Official title: Clinical Study on Predicting Lymph Node Metastasis of High-risk Prostate Cancer Based on Artificial Intelligence Multi-omics Analysis：A Multicenter, Prospective and Observational Clinical Study

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Project name: Clinical Study on Predicting Lymph Node Metastasis of High-risk Prostate Cancer Based on Artificial Intelligence Multi-omics Analysis：A Multicenter, Prospective and Observational Clinical Study

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Participating units: Cancer Hospital of Chinese Academy of Medical Sciences

Participating units: The First Affiliated Hospital of Bengbu Medical University

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Program summary

Project name	Clinical Study on Predicting Lymph Node Metastasis of High-risk Prostate Cancer Based on Artificial Intelligence Multi-omics Analysis：A Multicenter, Prospective and Observational Clinical Study
Research purpose	Based on artificial intelligence technology and combining clinical, pathological, and imaging data to build a prediction model for lymph node metastasis of high-risk prostate cancer
research design	Retrospective, prospective, multicenter, diagnostic accuracy study
study population	Patients with high-risk prostate cancer (PSA≥20ng/ml or Gleason≥8)
Total number of cases	2000 cases

Case selection	*Inclusion criteria* ● Age≥50 years old ● Patients must have histologically or cytologically confirmed prostate adenocarcinoma ● PSA≥20 of/ml or Gleason≥8 ● Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0-2 ● Life expectancy ≥6 months ● Normal bone marrow function: absolute neutrophil count ≥1.5×109/L; platelets ≥75×109/L; hemoglobin ≥90 g/L; white blood cell count ≥ 3.0×109/L ● Normal liver function: alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≤ 2.5 times the upper limit of normal (ULN); for patients with liver metastases, ALT/AST can be ≤ 5 times the ULN ● Total bilirubin ≤ 1.5 times ULN or total bilirubin > 1.5 times ULN and direct bilirubin ≤ ULN; ● Normal coagulation function: INR ≤ 1.5, partial thromboplastin time (APTT) ≤ 1.5 times ULN, prothrombin time (PT) < ULN + 4 seconds ● Normal heart function: left ventricular ejection fraction (LVEF) ≥50%; QTc for men <450ms, for women <470ms, serum potassium ≥3.5mmol/L ● Normal blood pressure: systolic blood pressure <160mmHg, diastolic blood pressure <95mmHg; patients with stable blood pressure assessment after appropriate clinical treatment can be enrolled. ● Normal renal function: serum creatinine ≤1.5 times ULN, and creatinine clearance ≥50 mL/min ● Prospective subjects can understand and be willing to sign the informed consent form ● Ability to adhere to study visit schedules and other protocol requirements

	Exclusion criteria ● There are contraindications for MRI examination, such as metal implants in the body, claustrophobia, etc. ● Patients with any missing items in baseline clinical and pathological information ● Have a clear history of neurological or mental disorders, such as dementia, epilepsy, or seizure proneness ● In the researcher's judgment, there are factors that seriously endanger the safety of the subjects, or concomitant diseases that affect the subjects' completion of this study (such as severe diabetes, thyroid disease, and mental illness, etc.), or there are factors that affect the safety of the patients or serious and/or unstable medical, mental, or other conditions (including abnormal laboratory tests) that affect the patient's ability to provide informed consent, or there are any psychological, family, sociological, or geographical conditions that affect the research plan and follow-up plan. ● The researcher believes that he is not suitable to participate in this clinical trial for any reason ● Unable to provide informed consent
intervention plan	1. Retrospective patient analysis and training set construction: 600 patients who underwent radical prostatectomy plus pelvic lymph node dissection in the First Affiliated Hospital of Anhui Medical University were collected as a training set (360 cases) and an internal validation set (240 cases) in a ratio of 3:2. MRI images of the patients' primary lesions, pathological images of puncture tissues, and clinical data (such as age, BMI, pre-puncture PSA, and preoperative imaging magnetic resonance PI-RADS score, whether neoadjuvant treatment was performed before surgery, postoperative Gleason score, TNM stage, whether the resection margin was positive, whether seminal vesicle invasion, whether lymph node metastasis, number of lymph node metastases (left/right), whether nerve invasion and other data, whether the patient had biochemical

	recurrence after surgery, survival period, etc.), combined with artificial intelligence deep learning to build a prediction model for lymph node metastasis in high-risk prostate cancer patients. A multi-omics lymph node metastasis prediction model for prostate cancer patients was constructed by combining pathomics, radiomics, and clinical data. 2. Retrospective patient validation set validation: The constructed multi-omics lymph node metastasis prediction model for prostate cancer patients will be externally validated in the internal validation cohort of the First Affiliated Hospital of Anhui Medical University (300 cases), the external validation cohort of Nanjing Drum Tower Hospital (400 cases), the external validation cohort of the Cancer Hospital of the Chinese Academy of Medical Sciences (400 cases), and the external validation cohort of the First Affiliated Hospital of Bengbu Medical University (300 cases). 3. Prospective patient validation set validation: A prospective cohort (300 cases) of high-risk prostate cancer who underwent radical surgery plus lymph node dissection in the First Affiliated Hospital of Anhui Medical University was established. The multi-omics lymph node metastasis prediction model of prostate cancer patients was used to analyze whether the patients had lymph node metastasis before surgery. Radical prostatectomy plus pelvic lymph node dissection was performed on the patients. Primary tumor specimens and Transcriptome and single-cell omics sequencing of pelvic lymph nodes were performed in some cases, combined with postoperative pathologists' reading of lymph node specimen tissue to determine whether there was lymph node metastasis, the accuracy of the artificial intelligence deep learning model in predicting lymph node metastasis was analyzed, and the effectiveness of the multi-omics artificial intelligence deep learning model combined with genomics in predicting lymph node metastasis was analyzed.

Evaluation index	Effectiveness evaluation index ● Diagnostic accuracy: sensitivity, specificity, positive predictive value, negative predictive value, overall diagnostic accuracy, etc. ● Clinical efficacy: progression-free survival (PFS), overall survival (OS), biochemical recurrence rate, and meaningless lymph node dissection rate of prostate cancer patients.
	Safety evaluation index ● The incidence of postoperative complications, such as the incidence of postoperative lymphadenopathy due to lymph node dissection ● Incidence of puncture- and surgery-related adverse events (AE) and serious adverse events (SAE)
Statistical methods	Descriptive statistical analysis, hypothesis testing, non-parametric testing, regression analysis, survival analysis, covariance analysis, missing data processing
Study period	August 2025 - July 2026

Chapter 1 Research background

Prostate cancer is the most common cancer in men, and most patients present with low-risk characteristics[1, 2], the global incidence rate is close to 20%[3]. Prostate cancer has the highest five-year survival rate of any cancer[4]. According to statistics, high-risk prostate cancer accounts for 12% to 15% of prostate cancer detected abroad.[5]. At present, the incidence rate of prostate cancer has surpassed that of bladder cancer, ranking first among malignant tumors of the male genitourinary system in my country. There are obvious regional differences in the composition of prostate cancer in my country, with urban areas being higher than rural areas, and eastern areas being higher than central and western areas.[6]. But overall, the proportion of high-risk prostate cancer is still relatively high[7], and the prognosis of high-risk prostate cancer patients is poor. As the population ages, the incidence of prostate cancer is on the rise. In 2020, the incidence and mortality rates of prostate cancer in my country will be as high as 8.92% and 13.37% respectively, ranking first among malignant tumors.[8], has become one of the important diseases threatening men's health.

According to the National Comprehensive Cancer Network guidelines and D'Amico risk stratification definition, one of the following criteria is met: tumor stage T2c~T4, serum prostate-specific antigen (PSA) >20ng/ml, Gleason score≥ a score of 8 falls into the category of high-risk prostate cancer.[9]. Most patients with low-risk prostate cancer can benefit from radical prostatectomy, but a subset of high-risk patients do not appear to benefit from radical prostatectomy.[10]; and are more likely to die from disease due to metastasis[5]. Lymph node dissection (LND) has been a routine part of cancer surgery for more than a century[11], the current view is that the treatment of high-risk prostate cancer should adopt a comprehensive treatment plan with radical prostatectomy combined with extended pelvic lymph node dissection as the initial treatment; this plan can improve the accuracy of staging and reduce the long-term risk of prostate cancer-specific death.[12, 13], there are also studies showing that pelvic lymph node dissection can reduce the probability of biochemical recurrence[14], has some oncological benefit; another study showed that patients who had pelvic lymph node dissection had a 9% lower 10-year risk of death compared with patients who had no pelvic lymph node dissection or limited lymph node dissection.[15], therefore the diagnostic value of pelvic lymph node dissection is undeniable. However, the positive rate of lymph nodes in clinical patients with high-risk prostate cancer is low. The positive rate of lymph node dissection in patients with high-risk prostate cancer is about

12%.[16], meaning that most patients undergoing this procedure have pointless dissections[17]. And pelvic lymph node dissection may lead to some poor perioperative complications, including intraoperative ureteral injury, vascular injury, obturator nerve injury, postoperative asymptomatic lymphocele and deep vein thrombosis, postoperative lymphatic leakage leading to delayed drainage tube removal, etc.[18]. Asymptomatic lymphocele is the longest postoperative complication[19, 20]. According to the Briganti 2017 nomogram prediction model recommendation, lymph node dissection should be performed in patients with lymph node infiltration index greater than 5%.[21], has higher prediction accuracy than Briganti 2012 and MSKCC, but it does not incorporate imaging data and genomic data.

In recent years, artificial intelligence (AI) technology has been increasingly used in the medical field, especially in disease diagnosis, treatment decision-making, efficacy evaluation, etc., showing great potential[22]. Since the 1970s, medical imaging technology has made new breakthroughs, various medical imaging models have gradually matured, and medical image recognition has become an important research direction for artificial intelligence diagnosis and treatment of diseases.[23]. Current methods for diagnosing prostate cancer mainly include serum prostate-specific antigen (PSA) testing[24], prostate multi-parameter magnetic resonance PI-RADS score, prostate biopsy tissue pathological section examination, etc.[25]; Conventional images such as computed tomography or magnetic resonance imaging (MRI) have poor accuracy in identifying lymph node invasion[26]. Although lymph node size can help identify lymph node invasion, its sensitivity is low because normal-sized lymph nodes may contain smaller metastatic disease[27]. Currently, artificial intelligence shows potential benefits in developing automatic or semi-automatic diagnostic tools and has been applied in many medical scenarios[28-30]. There are currently studies that combine prostate cancer pathology slide images with artificial intelligence to predict prostate cancer lymph node metastasis.[31]. However, this method still relies on postoperative pathology to help doctors determine whether lymph node metastasis has occurred. It cannot improve the accuracy of doctors' preoperative judgment of whether lymph node metastasis has occurred in patients and help patients avoid meaningless pelvic lymph node dissection. By combining artificial intelligence with imaging data, the research team has successfully built a lymph node pathology prediction model for high-risk prostate cancer patients based on clinical data, imaging data, pathology data and other multi-omics data of approximately 460 patients in the early stage, combined with

artificial intelligence deep learning, and achieved good prediction performance in the internal validation set. On this basis, this study plans to take the lead in conducting a retrospective combined with prospective multi-center study to further confirm the performance of the prediction model in an external validation set, and combine it with multi-omics artificial intelligence deep learning of prospective genomics to build a more accurate prediction model for lymph node metastasis. The developed prostate cancer lymph node metastasis prediction model was compared with pathological results to explore its clinical utility.

Chapter 2 Research purpose

2.1 Main purpose:

Based on artificial intelligence technology, by delineating the quantitative mapping correlation between prostate cancer tissue whole-section annotation images, MRI and pelvic lymph node metastasis, clarify common characteristics and establish a prediction model for lymph node metastasis in patients with high-risk prostate cancer. First, the model can accurately predict whether pelvic lymph nodes have metastasized before surgery, eliminating unnecessary pelvic lymph node dissection and avoiding postoperative complications caused by pelvic lymph node dissection, such as lymph leakage, lymph fluid infection, etc.; second, the established artificial intelligence prediction model can accurately diagnose the probability of lymph node metastasis, invasiveness, grade, subtype, and biochemical recurrence of prostate cancer by predicting the subject's MRI images and puncture pathological tissue images before surgery or puncture.

2.2 Secondary purpose:

Establish a model based on artificial intelligence technology to develop a personalized treatment plan for the patient before surgery or puncture; perform three-dimensional reconstruction of the prostate and lesions to clarify the exact location of the lesions and guide puncture or surgical treatment.

Chapter 3 Research methods

3.1 Research design

This study adopts a retrospective, prospective, multi-center, diagnostic accuracy research design, and is planned to be mainly conducted at the First Affiliated Hospital of Anhui Medical University. It is expected to cooperate with Anhui University, Nanjing Drum Tower Hospital, Cancer Hospital of the Chinese Academy of Medical Sciences, and the First Affiliated Hospital of Bengbu Medical University. Departments include urology, medical imaging, and pathology for multidisciplinary collaborative research.

600 patients who underwent radical prostatectomy plus pelvic lymph node dissection in the First Affiliated Hospital of Anhui Medical University were used as a training set (360 cases) and an internal validation set (240 cases) in a ratio of 3:2. MRI images of the patients' primary prostate lesions were collected, including multi-parameter magnetic resonance T1-weighted, T2-weighted, DWI diffusion-weighted images and other multi-parameter data, pathological images of punctured tissues and clinical data (such as age, BMI, pre-puncture) PSA, preoperative MRI PI-RADS score, whether neoadjuvant treatment was performed before surgery, postoperative Gleason score, TNM stage, whether the resection margin is positive, whether seminal vesicle invasion, whether lymph node metastasis, number of lymph node metastases (left/right), whether nerve invasion and other data, whether the patient has biochemical recurrence after surgery, survival time, etc.), combined with artificial intelligence deep learning to build a prediction model for lymph node metastasis in high-risk prostate cancer patients. A multi-omics lymph node metastasis prediction model for prostate cancer patients was constructed by combining pathomics, radiomics, and clinical data.

Research evaluation is specifically divided into the following parts:

(1)Evaluation of pathological diagnosis accuracy: Compare the diagnostic results of prostate cancer lymph node metastasis of the artificial intelligence model with pathological examination (gold standard) to evaluate the accuracy of the model in diagnosing postoperative pathology of lymph node metastasis in patients with prostate cancer.

(2)Prediction model evaluation: Evaluate the accuracy of the artificial intelligence model in predicting lymph node metastasis, lymph node metastasis, invasiveness, grade and subtype of prostate cancer based on preoperative MRI.

(3)Personalized evaluation of treatment options: Compare differences in treatment options, treatment effects, and patient prognosis among patients with high-risk prostate cancer.

(4)Three-dimensional reconstruction accuracy evaluation: Evaluate the accuracy of the artificial intelligence model in three-dimensional reconstruction of the prostate and lesions, including the accuracy of lesion location, size and shape.

(5)Clinical utility evaluation: Evaluate the feasibility, efficiency, and cost-effectiveness of artificial intelligence models in actual clinical applications. Assess medical staff's acceptance and satisfaction with artificial intelligence models through questionnaires, interviews, etc.

3.2 Study population

This study plans to include retrospectively before June 2025 and prospectively between June 2025 and May 2026 patients with high-risk prostate cancer who underwent radical prostatectomy plus pelvic lymph node dissection. Patients with high-risk prostate cancer from the Department of Urology of our hospital and cooperative units who met the enrollment conditions and signed informed consent forms will be included. Due to the heterogeneity of prostate cancer and the robustness of the model, in order to achieve the expected test performance and statistical power, the total number of subjects planned to be included is 2,000.

3.2.1 Inclusion criteria

Inclusion criteria

- Age≥50 years old
- Patients must have histologically or cytologically confirmed prostate adenocarcinoma
- PSA≥20 of/ml or Gleason≥8
- Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0-2
- Life expectancy ≥6 months
- Normal bone marrow function: absolute neutrophil count ≥1.5×109/L; platelets ≥75×109/L; hemoglobin ≥90 g/L; white blood cell count ≥ 3.0×109/L
- Normal liver function: alanine aminotransferase (ALT) or aspartate

aminotransferase (AST) ≤ 2.5 times the upper limit of normal (ULN); for patients with liver metastases, ALT/AST can be ≤ 5 times the ULN

- Total bilirubin ≤ 1.5 times ULN or total bilirubin > 1.5 times ULN and direct bilirubin ≤ ULN;
- Normal coagulation function: INR ≤ 1.5, partial thromboplastin time (APTT) ≤ 1.5 times ULN, prothrombin time (PT) < ULN + 4 seconds
- Normal heart function: left ventricular ejection fraction (LVEF) ≥50%; QTc for men <450ms, for women <470ms, serum potassium ≥3.5mmol/L
- Normal blood pressure: systolic blood pressure <160mmHg, diastolic blood pressure <95mmHg; patients with stable blood pressure assessment after appropriate clinical treatment can be enrolled.
- Normal renal function: serum creatinine ≤1.5 times ULN, and creatinine clearance ≥50 mL/min
- Prospective subjects can understand and be willing to sign the informed consent form
- Ability to adhere to study visit schedules and other protocol requirements

3.2.2 Exclusion criteria

- There are contraindications for MRI examination, such as metal implants in the body, claustrophobia, etc.
- Patients with any missing items in baseline clinical and pathological information
- Have a clear history of neurological or mental disorders, such as dementia, epilepsy, or seizure proneness
- In the researcher's judgment, there are factors that seriously endanger the safety of the subjects, or concomitant diseases that affect the subjects' completion of this study (such as severe diabetes, thyroid disease, and mental illness, etc.), or there are factors that affect the safety of the patients or serious and/or unstable medical, mental, or other conditions (including abnormal laboratory tests) that affect the patient's ability to provide informed consent, or there are any psychological, family, sociological, or geographical conditions that affect the research plan and follow-up plan.
- The researcher believes that he is not suitable to participate in this clinical trial for any reason

Unable to provide informed consent

3.2.3 Exit criteria

- Subject withdraws informed consent and voluntarily withdraws
- Serious adverse events occur, and the researcher determines that withdrawal is to protect the subjects.
- Lack of efficacy or disease progression, and the investigator determines that withdrawal is beneficial to the subject
- If the subject is lost to follow-up, the researcher needs to record the contact process
- Subject died
- Other reasonable reasons (the occurrence of concurrent diseases or the need for treatment with banned drugs, etc.)

3.2.4 Termination/suspension criteria

- If a serious adverse event occurs, there is a risk that the subject will suffer serious harm, and the trial protocol needs to be suspended.
- Serious protocol violations occurred, affecting the safety of subjects
- Ethics committee calls for suspension
- Suspension requested by the Food and Drug Administration or national competent authority
- The sponsor terminates the trial study or other reasonable reasons

3.3 Research interventions

3.3.1 Research interventions

Intervention was carried out for patients in the experimental group, and the intervention measures were as follows:

(1) Retrospective patient analysis and training set construction:

600 patients who underwent radical prostatectomy plus pelvic lymph node dissection in the First Affiliated Hospital of Anhui Medical University were collected as a training set (360 cases) and an internal validation set (240 cases) in a ratio of 3:2. MRI images of the patients' primary lesions, pathological images of puncture tissues, and clinical data (such as age, BMI, pre-puncture PSA, and preoperative imaging magnetic resonance PI-RADS score, whether neoadjuvant treatment was performed

before surgery, postoperative Gleason score, TNM stage, whether the resection margin was positive, whether seminal vesicle invasion, whether lymph node metastasis, number of lymph node metastases (left/right), whether nerve invasion and other data, whether the patient had biochemical recurrence after surgery, survival period, etc.), combined with artificial intelligence deep learning to build a prediction model for lymph node metastasis in high-risk prostate cancer patients. A multi-omics lymph node metastasis prediction model for prostate cancer patients was constructed by combining pathomics, radiomics, and clinical data.

(2) Retrospective patient validation set verification:

The constructed multi-omics lymph node metastasis prediction model for prostate cancer patients will be externally validated in the internal validation cohort of the First Affiliated Hospital of Anhui Medical University (300 cases), the external validation cohort of Nanjing Drum Tower Hospital (400 cases), the external validation cohort of the Cancer Hospital of the Chinese Academy of Medical Sciences (400 cases), and the external validation cohort of the First Affiliated Hospital of Bengbu Medical University (300 cases).

(3) Prospective patient validation set verification:

A prospective cohort (300 cases) of high-risk prostate cancer who underwent radical surgery plus lymph node dissection in the First Affiliated Hospital of Anhui Medical University was established. The multi-omics lymph node metastasis prediction model of prostate cancer patients was used to analyze whether the patients had lymph node metastasis before surgery. Radical prostatectomy plus pelvic lymph node dissection was performed on the patients. Primary tumor specimens and Transcriptome and single-cell omics sequencing of pelvic lymph nodes were performed in some cases, combined with postoperative pathologists' reading of lymph node specimen tissue to determine whether there was lymph node metastasis, the accuracy of the artificial intelligence deep learning model in predicting lymph node metastasis was analyzed, and the effectiveness of the multi-omics artificial intelligence deep learning model combined with genomics in predicting lymph node metastasis was analyzed.

The control group will follow the routine clinical diagnosis and treatment process: radical prostatectomy + pelvic lymph node dissection based on the patient's preoperative Gleason score or PSA, and immunohistochemistry will be performed based on the postoperative pathological conditions. And continue to follow up the patient's prognosis.

3.3.2 Combined medication

In clinical trials, clarifying the drugs that can be used together and the drugs that are prohibited from being used together is crucial to ensure the safety of the study and the validity of the results. The following are concomitant medication requirements based on research on prostate cancer diagnosis and treatment:

(1)Drugs that can be used together

1 Conventional drug treatment:

- Anti-infective drugs: used to treat or prevent infections.
- Painkillers: Used to relieve mild to moderate pain.
- Antacids: Used to treat symptoms related to hyperacidity in the stomach.

2 Chronic Disease Management Medications:

- Hypertension drugs: such as ACE inhibitors, ARBs, calcium channel blockers, etc.
- Diabetes drugs: such as insulin, oral hypoglycemic drugs, etc.
- Heart disease drugs: such as beta-blockers, antiplatelet drugs, etc.

3 Supportive treatment:

- Vitamin and mineral supplements: Use only as recommended by your doctor.
- Hormone replacement therapy: For example, used to treat symptoms associated with hormone deficiency.

(2)Drugs that are not allowed to be used together

1 Medications that may affect study results:

- Other anti-tumor drugs: may affect tumor growth and assessment of treatment efficacy.
- Drugs with strong immunomodulatory effects: may affect the natural course of the disease and the effectiveness of treatment.
- Drugs that may cause serious adverse reactions:

2 Unapproved medicines:

- Any medicine not approved by the drug regulatory authority.

(3)Special instructions

Before the start of the study, all medications the subject is taking will be documented in detail and the possibility of continued use will be discussed with the study physician. Any new medications or dosage changes during the study will be reported to the study physician and their impact on the study will be assessed. Subjects should avoid self-medication during the study, and the use of all drugs should be under

the guidance of the study doctor.

3.4 Evaluation indicators/research endpoints

3.4.1 Study Endpoints/Effectiveness Evaluation—Diagnostic Accuracy:

- Model sensitivity (true positive rate): The model's ability to correctly identify lymph node metastases in patients with high-risk prostate cancer.
- Model specificity (true negative rate): The model's ability to correctly identify non-metastatic lymph nodes in patients with high-risk prostate cancer.
- Positive predictive value: The proportion of positive predictions predicted by the model that are actually positive.
- Negative predictive value: The proportion of negative predictions predicted by the model that are actually negative.
- Overall diagnostic accuracy: the ratio of the total number of cases correctly diagnosed by the model to the total number of cases.

3.4.2 Secondary research endpoints/comprehensive efficacy evaluation and safety evaluation:

1 Clinical efficacy:

- Progression-free survival (PFS) in prostate cancer patients.
- Overall survival (OS).
- Disease recurrence rate.

2 Treatment safety:

- Postoperative complication rates.
- Surgery-related serious adverse events (SAEs).

3 Patient satisfaction:

- Patient ratings of satisfaction with treatment options.

3.4.3 Follow-up time:

- Short-term observation: from subject enrollment to the initial recovery period after surgery/puncture, usually 7-30 days. Evaluate surgical recovery and record pathological results and complications.
- Mid-term observation: 1 year after surgery/puncture (1 month, 3 months, 6 months, 12 months after surgery): Regular follow-up of PSA level, imaging examination,

liver and kidney function, and blood routine to evaluate the long-term effect and safety of treatment.

- Long-term observation: From 1 year to 5 years or more, PSA level testing, imaging examinations, and liver and kidney function tests should be performed every 6 months to evaluate long-term survival and disease control.

3.4.4 Record and analyze

1 Record

- Basic patient information: including age, gender, weight, prostate-specific antigen (PSA) level, etc.
- Diagnosis and treatment data: including MRI results, artificial intelligence model diagnosis results, treatment plans, surgery or puncture details, pathology reports, etc.
- Follow-up data: recurrence status (clinical indicators, including PSA level, DRE results, imaging examinations (MRI, CT, bone scan, etc.), survival status (quality of life assessment, assessing the patient's quality of life through questionnaires or other methods), complications and adverse event records.

2 analyze

(1) Effectiveness evaluation:

- Compare the coincidence rate of artificial intelligence model diagnosis results with pathological examination.
- Analyze the consistency between the treatment plan recommended by the model and the actual treatment plan.
- Use statistical methods (such as Kaplan-Meier curves, Cox regression analysis) to evaluate clinical efficacy indicators.

(2) Safety evaluation:

- The incidence rates of postoperative complications and SAEs were counted.
- Analyze the relationship between adverse events and AI model diagnosis and treatment.

(3) Comprehensive efficacy evaluation:

- Combined with diagnostic accuracy, treatment plan compliance rate, clinical efficacy and safety data, the value of artificial intelligence models in the diagnosis and treatment of prostate cancer is comprehensively evaluated.

- Conduct a cost-benefit analysis to assess the economic impact of AI models.

3.5 Schedule of visits and data collection during the study

3.5.1 Main efficacy endpoints

1 Diagnostic accuracy (for prostate cancer diagnosis)

- Precise prediction results such as whether prostate cancer has lymph node metastasis, grade, subtype, etc.
- Detailed pathological information results after radical prostatectomy.
- The accuracy of the treatment plan recommended by the artificial intelligence model and the actual treatment plan implemented.

3.5.2 secondary efficacy endpoints

1 clinical efficacy

- Progression-free survival (PFS)
- Overall survival (OS)
- disease recurrence rate
- Postoperative review of PSA indicators
- Imaging tests (such as MRI, CT, bone scan) to evaluate tumor progression or recurrence

2 patient satisfaction

- Assessing patient satisfaction through questionnaires

3.5.3 Security evaluation indicators

1 Postoperative complications (such as urinary incontinence, erectile dysfunction, urinary tract infection, etc.) rate

2 Incidence of serious adverse events (SAEs)

3.5.4 Detection time point

1 Baseline (at enrollment):

- PSA level.

- DRE results.
- Imaging examinations (MRI, CT, etc.).
- Blood routine, liver and kidney function, electrocardiogram.

2 Before making a treatment decision:

- Check the PSA level again.
- Additional imaging studies as needed.

3 After treatment:

- One week after surgery: Evaluate surgical recovery and record pathological results and complications.
- One month after surgery: PSA level, imaging examination to evaluate treatment effect.
- 3 months, 6 months, and 12 months after surgery: Regular follow-up of PSA level, imaging examination, liver and kidney function, and blood routine to evaluate the long-term effect and safety of treatment.

4 Long-term follow-up:

- PSA level testing, imaging studies, and liver and kidney function tests are performed every 6 months for 5 years or more to assess long-term survival and disease control.

Chapter 4 Statistical processing

4.1 Statistical software:

- R: A free, open source statistical programming language and software environment for complex statistical analysis and data visualization. Versions are constantly updated, such as R 4.0.x, R 4.1.x, etc.
- STATA: A comprehensive statistical software for data analysis, data management, graphing, simulation and custom programming. Common versions include STATA 15, STATA 16, etc.

4.2 Statistical analysis method content

1 Descriptive statistical analysis:

- Measurement data: Calculate mean, standard deviation, median, minimum value, maximum value, quartile, etc.

- Counting data: Calculate frequencies, percentages, etc.

2 Hypothesis testing:

- t test: used to compare the difference in means between two groups, suitable for continuous variables.
- ANOVA (Analysis of Variance): Used to compare differences in means of two or more groups.
- Chi-square test: used to compare the association between two or more categorical variables.

3 Non-parametric test:

- It is used when the data does not meet the prerequisites of parametric tests, such as Mann-Whitney U test, Kruskal-Wallis H test, etc.

4 Multiple comparisons:

- When comparing multiple groups, use methods such as Tukey's HSD and Bonferroni correction to control the Type I error rate.

5 Regression analysis:

- Linear Regression: Used to predict continuous dependent variables.
- Logistic regression: used to predict binary dependent variables.

6 Survival analysis:

- Used to analyze data from time to event, such as Kaplan-Meier curves and Cox proportional hazards models.

7 Analysis of covariance (ANCOVA):

- Used to compare the difference in means between two or more groups while controlling for one or more covariates.

8 Missing data handling:

- Use methods such as multiple imputation and maximum likelihood estimation to handle missing data.

Chapter 5 Reporting and handling of adverse events and emergency plans

An adverse event refers to any adverse medical event that occurs to a subject during a clinical trial, regardless of whether there is a causal relationship with the trial intervention.

Regarding the diagnosis of lymph node metastasis of high-risk prostate cancer in

this study, there may be the following expected adverse events and corresponding emergency treatments:

1. Prediction errors of artificial intelligence models lead to misdiagnosis or misdiagnosis: Due to poor image quality, software errors, or operator errors in pathological slide annotation, MR, and pathological mapping, at the same time, artificial intelligence models may cause errors in the prediction of lymph node metastasis of high-risk prostate cancer due to algorithm limitations, data bias, or insufficient training, leading to harm to subjects caused by errors in the selection and implementation of treatment plans.

Emergency treatment: ① Case re-evaluation: We will quickly organize a professional medical team to re-evaluate the subject's case to ensure accurate diagnosis, and formulate or adjust treatment plans based on the latest evaluation results. ②Medical intervention: For injuries to subjects caused by misjudgment, we will take necessary medical intervention measures, including but not limited to drug treatment, surgical treatment, psychological counseling, etc., to protect the physical and mental health of the subjects. ③Compensation mechanism: On the premise of ensuring that the subjects receive proper treatment, we will activate the compensation mechanism and compensate the subjects in accordance with the law based on the actual degree of damage suffered by the subjects. The scope of compensation includes medical expenses, lost work expenses, nursing expenses, transportation expenses, accommodation expenses, mental damage solatium, etc. ④ Model adjustment and optimization: In response to this incident, we will conduct an in-depth analysis of the artificial intelligence model to find out the root cause of the prediction error and make corresponding adjustments and optimizations. At the same time, data quality management should be strengthened to ensure the accuracy and diversity of training data and improve the prediction accuracy of the model. ⑤Supervision and tracking: After the compensation matters are processed, we will also conduct long-term tracking of the subjects to ensure that their health status receives continuous attention. At the same time, supervision of artificial intelligence models will be strengthened to prevent similar incidents from happening again.

2. Information leakage or privacy invasion: In clinical trials, due to negligence in data processing or transmission, subjects' personal information, medical records or imaging data may be accessed or leaked by unauthorized third parties.

Emergency handling: Stop data transmission immediately, investigate the cause of the leak, notify affected subjects, and take legal measures.

3. Adverse reactions of patients to MR imaging: Some patients may have adverse reactions to MR imaging, such as allergic reactions or other systemic reactions.

Emergency treatment: Stop the examination immediately, take emergency medical measures, and closely monitor the subjects.

4. Pathological slide processing errors or analysis errors: Technical problems may occur during the production, staining, or analysis of pathological slides, such as incomplete sections, uneven staining, or reading errors.

Emergency treatment: Reanalyze the slices, retake samples if necessary, and notify the subject.

For adverse events that have occurred, assessment tools will be used: such as Karch Lasagna rating scale, Naranjo rating scale, and independent experts or data monitoring committees (DMC) will be invited to review adverse events:

1. Chronology: Whether the adverse event occurred after the intervention and is temporally reasonable.

2. Consistency: Whether an adverse event occurs repeatedly across multiple subjects and is related to the intervention.

3. Specificity: Whether the adverse event is consistent with known side effects of the intervention.

4. Dose-response relationship: whether the severity of an adverse event is related to the dose of the intervention.

5. Dechallenge and rechallenge: whether the adverse event disappears or diminishes after the intervention is discontinued, and whether it reappears after the intervention is reintroduced.

This study will record the occurrence time, nature, severity, duration, treatment measures and results of adverse events in detail, and save all original records related to adverse events, including medical records, laboratory reports, imaging data, etc.

When a serious adverse event occurs, this study will promptly report it through the electronic system or paper in accordance with the regulations of the regulatory agency (no more than 24 hours), and take necessary medical measures for the serious adverse event to protect the health and safety of the subjects. Adverse events will continue to

be monitored until the incident is resolved. In the follow-up, subjects will be followed up regularly to prevent recurrence or deterioration. Adverse events that occurred this year will be summarized and analyzed in the annual report.

Chapter 6 Quality control and quality assurance of research

6.1 Laboratory indicator testing:

Pathological slice quality control: Establish a standardized pathological slice preparation process to ensure that the quality of the slices meets research requirements. Pathologists undergo unified training to ensure accuracy and consistency in slide reading.

MRI image quality control: Develop a standardized process for MRI image acquisition and transmission to ensure that image quality meets the needs of artificial intelligence model training and prediction. Radiologists undergo uniform training to ensure consistency in image acquisition.

6.2 Execute relevant SOPs:

Develop and implement detailed research protocols and operating procedures (SOPs): Ensure that all research steps are carried out according to established procedures to avoid human errors.

Regularly conduct SOP training for researchers: Ensure that researchers are familiar with and master SOP, and ensure the standardization and consistency of research operations.

6.3 Researcher training:

Provide unified training to all researchers involved in the study: including the use of artificial intelligence models, data analysis methods, ethical knowledge, etc., to ensure that researchers have the skills and knowledge required to conduct research.

Regularly organize seminars and exchange meetings: Promote communication and exchanges among researchers and solve problems encountered in research in a timely manner.

6.4 Subject compliance:

Establish a complete subject follow-up mechanism: ensure that subjects participate

in follow-up visits on time and collect follow-up data in a timely manner.

Regularly communicate and educate subjects: improve subjects' awareness and understanding of the study and enhance subjects' compliance.

6.5 Research monitoring:

Establish an independent Data Monitoring Committee (DMC): Regularly monitor research data to ensure data integrity and accuracy.

Conduct regular on-site inspections: Ensure that the implementation of the research plan meets the requirements, and promptly discover and solve problems existing in the research.

6.6 Other quality control measures:

Establish a data quality control system: including data entry, data cleaning, data backup, etc. to ensure data integrity and security.

Establish an adverse event reporting and handling mechanism: timely discover and handle adverse events that occur during research to ensure the safety of subjects.

Chapter 7 Data Security Monitoring

Clinical research will develop corresponding data safety monitoring plans based on the level of risk. All adverse events need to be recorded, processed and tracked until they are properly resolved or the condition is stable. Serious adverse events and unexpected events, etc., should be reported to the ethics committee, competent authorities, sponsors and drug regulatory authorities in accordance with regulations and time; the principal investigator will regularly conduct a cumulative review of all adverse events, and convene an investigator meeting when necessary to evaluate the risks and risks of the study. Benefits; double-blind trials can be unblinded urgently when necessary to ensure the safety and rights of subjects; low-risk studies will arrange independent data monitors to monitor the research data, and high-risk studies will establish an independent data safety monitoring committee to monitor the accumulated safety data and effectiveness data to make recommendations on whether the study should continue.

Chapter 8 Reporting format of research results

Research data will be integrated through a data collection form, and an annual report for that year will be written at the end of each year (specifically including trial progress and a summary of adverse events);

Subsequent research results will be presented in the form of articles.

Chapter 9 Ethics of clinical research

Clinical research will comply with relevant regulations such as the Declaration of Helsinki of the World Medical Assembly. Before the start of the study, the clinical study will be implemented after the ethics committee approves the research protocol. Before each subject is enrolled in this study, the researcher is responsible for fully and comprehensively introducing the purpose, procedures and possible risks of this study to the subject or his/her legal guardian, and signing a written informed consent form. Subjects should be informed that they have the right to withdraw from this study at any time, and the informed consent should be retained as a clinical research document for future reference. The personal privacy and data confidentiality of the subjects will be protected during the research process.

Chapter 10 Supplementary instructions for sample collection and biobanking

10.1Sample type and processing:

This study will collect prostate magnetic resonance imaging (MRI) images and prostate tissue pathological sections from subjects. MRI images will be used to build artificial intelligence models to predict lymph node metastasis, aggressiveness, grade and subtype of prostate cancer. Pathological sections of prostate tissue will be used to map with MRI images to verify the accuracy of the artificial intelligence model and assist in clinical immunohistochemical diagnosis.

This study plans to include 2,000 subjects. Each patient will have one MR image and one pathology section of prostate tissue. It is expected to collect 2,000 MRI images and 2,000 pathology sections. Each MRI image will contain a complete image sequence, including T1-weighted, T2-weighted, DWI and ADC image sequences. Each pathological section will contain complete tissue sections and undergo HE staining and

immunohistochemical staining.

10.2Sample source and testing unit:

The samples for this study are from planned collection, which plans to collect MR images of patients after admission and pathological sections of prostate tissue after surgery. All collected samples will be processed and stored at the First Affiliated Hospital of Anhui Medical University or cooperative units, MRI images will be collected and processed in the Radiology Department or Nuclear Medicine Department, and prostate tissue pathological sections will be stained and pathologically diagnosed in the Pathology Department. The MR images and pathological slide scans will be subsequently collected and sent to the First Affiliated Hospital of Anhui Medical University, Anhui, and part of the image information will be sent to Anhui University to build or optimize artificial intelligence models.

10.3Processing and destruction of remaining samples:

After the study is completed, according to the wishes of the subjects in the signed informed consent form, some permissionImages and digital information may be reused; the restMRI images and prostate tissue pathology sections will be destroyed uniformly. The destruction method will be implemented in accordance with the relevant regulations of the cooperative unit or the First Affiliated Hospital of Anhui Medical University, and the samples will be processed safely and harmlessly.

Chapter 11 Research Progress

June 2025-December 2025: Recruitment starts, patients are enrolled and mid-term evaluation is completed

June 2025-May 2026: Complete enrollment, final follow-up and data collection

April 2026 - May 2026: Data cleaning and quality control, statistical analysis, writing relevant research papers

Chapter 12 Participants

Name	Professional title/major	Task	GCP training

			(time)
Sheng Tai	chief physician	Project design, evaluation, revision	April 1, 2021

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