Research overview

Title: Prospective phase II clinical study of neoadjuvant chemotherapy combined with preoperative radiotherapy in locally advanced breast cancer

Applicant: Shenzhen Hospital, Cancer Hospital, Chinese Academy of Medical Sciences

Type of study: A clinical trial initiated by the investigator

Study subjects: Patients with locally advanced luminal (HER2-) and triple-negative breast cancer

Objective: To prospectively evaluate the efficacy and safety of neoadjuvant chemotherapy combined with preoperative large fraction radiotherapy followed by surgery in breast cancer

Study design: A prospective phase II clinical study

Number of patients planned to be enrolled: 50

Clinical trial unit: Shenzhen Hospital, Cancer Hospital, Chinese Academy of Medical Sciences

Radiotherapy: whole breast irradiation of the affected side ± chest wall + regional lymph node drainage area irradiation: whole breast ± chest wall + supraclavicular and internal mammary regional lymph node drainage area 40.05Gy/152.67Gy/ day per session, axillary regional lymph node drainage area 37.5Gy/15f,2.5Gy/ per session

Primary endpoint: pCR rate (pathological complete response rate)

Secondary safety indicator 1: incidence of acute adverse reactions during chemoradiotherapy (including acute skin injury, breast swelling and pain, local infection, radiation mucositis, radiation pneumonia, upper limb lymphedema rate, blood biochemical reaction and systemic reaction, etc.)

Secondary safety indicators 2: Postoperative complication incidence (delayed incision healing rate, secondary surgery rate), late adverse reaction incidence (including late skin injury, breast swelling and pain, cosmetic effect, radiation cardiomyopathy,

pulmonary fibrosis, radiation brachial plexus injury, upper limb lymphedema), quality of life (EORTC QLQ30, BR-23 questionnaire)

Secondary efficacy indicators: local recurrence rate, regional recurrence rate, pathological response rate (according to Miller-Payne classification), npCR (near npCR) rate, RCB (residual tumor burden) rate, event-free survival rate, distant metastasis rate, tumor-free survival rate, and overall survival rate

Biological research: Collect fresh tumor tissue and peripheral blood before treatment and surgery as test samples to evaluate biological markers such as tumor gene expression, circulating tumor cells, inflammatory factors, ctDNA, etc.

Imaging studies: analysis of breast ultrasound and breast MR imaging parameters before and after treatment.

Follow-up time: 5 years

Statistical methods: Based on the principle of intention processing analysis, pCR rate and incidence of adverse reactions were calculated. Kaplan-Meier was used to calculate local recurrence rate, regional recurrence rate, distant metastasis rate, tumor-free survival rate and total survival rate.

technology roadmap ：

2 cycles of neoadjuvant chemotherapy followed by reduction of primary lesion volume less than 50% + 3 weeks of neoadjuvant radiotherapy + 4 cycles of neoadjuvant chemotherapy + surgery ± adjuvant therapy

1. Research Background

Breast cancer (BC) is the leading malignancy among women globally, ranking first in cancer-related deaths and fifth in overall cancer mortality. In 2020, it surpassed lung cancer to become the world's most common malignancy. China accounts for 416,000 new cases annually, making it the primary cause of cancer-related deaths among women and representing 18.4% of the global breast

cancer burden.1 Non-metastatic locally advanced breast cancer (LABC) patients in China account for about 12-15%, significantly higher than that in the United States and Europe (about 8.5% in the United States and 4% in Europe).Due to its lower overall survival rate, higher recurrence rate, and risk of distant metastasis, Localized Axillary Breast Cancer (LABC) significantly impacts the overall efficacy and survival of breast cancer patients. The current treatment model for LABC patients remains a major clinical challenge. In clinical practice, these patients typically undergo primary systemic therapy (PST) to achieve tumor downstaging before surgery, followed by adjuvant treatments (primarily radiotherapy and medication). Radiotherapy serves as a crucial therapeutic approach to reduce local recurrence rates and improve overall survival rates.

Preoperative neoadjuvant radiotherapy (NART) is a common treatment option for many tumors (such as esophageal carcinoma, rectal carcinoma, soft tissue sarcoma, etc.). By bringing radiotherapy and systemic chemotherapy to the preoperative stage to strengthen preoperative treatment, reduce the stage and eliminate micro-metastatic lesions early to improve overall survival.3. In breast cancer patients, NART has the opportunity to further shrink the tumor, improve the probability of breast preservation, and may not need to perform axillary lymph node dissection, allowing axillary surgery downgrading.4 Many studies have found that patients with pCR after neoadjuvant therapy (NAT) generally have a better prognosis than non-pCR, and NART is also one of the treatment options to reduce the stage.But this combination has not yet been widely used in breast cancer patients.

In all breast cancers, luminal breast cancer accounts for 50-70% (LuminalA type about 30-40%, LuminalB type about 20-30%), among which HR+HER2-type breast cancer patients have poor efficacy of neoadjuvant chemotherapy, with objective response rate (ORR) about 40-60%, partial response rate (PR) about 30-50%, and complete response rate (CR) usually not more than 15%.When neoadjuvant chemotherapy shows poor efficacy, these patients typically opt for direct surgical

intervention followed by adjuvant radiotherapy and endocrine therapy. Research has demonstrated that postoperative adjuvant chemotherapy can still improve prognosis in axillary lymph node-positive LuminalA breast cancer patients. Therefore, even with suboptimal short-term response rates, neoadjuvant therapy may still offer significant benefits to the prognosis of axillary lymph node-positive Luminal breast cancer patients.In terms of drug therapy, chemotherapy remains the primary treatment approach, though options remain limited. Conventional neoadjuvant therapies such as anthracycline, taxane, and platinum-based regimens have reached their therapeutic limits, with pathological complete response (pCR) rates hovering around 35%. For triple-negative breast cancer patients, studies have shown that improved pCR rates translate to enhanced event-free survival (EFS) and overall survival (OS). Recent years have witnessed active exploration of neoadjuvant therapies targeting triple-negative breast cancer (TNBC) to achieve therapeutic breakthroughs. Current research indicates that while chemotherapy combined with immunotherapy can improve pCR rates and short-term survival advantages, the drug combination also brings significant toxic side effects including mortality, potentially preventing many patients from completing their planned treatment regimens (up to 10-20%). The long-term tolerance of certain immunotherapy-related adverse reactions still requires extended clinical follow-up, while the rising economic burden remains an unavoidable challenge.In LABC, most patients require both chemotherapy and radiotherapy, and the optimal delivery sequence is not known.In conclusion, for HR+/HER2-triple-negative LABC patients with poor response to neoadjuvant chemotherapy, radiotherapy can be considered as a precursor to improve the probability of conversion to operable state, breast preservation and pCR.[7－10]

Poleszczuk et al. analyzed breast cancer patients in the SEER database and found that preoperative radiotherapy was safe and effective, associated with increased disease-free survival (DFS). The study also revealed that preoperative radiotherapy reduced the incidence of secondary primary tumors compared to postoperative radiotherapy. The authors suggested that neoadjuvant radiotherapy (NART) could

directly target tumors for precision treatment, thereby activating specific immune responses against cancer cells. This radiation-induced immunity not only helps eliminate primary tumors but also removes microscopic lesions in both ipsilateral and contralateral breasts, while reducing the risk of distant metastasis—effects absent in postoperative radiotherapy. Additionally, neoadjuvant radiotherapy allows for preservation of primary breast tumors, making tumor localization easier and improving target delineation consistency. The preoperative APBI (Preoperative Prostate Biopsy Imaging) and postoperative APBI (Postoperative Prostate Biopsy Imaging) trials enrolled patients with similar tumor sizes, but the former demonstrated significantly smaller target volumes (average PTV: 122cm³ vs. 296 cm³). The reduced tumor target volume also minimized radiation-induced damage to normal tissues.13. In addition, the hypoxia caused by surgical injury to the blood supply of tumor tissue may increase the resistance of tumor cells to radiotherapy, resulting in weakened anti-tumor effect and reduced efficacy of postoperative radiotherapy.In addition to causing damage to BC tissues and cells, NART can also affect normal tissues exposed to radiation 14.The results showed that tumors in the irradiated area grew faster, while those in the non-irradiated area were halved in size. The authors believe that this study altered the cellular or molecular biology of normal tissues through preoperative radiotherapy, making the local environment unsuitable for cancer cell proliferation and thus reducing the likelihood of tumor recurrence and metastasis15.

Current clinical research shows insufficient exploration in the NART field. One reason may be that NART may cause excessive fibrosis in tumor tissues and blood vessels, complicating intraoperative tumor dissection. Clinicians generally believe this could lead to more surgical complications. Additionally, balancing the timing between radiotherapy and surgery poses challenges—safety must be ensured while minimizing risks of tumor regrowth and metastasis, along with sufficient time for pathological shrinkage and staging reduction. Therefore, strict control over the target area, dose, duration of NART, and intervals between radiotherapy and surgery is essential.

Studies have found that immediate surgery or intervals exceeding three months may increase wound healing issues and postoperative complications. Since potential fibrosis may complicate surgery and postoperative care, excessively extending intervals between radiotherapy and surgery could heighten metastasis risks and delay adjuvant therapy implementation, thereby reducing tumor recurrence likelihood. Most reported intervals between NART and surgery range from 6 to 12 weeks.According to the study of Chidley et al., NART can help to initially reduce tumor volume, thus improving postoperative aesthetic effect.For patients, breast reconstruction surgery can be performed at any time without causing tissue loss. From a oncology perspective, it also avoids delays in postoperative radiotherapy due to complications or prolonged recovery. Moreover, achieving overall target dose coverage becomes easier without reconstruction implants.[16]

In terms of dose fractionation, clinical studies have shown that the α/β value of breast cancer is lower than that of general tumors. Yarnold et al. calculated that the α/β value of breast cancer sensitivity to a single dose was about 4.1Gy, which was similar to 3.6Gy for advanced normal tissues.17 After 10 years of results from the START study, the meta-analysis of RMH/GOC and START A studies provided adjusted α/β values of 3.5Gy for locally recurrent breast cancer tissue and 3.1Gy for normal reactive breast tissue.

Since the 1990s, randomized clinical trials including RMH/GOC, START A, START B, Ontario Canada, DBCG HYPO, MDACC, and China Multicenter studies have demonstrated that large-field radiotherapy (LFR) following breast-conserving surgery is as effective as conventional fractionated radiotherapy without increasing adverse events. These approaches not only reduce treatment costs and medical resource consumption but also improve patients' quality of life. Notably, among 864 participants (14.7%) in the START series and 28 cases (3.8%) in the China Multicenter study receiving regional lymph node irradiation, LFR was found to cause no additional upper limb edema or shoulder joint dysfunction.Both the 2020 St Gallen

International Guidelines and the 2022 European Society of Radiation Oncology consensus recommend that regional lymph node irradiation can be performed with large fractionation.Research indicates that whole breast fractionated radiotherapy (WMFRT) delivers cosmetic outcomes comparable to conventional fractionated radiotherapy without increasing breast-related adverse reactions. Studies further demonstrate that WMFRT significantly improves the excellent-to-excellent cosmetic response rate and reduces the incidence of grade 2 or higher acute radiation dermatitis. The RMH/GOC and START studies revealed that late-stage adverse reactions in patients receiving 39-40Gy of WMFRT were lower than those in conventional fractionated radiotherapy groups, which may be attributed to the reduced equivalent dose (EQD2=46 Gy) in WMFRT regimens.Yarnold18 et al. found that compared with the dose effect curve of local control of breast cancer tumors, the dose effect curve of normal breast tissue was steeper. The EQD2 of whole breast radiotherapy decreased from 50Gy to 46Gy, and the local recurrence rate of tumor increased by ⩽1.5%, but the incidence of moderate and severe adverse reactions could be reduced by 30%.[19]

Combined regional lymph node irradiation (supraclavicular ± axillary lymph drainage area) increases the incidence of upper limb lymphedema compared to simple breast radiation therapy. In the START series study involving 864 patients receiving regional lymph node irradiation, only one case of brachial plexus injury occurred. Moreover, there was no significant difference in the incidence of upper limb lymphedema between the large-field radiotherapy group and conventional fractionation group (with similar rates at 5-year and 10-year follow-up). A Chinese study on modified radical postoperative large-field radiotherapy for stage III disease, which included chest wall and regional lymph nodes, also showed that large-field radiotherapy did not significantly increase the incidence of upper limb lymphedema compared to conventional fractionation—20.2% versus 20.5%, respectively.The Korean study reported by 23Byun et al showed that the incidence of upper limb lymphedema in the three-year period was significantly lower in the large fractionation group than in the conventional fractionation group (6.8% vs. 13.5%), which may be

related to the lower EQD2 (45-46Gy) in the large fractionation group.In 24 studies on large-field radiotherapy after breast-conserving surgery and modified radical mastectomy in China, no patients developed brachial plexus injury. Leong et al. analyzed 708 patients across two centers, revealing that medium-dose large-field radiotherapy did not increase upper limb dysfunction or brachial plexus injury compared to conventional fractionation, while showing lower incidence of shoulder joint dysfunction in the large-field group. All these studies employed medium-dose large-field radiotherapy with a single dose <3Gy.25Johansson et al. reported that the incidence of brachial plexus injury was almost zero when EQD2 was less than 57Gy, so regional lymph node prophylactic irradiation with an appropriate dose of medium large fraction theoretically would not increase brachial plexus injury.26 Studies reported that the risk of lymphedema was significantly higher with axillary lymph node dissection than with sentinel lymph node biopsy + regional lymph node irradiation (supraclavicular and axillary I-III groups).In a prospective randomized controlled phase 2 study published in JAMA by Schaverien et al., comparing preoperative large-field radiotherapy (LFR) with conventional fractionated radiotherapy, 50 breast cancer patients (25 each group) were enrolled in a 1:1 randomized trial. The treatment regimen included whole breast irradiation and regional lymph node dissection (supraclavicular, internal mammary, and axillary). Among these 45 patients, 60 underwent axillary lymph node dissection. The LFR group received 40.05Gy/15f radiation for the entire breast and 37.5Gy/15 sessions for regional lymph nodes. Notably, 6% (13%) of all patients developed clinical lymphedema 8.3 months post-radiation completion.A total of 29 patients developed Grade 1 CTCAE dermatitis, with 10 cases progressing to Grade 2 dermatitis upon completion of radiotherapy (1 case in the large-field group versus 9 in the conventional-field group; P=0.02). No patients experienced Grade 3 or higher radiation-related toxicities or required treatment discontinuation. The study indicates that the risk of lymphedema following axillary dissection after preoperative axillary lymph node drainage with large-field radiotherapy remains safe and manageable for

breast cancer patients. However, further evidence and extended follow-up periods are needed to confirm these findings.

Current research remains inconclusive regarding the indications for internal mammary radiation therapy (IMRT). Intraoperative IMR lymph node biopsy studies indicate that the positive rate of internal mammary lymph nodes varies between 10% to 40% depending on the tumor's quadrant, while the recurrence rate after systemic treatment is less than 5%. Recent prospective multicenter postoperative adjuvant radiotherapy studies have incorporated the internal mammary field into treatment targets, demonstrating local control and long-term survival benefits. Guidelines recommend IMR irradiation for patients with high preoperative imaging-predicted internal mammary lymph node metastasis risk, primary tumors in the medial quadrant or central region with axillary lymph node metastasis, or other high-risk internal mammary lymph node metastasis probabilities. Notably, for patients with tumors in the internal quadrant or central region and N2 status, the internal mammary lymph node metastasis rate can exceed 40%.This study aims to perform intraluminal radiotherapy in the inner quadrant/central region under full informed consent of patients.

Currently, there are no definitive biomarkers in clinical practice to predict whether breast cancer patients will benefit from radiotherapy. In translational research, NART can directly evaluate the effectiveness of radiotherapy for breast tumors, identify predictors and biomarkers of radiation response, advance radiobiological studies of tumors and normal tissues, and guide personalized treatment plans for breast cancer patients. A secondary objective of the PAPBI13 trial was to explore gene expression profiles that predict radio-sensitivity. Significant changes in gene expression were observed in specimens before and after radiotherapy, primarily involving p53 signaling, cell cycle regulation, DNA repair, and inflammatory responses. However, no clinically significant differences in gene expression were found between patients who received radiotherapy and those who did not.Tanic et al. identified differential expression of APOA1, MAP3K4, and MMP14 genes between tumors with and without NRT response. Mitogen-activated protein kinase kinase 4

(MAP3K4), a mitogen-activated protein kinase, was identified as a radiotherapy-specific biomarker for predicting survival in patients with adjuvant radiotherapy without distant metastasis. These findings suggest that MAP3K4 may serve as a biomarker for predicting preoperative and postoperative radiotherapy responses, as well as a potential target for combined radiotherapy-sensitizing therapy. This suggests the need for further preclinical studies and prospective clinical trials to validate these findings.The limitations of most studies lie in the selection of biomarkers not based on their radiotherapy specificity, predominantly relying on cell and animal models for foundational research, while clinical studies require large-scale long-term follow-up. This study also contributes to organoid development: Organoids are 3D cultured in vitro tissues that closely mimic cellular components, structural features, and functional properties of in vivo tissues. Widely used in both basic and translational research, they facilitate investigations into cancer onset, progression, tumor heterogeneity, gene-drug interactions, target prediction, and drug sensitivity testing. Compared with traditional 2D cell culture models, organoids exhibit complex spatial configurations simulating differentiated tissues, demonstrating interactions between cells and their surrounding matrix, as well as spatial positioning patterns. They also exhibit physiological responses similar to in vivo differentiated organs and maintain high similarity to their source tissues. These characteristics make organoids highly promising for both basic research and clinical applications. The resulting organoid disease models exhibit organ-specific structures and functions while replicating in vivo disease manifestations, effectively avoiding species-specific variations. Patient-derived tumor organoids (PDTOs) refer to models established through in vitro cultivation of patients 'tumor tissues (typically malignant specimens obtained via surgical resection or biopsy), which closely resemble the patient's tumor type. Compared with other tumor models, PDTO (Patient-Derived Tumor Oligonuclear Organoids) largely preserves the histological and genetic information of original tumor tissues, maintaining structural similarity to the primary tumor. This makes it an excellent in vitro "surrogate" for patient tumors. Additionally, tumor organoids offer advantages such as ease of operation, minimal required tissue volume,

rapid model establishment, convenient in vitro observation, and stable genome stability. These features make them highly promising for disease modeling, clinical cancer research, and drug sensitivity testing. With moderate costs, short culture timelines, high success rates, and conversion rates, they are expected to provide a new cost-effective platform for drug screening and development. Furthermore, continuous monitoring of pre-and post-radiotherapy tumor biopsy specimens from patients with known clinical responses can help evaluate radiobiological characteristics, specific biomarkers/imaging markers, and optimal radiotherapy-drug combination regimens for breast tumors/normal tissues. This approach promotes more effective treatment selection, avoids overtreatment, and reduces associated medical expenses.4,30 – 32

In imaging evaluation, conventional breast X-ray and two-dimensional grayscale ultrasound primarily assess neoadjuvant therapy efficacy based on tumor morphology and size changes. However, relying solely on morphological and dimensional measurements may fail to comprehensively evaluate neoadjuvant treatment outcomes, as some tumors may show minimal volume changes while exhibiting diminished or lost cellular activity. Compared to other breast imaging modalities, MRI demonstrates advantages in multi-parameter, multi-slice functional imaging capabilities. Its dynamic contrast enhancement, diffusion-weighted imaging (DWI), and magnetic resonance spectroscopy (MRS) allow comprehensive assessment of tumor morphology, hemodynamic changes, and functional metabolic alterations. Studies confirm that breast MRI shows high consistency with histopathological evaluation of neoadjuvant therapy efficacy in breast cancer, closely approximating the extent of pathological changes compared to histological findings. Additionally, MRI outperforms clinical palpation, X-ray, and ultrasound in distinguishing residual lesions from fibrosis and detecting microscopic residual lesions post-neoadjuvant therapy. As a standard imaging method, MRI has become the primary assessment tool for evaluating breast cancer's response to neoadjuvant therapy. Conventional breast MRI evaluation metrics include tumor morphology and size, time-signal intensity curve patterns after dynamic contrast enhancement, MRI semi-quantitative/quantitative parameters, ADC values from DWI, and cholinergic

peak variations from MRS. While non-invasive tumor prognosis assessment and predictive biomarkers hold significant advantages, their clinical validation and application progress remain slow. Preoperative treatment studies demonstrate advantages in validating imaging biomarkers, enabling correlation analysis between preoperative/interoperative imaging features and pathological/molecular endpoint indicators. Advances in imaging-based tumor biology capabilities could, in turn, facilitate research into adaptive radiotherapy using strategies such as dose-fine distribution, individualized dose and fractionation plans, and radiotherapy combined with drugs.4,33,34

The evolving multidisciplinary management paradigm for breast cancer has driven our efforts to develop personalized treatment strategies and integrate various approaches. As a non-mainstream treatment modality, preoperative high-dose neoadjuvant radiotherapy (NART) has emerged as a novel therapeutic option for locally advanced luminal (HER2-)-and triple-negative breast cancer patients who have demonstrated suboptimal response to neoadjuvant chemotherapy. This study aims to investigate the efficacy and safety of combining neoadjuvant chemotherapy with preoperative high-dose radiotherapy in locally advanced luminal (HER2-)-and triple-negative breast cancer patients through a prospective Phase II single-arm trial.

2. Objective of the study

2.1 Research task: Prospective enrollment of eligible patients with locally advanced breast cancer, using intensity-modulated technology to implement preoperative large fraction of whole breast ± chest wall + supraclavicular lymph node drainage area + axillary lymph node drainage area ± internal mammary lymph node drainage area. Follow-up observation of radiation toxicity and efficacy.

2.2 Research objectives: To clarify the safety and feasibility of preoperative large fraction radiotherapy for patients with locally advanced breast cancer, and to provide a basis for future phase III clinical studies.

2.1 Objectives: Eligible patients with locally advanced breast cancer were prospectively enrolled. Imrt was used to perform preoperative hypofractionated whole breast irradiation ± chest wall + supraclavicular lymph node irradiation+axillary lymph node irradiation ± internal mammary lymph node irradiation. The toxicity and efficacy of radiotherapy were followed up.

3. Study endpoints

3.1 Primary study endpoint: pCR rate (pathological complete response rate)

3.2 Secondary study endpoints:

3.2.1 Early adverse reactions: acute skin injury, breast swelling and pain, local infection, radiation mucositis, radiation pneumonia, upper limb lymphedema rate, blood biochemical reaction and systemic reaction

3.2.2 Other adverse events:

Postoperative complication rate (delayed incision healing rate, secondary surgery rate), late adverse reaction rate (including late skin injury, breast swelling and pain, cosmetic effect, radiation cardiomyopathy, pulmonary fibrosis, radiation brachial plexus injury, upper limb lymphedema), quality of life (EORTC QLQ30, BR-23 questionnaire)

3.2.3 Efficacy indicators: local recurrence rate, regional recurrence rate, pathological response rate (according to Miller-Payne classification), npCR (near npCR) rate, RCB (residual tumor burden) rate, event-free survival, distant metastasis rate, tumor-free

survival rate, and overall survival rate

3.2.4 Transformation research indicators:

Tissue samples: including pre-adjuvant therapy and surgical specimens. Some are used for organoid construction; others are used for single-cell sequencing; paraffin tissue samples: some tissue blocks are fixed in centrifuge tubes containing formalin, which can be used for spatial transcriptome sequencing.

Peripheral blood samples: The plasma part can detect immune factors and ctDNA, and PBMC (peripheral blood mononuclear cells) can be enriched by flow or mass spectrometry, as well as T and NK. CTDNA is planned to collect 5-10 patients.

（1）Fecal Sample Collection Protocol: Collect stool samples at the following intervals: 1 week prior to radiotherapy, within 3 days after radiotherapy completion, 1 week before initial chemotherapy, 1 week before the 3rd and 5th chemotherapy cycles, 1 week preoperatively, and 1 week postoperatively. (Pre-collection instructions: Record the patient's oral antibiotic or probiotic intake history from 2 weeks prior to the designated collection time. Instruct patients to collect stool in a clean container (e.g., disposable toilet bowl), avoiding contamination from urine or toilet surfaces. Collect fresh mid-to-late portion stool using a sterile spoon inside the collection tube (1-3 scoops, 1g). Store samples in-80℃ ° C freezer within 30 minutes after collection. Sample labeling: Tumor type code + medical record number + collection date. If possible, collect family members' samples as paired control specimens.)

（2）Saliva: Samples are collected one week before radiotherapy, within three days after radiotherapy completion, one week before the first chemotherapy session, one week before the 3rd and 5th chemotherapy cycles, and one week before surgery. (Pre-collection instructions: Record the patient's history of oral antibiotics, probiotics, and dental procedures (like fillings or extractions) two weeks prior to the designated time point. Ensure the patient rinses their mouth with 30ml of purified water for 30 seconds on the collection day, then empties it into the saliva sampling cup. Store samples in an-80℃ freezer within 30 minutes after collection. Document detailed sampling times (Sample code: Tumor type code + Medical record number + Collection time). If possible, collect family members as paired control samples.)

（3）Imaging: Breast MR was performed 2 weeks before radiotherapy, 1 week before the first chemotherapy, 1 week before the 3rd and 5th cycle of chemotherapy, and 1 week before surgery

（4）Imaging: Breast MR was performed 2 weeks before radiotherapy, 1 week before the first chemotherapy, 1 week before the 3rd and 5th cycle of chemotherapy, and 1 week before surgery

（5）Imaging part: MRI of breast was performed 2 weeks before radiotherapy, 1 week before the first chemotherapy, 1 week before the third and fifth cycles of chemotherapy, and 1 week before surgery

4. Enrollment screening criteria

Entry criteria

Women aged 18 to 70

1) The pathological diagnosis was invasive breast cancer
2) cT3-4, cN1-2, M0 (AJCC Cancer Staging Manual, 8th edition), molecular subtype was luminal (HER2-) and triple-negative, and the primary lesion volume was reduced by less than 50% after 2 cycles of neoadjuvant chemotherapy
3) Generally in good condition (KPS>70 points), organ function is tolerable, and no severe complications of poor control
4) Generally in good condition (KPS>70 points), organ function is tolerable, and no severe complications of poor control
5) MRI scans are acceptable
6) Be eligible for follow-up
7) Sign the informed consent form

Exclusion criteria

1) gestation period
2) Simultaneous bilateral breast cancer
3) History of chest radiotherapy
4) There is active connective tissue disease
5) Inflammatory breast cancer

6) Breast size is large (>600ml) and BMI>30
7) diabetes mellitus
8) The upper and lower regions of the clavicle and the internal mammary region showed positive lymph nodes
9) Use of immunotherapy drugs such as PD-1/PD-L1
10) History of other malignancies, excluding but not limited to curable early papillary thyroid carcinoma, non-malignant melanoma skin cancer, cervical carcinoma in situ, contralateral breast carcinoma in situ, early lung cancer, etc

Standards for shedding

1) Significant tumor progression during radiotherapy or systemic therapy
2) Serious non-oncological disease that makes continued treatment or routine follow-up impossible
3) The patient volunteered to withdraw

Baseline check, Table 1

Pre-entry screening (mandatory)		testing time
History and physical examination	General condition score, height, weight	Within 1 week prior to treatment
Pathological review	Postoperative pathological sections from the external hospital (lower level hospital) need to be reviewed again by the research unit, including relevant immunohistochemistry	Pre-treatment preparations
routine blood test	Absolute white blood cell, lymphocyte and neutrophil count, hemoglobin, platelets	Within 1 week prior to treatment
Liver and kidney function	AST, ALT, total bilirubin, creatinine	
blood fat	Triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C)	
myocardial enzyme	CK-MB,TNI, NT-proBNP	

iconography	Breast X-ray/ breast B-ultrasound + breast MRI; Chest enhanced CT (preferably thin layer) in our hospital; B-ultrasound of bilateral axilla, neck, supraclavicular and inner breast; Liver ultrasound/or enhanced CT/or MRI; brain MR; Whole-body bone scan Whole-body PETCT (optional)	Within 2 weeks (maximum 4 weeks) prior to whole body treatment
Toxicological evaluation	Baseline toxicity assessment, photos (beauty evaluation), and written records	1 week before radiotherapy
Table of breast beauty effect and quality of life	BREAST-Q questionnaire	
Thyroid examination	T3, T4, FT3, FT4, TSH; thyroid ultrasound	Within 1 month before treatment, after every 2 cycles of chemotherapy, preoperatively, and 3 months, 6 months, and 1 year after surgery
cardio-pulmonary function	Twelve lead electrocardiogram	
	echocardiogram	
	lung function	Two weeks before surgery (maximum 1 month)
Organizational specimens, blood specimens, urine and stool specimens	Fine needle aspiration tissue and surgical specimens before radiotherapy	
	Blood specimens and urine specimens were collected within 2 weeks before radiotherapy, on the day of radiotherapy end, before the first, third and fifth cycles of chemotherapy, preoperatively, postoperatively, 3 months after surgery and 1 year after surgery	

5. Experimental design and statistical processing

5.1. Sample size

This exploratory study investigates the design and optimization of neoadjuvant chemotherapy combined with preoperative medium-dose fractionated radiotherapy (MFRT) in breast cancer. The prospective single-arm Phase II clinical trial aims to compare pathological complete response rates (PBR) and acute-phase adverse event (AEE) rates between this regimen and standard treatment protocols. Specifically, it evaluates the efficacy of preoperative MFRT in locally advanced luminal HER2-negative triple-negative breast cancer patients. Given the exploratory nature of the study and limited prior research on preoperative MFRT, a total sample size of 50 cases was determined to ensure robust analysis.

5.2 Statistical analysis

1) Kaplan-Meier statistical analysis was used for survival analysis, log-rank test was used to compare survival curves, and Pearson's chi-square test was used to compare categorical variables. When the p value was less than 0.05, the difference was considered statistically significant.

6. Radiation therapy

Prior to initiating treatment, the primary tumor site should be marked with photographic documentation for subsequent resection. The primary lesion may be identified using tattoos, superficial projections, or metallic markers. These metallic markers can be placed at the tumor center and/or margins. When no intramammary markings are available, external identifiers such as superficial tattoos, mass projections, or diagrams may be employed. Clinical examinations combined with ultrasound imaging should thoroughly evaluate axillary lymph nodes. If ultrasound suggests suspicious axillary lymph nodes during baseline assessment, a guided ultrasound-assisted biopsy should be performed to determine the status of these lymph nodes.

Photography (front, left oblique, right oblique): 1 week before radiotherapy, the day

of radiotherapy end, 1 week before surgery, 1 month after surgery

Radiotherapy: CT positioning is used to delineate the target area and normal tissue for dose assessment.

6.1 Simulation positioning

CT positioning: The patient is placed in a supine position with the affected upper limb abducted to grasp the rod. Positioning fixation is performed using a breast support frame combined with a headplate or a cervical-thoracic integrated frame with cervical-thoracic membrane (TOMO treatment requires prolonged duration and necessitates the use of a cervical-thoracic integrated frame with membrane fixation). A plain CT scan is performed, with the scanning range extending from below the jawline at the upper boundary to encompass the entire liver at the lower boundary, using 5mm layer thickness and lead wire markers for the entire breast area

6.2 Exposure range

Target delineation:

CTV: The whole breast on the affected side, up to 5mm subcutaneously

PTV: CTV extends 5mm outward and retracts to 5mm subcutaneously

CTVsc: Suprascapular lymph node drainage area

CTVax: Axillary lymph node drainage area

CTVim: Internal mammary lymph node drainage area

PTVsc/ax/im: CTVsc/ax/im were expanded 0.5cm outward in three dimensions and retracted 0.5cm subcutaneously

Usage of bolus: Patients with skin invasion used 0.5cm thick bolus throughout the whole course to improve the dose on the skin surface, and the size was 2cm wider than the affected skin.

Table 2. Reference anatomical boundaries for CTV delineation of the whole breast, chest wall, above and below clavicle, axilla, and inner breast

	upper	the world of	mental	the external	prezong	The backside

	bound	mortals	state	world		
mammary gland	Clinical border / level of the second anterior rib	Clinical border/CT seen disappearance of breast layer	The junction of the ribs and sternum	Breast folds/adenoids seen on CT	Under 5mm of skin	Excluding the pectoral muscles, chest wall muscles and ribs
walls of the chest	Lock the lower edge of the bone horizontally	Breast underscoring level	Within 1cm of the midline	midaxillary line	The posterior boundary of the breast	Posterior border of pectoralis minor
Above the collarbone	Lower edge of the annular cartilage	0.5cm below the lower margin of the subclavian vein	The inner edge of the internal carotid artery or vein	Medial border of trapezius muscle, medial border of pectoralis minor	Superficial to the sternocleidomastoid muscle and deep to the platysma	The ventral surface of the scalene muscle
Axillary Group III (subclavicular area)	Start level of pectoralis minor	0.5cm below the axillary vein in the medial border of the pectoralis minor muscle	The junction of the subclavian vein and the jugular vein	Medial border of pectoralis minor	Posterior border of pectoralis major	Superior 5mm of the subclavian vein or muscle at the rib/intercostal space
Axillary I group	The axillary vein runs along the	The pectoralis major fused with the ribs	Medial border of pectoralis minor	The medial border of the latissimus dorsi	The anterior border of the pectoralis major or latissimus dorsi muscle	The anterior border of the subscapular muscle

	lateral border of the pectoralis minor muscle					
Axillary II group	The axillary vein runs along the medial border of the pectoralis minor muscle	The axillary vein runs along the lateral border of the pectoralis minor muscle	Medial border of pectoralis minor	Medial border of pectoralis minor	The anterior border of the pectoralis minor	The ribs or intercostal muscles
endosperm	0.5cm below the lower margin of the subclavian vein	The upper edge of the fourth anterior rib cartilage	Intrauterine blood vessels are 0.5cm	0.5cm inside the breast and outside the blood vessel	0.5cm anterior to the internal mammary vein (resection at the chest wall)	0.5cm posterior to the internal mammary vein (resection at the lung tissue site)

Normal: Threatened organs: contralateral breast, ipsilateral and contralateral lungs, heart, coronary arteries (left anterior descending artery LAD, right coronary artery RA), affected shoulder joint, esophagus, thyroid gland, brachial plexus, liver, stomach, spinal cord (spinal cord PRV)

Normal tissue delineation:

Heart (heart): Upper boundary: the lower edge of the right pulmonary artery trunk, lower boundary to the heart tip, corresponding level including the pericardium.

Left anterior descending artery (LAD): The LAD originates from the aortic root and extends as the left main trunk of the coronary artery, approximately 2cm long. It then branches into the LAD, which runs along the interventricular groove. The LAD does not include the left main trunk and descends to outline the entire length up to the cardiac apex. Considering the PRV caused by cardiac pulsation, the LAD is outlined with a uniform diameter of 1cm.

Right main coronary artery (RA): Originates from the aortic origin, runs along the right atrioventricular septum, and delineates the full length. Considering the PRV and LAD of the heart beat, delineate a uniform diameter of 1cm.

Left and right lungs (lung L, lung R): The lung window is drawn separately, and the left and right lungs are drawn separately. It includes the small blood vessels extending into the lung tissue from the hilum, but does not include the mediastinum structures such as the hilum, trachea and bronchus.

Spinal cord (cord): delineates the boundary of the spinal cord. 5mm is expanded in front, back, left and right to form the spinal cord PRV (cord PRV). The upper boundary is to the base of the skull and the lower boundary is to the lower edge of the second lumbar vertebra.

The affected shoulder joint (L/R): outlined on the bone window, including the entire humeral head and the joint cavity and articular surface

Affected brachial plexus (L/R): According to the RTOG delineation guidelines, the brachial plexus is delineated from C4-5 to 1cm below the olecranon bone, and the brachial plexus is delineated from the intervertebral foramen to the anterior and middle scalene muscle space, with a uniform diameter of 0.6cm.

Thyroid (thyroid): outline the left and right lobes and isthmus of the thyroid gland.

Esophagus (esophagus): from the entrance of the esophagus to the lower border of the aortic arch.

Liver: The whole liver is required.

The stomach (stomach) is required to be delineated at 2cm below the PTV.

6.3 Radiation therapy plan requirements

Table 3

<table>
<tr><td colspan="2">target section</td><td>Limitations</td><td colspan="2">Lymph node drainage area of whole breast/thoracic wall + supraclavicular and internal breast area: 40.05Gy/15 times, 2.67Gy/time/day; lymph node drainage area of axillary area: 37.5Gy/15f,2.5Gy/ times/day; both are 5 times per week and completed in 3 weeks;</td></tr>
<tr><td colspan="2"></td><td></td><td>Dosage indicators</td><td>Limitations</td></tr>
<tr><td rowspan="6">ensemble PTV（PTVsc/PTVax/PTVim/PTV）</td><td rowspan="2">Prescription dosage</td><td>target</td><td>D95%</td><td>40.05Gy</td></tr>
<tr><td>Offset is acceptable</td><td>V40.05</td><td>90%</td></tr>
<tr><td rowspan="2">Hotspot doses</td><td>objective</td><td>Dmax</td><td><48Gy</td></tr>
<tr><td>Offset is acceptable</td><td>V48</td><td><1ml</td></tr>
<tr><td rowspan="2">Hotspot doses</td><td>target</td><td>V44</td><td><5%</td></tr>
<tr><td>Offset is acceptable</td><td>V48</td><td><1%</td></tr>
<tr><td>Whole body</td><td>Hotspot</td><td>target</td><td>Dmax</td><td><48Gy</td></tr>
</table>

organization (Including organized and unorganized sketches)	doses	Offset is acceptable	V48	<1ml

Normal tissue limit:

normal structure	Limitations	Lymph node drainage area of whole breast/corctaneous wall + supraclavicular and internal mammary region: 40.05Gy/15 times, 2.67Gy/time/day; lymph node drainage area of axillary region: 37.5Gy/15f,2.5Gy/ times/day; both are 5 times per week and completed in 3 weeks;	
		Dosage indicators	Limitations
Heart (left breast cancer)	target	mean dose	<8Gy
	Offset is acceptable		<10Gy
	objective	V5	<45%
	Offset is acceptable		<50%

Heart (right breast cancer)	objective	mean dose	<5Gy
	Offset is acceptable		<6Gy
	target	V5	<30%
	Offset is acceptable		<35%
LAD	objective	V40	<20%
	Offset is acceptable		<25%
RA	objective	V40	<20%
	Offset is acceptable		<25%
Lung on the affected side	objective	mean dose	<15Gy
	Offset is acceptable		<16Gy
	objective	V20	<30%
	Offset is acceptable		<32%
	target	V5	<55%
	Offset is acceptable		<60%
Healthy side of lung	target	V5	<20%
	Offset is acceptable		<25%

Healthy breast	objective	mean dose	<5Gy
	Offset is acceptable		<8Gy
medulla spinalis PRV	target	maximum dose	<30Gy
	Offset is acceptable		<32Gy
Inferior brachial plexus injury	objective	maximum dose	<48Gy
oesophagus	objective	V30	<48Gy
	Offset is acceptable		<50Gy
The shoulder joint on the affected side	target	V30	<30%
	Offset is acceptable		<35%
thyroid gland	objective	mean dose	<28Gy
	Offset is acceptable		<32Gy
Liver (right breast cancer)	objective	V5	<25%
	Offset is acceptable		<30%
Stomach (left breast cancer)	target	V5	<25%
	Offset is acceptable		<30%
Liver (left breast	target	V5	<10%

cancer)	Offset is acceptable		<15%
Stomach (right breast cancer)	objective	V5	<10%
	Offset is acceptable		<15%

6.4 Irradiation dose

1) Whole breast ± Thoracic wall + supraclavicular and internal mammary area lymph node drainage area: 40.05Gy/15 times, 2.67Gy/time/day; axillary area lymph node drainage area: 37.5Gy/15f,2.5Gy/ times/day;

2) If the primary lesion after surgery does not reach pCR, the tumor bed is increased to 13.35Gy/2.67Gy/5F (the total EQD2 of tumor bed = 60Gy);

3) If the axilla is non-R0 resection, postoperative residual lesions should be considered for additional treatment. Residual lymph nodes should be routinely divided with 26Gy/13F after surgery, and the total radiation dose before and after surgery should be 66Gy (EQD2)

6.5 Radiotherapy techniques

1) All of them adopt whole intensity modulation technology, which can be IMRT or VMAT

2) It is recommended that all left breast cancer patients choose respiratory control technology, and right breast cancer patients may consider using respiratory control technology.

3) All plans must be physically QA validated before treatment can begin

4) Daily CBCT location verification is required during treatment

7. Systemic treatment

The chemotherapy regimen, endocrine therapy, and other pharmacological treatments are standardized according to the latest domestic and international guidelines, along

with recommendations from oncology specialists. The treatment course consists of six cycles: two before radiotherapy and four after radiotherapy. If tumor shrinkage remains below 50% after four cycles of chemotherapy, surgical intervention may be considered. Endocrine therapy and other medications are initiated post-surgery.

8. Surgery

1) Breast-conserving surgery is preferred whenever possible; autologous flap transplantation may be considered if the patient strongly requests and accepts the associated risks. The specific procedure is determined by the surgeon
2) For patients with trans-quadrant multifocal tumors at diagnosis, radical mastectomy is recommended regardless of the response after neoadjuvant therapy; if it is multifocal in the same quadrant, surgical mode can be selected according to the surgeon's advice;
3) If no positive lymph nodes are detected during preoperative axillary assessment, sentinel axillary lymph node biopsy (SLND) may be performed first. For patients with cN1 stage before neoadjuvant therapy, if clinical evaluation after neoadjuvant therapy shows negative axillary lymph nodes and the biopsy retrieves $\geqslant 3$ sentinel lymph nodes with confirmed pathological negativity, axillary lymph node dissection (ALND) may be considered as an option. If metastasis is observed in any sentinel lymph node, ALND should be performed, with specific procedures guided by surgical consultation.

9. Surgical pathology

9.1 The size of the primary lesion must be measured

9.2 Primary tumors must be graded according to the Nottingham revision of the Bloom & Richardson grading system

9.3 The surgical margins must be assessed and reported

9.4 Routine immunohistochemical detection of ER, PR, Her2 and Ki67

9.5 Pathological response rate (must be classified according to Miller-Payne classification), pCR was defined as: ypt0/Tis ypN0

10. Onboarding process

10.1 The eligible patient shall sign the informed consent form

10.2 Complete radiotherapy as required

10.3 Fill in the CRF form

Any subsequent study-specific procedures or assessments may be performed after the subject signs the informed consent form (ICF)

Screening process schedule:

Research phase	**Screening period**
flow path	Day 14 to Day 1 (D-14 to D-1)
Sign the informed consent form/assign subject number	X
Demographic data and medical history, smoking history, alcohol consumption history	X
History of previous tumors and previous treatment	X
Inclusion/exclusion criteria	X
Clinical examination and evaluation	
Physical examination, height, weight, blood pressure	X
vital sign	X
Chest, abdomen and pelvis CT (PETCT can be omitted if complete)	X
Breast MRI, brain MRI	X
Whole body bone scan /PETCT	X
Cervical, thyroid and breast ultrasound	selectable
12 lead electrocardiogram	X
echocardiogram	X
ECOG physical score	X
Concomitant medication/treatment	X
Laboratory tests/assessments	Day 14 to Day 1 (D-14 to D-1)
Biopsy of breast mass, axillary lymph node biopsy	X
Pathological results	X

Cancer markers CEA, CA15-3, CA-125	X
routine blood test	X
Blood biochemistry	X
Urinalysis, stool analysis	X
Cardiac markers	X
Thyroid function tests (TSH, free T3, free T4)	X
Coagulation function tests	X
Pregnancy test, serum β hCG	X
HBV, HCV, HIV virus screening	X
Tumor evaluation	
Imaging examination (breast enhanced MRI + whole body enhanced CT is preferred)	X
Tumor lesions were evaluated based on RECIST 1.1	X
Quality of life assessment	X

11. Efficacy evaluation

11.1 Long-term follow-up after surgery (see the follow-up table in Appendix for details).

11.2 The follow-up form should be completed for each follow-up visit, and any tumor recurrence, metastasis and death should be recorded on the follow-up form

11.3 Physical examination, breast and axillary, neck and supraclavicular ultrasound, neck, chest and abdominal CT every 3-6 months for the first two years, bone scan, cranial MR: routine once a year, timely review when relevant symptoms occur.

cCR criteria: Complete absence of enhancement in the primary tumor bed and primary metastatic lymph nodes on MRI after NAC is defined as complete imaging remission. In principle, surgical resection is still recommended for patients who achieve cCR. If resection is not willing for any reason, MDT should be discussed to make a decision.

12. Assessment of acute and late radiotherapy side effects

12.1 Postoperative follow-up for 5 years

12.2 The follow-up form should be completed for each follow-up visit, and all adverse reactions should be recorded on the follow-up form

12.3 The main adverse reactions include: swelling and pain of the breast on the affected side, acute radiation dermatitis, radiation pneumonia, breast beauty effect, radiation heart injury, radiation pulmonary fibrosis, lymphatic edema, brachial plexus neuralgia

12.4 Adverse reactions were graded using the CTCAE 3.0 standard, and lymphedema was assessed using the arm, shoulder and hand disability questionnaire (QuickDASH-9, clinical lymphedema was defined as at least one reported relative percentage difference of 10% or more compared to the baseline, healthy upper limb) and the arm symptom subscale of cancer treatment function assessment-breast +4 (FACT-B+4)

12.5 Physical examination

12.6 Breast beauty evaluation (including physical examination and breast photos)

13. Quality of life assessment

13.1 Chinese version of EORTC QLQ-C30 scale and QLQ-BR23 scale

13.2 Assessments are performed before, after and at 1, 3, 6, 12,18 and 24 months post-radiation therapy

Basic Requirements ：

1) The CRF should be completed for each follow-up visit, and all adverse reactions should be recorded on the follow-up form.

2) The evaluation criteria for acute and late radiotherapy adverse reactions were based on CTCAE 3.0.

3) Upper limb edema was compared with the baseline and the measurement of the circumference of the healthy upper arm. If the increase was more than 10%, edema was considered to exist.

4) For the evaluation of skin injury and upper limb dysfunction, photos and videos are preferred.

5) Adverse reactions occurring 3 months after the start of radiotherapy are considered as late adverse reactions.

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