Investigator-Initiated Retrospective Real-World Study

A Real-World Study on CDK4/6 Inhibitors for HR+/HER2- Advanced Breast Cancer and Subsequent Therapeutic Options After Drug Resistance

Study Protocol

Principal Investigator: Shi Yehui

Investigator Signature Page

Study Title:

A Real-World Study on CDK4/6 Inhibitors for HR+/HER2- Advanced Breast Cancer and Subsequent Therapeutic Options After Drug Resistance

I will faithfully perform the investigator's responsibilities in accordance with the provisions of the Good Clinical Practice (GCP) of China, and personally participate in or directly supervise this clinical study. We have read and confirmed this protocol, and agree with its scientificity and ethicality. We will fulfill the relevant responsibilities in accordance with the laws of China, the Declaration of Helsinki, the GCP of China and the provisions of this study protocol. Any modification to the protocol will only be made after notifying the sponsor, and can be implemented only after being approved by the Institutional Review Board (IRB), unless measures must be taken to protect the safety, rights and interests of the subjects.

We will keep this study protocol confidential.

Principal Investigator

Signature:_____ Date:_____/_____/_____

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1. Study Background

Since 2020, breast cancer has surpassed lung cancer to become the most incident malignant tumor worldwide [1]. Advanced breast cancer is almost incurable, so delaying disease progression and improving quality of life are of vital importance. Among advanced breast cancer cases, nearly 75% are hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2−) molecular subtypes. According to clinical guidelines, the standard first-line treatment regimen is cyclin-dependent kinase 4/6 inhibitors (CDK4/6 inhibitors) combined with endocrine therapy. To date, based on the results of multiple phase III clinical trials [2-5], palbociclib, abemaciclib, ribociclib and dalpiciclib have been approved by the U.S. Food and Drug Administration (FDA) and the National Medical Products Administration (NMPA) of China respectively, for the first-line treatment of HR+/HER2− advanced breast cancer patients and those with disease progression after prior endocrine therapy. However, for different HR+/HER2− advanced breast cancer patients, which CDK4/6 inhibitor is the optimal first-line treatment option? Which CDK4/6 inhibitor is more suitable for patients with progression after prior endocrine therapy? In addition, the real-world adverse reactions of different CDK4/6 inhibitors in clinical practice are all worthy of exploration as clinical issues. The number of Asian populations in most landmark clinical trials of CDK4/6 inhibitors is limited [6], and the incidence of potential grade⩾3 adverse reactions is higher in Asian populations [7], which also poses challenges for selecting the most appropriate CDK4/6 inhibitor for the Chinese population. Moreover, despite the significant clinical benefits of these drugs, patients will eventually develop drug resistance. At present, there are no high-level guideline recommendations for therapeutic options after CDK4/6 inhibitor resistance. Therefore, the optimal treatment strategy after progression on CDK4/6 inhibitors has become an urgent clinical problem to be solved.

In recent years, real-world evidence derived from the analysis of real-world data including treatment patterns and outcomes has attracted increasing attention. Real-world studies usually cover a broader and more realistic patient population, and can generate new analyses and insights after phase III studies and the approval of treatment regimens. A real-world study based on the Flatiron Health database published by Rugo et al. indicated that in a real-world setting, first-line palbociclib combined with endocrine therapy, rather than endocrine therapy alone, can significantly prolong the overall

survival of HR+/HER2− advanced breast cancer patients [8]. In addition, real-world studies can often include populations that are overlooked in phase III studies, provide important clinical evidence, and reflect treatment patterns in real-world practice. For example, some real-world studies have focused on populations that are usually underrepresented in phase III studies, such as elderly patients [9-10]. Therefore, we designed this study to evaluate the real-world data of CDK4/6 inhibitors (including but not limited to the four currently marketed drugs: palbociclib, abemaciclib, ribociclib and dalpiciclib) in HR+/HER2− advanced breast cancer patients and the real-world clinical treatment options after drug resistance, so as to provide real-world practice insights for clinical decision-making in HR+/HER2− advanced breast cancer patients.

2. Study Objectives

This study aims to collect clinical data and case materials of HR+/HER2- advanced breast cancer patients treated with CDK4/6 inhibitors combined with endocrine therapy in real-world clinical practice, evaluate the real-world data of CDK4/6 inhibitors (including but not limited to the four currently marketed drugs: palbociclib, abemaciclib, ribociclib and dalpiciclib) in HR+/HER2− advanced breast cancer patients and the real-world clinical treatment options after drug resistance, so as to provide real-world practice insights for clinical decision-making in HR+/HER2− advanced breast cancer patients.

Primary Objectives:

1. To evaluate the differences in efficacy among different CDK4/6 inhibitors combined with endocrine therapy regimens in advanced patients.
   Endpoint: Real-world progression-free survival in the treatment line with CDK4/6 inhibitors (rwPFS1)；
2. To investigate the subsequent treatment regimens and their efficacy received by patients in the real world after progression on CDK4/6 inhibitors.
   Endpoint: Real-world progression-free survival in the treatment line after CDK4/6 inhibitor resistance (rwPFS2)；

Secondary Objectives:

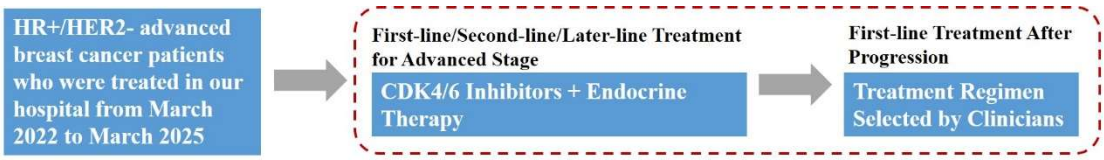
1. To truly reflect the differences in adverse reactions of different CDK4/6 inhibitors in clinical practice among the Chinese population through the collection of laboratory test results, imaging findings and other examination data；

2. To collect tumor samples or peripheral blood specimens from some patients for genomics and transcriptomics analyses, so as to lay the foundation for translational research on the resistance mechanisms of CDK4/6 inhibitors；

3. Study Design

This study is a single-center, retrospective, observational cohort study. It plans to enroll all HR+/HER2- advanced breast cancer patients who were treated in our hospital from March 2022 to March 2025 and received CDK4/6 inhibitors during the treatment of advanced stage. According to different CDK4/6 inhibitors, the patients will be divided into five cohorts: palbociclib cohort, abemaciclib cohort, ribociclib cohort, dalpiciclib cohort and other investigational CDK4/6 inhibitor cohort.

4. Technical Roadmap



Obtain patients' clinical case information, laboratory and imaging examination data, specimens and follow-up data through the hospital's electronic medical record system, combined with the imaging department, laboratory department, pathology department, follow-up center, etc.

5. Inclusion and Exclusion Criteria

Inclusion Criteria

1. Aged ⩾ 18 years old.
2. Patients diagnosed with HR+/HER2- (defined according to ASCO/CAP guidelines) advanced breast cancer who were treated in our hospital from March 2022 to March 2025.
3. Received CDK4/6 inhibitors (including but not limited to the four currently marketed drugs: palbociclib, abemaciclib, ribociclib and dalpiciclib) for at least one cycle as the treatment for advanced stage.
4. Have complete medical records, including demographic information, pathological reports, treatment records, laboratory test results, imaging examination reports, etc.

Exclusion Criteria

1. Incomplete medical records.
2. Received only CDK4/6 inhibitors monotherapy.
3. Received CDK4/6 inhibitors as neoadjuvant therapy or postoperative adjuvant consolidation therapy.

6. Data Collection and Statistical Analysis

Referring to the results of previous similar real-world studies, assuming that the median progression-free survival (PFS) of CDK4/6 inhibitor treatment is 12 months, the expected absolute error is 1.5 months, α=0.05 (two-sided), β=0.2 (statistical power 80%), the minimum sample size required is calculated to be 220 cases using the sample size estimation formula for survival analysis. Considering the loss to follow-up rate

during the follow-up period (expected 10%), the final minimum sample size is determined to be 245 cases. According to different CDK4/6 inhibitors, the study will be divided into five cohorts: palbociclib cohort, abemaciclib cohort, ribociclib cohort, dalpiciclib cohort and other investigational CDK4/6 inhibitor cohort. The clinical treatment regimens combined with CDK4/6 inhibitors and the treatment regimens after CDK4/6 inhibitor resistance in clinical practice will be recorded truthfully.。

Data Source: The hospital's Hospital Information System (HIS) electronic medical records, combined with the imaging department, laboratory department, follow-up center and other systems to obtain patients' case data.

Specimen Source: Residual blood, urine and preserved tumor tissue sections stored in our hospital will be collected from patients, and no additional biological sample collection will be performed on patients.

Data and Specimen Collection Methods: A unified data collection form will be formulated, and personnel involved in the study will be trained to ensure the accuracy and consistency of data and specimen information collection. Data will be entered into an electronic database using double data entry and cross-checking methods, and logical data verification rules will be set to detect and correct entry errors in a timely manner. A data management specialist will be appointed to be responsible for the daily management of data, quality verification and communication of doubts. All patient data will be anonymized, and access permissions will be set for the database to strictly protect patient privacy. Unauthorized disclosure is prohibited.

Statistical Analysis Methods: SPSS 26.0 and R 4.0.0 statistical software will be used for data analysis.

1. Descriptive Statistics: Measurement data conforming to normal distribution will be expressed as mean ± standard deviation (x±s), while those not conforming to normal distribution will be expressed as median (interquartile range) [M (Q1, Q3)]. Count data will be expressed as frequency (percentage) [n (%)].

2. Survival Analysis: Kaplan-Meier method will be used to draw survival curves, and the median PFS1, PFS2 and their 95% confidence intervals (CI) will be calculated. Log-rank test will be used to compare survival differences among different subgroups (such as different age groups, different comorbidities, different treatment regimens after drug resistance, etc.). Cox proportional hazards regression model will be used for multivariate analysis of independent risk factors affecting survival.

3. Correlation Analysis: Spearman or Pearson correlation analysis will be used to explore the correlation between biomarkers and efficacy as well as drug resistance.

4. Safety Analysis: The incidence of adverse reactions and their 95% CI will be calculated. Chi-square test or Fisher's exact test will be used to compare the differences in the incidence of adverse reactions among different subgroups.

5. All statistical tests are two-sided, and a P value < 0.05 will be considered statisticallysignificant.

References:

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[7] Braal CL, Jongbloed EM, Wilting SM, et al. Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: similarities and differences. Drugs 2021; 81: 317–331.

[8] Rugo HS, Brufsky A, Liu X, et al. Real-world study of overall survival with palbociclib plus aromatase inhibitor in HR+/HER2− metastatic breast cancer. NPJ Breast Cancer 2022; 8: 114.

[9] Pla H, Felip E, Obadia V, et al. Elderly patients with hormone receptor-positive HER2-negative metastatic breast cancer treated with CDK4/6 inhibitors in a multicentre cohort. Clin Transl Oncol 2024; 26: 1748–1758.

[10] Rugo HS, Liu X, Li B, et al. Real-world effectiveness of palbociclib plus aromatase inhibitors in African American patients with metastatic breast cancer. Oncologist 2023; 28: 866–87

Appendix:

Informed Consent Form

Dear Patient,

We sincerely invite you to participate in a study entitled "A Real-World Study on CDK4/6 Inhibitors for HR+/HER2- Advanced Breast Cancer and Subsequent Therapeutic Options After Drug Resistance". This study will be conducted at Tianjin Medical University Cancer Institute and Hospital, with Professor Shi Yehui as the principal investigator. After full discussion by an expert panel, the clinical trial protocol has been formulated. It has been reviewed and approved by the Institutional Review Board of Tianjin Medical University Cancer Institute and Hospital, and the clinical study is allowed to be carried out in accordance with the formulated trial protocol. This study will strictly comply with the Declaration of Helsinki and the relevant laws and regulations of China.

Before you decide whether to participate in this clinical study, please carefully read the following content, which can help you understand the purpose of this study, the procedures and duration of the study, as well as the potential benefits, risks and discomforts that may be brought to you after participating in the study. You can discuss it with your relatives and friends, or consult the doctor in charge of the study, who will explain the relevant questions to you and help you make a final decision.

1. Background and Purpose of the Study:

Since 2020, breast cancer has surpassed lung cancer to become the most incident malignant tumor worldwide. Advanced breast cancer is almost incurable, so delaying disease progression and improving quality of life are of vital importance. Among advanced breast cancer cases, nearly 75% are hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2−) molecular subtypes. According to clinical guidelines, the standard first-line treatment regimen is cyclin-dependent kinase 4/6 inhibitors (CDK4/6 inhibitors) combined with endocrine therapy.

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This study aims to collect clinical data and case materials of HR+/HER2- advanced breast cancer patients treated with CDK4/6 inhibitors combined with endocrine therapy in real-world clinical practice, evaluate the real-world data of CDK4/6 inhibitors (including but not limited to the four currently marketed drugs: palbociclib, abemaciclib, ribociclib and dalpiciclib) in HR+/HER2− advanced breast cancer patients and the real-world clinical treatment options after drug resistance, so as to provide real-world practice insights for clinical decision-making in HR+/HER2−

advanced breast cancer patients.

2. Content and Process of Participating in the Study:

If you agree to participate in this study, each subject will be assigned a unique identification number and a medical record file will be established. During the study, we need to collect the imaging data, laboratory test results, pathological specimens and other materials stored in our hospital. Your specimens will only be used for the clinical research of this project.

3. Potential Benefits of the Study

The analysis of your clinical case data and specimens will provide real-world data for Chinese HR+/HER2− advanced breast cancer patients, as well as the real-world clinical treatment options after drug resistance, so as to provide real-world practice insights for clinical decision-making in HR+/HER2− advanced breast cancer patients.

4. Risks and Compensation Measures of Participating in the Study:

For your participation in this study, we will use the residual specimens and tissues preserved in our hospital, and no additional sample collection will be performed on you. This study will not cause harm to your psychology and social relations, nor will it have a negative impact on the diagnosis and treatment of your disease. The entire study process will be supervised by the Institutional Review Board of Tianjin Medical University Cancer Institute and Hospital. If you have any questions during the study, you can consult the researchers.

5. Privacy Issues:

If you decide to participate in this study, your participation in the trial and your

personal data during the trial will be kept confidential. Your medical records will be stored in the hospital. To ensure that the study is conducted in accordance with the regulations, the competent government departments or members of the Institutional Review Board may be allowed to review your personal data at the research site in accordance with the regulations when necessary. Any public report on the results of this study will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical data to the extent permitted by law. At any time, you can request to access your personal information and modify it if necessary.

Your signature on this informed consent form indicates that you agree to your personal and medical information being used for the purposes described above. Your files will be stored in the filing cabinet of Tianjin Medical University Cancer Institute and Hospital, and will only be accessible to the researchers.

Your personal medical data will not be disclosed when the results of this study are published.

6. Your Rights:

Your participation in the trial is completely voluntary. You can withdraw from the trial at any time without giving any reason, and it will never affect your relationship with medical staff and your future diagnosis and treatment. All your personal data and observation records are confidential and will only be used for this study. During the trial, you can obtain relevant information at any time. If you have any problems or need to consult relevant questions during the trial, you can contact the attending physician.

7. Contact Information of the Institutional Review Board:

If you have any questions related to the rights and interests of the subjects, or want to

provide opinions and suggestions related to this study, you can also contact the Institutional Review Board of Tianjin Medical University Cancer Institute and Hospital. Contact telephone number: _____.

Signature Page of the Informed Consent Form for

A Real-World Study on CDK4/6 Inhibitors for HR+/HER2- Advanced Breast Cancer and Subsequent Therapeutic Options After Drug Resistance

Subject's Statement

The researchers have explained the purpose, process, potential risks and benefits of this study to me in detail. I have carefully read the informed consent form, all my questions have been answered satisfactorily, and I have fully understood all the contents.

I agree to the researchers collecting and using my medical data. I agree that Tianjin Medical University Cancer Institute and Hospital can review my medical data and research results in this study for scientific research purposes. I agree that members of the Institutional Review Board and representatives of the competent government departments can review my medical data in accordance with their respective authorities on the premise of abiding by the principle of confidentiality. I understand that the review of these records is to ensure that the data collected from this study is true, complete and reliable.

I clearly understand that my participation in this study is voluntary. I can withdraw from the study at any time and it will not affect my subsequent medical treatment or legitimate rights and interests.

I have received a signed copy of the informed consent form. By signing this consent form, I do not waive any of my legitimate rights and interests.

I agree to the collection of my imaging data, laboratory test results, pathological specimens and other materials stored in Tianjin Medical University Cancer Institute and Hospital for this study:

Yes (Please tick the box) ☐

No (Please tick the box) ☐

Subject's Signature: _____ Date: _____

Legal Representative's Signature*: _____ Date: _____Relationship with the Subject: _____

*A legal representative's signature is not required unless the subject is unable to read (e.g., illiterate or blind) or unable to sign by himself/herself for other reasons.

Investigator's Statement

I promise to strictly abide by the GCP principles, relevant regulations of the state and Tianjin Medical University Cancer Institute and Hospital in the clinical study, protect the rights and interests of the subjects, and ensure the research time and the truthfulness, completeness and reliability of the research data. I agree that Tianjin Medical University Cancer Institute and Hospital can review the medical data and research results of this study for scientific research purposes. I agree to return the remaining biological specimens of the study to Tianjin Medical University Cancer Institute and Hospital for preservation.

I have fully explained the purpose, process, potential risks and benefits of this study to the patient. The patient has obtained sufficient information to make a decision to participate in this study. I will provide the patient or his/her legal representative with a signed and dated copy of the informed consent form.

Investigator's Signature: _____ Date: _____

Investigator's Contact Information: _____