adeno subtypes, respectively. GSEA analysis comparing G7 vs. G1 yielded similar conclusions to those from the spatial transcriptome sequencing.

2. Screening of Homoharringtonine by Observing Enrichment of Protein Synthesis Pathways in C4/C5

Besides the many known pro-cancer and stemness pathways enriched in C4/C5-SCLPC identified in the spatial transcriptome analysis, the significant enrichment of ribosome biogenesis and translation pathways caught our attention. We found that protein synthesis pathways were significantly enriched in C4/C5, suggesting that targeting protein synthesis pathway activity might be a novel clinical strategy for treating malignant tumors. For this, we selected homoharringtonine, currently the only small-molecule translation elongation inhibitor approved by the U.S. FDA, to treat prostate cancer cells. Homoharringtonine is currently widely used in the treatment of various types of acute non-lymphocytic leukemia and also has certain efficacy in myelodysplastic syndromes, chronic myeloid leukemia, and polycythemia vera.

3. Preliminary In Vivo and In Vitro Validation of the Efficacy and Safety of Homoharringtonine in Prostate Cancer

For in vivo experiments, we used small cell-like PC3 xenograft tumors and AR-independent CRPC RM1 allograft tumors. The in vivo drug treatment results showed that various malignant biological behaviors of tumor cells (including proliferation, migration, invasion, and stemness) were significantly inhibited, demonstrating that homoharringtonine is a potential therapeutic strategy.

To further confirm the clinical relevance of this study, we conducted an investigator-initiated prospective single-arm clinical trial (IIT) using homoharringtonine. The results showed that a single dose of homoharringtonine effectively reduced PSA levels and alleviated cancer pain in CRPC patients, and inhibited the malignant progression and spread of metastatic lesions. Several patients were enrolled in this IIT, and their tumor burden was effectively controlled, indicating that homoharringtonine is a feasible treatment option for CRPC.

Available Experimental Conditions:

Southeast University is one of the first batch of "985" and "Double First-Class" universities

directly under the Ministry of Education. It hosts a National Key Research and Development & Innovation Platform for Medicine-Engineering Integration, the "Development and Genes" Key Laboratory of the Ministry of Education, the Jiangsu Provincial Key Laboratory for "Gene Diagnosis and Gene Therapy," and the Institute of Urology of Southeast University, which can provide the necessary experimental space for this project. The laboratory is equipped with state-of-the-art cell and molecular biology experimental equipment. The Department of Urology at Zhongda Hospital, Southeast University, is a National Key Clinical Specialty. To date, it has completed nearly 2,000 robot-assisted laparoscopic surgeries, performing over 200 radical prostatectomies annually. In preliminary work, the project team has accumulated extensive clinical data and follow-up information, establishing a solid data foundation for future research. In the past five years, the Department of Urology at Zhongda Hospital, Southeast University, has received research funding totaling approximately 15 million RMB. Prostate cancer-related research has been supported by the National Natural Science Foundation of China, the National Key R&D Program of China, the 科教强卫工程 (Science and Education for Health Project) – Innovation Team, research projects from the Ministry of Education, the "Six Talent Peaks" Project, the Natural Science Foundation of Jiangsu Province, and the Jiangsu Provincial Key R&D Program, among others. In the past three years, the department has published 58 SCI papers, 10 of which have impact factors > 10. The prostate cancer diagnosis and treatment technologies developed by the Department of Urology at Zhongda Hospital, Southeast University, have twice won the First Prize for Medical New Technology Introduction from Jiangsu Province.

Research Team Member List:

Name Gender Age Position/Title Department/Institution Professional Field Project Role
Xu Bin Male 42 Vice Dean, Director of Urology, Chief PhysicianDepartment of Urology, Zhongda Hospital, Southeast University Urology Project Design, Quality Control
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IV. Budget

The funding budget is self-funded. Related research operation fees, equipment fees, experimental material fees, laboratory renovation fees, collaboration fees, and project implementation fees are self-raised. Labor costs are approximately 5,000 RMB, subject to change based on project implementation.