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Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

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ABSTRACT

Introduction

Despite achieving axillary pathological complete response (apCR) after neoadjuvant systematic therapy (NST), a majority of breast cancer patients still undergo axillary lymph node dissection (ALND). Given that ALND may not contribute to local control or survival and could lead to increased arm morbidity, several studies have suggested less invasive axillary surgery procedures as an alternative. However, the application of these axillary de-escalation procedures is limited due to the absence of substantial evidence of long-term outcomes.

Methods and analysis

This prospective, single-arm, open-label, non-inferiority, monocentric phase II trial is designed for breast cancer patients who were initially diagnosed with axillary metastasis and achieved apCR after NST. The apCR is verified by a novel de-escalation procedure, Stained Region Lymph Node Biopsy (SrLNB), which was developed and tested in our preliminary study. We anticipate enrolling a total of 92 patients who will be exempted from ALND after SrLNB, followed by complete regional node irradiation (RNI) including the axilla. The primary endpoint is the 3-year invasive disease-free survival (iDFS). Secondary endpoints include local-regional recurrence (LRR), incidence of breast cancer-related lymphedema (BCRL), and patient-reported outcomes (PRO). The trial was initiated on September 11, 2023, with the first patient enrolled on September 25, 2023, and is scheduled to end in 2026.

Ethics and dissemination The trial protocol received approval from the Human Research Ethics Committee of The First Affiliated Hospital with Nanjing Medical University in May 2023 (Reference 2023-SR-169). All participants will provide informed consent.The study results will be disseminated through international peer-reviewed scientific journals, presentations at international scientific conferences, and public lectures.

Trial registration NCT05939830

Key words: Breast cancer, Neoadjuvant Systemic Therapy, Axillary Surgery , Axillary lymph node dissection, Disease-free survival, Breast Cancer Related Lymphedema, Quality of life

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The SrLNB procedure, which combines the advantages of both localization clips and carbon tattooing, is more feasible, and can be considered a further de-escalation of the standardized SLNB procedure.
- Our previous research (NCT04482803) on the SrLNB procedure confirmed a high identification rate (IR) of 100% and a low FNR rate of 5.3%.
- The SrLNB study places greater emphasis on arm morbidity and quality of life (QoL), with multiple objective and subjective measurements.
- This is a single-center study with a limited sample size. We hope to expand the experimental center and increase the number of enrollments after achieving preliminary results.

INTRODUCTION

Axillary lymph node status is pivotal for locoregional and systemic treatment decisions and serves as a prognostic factor for long-term survival. Due to significant morbidity, ALND has been replaced by de-escalation axillary surgeries like Sentinel Lymph Node Biopsy (SLNB) in recent years. These procedures spare patients from surgical morbidity (i.e., lymphedema, chronic pain, mobility restrictions) and improve their quality of life (QoL). Complete ALND has been the gold standard surgical management of the axilla in patients with node-positive breast cancer (cN+) at the time of diagnosis, while for patients without metastatic lymph nodes (cN0), ALND has been replaced by SLNB.

With the widespread use of Neoadjuvant Systemic Therapy (NST) and advances in chemotherapy and targeted agents, axillary pathologic complete response (apCR) has been observed in approximately 40%[1] of patients with proven lymph node metastases at the time of diagnosis. This means their axillary lymph nodes will downstage to negative (ypN0) after NST. The need for conventional ALND for these responders to NST has been questioned due to its unproven survival benefit and high risk of arm morbidity, which is associated with reduced QoL. Therefore, alternative, less invasive surgical techniques to exempt patients from ALND have been considered to reduce complications such as lymphedema and upper limb dysfunction associated with ALND.

To be exempt from ALND, one of the key points lies in the accuracy of de-escalation axillary procedures, which means identifying patients with ypN0 from those who

remain positive after NST (ypN+). When SLNB is administered in a neoadjuvant context, studies show that there will be a high risk of leaving the tumor behind, with a false negative rate (FNR) of 13%[2]. ACOSOG Z1071[1], SN FNAC[3], GANEA[4], and SENTINA[5] studies modified SLNB by the use of dual tracing agents and removal of ≥3 sentinel nodes, and reported an improvement in diagnostic performance with an FNR below the threshold target of 10%. Targeted Axillary Dissection (TAD) has also been proposed by the University of Texas M.D. Anderson Cancer Center[6], where a suspicious lymph node is marked by various methods to be a targeted lymph node (TLN) before NST and will be biospied (TLNB) along with SLNB, which can reduce the FNR to 2%.

Considering that both TAD and standardized SLNB with dual tracers and removal of ≥3 lymph nodes extend the scope of surgery, they are essentially upgrades of SLNB. However, a study by Donker[7] proposed a novel procedure, marking the axillary lymph node with radioactive iodine (^{125}I) seeds (MARI), where TLN were dissected without SLNB, yielding an acceptable FNR of 7%. A meta-analysis[8] demonstrated that the FNR of the TAD procedure was 6.28%, whereas that of targeted lymph node biopsy (TLNB, excision of marked lymph nodes only, without SLNB) was 5.18%. Their FNR did not differ statistically significant (p = 0.484), with an absolute difference of 1.1%. In this regard, TLNB can be considered as an option for de-escalation axillary surgery following NST. To date, various methods of marking and localizing metastatic lymph nodes have been developed, such as iodine-125 seed[9], carbon tattooing[10 11], and Magseed[12] for marking positive lymph nodes, and gamma probes[9],

guide wires, and intraoperative ultrasound for localizing marked lymph nodes. The optimal procedure to minimize surgical treatment of ypN0 patients is currently unclear, and various marking and localizing methods for TLNB are under discussion due to a lack of comparative data.

Since NST is primarily used in local advanced patients with a high tumor burden, the lymphatic vessels of positive lymph nodes are more likely to be obstructed by cancer cells.. Meanwhile, the subsequent response to NST can cause fibrosis and distort the drainage pathways. Dyes will be blocked from positive lymph nodes, reaching the negative lymph nodes with clear lymphatic vessels instead. This partly explains the discordance between sentinel nodes and metastatic nodes, which is reflected in a high FNR of SLNB. Additionally, the use restriction of radioactive particles makes standardized SLNB, TAD, and relevant TLNB unrealistic in China and some other countries.

Drawing on the studies mentioned above and clinical practice in China, our research group developed a novel procedure, Stained Region Lymph Node Biopsy (SrLNB), to modify TLNB. This dual-localization technique employs a titanium clip and carbon tattoo. Before NST, a titanium clip was placed in the metastatic nodes confirmed by FNA, and a suspension of carbon nanoparticles (0.1–0.2 mL, LUMMY®, Chongqing, China) was injected into the cortex of the surrounding suspicious nodes. During axillary surgery, SrLNB was performed first, followed by ALND to assess the accuracy of the procedure. Data from our previous research (unpublished) indicated that SrLNB has a high IR of 100.0% (159/159), with a FNR of 5.3% (5/94), which is

below the threshold value (10%). This suggests good diagnostic performance and the potential to downstage axillary surgery. The SrLNB procedure, which combines the advantages of both localization clips and carbon tattooing, offers several benefits: it eliminates the need for a second localization prior to surgery, is free of radioactive particles, allows for the completion of axillary surgery at one time, and provides a long identifiable period (up to half a year) for black-stained lymph nodes by carbon nanoparticles.

Nevertheless, oncology outcomes, including local control and survival, must be considered when only de-eacalation axillary surgery such as SLNB, TAD, TLNB, or SrLNB is performed without ALND. To date, several retrospective and prospective studies[13-18] have reported prognostic data for only SLNB or TAD performed without ALND in apCR patients. A meta-analysis reported a low axillary recurrence of 0%–3.4%[19 20], a pooled 5-year DFS of 86% with no significant difference to the ALND group (p = 0.24), and a 5-year OS of 93.1%[20]. In Si-Yu Wu's study[18], 152 patients who received TAD had a 3-year recurrence-free survival of 100%, with no significant difference from the ALND group (p = 0.254). The SenTa study[17]also reported a 3-year iDFS of 91.2% in 119 patients, and only TAD without ALND was not associated with the risk of recurrence or death (p = 0.69, p = 0.91, respectively). However, this was an observational study without treatment intervention, and axillary metastasis was clinically diagnosed without pathological examination. Regarding TLNB, there is very little high-quality evidence of long-term prognostic outcome.

The impact of de-escalation surgery on oncology safety remains unconfirmed,

limiting its clinical application. Therefore, the SrLNB study is designed to assess the feasibility and oncology safety of axillary de-escalation procedures to minimize surgery after NST. Breast cancer patients with pathological axillary metastasis (pN+) and clinically downstaged to negative (ycN0) after NST will be enrolled, among whom ypN0 patients identified by SrLNB will be exempt from ALND, while ypN+ patients will be excluded and undergo ALND. The detailed protocol is as follows: specimens excised by the SrLNB procedure will be sent for intraoperative rapid pathological examination, which will determine whether ALND will be performed. Those with residual axillary disease will ungergo ALND and be excluded from the trial, while those with no remaining tumor in the axilla will be exempt from ALND and subsequently undergo regional lymph node radiotherapy (RNI) including the axilla. The safety and feasibility of SrLNB will be primarily evaluated by a 3-year follow-up of invasive disease-free survival (iDFS) and locoregional recurrence rate (LRR). Additional focus will be given to the incidence of breast cancer-related lymphedema (BCRL) and QoL.

METHODS AND ANALYSIS

Study design and setting

The SrLNB is a prospective, single-arm, single-center, open-label phase II clinical trial. It is currently being conducted by the First Affiliated Hospital with Nanjing Medical University in China and aims to enroll 92 patients. Women with FNA confirmed lymph node metastases that have downstaged to ypN0, as confirmed by

SrLNB after NST, will be enrolled and exempt from ALND. Following the procedure, regional lymph node radiotherapy (RNI) including the axilla and adjuvant therapy will be completed as part of the routine regimen, with a 3-year follow-up. Enrollment is expected to be complete within 2 years, with follow-up scheduled to complete in 2026.

The primary aim of this study is to assess the 3-year invasive disease-free survival (iDFS) of ypN0 patients receiving SrLNB without ALND. Secondary endpoints include the LRR, BCRL rate, QoL, and upper extremity function. These will be used to comprehensively evaluate the feasibility and safety of the SrLNB procedure. The SrLNB is designed to assess whether de-escalation axillary surgery will impair local control, reduce complications, and improve quality of life.

The study was reported according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) checklist[21] . The detailed flow chart of the study is shown in Figure 1. The trial schedule based on SPIRIT checklist is presented in Table 1.

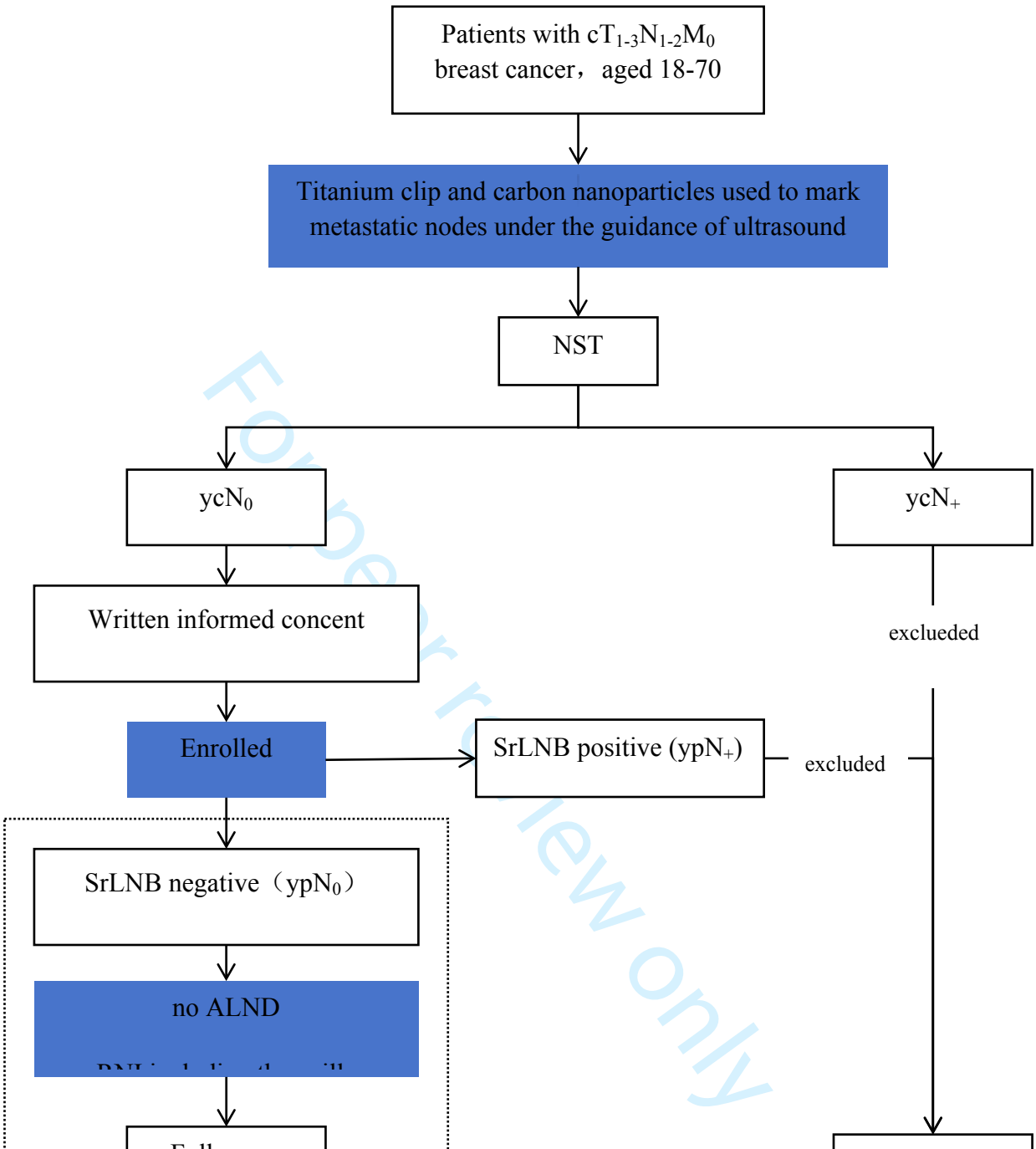

Figure1. Flow chart of SrLNB trial. cTNM, clinical classification of TNM; NST, neoadjuvant systematic therapy; ycN0, clinically assessed lymph node negative after NST; ycN+, clinically assessed lymph node positive after NST; SrLNB, Stained Region Lymph Node Biopsy; ypN0, pathologically assessed lymph node negative after NST; ycN+, pathologically assessed lymph node positive after NST; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy.

Table1. The schedule of enrolment, interventions, and follow-up for each participants.

Period	**Screening**	**Enrolment**	**Follow-up**							
Timepoint	-1w (before SrLNB)	0 (after SrLNB)	1w	1m	3m	6m	1y	1.5y	2y	3y
ENROLMENT:										
Eligibility screen*	×	×								
Informed consent†	×									
Physical examination†	×									
Radiological examination†	×				×	×	×	×	×	×
Intraoperative rapid pathological examination to SrLNB specimen‡		×								
INTERVENTIONS:										
SrLNB procedure without ALND		×								
RNI including the axilla		×		×	×					
Breast surgery		×								
Adjuvant systemic therapy		×		×	×	×	×	×	×	×
ASSESSMENTS:										
Survival outcome and disease status			×	×	×	×	×	×	×	×
BCRL assessment (Arm circumference and SFBIA)§	×		×	×	×	×	×	×	×	×
QoL questionnaires (FACT-B and QuickDASH)§	×		×	×	×	×	×	×	×	×

*Eligibility screen includes confirmation of positive axillary lymph nodes successfully marked by carbon nanoparticle and titanium clip, and completion of NST.

†Physical examination and radiological examination are used to screen patients response to NST. They will be pre-enrolled, and sign an written informed consent.

‡Pre-enrolled patiemts with negative result in intraoperative rapid pathological examination to SLNB specimen will be enrolled, while with residue disease in the axilla will be excluded.

§BCRL assessment and QoL questionnaires will be administered in pre-enrolled patients before surgery as baseline character.

SrLNB, Stained Region Lymph Node Biopsy; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy; BCRL, breast cancer-related lymphedema; SFBIA, single-frequency bioimpedance analysis; QoL, quality of life; NST, neoadjuvant systematic therapy.

Inclusion criteria

Patients who meet the following criteria are eligible for inclusion:

1. females, aged between 18 and 70;

2. pathologically confirmed invasive breast cancer (regardless of pathological type) with a clinical stage of cT1-3;

3. pathologically confirmed positive axillary lymph nodes with a clinical stage of N1-3;

4. received a full course of NST (including neoadjuvant chemotherapy, neoadjuvant targeted therapy, neoadjuvant immunotherapy);

5. positive axillary lymph nodes successfully stained by carbon nanoparticle injection with/without titanium clip marking;

6. all patients are required to undergo immunohistochemical staining (IHC) for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor2 (HER2), Ki-67 proliferation index, and further fluorescence in situ hybridization should be performed in HER2 2+ cases;

7. preoperative clinical assessment (including physical examination, imaging, with or without nomogram assessment) suggests positive axillary lymph nodes converted to negative (ycN0);

8. ECOG score of 0–1;

9. patients voluntarily participated in this study and signed the informed consent form.

Exclusion criteria

Patients will be excluded on any of the following grounds:

1. bilateral breast cancer;

2. breast cancer during lactation or pregnancy;

3. presence of distant metastases confirmed by physical examination or imaging examination;

4. previous history of a malignant tumor;

5. history of previous surgery on the affected axilla, or history of surgery affecting the function of the upper extremity;

6. history of radiation therapy to the breast or chest;

7. positive incision margins for breast-conserving surgery/mastectomy;

8. positive results of intraoperative rapid freeze pathology (including isolated tumor cells and micrometastases) for SrLNB (ypN+);

9. inability to complete the full course of adjuvant therapy as prescribed;

10. aspartate transaminase (AST) or alanine transaminase (ALT) ≥1.5 times the upper limit of normal, alkaline phosphatase (ALP) ≥2.5 times the upper limit of normal, total bile ≥ 1.5 times the upper limit of normal, serum creatinine ≥1.5 times the upper limit of normal; left ventricular ejection fractions (LVEF) <50% on cardiac ultrasound; severe coagulation dysfunction, serious systemic disease, or uncontrolled infection;

11. lack of personal freedom and independent civil capacity;

12. presence of mental disorders, addictions, and those deemed ineligible by the investigator.

Intervention

NST

All enrolled patients will receive NST, which will be determined by the molecular subtype as clarified by the status of ER, PR, HER2, and Ki67 index. The chemotherapy regimen is primarily based on sequential paclitaxel with anthracycline, and targeted therapy (trastuzumab and/or patuzumab) is added in HER2-positive patients. The course of NST typically spans 4–8 cycles and will be individually adjusted.

Breast surgery

The choice between breast-conserving surgery or mastectomy depends on the condition of the tumor and the patient's preference. Patients who undergo breast-conserving surgery will receive whole-breast radiotherapy (WBI) afterwards.

Axillary surgery

SrLNB procedure

Before NST, positive lymph nodes will be marked with titanium clips under ultrasound guidance. A small amount (usually 0.1–0.2 mL) of carbon nanoparticle suspension (LUMMY®, Chongqing, China) will be injected into the cortex of the positive lymph nodes and surrounding suspicious lymph nodes for staining. The carbon nanoparticle suspension is injected using a 1 mL syringe, which will be replaced per lymph node. Lymph nodes will turn black after staining. During surgery,

marked and stained lymph nodes will be removed and biopsied by intraoperative rapid frozen pathology. Those with negative nodes will be enrolled, while those with macro-metastases, micrometastases, or isolated tumor cells confirmed by intraoperative pathology or postoperative routine pathology will be excluded from this study.

Axillary lymph node dissection

Enrolled patients will be exempted from ALND, which includes axillary lymph nodes in region I and II. However, patients with residual axillary disease will routinely undergo ALND.

Radiotherapy

Those undergoing breast-conserving surgery will receive whole-breast radiothera py (WBI), with a boost to the tumor bed delivered sequentially immediately af ter. The prescribed dose for conventional fractionated irradiation is 50 Gy in 2 5 fractions with a single dose of 2 Gy, administered 5 times a week. The dos e to the tumor bed is 60 Gy in 25 fractions with a single dose of 2.4 Gy. Al ternatively, when a hypofractionated regimen is applied, the dose will be 43.5 Gy in 15 fractions of 2.9 Gy over 3 weeks, and the dose to the tumor bed w ill be 52.5 Gy in 18 fractions of 2.9 Gy.

Postmastectomy radiotherapy (PMRT) is defined as radiotherapy to the chest w all for those undergoing mastectomy. The dose is 50 Gy/25 F for conventional fractionated irradiation or 43.5 Gy/15 F for hypofractionated irradiation.

All enrolled patients will receive regional lymph node radiotherapy (RNI), which includes the axilla. RNI including the axilla covers the supraclavicular region and the entire axillary lymphatic drainage area (region I, II, and III), with a dose of 50 Gy/25F. Prophylactic irradiation of the internal mammary node (IMN) drainage area will be added for those who meet the following criteria: (1) ≥4 positive axillary lymph nodes; (2) primary tumor located centrally or in the medial quadrant of the breast, or posterior to the nipple; (3) imaging-diagnosed suspicious metastasis of IMN, or pathologically confirmed metastatic IMN.

Adjuvant systemic treatment

Systemic adjuvant therapies will be administered in accordance with local and international guidelines.These therapies include chemotherapy, targeted therapy, endocrine therapy, and immunotherapy.

Follow-up

Follow-up will commence after surgery, with a 3-year follow-up as the primary endpoint. The schedule for follow-up examinations is as follows:

1. Physical examination: every 6 months;

2. Imaging examination: bilateral breast and axillary ultrasound every 6 months ; mammography with or without MRI annually for patients who have undergon e breast-conserving surgery; Chest X-ray or CT, abdominal ultrasound, bone sc anning, and head MRI will be conducted annually when necessary;

3. Objective assessment of BCRL: Relative Volume Change (RVC) and single-frequency bioimpedance analysis (SFBIA) will be tested and calculated at one week, 1 month, 3 months, 6 months, 12 months, 24 months, and 36 months p ost-operation;

4. PRO: FACT-B and QuickDASH will be surveyed at one week, 1 month, 3 months, 6 months, 12 months, 24 months, and 36 months postoperatively.

Sample size

This study is a single-arm objective performance criteria study, with the 3-year iDFS of ypN0 patients receiving standard treatment (ALND and RNI) as the t arget, which is expected to be 90% as described in previous studies[13-20 22-25]. A non-inferiority test will be used to evaluate the 3-year iDFS against the margi n value of 10%, with a significance level of 0.05 and power of 0.8 . Assumin g a 10% rate of loss to follow-up, the required sample size is 92 (calculated by PASS 15.0 software). In light of this, a 3-year iDFS >80% for enrolled pat ients who underwent SrLNB is considered non-inferior to ALND in terms of o ncology safety, and SrLNB followed by RNI including the axilla is non-inferio r to the standard of care.

Endpoints

Primary endpoint

The primary endpoint of the trial is invasive disease-free survival (iDFS), defin

ed as the time interval from the surgery to invasive LRR, distant metastasis, c ontralateral invasive breast cancer, or death from any cause. iDFS will be calc ulated after a 3 year follow-up. Patients without an event will be censored at t he date of the last available assessment.

Secondary endpoint

1. LRR

This includes both local and regional recurrence. Local recurrence is defined as recurrence in the ipsilateral breast, chest wall, skin, or surgical scar. Regional recurrence is defined as recurrence in the affected lymphatic drainage area, in cluding the axilla, supraclavicular region, subclavicular region, and IMN area. LRR will be calculated after a 3 year follow-up.

2. Breast Cancer Related Lymphedema (BCRL)

In our study, two objective methods will be used to assess upper limb lymphedema. If either criterion is met, BCRL will be diagnosed. In the event of inconsistency, the former results will prevail.

(1) RVC >10% in the affected upper extremity.

The volume of the upper limb is calculated based on arm circumference, where 5 points were taken to measure at 10cm intervals from ulnar styloid to 40cm away. Five points divide upper limb into 4 truncated cones. For each truncated cones, arm circumference of both ends are recorded as C1 and C2, the volume formula is[26]: $V = h(C_1^2 + C_2^2 + C_1C_2)/12\pi$ (h is the length, which was 10cm in the study). The volume of

the upper limb is the sum of four truncated cones. The baseline volume of both upper limbs is measured and calculated preoperatively, with the volume of the affected arm recorded as A1 and the unaffected arm as U1. At the time of follow-up, the arm volume is calculated in the same way, with the affected arm recorded as A2 and the unaffected arm as U2. Then, the RVC can be calculated as follows: $RVC = (A_2/U_2)(A_1/U_1) - 1$. When RVC＞10%, BCRL will be diagnosed[27]. Follow-up of RVC will be conducted at one week and 1, 3, 6, 12, 18, 24, 36 months after the operation.

(2) Bioelectrical Impedance Analysis (BIA)

The SFBIA ratio is defined as the ratio of the impedance of the unaffected upper limb to the affected one. BCRL is diagnosed when the impedance ratio exceeds the threshold value of the mean ± 2SD (standard deviation) of healthy controls. The impedance of both arms is measured by Inbody device at 1 kHz and 5 kHz, and the ratio is calculated. Referring to the study by Liu et al.[28], a diagnosis will be made when any one of the following criteria is met: when the affected arm is the dominant hand, its impedance ratio >1.067 at 1 kHz or >1.068 at 5 kHz; when the affected arm is the non-dominant hand, the ratio > 1.043 at 1 kHz or >1.044 at 5 kHz suggests edema.

3. PRO

PRO will be reported using the Chinese version of FACT-B (V4.0) and Quick DASH. These scales will be used to subjectively assess postoperative upper ext remity complications and QoL. They will be surveyed before surgery as a base

line measurement, and follow-up will be conducted at one week and 1, 3, 6, 1 2, 24, and 36 months postoperative.

Data collection and management

This study employs an electronic case report form (eCRF) for data management and recording. The principal investigator oversees all projects in this trial. Investigators or authorized researchers are tasked with data collection, entry, corrections, and revisions. To ensure data accuracy, two data managers will independently perform double entry and proofreading.

Clinical supervisors and administrators will verify and audit the data to assess its completeness, consistency, and standardization. They will challenge any data that is nonsensical or non-standardized. Researchers are expected to respond to and address these challenges promptly, recording the reasons for any modifications. If a subject withdraws their informed consent during participation, no further data will be collected. However, study-related data collected prior to discontinuation will be retained and used for up to three years. At the study's conclusion, the principal investigator will submit a final report for review and written approval by the Ethics Committee.

Statistical analysis

Baseline characteristics, including demographics, pathology features, treatment re gimen, BCRL rate, and scores of QoL scales, will be described. For continuou

s variables, data will be presented as mean, standard deviation, or median and interquartile range. For categorical variables, data will be analyzed using the chi-square test or Fisher's exact test, and presented as totals, percentiles, and frequencies. Non-parametric tests will be analyzed using the Student's t test or the Mann–Whitney U test.

Kaplan–Meier curves will be established to analyze iDFS and LRR, and HR and 95% CI will be calculated. For iDFS with a single-group target value, a non-inferiority test will be performed: the target value of iDFS for the experimental group is set at 90%, with a non-inferiority cut-off value of 10%, $\alpha = 0.05$, $\beta = 0.2$, and an estimated 10% loss rate of follow-up. Univariate and multivariate Cox regression models will be used to analyze impact factors on iDFS and LRR. If the LRR events are few, they could be reported as a crude rate. Differences are considered statistically significant with $p < 0.05$.

Data monitoring

An independent decision management committee will be established for this study and will undergo review every six months. Any SAEs that occur during the trial will be documented following the Common Terminology Criteria for Adverse Events 5.0 (CTCAE v5.0). Investigators are required to report all adverse reactions, suspected adverse reactions, unexpected adverse reactions, and unanticipated events during the trial, in writing to the sponsor and the Ethics Committee within 24 hour of the occurrence. Clinical data administrators will write a data review report at the end of

data cleansing. This report will be discussed at a data review meeting by the principal investigator, statistician, project manager, medical staff, and data management staff, and the statistical analysis population will be identified.

Monitoring of adverse event

An adverse event (AE) is defined as any unfavorable experience that participants encounter during the study period, regardless of its relation to the treatment. Predefined AEs specific to the axilla include postoperative bleeding or infection, lymphedema of the upper limb or chest wall, neuralgia, sensory abnormalities, decreased mobility, muscle weakness, or pain in the arm and shoulder. The severity of AEs is categorized as mild, moderate, or severe, based on the CTCAE 5.0 criteria (Common Terminology Criteria for Adverse Events V.5.0). A serious adverse event (SAE) is defined as an adverse medical occurrence or effect related to surgical treatment that results in hospitalization, prolonged hospital stay, or death.

In this study, the procedural risk is low, and the likelihood of an SAE is minimal. In the event of BCRL and radiotherapy-related adverse effects, symptomatic treatment is typically sufficient. The principal investigators of this study are responsible for evaluating SAE, which should be reported to the approved medical ethics committee within 15 days of occurrence. For fatal or life-threatening cases, there is a maximum of 7 days for an initial report and 8 days for a completed report. All SAEs should be followed up until they have subsided or reached a steady state. Follow-up may necessitate additional tests or medical procedures and/or referral to a general

practitioner or medical specialist, depending on the specific event.

Ethics and dissemination

The study will be conducted in strict compliance with the principles of the Declaration of Helsinki, and all patient data will be processed anonymously. The study has received approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Ethical review number: 2023-SR-169). Any amendments to the protocol, informed consent, and other study materials will only be implemented after receiving approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, and these changes will be notified to the trial registry. The findings from the study will be disseminated through academic conferences and published in peer-reviewed journals.

DISCUSSION

In clinical practice, the management of axillary surgery in patients with apCR after NST exhibits significant heterogeneity. The question of whether patients with ypN0 can be exempt from ALND remains a controversial issue. A recent survey[13] conducted by the European Breast Cancer Research Association of S urgical Trialists, which included 349 breast surgeons and oncological radiologist s across 45 countries, revealed that a substantial proportion of surgeons still ad vocate for ALND in patients with ypN0 (22.4% support in cN1 patients, 45.1 % in cN2). The least advocated surgery is TLNB (2.5% in cN1, 2.1% in cN2)

, due to the lack of long-term prognostic data on local control and survival in axillary de-escalation procedures such as SLNB, TAD, and TLNB.

Several studies, such as those by Sabrina Kahler-Ribeiro-Fontana[14], Andrea V. Barrio[15], Si-Yu Wu[18], and MARI[16], SenTa[17], study have published data on recu rrence or survival rates in patients with apCR after NST who were exempt fro m ALND and only underwent SLNB or TAD. However, there are few prospec tive studies that have reported safety data for the TLNB procedure, indicating an urgent need for such studies.

Several ongoing trials, including ATNEC (NCT04109079), TAXIS[29], AXSANA[30], and our SrLNB, are expected to provide safety evidence for axillary de-escal ation surgery and expedite its clinical application. The ATNEC study enrolls p atients with cN1 and performs SLNB without ALND in apCR patients. In cont rast, the TAXIS trial includes cN1-3 patients and employs the TATTOO techni que to conduct TAD. The AXSANA study utilizes a variety of marking techni ques with the goal of identifying the optimal procedure.

Compared to these studies, our SrLNB study offers several advantages: First, o nly patients with axillary lymph node metastasis confirmed by FNA before NS T are initially included in our study, thereby eliminating false-positive axillary metastasis from imaging diagnosis. Second, SrLNB combines the benefits of bo th localization clips and carbon tattooing, enhancing its feasibility. This include s eliminating the need for a second localization prior to surgery, avoiding the use of radioactive particles, and providing a long, identifiable period of black-s

tained lymph nodes by carbon nanoparticles that lasts up to half a year. In fac t, it represents a further downstaging of the standardized SLNB procedure in te rms of surgical extent and trauma to the axilla. More importantly, our prelimin ary experiments with the SrLNB procedure have confirmed a high detection rat e of 100.0% (159/159) and a low FNR rate of 5.3% (5/94) (unpublished). The procedure has been successfully performed multiple times in our center, and t he surgeons have gained proficiency in this procedure, enhancing its feasibility in our center. Additionally, our study places greater emphasis on axillary mor bidity and QoL. BCRL rate and PRO will be reported using multiple objective and subjective measurements.

Trial status and Time plan

The protocol version number and date are V3.0 and May 16, 2023, respectivel y. The study was conceived and designed in 2022. The first patient was enroll ed on September 11, 2023, and the study is currently in the patient recruitmen t phase. Enrollment is projected to be completed by August 2025, followed by a 3-year follow-up period, which is expected to be preliminary completed by October 2026.

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Contributors

All authors are from The First Affiliated Hospital with Nanjing Medical University. Jue Wang and Xiaoming Zha were the principal investigators of this study, they proposes the preliminary conception and conduct a tentative design, financial support is also provided. Lingjun Ma and Xuan Li developed the detailed protocol, Rui Chen and Mingyu Wang helped to register the trial and obtain ethical approval. Lexin Wang, Ran Zheng，Jingjing Ding，Hao Yao and Xingye Sheng were responsible for patient recruitment and data collection, Yichun Gong, Jingjing Ding and Yuanyuan Wang were responsible for data checking and data monitoring. Lingjun Ma drafted the initial manuscript. All authors critically revised the manuscript and approved the final study protocol.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Not applicable.

Data availability statement

Data sharing is currently not applicable to this article as the study is still open for inclusion of patients. Only principal investigators have access to the final trial datase. We are willing to consider requests for data sharing on a case-by-case basis. Researchers interested in accessing the data should contact the corresponding author with a detailed proposal outlining the purpose of their request.

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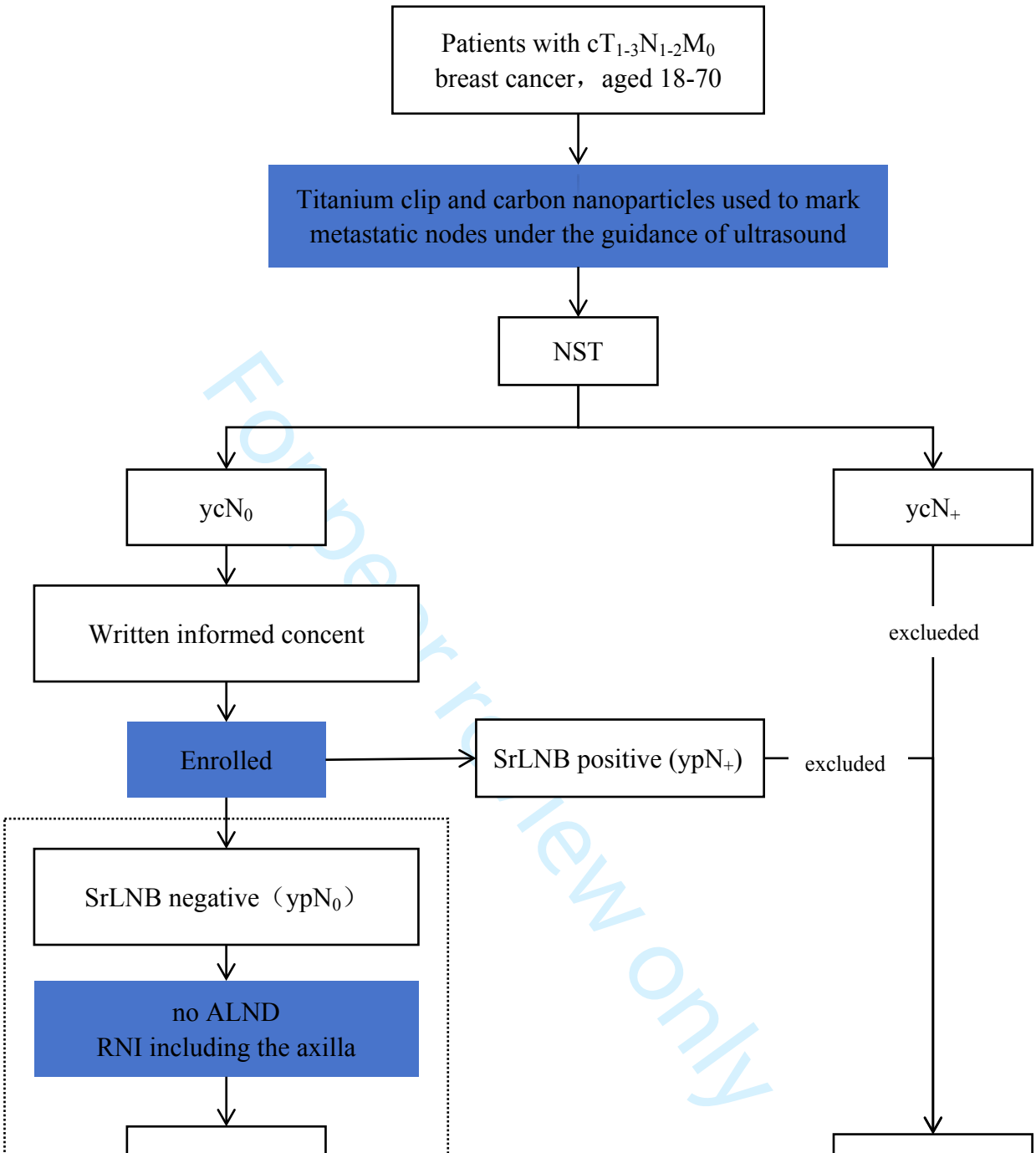

Figure1. Flow chart of SrLNB trial. cTNM, clinical classification of TNM; NST, neoadjuvant systematic therapy; ycN0, clinically assessed lymph node negative after NST; ycN+, clinically assessed lymph node positive after NST; SrLNB, Stained Region Lymph Node Biopsy; ypN0, pathologically assessed lymph node negative after NST; ycN+, pathologically assessed lymph node positive after NST; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy.

Table1. The schedule of enrolment, interventions, and follow-up for each participants.

Period	**Screening**	**Enrolment**	**Follow-up**							
Timepoint	-1w (before SrLNB)	0 (after SrLNB)	1w	1m	3m	6m	1y	1.5y	2y	3y
ENROLMENT:										
Eligibility screen*	×	×								
Informed consent†	×									
Physical examination†	×									
Radiological examination†	×				×	×	×	×	×	×
Intraoperative rapid pathological examination to SrLNB specimen‡		×								
INTERVENTIONS:										
SrLNB procedure without ALND		×								
RNI including the axilla		×		×	×					
Breast surgery		×								
Adjuvant systemic therapy		×		×	×	×	×	×	×	×
ASSESSMENTS:										
Survival outcome and disease status			×	×	×	×	×	×	×	×
BCRL assessment (Arm circumference and SFBIA)§	×		×	×	×	×	×	×	×	×
QoL questionnaires (FACT-B and QuickDASH)§	×		×	×	×	×	×	×	×	×

*Eligibility screen includes confirmation of positive axillary lymph nodes successfully marked by carbon nanoparticle and titanium clip, and completion of NST.

†Physical examination and radiological examination are used to screen patients response to NST. They will be pre-enrolled, and sign an written informed consent.

‡Pre-enrolled patiemts with negative result in intraoperative rapid pathological examination to SLNB specimen will be enrolled, while with residue disease in the axilla will be excluded.

§BCRL assessment and QoL questionnaires will be administered in pre-enrolled patients before surgery as baseline character.

SrLNB, Stained Region Lymph Node Biopsy; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy; BCRL, breast cancer-related lymphedema; SFBIA, single-frequency bioimpedance analysis; QoL, quality of life; NST, neoadjuvant systematic therapy.

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Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

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Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

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ABSTRACT

Introduction

Despite achieving axillary pathological complete response (apCR) after neoadjuvant systematic therapy (NST), a majority of breast cancer patients still undergo axillary lymph node dissection (ALND). Given that ALND may not contribute to local control or survival and could lead to increased arm morbidity, several studies have suggested less invasive axillary surgery procedures as an alternative. However, the application of these axillary de-escalation procedures is limited due to the absence of substantial evidence of long-term outcomes.

Methods and analysis

This prospective, single-arm, open-label, non-inferiority, monocentric phase II trial is designed for breast cancer patients who were initially diagnosed with axillary metastasis and achieved apCR after NST to validate the oncological safety of SrLNB procedure. Stained Region Lymph Node Biopsy (SrLNB) is a novel de-escalation axillary surgery, which was developed and tested in our preliminary study. The primary endpoint of this trial is the 3-year invasive disease-free survival (iDFS). Secondary endpoints include local-regional recurrence (LRR), incidence of breast cancer-related lymphedema (BCRL), and patient-reported outcomes (PRO). It is expected that the 3 year iDFS in patients undergoing ALND is about 90%, and a non-inferiority margin value of 10%, with a significance level of 0.05 and power of 0.8, loss to follow-up rate of 10%, the planned enrollment is 92 patients. The trial was initiated on September 11, 2023, with the first patient enrolled on September 25,

2023, and is scheduled to end in 2026.

Ethics and dissemination The trial protocol received approval from the Human Research Ethics Committee of The First Affiliated Hospital with Nanjing Medical University in May 2023 (Reference 2023-SR-169). All participants will provide informed consent.The study results will be disseminated through international peer-reviewed scientific journals, presentations at international scientific conferences, and public lectures.

Trial registration NCT05939830

Key words: Breast cancer, Neoadjuvant Systemic Therapy, Axillary Surgery , Axillary lymph node dissection, Disease-free survival, Breast Cancer Related Lymphedema, Quality of life

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The SrLNB procedure is economical and easy to conduct with the use of carbon tattooing, and can be considered a further de-escalation of the standardized SLNB procedure.
- The SrLNB study places greater emphasis on arm morbidity and quality of life (QoL) with multiple objective and subjective measurements.
- The small sample size and the monocentric design of the study may lead to some limitations in the extrapolation of the results.

INTRODUCTION

Axillary lymph node dissection (ALND) has been replaced by Sentinel Lymph Node Biopsy (SLNB) to cN0 patients (clinically axillary lymph node negative) due to significant morbidity since decades ago. The de-escalation surgery to axillary can reduce surgical morbidity rate (i.e., lymphedema, chronic pain, mobility restrictions) and improve patients' quality of life (QoL).

For patients with cN+ at the time of diagnosis, neoadjuvant systematic therapy (NST) is often the option. With great advances in Neoadjuvant Systemic Therapy (NST), axillary pathologic complete response (apCR) has been observed in 13-60%[1] of patients with axillary metastases at the time of diagnosis in different molecular subtypes, especially in 59% of HER2+ patients and 48% of triple negative patients (TNBC)[1]. To those with axillary node remains clinically positive (ycN+) after NST, ALND of level I-II is still the standard management of the axilla, while for patients with lymph nodes downstaging to negative (ycN0), the need for conventional ALND has been questioned. To date, ALND has been replaced by de-escalation procedures like standardized SLNB or TAD.

To replace ALND with de-escalation procedures, one of the key points lies in the false negative rate (FNR). ACOSOG Z1071[2], SN FNAC[3], GANEA[4], and SENTINA[5] studies modified SLNB by use of dual tracing agents and removal of ≥3 sentinel nodes to make FNR below the threshold target of 10%. In targeted axillary dissection (TAD) procedure[6], suspicious lymph node marked by various methods before NST will be biospied (targeted lymph node biospied, TLNB) along with SLNB, which can

reduce the FNR to 2%. A study by Donker et al.[7] proposed MARI procedure, marking the axillary lymph node with radioactive iodine (^{125}I) seeds, where only TLNB were dissected without SLNB, with an acceptable FNR of 7%, thus TLNB alone can be considered as an option for de-escalation procedure. In adition, various methods of marking and localizing metastatic lymph nodes have been developed, such as iodine-125 seed[8], carbon tattooing[9 10], Magseed[11]. However, the optimal marking and localizing methods for TLNB are under discussion due to a lack of comparative data. Additionally, the use restriction of radioactive particles makes standardized SLNB, TAD, and relevant TLNB unrealistic in China and some other countries.

Drawing on the studies mentioned above and clinical practice in China, our research group developed a novel procedure, Stained Region Lymph Node Biopsy (SrLNB), to modify TLNB. When conducting fine-needle aspiration (FNA), a suspension of carbon nanoparticles (0.1–0.2 mL, LUMMY®, Chongqing, China) will be injected into the cortex of the biopsied nodes immediately. SrLNB requires removing all lymph nodes within the stained region and retrieving abnormally palpated lymph nodes near the stained region. Data from our previous research indicated that SrLNB has a high detection rate and a FNR below the threshold value (10%). This suggests good diagnostic performance and the potential to downstage axillary surgery. The SrLNB procedure offers several benefits with the use of carbon tattooing: eliminating the need for a second localization prior to surgery, free of radioactive particles, allowing for the completion of axillary surgery at one time, and providing a long identifiable period (up to half a year) for black-stained lymph nodes by carbon

nanoparticles.

Nevertheless, oncology outcomes, including local control and survival, must be considered when only de-escalation axillary surgery such as SLNB, TAD, TLNB, or SrLNB is performed without ALND. To date, several retrospective and prospective studies[12-17] have reported prognostic data for standardized SLNB or TAD, while TLNB still lack data of oncological outcome. A review reported a low axillary recurrence of 0%–3.4%[18], and a meta-analysis[19] reported a pooled 5-year DFS of 86% with no significant difference to the ALND group (p = 0.24). In the study of Si-Yu Wu et al.[17], 152 patients who received TAD had a 3-year recurrence-free survival of 100%, with no significant difference from the ALND group (p = 0.254). The SenTa study[16] also reported a 3-year iDFS of 91.2% in 119 patients, and only TAD without ALND was not associated with the risk of recurrence or death (p = 0.69, p = 0.91, respectively). However, this was an observational study without treatment intervention, and axillary metastasis was clinically diagnosed without pathological examination.

Regarding TLNB, there is very little high-quality evidence of long-term prognostic outcome, limiting its clinical application. Therefore, this study is designed to assess the oncological safety of SrLNB procedures. Breast cancer patients with pathological confirmed axillary metastasis (pN+) and clinically downstaged to negative (ycN0) after NST will be enrolled, among whom ypN0 patients identified by SrLNB will be exempt from ALND and subsequently undergo regional lymph node radiotherapy (RNI) including the axilla, while ypN+ patients will be excluded and undergo ALND.

The oncological safety of SrLNB will be primarily evaluated by a 3-year follow-up of invasive disease-free survival (iDFS) and locoregional recurrence rate (LRR). This will also add evidence to the oncological safety of TLNB. Additional focus will be given to the incidence of breast cancer-related lymphedema (BCRL) and QoL, which will provide subjective perspective to assess the potential of SrLNB in reducing arm morbidity and QoL.

METHODS AND ANALYSIS

Study design and setting

The SrLNB is a prospective, single-arm, single-center, open-label phase II clinical trial. It is currently being conducted by the First Affiliated Hospital with Nanjing Medical University in China. Women with FNA confirmed lymph node metastases and downstaged to ypN0 after NST confirmed by SrLNB, will be enrolled and exempt from ALND, regional lymph node radiotherapy (RNI) including the axilla (covers the supraclavicular region and the axillary region I, II, and III) will be completed subsequently, with a 3-year follow-up. Prophylactic irradiation of the internal mammary node (IMN) drainage area will be added when necessary. It is expected that the 3-year iDFS in patients undergoing ALND is about 90%, and a non-inferiority margin value of 10%, with a significance level of 0.05 and power of 0.8, loss to follow-up rate of 10%, the planned enrollment is 92 patients. Enrollment is expected to be complete within 2 years, with follow-up scheduled to complete in 2026.

The primary aim of this study is to assess the 3-year invasive disease-free survival

(iDFS) of ypN0 patients receiving SrLNB without ALND. Secondary endpoints include the LRR, BCRL rate, QoL, and patient-reported arm morbidity. BCRL will be assessed by relative volume change (RVC) and single-frequency bioimpedance analysis (SFBIA). QoL will be assessed by FACT-B (V4.0) and patient-reported arm morbidity will be assessed by QuickDASH questionnaire. The SrLNB is designed to assess whether de-escalation axillary surgery will impair local control, reduce complications, and improve quality of life.

The study was reported according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) checklist[20] . The detailed flow chart of the study is shown in Figure 1. The trial schedule based on SPIRIT checklist is presented in Table 1.

Table1. The schedule of enrolment, interventions, and follow-up for each participants.

	Study Period										
	Screening	Enrollment and treatment		Follow-up							
Timepoint	**-1w**	**0**	**(SrLNB)**	**1w**	**1m**	**3m**	**6m**	**1y**	**1.5y**	**2y**	**3y**
Enrollment											
Eligibility screen*	×		×								
Informed consent†	×										
Physical examination†	×					×	×	×	×	×	×
Imaging examination†	×					×	×	×	×	×	×
Frozen section to SrLNB specimen‡			×								
Interventions											
SrLNB without ALND			×								
RNI including the axilla			×								
Breast surgery			×								
Adjuvant systemic therapy			×								
Assessments											
Survival outcome				×	×	×	×	×	×	×	×
Disease status											
Arm circumference											
SFBIA											
Questionnaire FACT-B	×			×	×	×	×	×	×	×	×
Questionnaire QuickDASH	×			×	×	×	×	×	×	×	×

*Eligibility screen includes confirmation of positive axillary lymph nodes successfully marked by carbon nanoparticles, and completion of NST.

†Physical examination and imaging examination are used to screen patients response to NST. They will be pre-enrolled, and sign an written informed consent. Physical examination mainly includes palpation of the breast and axillary lymph node. Imaging examination mainly includes breast and axillary ultrasound and MRI.

‡Pre-enrolled patients with negative result in intraoperative rapid pathological examination to SLNB specimen will be enrolled, while with residue disease in the axilla will be excluded.

SrLNB, Stained Region Lymph Node Biopsy; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy; SFBIA, single-frequency bioimpedance analysis;

Trial participants and inclusion/exclusion criteria

Inclusion criteria

Patients who meet the following criteria are eligible for inclusion:

1. females, aged between 18 and 70;
2. pathologically confirmed invasive breast cancer (regardless of pathological type) with a clinical stage of cT1-3;
3. pathologically confirmed positive axillary lymph nodes with a clinical stage of N1-3;
4. received a full course of NST (including neoadjuvant chemotherapy, neoadjuvant targeted therapy, neoadjuvant immunotherapy);
5. positive axillary lymph nodes successfully marked stained by carbon nanoparticle suspension;
6. all patients are required to undergo immunohistochemical staining (IHC) for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor2 (HER2), Ki-67 proliferation index, and further fluorescence in situ hybridization should be performed in HER2 2+ cases;
7. metastatic axillary lymph nodes downstage to clinically negative after NST (ycN0) assessed by ultrasound and MRI;
8. ECOG score of 0–1;
9. patients voluntarily participated in this study and signed the informed consent form.

Exclusion criteria

Patients will be excluded on any of the following grounds:

1. bilateral breast cancer;

2. breast cancer during lactation or pregnancy;

3. presence of distant metastases confirmed by physical examination or imaging examination;

4. previous history of a malignant tumor;

5. history of previous surgery on the affected axilla, or history of surgery affecting the function of the upper extremity;

6. history of radiation therapy to the breast or chest;

7. positive incision margins for breast-conserving surgery/mastectomy;

8. positive results of intraoperative frozen-section evaluation (including isolated tumor cells and micrometastases) for SrLNB (ypN+);

9. inability to complete the full course of adjuvant therapy as prescribed;

10. aspartate transaminase (AST) or alanine transaminase (ALT) ≥1.5 times the upper limit of normal, alkaline phosphatase (ALP) ≥2.5 times the upper limit of normal, total bile ≥ 1.5 times the upper limit of normal, serum creatinine ≥1.5 times the upper limit of normal; left ventricular ejection fractions (LVEF) <50% on cardiac ultrasound; severe coagulation dysfunction, serious systemic disease, or uncontrolled infection;

11. lack of personal freedom and independent civil capacity;

12. presence of mental disorders, addictions, and those deemed ineligible by the investigator.

Sample size

This study is a single-arm objective performance criteria study, with the 3-year iDFS of ypN0 patients receiving standard treatment (ALND and RNI) as the target, which is expected to be 90% as described in previous studies[12-19 21-24]. A non-inferiority test will be used to evaluate the 3-year iDFS against the margin value of 10%, with a significance level of 0.05 and power of 0.8 . Assuming a 10% rate of loss to follow-up, the required sample size is 92 (calculated by PASS 15.0 software). In light of this, a 3-year iDFS >80% for enrolled patients who underwent SrLNB is considered non-inferior to ALND in terms of oncology safety, and SrLNB followed by RNI including the axilla is non-inferior to the standard of care.

Intervention

NST

All enrolled patients will receive NST, which will be determined by the molecular subtype as clarified by the status of ER, PR and HER2. ER and PR positive are both defined as no less than 1% of the invasive tumor cells staining positive via IHC. HER2 positive was defined as HER2 3+ and HER2 2+ by IHC with the presence of HER2 amplification by FISH (ISH+). The chemotherapy regimen is primarily based on sequential paclitaxel with anthracycline, and targeted therapy (trastuzumab and/or pertuzumab) is added in HER2-positive patients. The course of NST typically spans 6–8 cycles and will be individually adjusted.

Breast surgery

The choice between breast-conserving surgery or mastectomy depends on the condition of the tumor and the patient's preference. Patients who undergo breast-conserving surgery will receive whole-breast radiotherapy (WBI) afterwards.

Axillary surgery

SrLNB procedure

Before the NST, the most abnormal lymph nodes will be selected for FNA. At the same time, carbon nanoparticle suspension injection (0.5 mL:25 mg, H20073246, Chongqing Lummy Pharmaceutical Co. Ltd, China) will be diluted (concentration: 0.05 mL per 0.15 mL saline) and injected into the cortex of the biopsied lymph node under ultrasound guidance to ensure that the biopsied lymph node is accurately stained and marked. Only when there are many abnormal lymph nodes with a wide distribution, the region will be marked by staining the closest and most distal lymph nodes. Lymph nodes will turn black after staining. During surgery, SrLNB requires removing all lymph nodes within the stained region and retrieving abnormally palpated lymph nodes near the stained region. Those with negative results of intraoperative pathology will be enrolled, while those with macro-metastases, micrometastases, or isolated tumor cells will be excluded from this study.

Axillary lymph node dissection

Enrolled patients will be exempted from ALND, which includes axillary lymph nodes in region I and II. However, patients with residual axillary disease will routinely

undergo ALND.

Radiotherapy

All patients enrolled will undergo whole-breast radiotherapy (WBI) or postmast ectomy radiotherapy (PMRT) according to breast surgery, and regional lymph n ode radiotherapy (RNI) including the axilla, regardless of treatment response to systemic therapy. Part of patients will receive prophylactic irradiation of the i nternal mammary node (IMN) drainage area when necessary.

Those undergoing breast-conserving surgery will receive WBI, with a boost to the tumor bed delivered sequentially immediately after. The prescribed dose for conventional fractionated irradiation is 50 Gy in 25 fractions with a single do se of 2 Gy, administered 5 times a week. The dose to the tumor bed is 60 G y in 25 fractions with a single dose of 2.4 Gy. Alternatively, when a hypofrac tionated regimen is applied, the dose will be 43.5 Gy in 15 fractions of 2.9 G y over 3 weeks, and the dose to the tumor bed will be 52.5 Gy in 18 fractio ns of 2.9 Gy.

PMRT is defined as radiotherapy to the chest wall for those undergoing maste ctomy. The dose is 50 Gy/25 F for conventional fractionated irradiation or 43. 5 Gy/15 F for hypofractionated irradiation.

RNI including the axilla covers the supraclavicular region and the entire axillary lymphatic drainage area (region I, II, and III), with a dose of 50 Gy/25F.

IMN will be added for those who meet the following criteria: (1) ≥4 positive axillary

lymph nodes; (2) primary tumor located centrally or in the medial quadrant of the breast, or posterior to the nipple; (3) imaging-diagnosed suspicious metastasis of IMN, or pathologically confirmed metastatic IMN.

Adjuvant systemic treatment

Systemic adjuvant therapies will be administered in accordance with local and international guidelines. These therapies include chemotherapy, targeted therapy, endocrine therapy, and immunotherapy. Regimen to luminal patients is mainly epirubicin and cyclophosphamide followed by docetaxel (EC-T) combined with endocrine drugs. HER2+ patients mainly receive epirubicin and cyclophosphamide followed by docetaxel, trastuzumab and pertuzumab (EC-THP), or docetaxel, carboplatin, trastuzumab and pertuzumab (TCbHP). To TNBC, docetaxel and carboplatin, followed by epirubicin and cyclophosphamide, with or without pembrolizumab, is the main regimen. The regimen will be individually adjusted based on the actual situation.

Follow-up

Follow-up will commence after surgery, with a 3-year follow-up as the primary endpoint. The schedule for follow-up examinations is as follows:

1. Physical examination: every 6 months;

2. Imaging examination: bilateral breast and axillary ultrasound every 6 months ; mammography with or without MRI annually for patients who have undergon

e breast-conserving surgery; Chest X-ray or CT, abdominal ultrasound, bone sc anning, and head MRI will be conducted annually when necessary;

3. Objective assessment of BCRL: Relative Volume Change (RVC) and single-frequency bioimpedance analysis (SFBIA) will be tested and calculated at one week, 1 month, 3 months, 6 months, 12 months, 24 months, and 36 months p ost-operation;

4. Patient-reported questionnaires: FACT-B and QuickDASH will be surveyed a t one week, 1 month, 3 months, 6 months, 12 months, 24 months, and 36 mo nths postoperatively.

Endpoints

Primary endpoint

The primary endpoint of the trial is invasive disease-free survival (iDFS), defin ed as the time interval from the surgery to invasive LRR, distant metastasis, c ontralateral invasive breast cancer, or death from any cause. iDFS will be calc ulated after a 3 year follow-up. Patients without an event will be censored at t he date of the last available assessment.

Secondary endpoints

1. LRR

This includes both local and regional recurrence. Local recurrence is defined as recurrence in the ipsilateral breast, chest wall, skin, or surgical scar. Regional recurrence is defined as recurrence in the affected lymphatic drainage area, in

cluding the axilla, supraclavicular region, subclavicular region, and IMN area. LRR will be calculated after a 3 year follow-up.

2. Breast Cancer Related Lymphedema (BCRL)

In our study, two objective methods will be used to assess upper limb lymphedema. If either criterion is met, BCRL will be diagnosed. In the event of inconsistency, the former results will prevail.

(1) RVC >10% in the affected upper extremity.

The volume of the upper limb is calculated based on arm circumference, where 5 points were taken to measure at 10cm intervals from ulnar styloid to 40cm away. Five points divide upper limb into 4 truncated cones. For each truncated cones, arm circumference of both ends are recorded as C1 and C2, the volume formula is[25]: $V = h(C_1^2+C_2^2+C_1C_2)/12\pi$ (h is the length, which was 10cm in the study). The volume of the upper limb is the sum of four truncated cones. The baseline volume of both upper limbs is measured and calculated preoperatively, with the volume of the affected arm recorded as A1 and the unaffected arm as U1. At the time of follow-up, the arm volume is calculated in the same way, with the affected arm recorded as A2 and the unaffected arm as U2. Then, the RVC can be calculated as follows: $RVC = (A_2/U_2)(A_1/U_1) - 1$. When RVC＞10%, BCRL will be diagnosed[26]. Follow-up of RVC will be conducted at one week and 1, 3, 6, 12, 18, 24, 36 months after the operation.

(2) Bioelectrical Impedance Analysis (BIA)

The SFBIA ratio is defined as the ratio of the impedance of the unaffected upper limb to the affected one. BCRL is diagnosed when the impedance ratio exceeds the

threshold value of the mean ± 2SD (standard deviation) of healthy controls. The impedance of both arms is measured by Inbody device at 1 kHz and 5 kHz, and the ratio is calculated. Referring to the study by Liu et al.[27], a diagnosis will be made when any one of the following criteria is met: when the affected arm is the dominant hand, its impedance ratio >1.067 at 1 kHz or >1.068 at 5 kHz; when the affected arm is the non-dominant hand, the ratio > 1.043 at 1 kHz or >1.044 at 5 kHz suggests edema.

3. QoL

PRO will be reported using the Chinese version of FACT-B (V4.0). These scales will be used to subjectively assess QoL. They will be surveyed before surgery as a baseline measurement, and follow-up will be conducted at one week and 1, 3, 6, 12, 24, and 36 months postoperative.

4. Patient-reported arm morbidity

Patient-reported arm morbidity will be reported using the Chinese version of Quick Disability of the Arm, Shoulder, and Hand (QuickDASH) questionnaires, They will be surveyed before surgery as a baseline measurement, and follow-up will be conducted at one week and 1, 3, 6, 12, 24, and 36 months postoperative.

Data collection and management

This study employs an electronic case report form (eCRF) for data management and recording. The principal investigator oversees all projects in this trial. Investigators or authorized researchers are tasked with data collection, entry, corrections, and

revisions. To ensure data accuracy, two data managers will independently perform double entry and proofreading.

Clinical supervisors and administrators will verify and audit the data to assess its completeness, consistency, and standardization. They will challenge any data that is nonsensical or non-standardized. Researchers are expected to respond to and address these challenges promptly, recording the reasons for any modifications. If a subject withdraws their informed consent during participation, no further data will be collected. However, study-related data collected prior to discontinuation will be retained and used for up to three years. At the study's conclusion, the principal investigator will submit a final report for review and written approval by the Ethics Committee.

Statistical analysis

Baseline characteristics, including demographics, pathology features, treatment re gimen, BCRL rate, and scores of QoL scales, will be described. For continuou s variables, data will be presented as mean, standard deviation, or median and interquartile range. For categorical variables, data will be analyzed using the ch i-square test or Fisher's exact test, and presented as totals, percentiles, and freq uencies. Non-parametric tests will be analyzed using the Student's t test or the Mann–Whitney U test.

Kaplan–Meier curves will be established to analyze iDFS and LRR, and HR and 95% CI will be calculated. For iDFS with a single-group target value, a non-inferiority test

will be performed: the target value of iDFS for the experimental group is set at 90%, with a non-inferiority cut-off value of 10%, $\alpha = 0.05$, $\beta = 0.2$, and an estimated 10% loss rate of follow-up. Univariate and multivariate Cox regression models will be used to analyze impact factors on iDFS and LRR. If the LRR events are few, they could be reported as a crude rate. Differences are considered statistically significant with $p < 0.05$.

Data monitoring

An independent decision management committee will be established for this study and will undergo review every six months. Any SAEs that occur during the trial will be documented following the Common Terminology Criteria for Adverse Events 5.0 (CTCAE v5.0). Investigators are required to report all adverse reactions, suspected adverse reactions, unexpected adverse reactions, and unanticipated events during the trial, in writing to the sponsor and the Ethics Committee within 24 hour of the occurrence. Clinical data administrators will write a data review report at the end of data cleansing. This report will be discussed at a data review meeting by the principal investigator, statistician, project manager, medical staff, and data management staff, and the statistical analysis population will be identified.

Monitoring of adverse event

An adverse event (AE) is defined as any unfavorable experience that participants encounter during the study period, regardless of its relation to the treatment.

Predefined AEs specific to the axilla include postoperative bleeding or infection, lymphedema of the upper limb or chest wall, neuralgia, sensory abnormalities, decreased mobility, muscle weakness, or pain in the arm and shoulder. The severity of AEs is categorized as mild, moderate, or severe, based on the CTCAE 5.0 criteria (Common Terminology Criteria for Adverse Events V.5.0). A serious adverse event (SAE) is defined as an adverse medical occurrence or effect related to surgical treatment that results in hospitalization, prolonged hospital stay, or death.

In this study, the procedural risk is low, and the likelihood of an SAE is minimal. In the event of BCRL and radiotherapy-related adverse effects, symptomatic treatment is typically sufficient. The principal investigators of this study are responsible for evaluating SAE, which should be reported to the approved medical ethics committee within 15 days of occurrence. For fatal or life-threatening cases, there is a maximum of 7 days for an initial report and 8 days for a completed report. All SAEs should be followed up until they have subsided or reached a steady state. Follow-up may necessitate additional tests or medical procedures and/or referral to a general practitioner or medical specialist, depending on the specific event.

Ethics and dissemination

The study will be conducted in strict compliance with the principles of the Declaration of Helsinki, and all patient data will be processed anonymously. The study has received approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Ethical review number: 2023-SR-169). Any

amendments to the protocol, informed consent, and other study materials will only be implemented after receiving approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, and these changes will be notified to the trial registry. The findings from the study will be disseminated through academic conferences and published in peer-reviewed journals.

DISCUSSION

Although de-escalation procedure like standardized SLNB and TAD is widely widely recognized, based on their acceptable FNR, lack of data on oncological outcome, especially TLNB, remains to be a problem. In clinical practice, the management of axillary surgery in patients with apCR exhibits significant heter ogeneity. A recent survey[12] conducted by the European Breast Cancer Research Association of Surgical Trialists, which included 349 breast surgeons and onc ological radiologists across 45 countries, revealed that a substantial proportion of surgeons still advocate for ALND in patients with ypN0 (22.4% support in cN1 patients, 45.1% in cN2), due to the lack of long-term prognostic data on local control and survival in axillary de-escalation procedures such as SLNB, T AD, and TLNB.

Several studies[13-17] have published data on recurrence or survival outcome in p atients with apCR after NST who were exempt from ALND and only underwe nt SLNB or TAD. However, there are few prospective studies that have report ed safety data for the TLNB procedure, indicating an urgent need for such stu

dies.

Ongoing trials, including ATNEC (NCT04109079), MINIMAX[28], AXSANA[29], a nd our SrLNB, are expected to provide safety evidence for axillary de-escalatio n surgery and expedite its clinical application. The ATNEC study enrolls patie nts with cN1 and performs SLNB without ALND in apCR patients. The MINI MAX trial includes cN1-3 patients and axillary de-escalation surgery will be e mployed according to local protocol. The AXSANA study utilizes a variety of marking techniques with the goal of identifying the optimal procedure.

Compared to these studies, our SrLNB study offers several advantages: First, S rLNB has the benefits of using carbon tattooing, including eliminating the need for a second localization prior to the surgery, avoiding the use of radioactive particles, and providing a long, identifiable period of black stained lymph node s by carbon nanoparticles that lasts up to half a year. Secondly, our preliminar y trial with the SrLNB procedure have confirmed a high detection rate and a FNR below the threshold value (10%). More importantly, our study places grea ter emphasis on axillary morbidity and QoL. BCRL rate and PRO will be repo rted using multiple objective and subjective measurements. This study also has several limitations. This is a single-center study with a limited sample size. W e hope to expand the experimental center and increase the number of enrollme nts after achieving preliminary results.

Trial status and Time plan

The protocol version number and date are V3.0 and May 16, 2023, respectively. The study was conceived and designed in 2022. The first patient was enrolled on September 11, 2023, and the study is currently in the patient recruitment phase. Enrollment is projected to be completed by August 2025, followed by a 3-year follow-up period, which is expected to be preliminary completed by October 2026.

Acknowledgement

We thank all participants who volunteered to take part in this study and all investigators who have contributed to this study.

Contributors

All authors are from The First Affiliated Hospital with Nanjing Medical University. Jue Wang and Xiaoming Zha were the principal investigators of this study, they proposes the preliminary conception and conduct a tentative design, financial support is also provided. Lingjun Ma and Xuan Li developed the detailed protocol, Rui Chen and Mingyu Wang helped to register the trial and obtain ethical approval. Lexin Wang, Ran Zheng，Jingjing Ding，Hao Yao and Xingye Sheng were responsible for patient recruitment and data collection, Yichun Gong, Jingjing Ding and Yuanyuan Wang were responsible for data checking and data monitoring. Lingjun Ma drafted the initial manuscript. Jue Wang is responsible for the overall content as guarantor. All authors critically revised the manuscript and approved the final study protocol.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Written consent obtained directly from patients.

Data availability statement

Data sharing is currently not applicable to this article as the study is still open for inclusion of patients. However, we are willing to consider requests for data sharing on a case-by-case basis. Researchers interested in accessing the data should contact the corresponding author with a detailed proposal outlining the purpose of their request.

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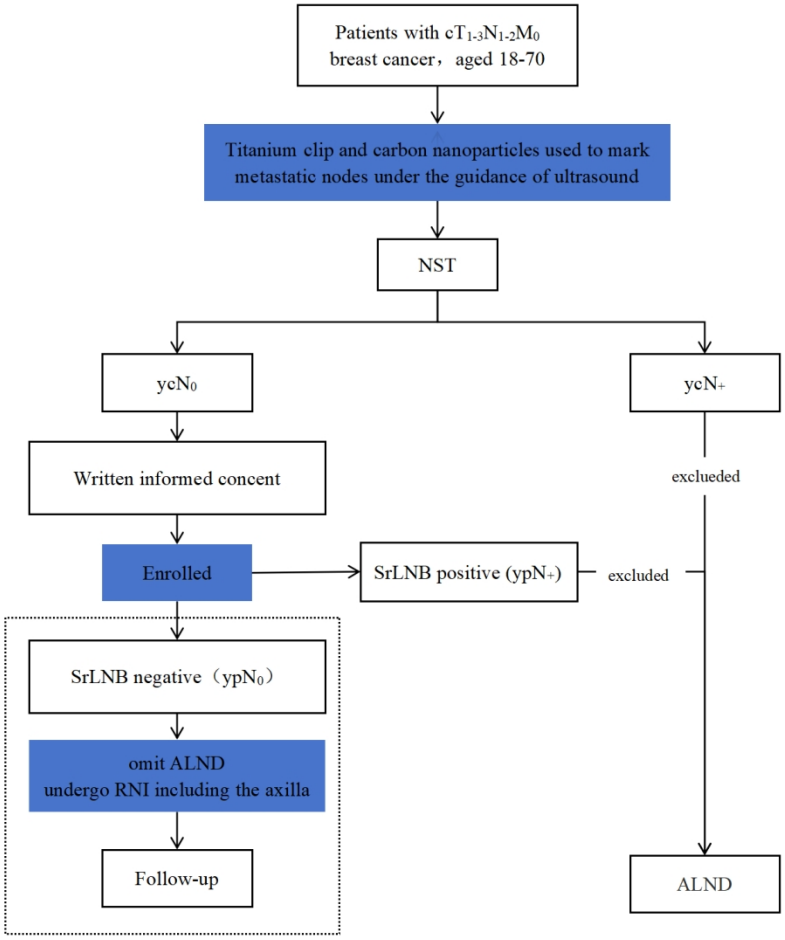
Figure Legends

Figure 1. Flow chart of SrLNB trial. cTNM, clinical classification of TNM; NST, neoadjuvant systematic therapy; SrLNB, Stained Region Lymph Node Biopsy; ypN0, pathologically assessed lymph node negative after NST; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy.



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Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

Informed Consent Form

Version:V2.0

Version date: June 5, 2023

Patient initials:

Patient study number:

Patient contact number:

Patient address:

Information Disclosure Page

Participant Information Sheet

Dear Patient,

We invite you to participate in the phase II clinical trial to exempt from axillary lymph node dissection (ALND) for breast cancer patients achieving axillary complete response (apCR) after neoadjuvant therapy. This study is initiated and led by Director Jue Wang and Xiaoming Zha, and will be conducted in the Department of Breast Diseases, Jiangsu Province Hospital. 92 subjects is expected to participate voluntarily. This study has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital). Ethical Review number is 2023-SR-169.

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

1. Why is this study conducted?

Up to 40% of patients with positive axillary lymph node breast cancer may convert to negative (ypN0) after NST. However, the current clinical guidelines still recommend ALND, which will bring about more complications, such as lymphedema, dysfunction, pain and numbness in affectedl upper limb and the axilla, to ypN0 patients. Actually, it is feasible to downgrade axillary surgery in such patients, with high requirements to technical and equipment, such as: (1) using dual tracers (dye and radionuclide) for Sentinel Lymph Node Biopsy (SLNB); (2) $\geqslant$3 axillary lymph nodes must be removed; (3) titanium clips must be used before neoadjuvant therapy to localize metastatic axillary lymph nodes and ensure that the localized lymph nodes will be removed at the time of surgery. However, radioactive particles are under strict supervision in most

countries and regions around the world (including our country), which prevents the vast majority of countries and regions from performing axillary downstaging procedures.

Team of Director Xiaoming Zha has been conducting clinical research since 2019, and successfully developed Stained Region Lymph Node Biopsy (SrLNB) to downstage axillary surgery. In SrLNB procedure, titanium clips is used to mark positive lymph node, and carbon nanoparticle suspension will also be injected under the guidance of ultrasound for staining at the time of diagnosis. When surgery after completion of NST, clipped and the stained lymph nodes will be biopsied. Data from the our previous study showed that the false-negative rate of stained-area lymph node biopsy was 5.3%, much lower than the generally recognized threshold of 10%, suggesting that SrLNB is safe and feasible.

In this study, team of Dr. Zha intends to enroll patients with positive axillary lymph nodes at diagnosis and receiving NST to undergo SrLNB. When SrLNB turns out to be negative, ALND is exempted, and then undergo axillary area radiotherapy after surgery. The physician team will further evaluate the efficacy and long-term safety of the procedure by following up and monitoring data such as local recurrence and complication rates.

2. Who is eligible (or not) to participate in the study?

The main inclusion criteria includes: (1) female breast cancer patients aged 18-70 years; (2) pathologically confirmed metastatic axillary lymph nodes at the time of diagnosis, with stage of cN1-3; (3) successful injection of carbon nanoparticle suspension to mark positive lymph nodes.

The main exclusion criteria includes: (1) bilateral breast cancer or during lactating/pregnant period; (2) clinically confirmed distant metastases; (3) unable to complete the subsequent adjuvant therapy in full course as prescribed for various reasons; (4) not eligible for enrollment as judged by the investigator.

3. What need to do if participate in the study?

If you are willing to participate in this study, the surgeon will perform SrLNB. When intraoperative rapid pathology shows positive results (with micrometastases and isolated tumor cells), then you will be excluded from this study and proceed with ALND, other adjuvant

treatments including chemotherapy, endocrine therapy and targeted therapy, will be performed according to guidelines. When intraoperative rapid pathology shows negative results, ALND will be omitted, postoperative regional lymph node radiotherapy (RNI) including axilla will be required, and other adjuvant treatments will be performed as prescribed. When the intraoperative pathology unable to clarify the presence of metastasis, the subsequent axillary will be suspended, and whether to add ALND will be depends on the routine pathology.

We will perform a follow-up within 3 years after surgery, which mainly includes: (1) breast and whole body imaging; (2) upper extremity circumference and bioelectrical impedance examination; (3) quality of life questionnaire.

4. What's the benefit to participate?

Patients who only undergo SrLNB are likely to experience less postoperative complications such as upper limb lymphedema, dysfunction, numbness and pain, and have improved quality of life,compared to ALND patients. In addition, our team will provide several examinations for free (see details in"Do I need to pay for participation in the study?") and closely monitor the results of subsequent visit to provide effective treatment and guidance timely.

5. What's the risk to participate?

SrLNB is the original and latest axillary downstaging procedure developed by team od Dr. Zha, which has been found with good operability and safety in our previous study. However, same as sentinel lymph node biopsy, there is possibility of false-negative results due to missed biopsy to metastatic lymph node, which may increase the possibility of recurrence in the axillary or even distant metastasis. Thus, all patients exempted from ALND in this study will undergo axillary radiotherapy to further eliminate tumor cell that may have been missed, reducing the risk of local recurrence and distant metastasis. Even with the boost of radiotherapy to the axilla and other subsequent systemic treatments, there may be an increased risk of local recurrence and distant metastasis. Our team will closely monitor recurrence and metastasis through regular follow-up, so that timely treatment measures (including complementary axillary surgery, etc.) can be taken.

In case of any discomfort and adverse reactions, please promptly contact the study physician. All agents of chemotherapy, targeted therapy, endocrine therapy, and radiation used in this study are

part of the routine clinical treatment of breast cancer. Even if you do not participate in this study, these side effects/adverse reactions may occur as long as you receive these treatments. In addition, any treatment may failed to take effect, as well as the disease continue to progress due to ineffective treatment or other coexisting diseases.

6. Do I need to pay for participation in the study?

To compensate for any inconvenience this study may cause, bioelectrical impedance analysis, follow-up of quality of life and monitoring instructions will be provided free of charge during this study. The cost of medications for routine breast cancer treatment and follow-up examinations will not be covered. The cost of treatments and tests required for other medical conditions, or switching to other treatments if the treatment failed to take effect, will not be covered as well. Transportation, lost wages, and nutritional expenses are not covered by this study. In case of trial-related damages, appropriate treatment and compensation will be provided in accordance with relevant national regulations.

7. Is personal information confidential?

Your medical records will be kept at the hospital, and only the investigator, research authorities, and ethics committee are allowed to access your medical records. Any public reporting of the results of this study will not disclose your personal identity. Every effort will be made to protect the privacy of your personal medical information to the extent permitted by law.

8. What other treatments are available if not participating in this study?

If not participate in this study, you will undergo ALND, and the incidence and severity of arm mobidity may be higher. However, the subsequent adjuvant treatment will be performed according to clinical guidelines without change.

If you participate in the study but drop out halfway through the follow-up, you will still receive the most standardized and regular treatment.

9. Do I have to participate in the study?

Participation in this study is completely voluntary, and you may refuse to participate or withdraw at any time during the study, which will not affect your treatment. If you decide to withdraw,

please contact the doctor, you may be asked to undergo related tests and complementary procedures (e.g., axillary lymph node dissection, etc.) that may be beneficial to protect your health.

If you have any questions related to your personal rights and interests, you can contact the Ethics Committee of our hospital at 025-68306360.

Informed Consent

Consent Signature Page

SUBJECT DECLARATION: I have read the above description of this study and fully understand the possible risks and benefits of participating in this study. I volunteer to participate in this study. I understand that I will be given a copy of this informed consent form.

I **agree□ or decline□** to other research utilizing my medical records and clinical specimens related to this study.

Patient Signature: _____Date of Signature: _____

Contact phone number: _____

Or

(To be used only if subject is incapacitated)

Signature of Legal Representative: _____

Date of Signature: _____

Relationship to the patient: _____

Contact phone number: _____

INVESTIGATOR DECLARATION: I confirm that I have explained the details of this study, particularly the possible risks and benefits of participating in this study, and answered all relevant questions from the subject, who has voluntarily agreed to participate in this study. This informed consent form is in duplicate, with one signed copy retained by the investigator and one by the subject.

Signature of Investigator: _____ Date of Signature: _____

Contact phone number: _____

知情同意书

尊敬的女士：

我们邀请您参加南京医科大学第一附属医院（江苏省人民医院）批准开展的“新辅助治疗后腋窝淋巴结阳性转阴性的乳腺癌患者豁免腋窝淋巴结清扫的 II 期临床研究”。本研究由乳腺病科查小明主任发起和负责，将在江苏省人民医院乳腺病科开展，预计将有 92 名受试者自愿参加。本研究已经得到南京医科大学第一附属医院（江苏省人民医院）伦理委员会的审查和批准，伦理审查编号：2023-SR-169。

为什么要开展本项研究？

腋窝淋巴结阳性的乳腺癌患者经新辅助治疗（Neoadjuvant Systemic Therapy，NST）后可有高达 40%的比例转为阴性（ypN0）。**然而即使淋巴结转为阴性，目前的临床指南仍然首推腋窝淋巴结清扫术（axillary lymph nodes dissection，ALND）**，而这也会带来更多的后遗症，如上肢淋巴水肿、上肢活动障碍、上臂内侧和腋窝疼痛麻木等。**实际上对此类患者进行腋窝淋巴结手术的降级是可行的，但是技术和设备的要求很高**，比如：(1) 使用双示踪剂（染料和放射性核素）进行前哨淋巴结活检（Sentinel Lymph Node Biopsy，SLNB）；(2) 必须切检≥3 枚腋窝淋巴结；(3) 必须在新辅助治疗前用钛夹定位转移的腋窝淋巴结，并且在前哨淋巴结手术时要确保取到钛夹定位的淋巴结。**由于放射性核素在全世界大多数国家和地区（包括我国）都要受到严格的监管，因此仅此一个要求就使绝大多数国家和地区无法开展腋窝的降级手术**。

查小明主任团队从 2019 年就开始开展临床研究，成功原创了染色区淋巴结活检术（Stained region Lymph Node Biopsy，SrLNB），即在初诊时经超声引导将纳米炭混悬注射液（卡纳琳）注射到腋窝阳性淋巴结的皮质中进行染色标记，在新辅助治疗完成后进行手术时活检染色区域的淋巴结。**前期临床研究数据初步显示染色区淋巴结活检术的假阴性率为 5.5%，远低于国际公认的 10%的阈值，初步证明染色区淋巴结活检术是安全可行的**。

在本研究中，查小明医生团队拟入组初诊腋窝淋巴结阳性且接受新辅助治疗的患者，行染色区淋巴结活检术，如为阴性则豁免腋窝淋巴结清扫手术，术后行腋窝区放疗。医生团队会通过随访和监测局部复发情况、并发症率等数据，**进一步评估染色区淋巴结活检手术的有效性和长期安全性**。

哪些人适（不）宜参加研究？

入选标准主要有：(1) 18-70 周岁的女性乳腺癌患者；(2) 初诊时经病理证实腋窝淋巴结

为阳性，N 分级为 cN1-3；(3) 阳性淋巴结经纳米炭混悬注射液成功注射标记。

排除标准主要有：(1) 双侧乳腺癌/哺乳期/孕期乳腺癌；(2) 临床或影像学证实存在远处转移；(3) 各种原因导致无法按医嘱足疗程完成后续辅助治疗者；(4) 经研究者判断不符合入组者。

如果参加研究，需要做什么？

如果您愿意参加本项研究，医生会在术中行染色区淋巴结活检术。如果术中快速病理显示腋窝淋巴结阳性（含微转移和孤立肿瘤细胞）则排除出本研究，并继续行腋窝淋巴结清扫手术，术后的其他综合治疗（如化疗、内分泌治疗、靶向治疗等）按照指南进行。如果术中快速病理显示腋窝淋巴结阴性则不做腋窝淋巴结清扫术，术后需要接受包含腋窝的区域淋巴结放疗（RNI），术后的其他综合治疗也按照指南进行。如果术中快速病理无法明确有无转移，则会暂停后续的腋窝淋巴结清扫术，等常规病理出来后再决定是否加做腋窝淋巴结清扫术。

我们会在手术治疗结束的 3 年内对您进行随访，随访内容主要包括：(1) 乳腺及全身影像学检查；(2)上肢周径和生物电阻抗检查；(3) 生活质量量表问卷调查等。

参加研究有哪些好处？

只接受染色区淋巴结活检术的患者，术后发生淋巴水肿、上肢功能障碍、麻木疼痛等后遗症的概率及其严重程度大概率会明显降低，生活质量也可能提高。另外，本团队也会提供部分免费检查（见“参加研究需要支付有关费用吗”），并密切关注复查结果，以便及时提供有效的治疗和指导。

参加研究有哪些风险？

染色区淋巴结活检术是查小明医生团队原创的、最新的腋窝淋巴结活检手术，前期研究发现其具有较好的操作性和安全性。但正如前哨淋巴结活检术一样，染色区淋巴结活检术同样会存在漏检导致假阴性结果的可能。因漏检而残留在腋窝的转移淋巴结有可能会增加腋窝区的复发率甚至远处转移率，因此本研究中所有豁免腋窝淋巴结清扫手术的患者都需要接受腋窝放疗，以进一步消灭可能漏检的阳性淋巴结，从而减少局部复发和远处转移的风险。当然，即使增加了放疗和后续其他全身治疗，也可能会出现局部复发和远处转移的风险增高，医生团队将会通过定期密切随访，及时监测疾病复发、转移等情况，以便及时采取治疗措施（包括补充腋窝手术等）。

如果出现任何不适和不良反应，请及时与研究医生联系。本研究方案中使用的所有化疗

药、靶向药、内分泌药物以及放疗等都属于临床治疗乳腺癌的常规方式，即使您不参加本临床研究，只要接受该治疗方法，就有可能发生这些副作用/不良反应。此外，任何治疗都可能出现无效的情况，以及因治疗无效或者因合并其他疾病等原因而导致病情继续发展。

参加研究需要支付有关费用吗？

为了补偿本研究可能给您带来的不便，本研究期间将免费提供生物电阻抗检测，免费进行生活质量随访和随访监测指导。乳腺癌常规的治疗药物以及随访检查费用不在免费范围之内。如果您同时合并其他疾病所需的治疗和检查，以及因治疗无效而改用其他治疗的费用，也不在免费范围之内。本研究不提供交通费、误工费、营养费等。如果出现试验相关的损害，将依据国家有关规定提供相应的治疗与补偿。

个人信息是保密的吗？

您的医疗记录将保存在医院，研究者、研究主管部门、伦理委员会将被允许查阅您的医疗记录。任何有关本项研究结果的公开报告将不会披露您的个人身份。我们将在法律允许的范围内，尽一切努力保护您个人医疗资料的隐私。

如果不参与本研究还有哪些治疗方法？

如果您不参加本项研究，则接受标准的腋窝淋巴结清扫手术，腋窝后遗症的发生率和严重程度可能会较高。但后续的综合治疗会按照临床指南的要求进行，不会有任何改变。

如果您参加了研究，但后续中途退出了，您仍会得到最标准正规的治疗。

我必须参加研究吗？

参加本项研究是完全自愿的，您可以拒绝参加研究，或在研究过程中的任何时间退出本研究，这都不会影响医生对您的治疗。如果您决定退出本研究，请与您的医生联系，您可能被要求进行相关检查和补充手术（如腋窝淋巴结清扫术等），这对保护您的健康是有利的。

如您有涉及个人权益方面的问题可与本院伦理委员会联系，联系电话：025-68306360。

受试者声明：我已经阅读了上述有关本研究的介绍，对参加本研究可能产生的风险和受益充分了解。我自愿参加本研究。我将获得一份签署姓名和日期的本知情同意书副本。

我同意□ 或拒绝□ 其他研究利用我的与本研究相关的医疗记录和临床标本。

受试者签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

受试者的联系电话：_____ 手机号：_____

（适用时）法定监护人/见证人签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

法定监护人/见证人联系电话：_____ 手机号：_____

研究者声明：我确认已向受试者解释了本研究的详细情况，特别是参加本研究可能产生的风险和受益，并回答了受试者的所有有关问题，受试者是出于自愿同意参加本研究。此知情同意书一式两份，研究者与受试者各留一份已签字的知情同意书。

研究医生签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

研究医生的工作电话：025-68308172 手机号：_____

BMJ Open

Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

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Primary Subject Heading:	Surgery
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Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathologic Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

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ABSTRACT

Introduction

Given that ALND may not contribute to local control or survival and could lead to increased arm morbidity, axillary de-escalation procedures have replaced ALND in patients achieving axillary pathologic complete response (apCR) after neoadjuvant systemic therapy (NST). However, the application of targeted lymph node biospy (TLNB), one of de-escalation procedures, remains limited due to a lack of long-term follow-up studies..

Methods and analysis

This prospective, single-arm, open-label, non-inferiority, single-center phase II trial targets breast cancer patients initially diagnosed with axillary metastasis who achieved apCR after NST. The study aims to validate the oncological safety of Stained Region Lymph Node Biopsy (SrLNB) procedure. SrLNB is a novel de-escalation axillary surgery, which was developed and tested in our preliminary study. The primary endpoint of this trial is the 3-year invasive disease-free survival (iDFS). Secondary endpoints include local-regional recurrence (LRR), incidence of breast cancer-related lymphedema (BCRL), and patient-reported outcomes (PRO). The 3-year iDFS in patients undergoing ALND is expected to be approximately 90%, with a non-inferiority margin of 10%, a significance level of 0.05, power of 0.8, and a loss-to-follow-up rate of 10%. The planned enrollment is 92 patients. The trial was initiated on September 11, 2023, with the first patient enrolled on September 25, 2023, and is scheduled to end in 2026.

Ethics and dissemination The trial protocol received approval from the Human Research Ethics Committee of The First Affiliated Hospital with Nanjing Medical University in May 2023 (Reference 2023-SR-169). All participants will provide informed consent. The study results will be disseminated through international peer-reviewed scientific journals, presentations at international scientific conferences, and public lectures.

Trial registration NCT05939830

Key words: Breast cancer, Neoadjuvant Systemic Therapy, Axillary Surgery , Axillary lymph node dissection, Disease-free survival, Breast Cancer Related Lymphedema, Quality of life

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The SrLNB procedure is economical and easy to conduct with the use of carbon tattooing, and can be considered a further de-escalation of the standardized SLNB procedure.
- The SrLNB study places greater emphasis on arm morbidity and quality of life (QoL) with multiple objective and subjective measurements.
- The small sample size and the single-center design of the study may lead to some limitations in the extrapolation of the results.

INTRODUCTION

Axillary lymph node dissection (ALND) has been replaced by Sentinel Lymph Node Biopsy (SLNB) for cN0 patients (clinically axillary lymph node negative), based on randomized studies showing that ALND does not improve oncological outcomes but is associated with substantial upper limb morbidity[1]. Axillary de-escalation surgery can reduce surgical morbidity rate (i.e., lymphedema, chronic pain, mobility restrictions) and improve patients' quality of life (QoL).

For patients with cN+ at the time of diagnosis, neoadjuvant systemic therapy (NST) is often the option. With great advances in NST, axillary pathologic complete response (apCR) has been observed in 13-60%[2] of patients with axillary metastases at the time of diagnosis in different molecular subtypes, especially in 59% of HER2+ patients and 48% of triple negative patients (TNBC)[2]. For patients with axillary nodes remaining clinically positive (ycN+) after NST, ALND of levels I-II remains the standard management, whereas in patients with lymph nodes downstaged to negative (ycN0), the necessity of conventional ALND has been questioned. To date, ALND has been replaced by de-escalation procedures like standardized SLNB or targeted axillary dissection (TAD).

To replace ALND with de-escalation procedures, one of the key points lies in the false negative rate (FNR). ACOSOG Z1071[3], SN FNAC[4], and SENTINA[5] studies modified SLNB by use of dual tracing agents and removal of ≥3 sentinel nodes to make FNR below the threshold target of 10%. In TAD procedure[6], suspicious lymph node marked by various methods before NST will be biopsied (targeted lymph node

biospy, TLNB) along with SLNB, which can reduce the FNR to 2%. A study by Donker et al.[7] proposed the MARI procedure, marking the axillary lymph node with radioactive iodine (^{125}I) seeds, where only TLNB were dissected without SLNB, with an acceptable FNR of 7%, thus TLNB alone can be considered as an option for de-escalation procedure. In addition, various methods of marking and localizing metastatic lymph nodes have been developed, such as iodine-125 seed[8], carbon tattooing[9] and Magseed[10]. However, the optimal marking and localizing methods for TLNB are under discussion due to a lack of comparative data. Additionally, the use restriction of radioactive particles makes standardized SLNB, TAD, and relevant TLNB unrealistic in China and some other countries.

Based on prior studies and clinical practices in China, we developed SrLNB as a modification of TLNB. When conducting fine-needle aspiration (FNA), a suspension of carbon nanoparticles (0.1–0.2 mL, LUMMY®, Chongqing, China) will be injected into the cortex of the biopsied nodes immediately. SrLNB requires removing all lymph nodes within the stained region and retrieving abnormally palpated lymph nodes near the stained region. Data from our previous research indicated that SrLNB has a high detection rate and a FNR below the threshold value (10%)[11]. This suggests good diagnostic performance and the potential to downstage axillary surgery. The SrLNB procedure offers several benefits with the use of carbon tattooing: eliminating the need for a second localization prior to surgery, free of radioactive particles, allowing for the completion of axillary surgery at one time, and providing a long identifiable period (up to half a year) for black-stained lymph nodes by carbon

nanoparticles. SrLNB also has the drawback of increased axillary tissue trauma due to the need for axillary dissection to visually identify the marked node. Additionally, it may cause black staining of axillary tissue and dye migration, although these risks are minimized by controlling the amount of carbon nanoparticles. Despite these limitations, SrLNB remains a highly promising approach for axillary de-escalation surgery.

Nevertheless, oncology outcomes, including local control and survival, must be considered when only de-escalation axillary surgery such as SLNB, TAD, TLNB, or SrLNB is performed without ALND. To date, several retrospective and prospective studies[12-17] have reported prognostic data for standardized SLNB or TAD, while robust evidence regarding the oncological prognosis of TLNB remains limited. A review reported a low axillary recurrence of 0%–3.4%[18], and a meta-analysis[19] reported a pooled 5-year DFS of 86% in SLNB group, with no significant difference to the ALND group (p = 0.24). In the study of Si-Yu Wu et al.[17], 152 patients who received TAD had a 3-year recurrence-free survival of 100%, with no significant difference from the ALND group (p = 0.254). The SenTa study[16] also reported a 3-year iDFS of 91.2% in 119 patients, and only TAD without ALND was not associated with the risk of recurrence or death (p = 0.69, p = 0.91, respectively). However, this was an observational study without treatment intervention, and axillary metastasis was clinically diagnosed without pathological examination.

Regarding TLNB, there is very little high-quality evidence of long-term prognostic outcome, limiting its clinical application. Therefore, this study is designed to assess

the oncological safety of SrLNB procedures. Breast cancer patients with pathologically confirmed axillary metastasis (pN+) who are clinically downstaged to negative (ycN0) after NST will be enrolled. Among them, ypN0 patients identified by SrLNB will not undergo ALND and will subsequently receive regional lymph node radiotherapy (RNI) including the axilla, while ypN+ patients will be excluded and undergo ALND. The oncological safety of SrLNB will be primarily evaluated by a 3-year follow-up of invasive disease-free survival (iDFS) and locoregional recurrence rate (LRR). This will also add evidence to the oncological safety of TLNB. Additional focus will be given to the incidence of breast cancer-related lymphedema (BCRL) and QoL, which will provide subjective perspective to assess the potential of SrLNB in reducing arm morbidity and QoL.

METHODS AND ANALYSIS

Study design and setting

The SrLNB is a prospective, single-arm, single-center, open-label phase II clinical trial. It is currently being conducted by the First Affiliated Hospital with Nanjing Medical University in China. Women with FNA-confirmed lymph node metastases, downstaged to ypN0 after NST and confirmed by SrLNB, will be enrolled and will not undergo ALND. Regional lymph node radiotherapy (RNI) covering the axilla (including the supraclavicular region and axillary regions I, II, and III) will be administered, followed by a 3-year follow-up. Prophylactic irradiation of the internal mammary node (IMN) drainage area will be added when necessary. It is expected that

the 3-year iDFS in patients undergoing ALND is about 90%, and a non-inferiority margin value of 10%, with a significance level of 0.05 and power of 0.8, loss to follow-up rate of 10%, the planned enrollment is 92 patients. Enrollment is expected to be complete within 2 years, with follow-up scheduled to complete in 2026.

The primary aim of this study is to assess the 3-year invasive disease-free survival (iDFS) of ypN0 patients receiving SrLNB without ALND. Secondary endpoints include the LRR, BCRL rate, QoL, and patient-reported arm morbidity. BCRL will be assessed by relative volume change (RVC) and single-frequency bioimpedance analysis (SFBIA). QoL will be assessed by FACT-B (V4.0) and patient-reported arm morbidity will be assessed by QuickDASH questionnaire. This study evaluates the local control and oncological outcomes of the SrLNB procedure, with additional focus on arm morbidities and quality of life.

The study was reported according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) checklist[20] . The detailed flow chart of the study is shown in Figure 1. The trial schedule based on SPIRIT checklist is presented in Table 1.

Table1. Schedule of enrolment, interventions, and follow-up for each participant.

	Study Period						
	Screening	Enrollment and treatment	Follow-up				
Timepoint	**-1w**	**0 (SrLNB)**	**1w**	**1m**	**3m**	**6m**	**1-3y**
Enrollment							
Eligibility screening*	×	×					
Informed consent†	×						
Physical examination†	×				×	×	×
Imaging examination†	×				×	×	×
Frozen section to SrLNB specimen‡		×					
Interventions							
SrLNB without ALND		×					
RNI including the axilla		×					
Breast surgery		×					
Adjuvant systemic therapy		×					
Assessments							
Survival outcome			×	×	×	×	×
Disease status			×	×	×	×	×
Arm circumference			×	×	×	×	×
SFBIA	×		×	×	×	×	×
Questionnaire FACT-B	×		×	×	×	×	×
Questionnaire QuickDASH	×		×	×	×	×	×

*Eligibility screen includes confirmation of positive axillary lymph nodes successfully marked by carbon nanoparticles, and completion of NST.

†Physical examination and imaging examination are used to screen patients' responses to NST. They will be pre-enrolled, and sign an written informed consent (see Supplementary File 1). Physical examination mainly includes palpation of the breast and axillary lymph node. Imaging examination mainly includes breast and axillary ultrasound and MRI.

‡Pre-enrolled patients with negative result in intraoperative rapid pathological examination to SLNB specimen will be enrolled, while with residual disease in the axilla will be excluded.

SrLNB, Stained Region Lymph Node Biopsy; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy; SFBIA, single-frequency bioimpedance analysis;

Trial participants and inclusion/exclusion criteria

Inclusion criteria

Patients who meet the following criteria are eligible for inclusion:

1. females, aged between 18 and 70.

2. histologically confirmed invasive breast cancer (regardless of pathological type) with a clinical stage of cT1-3.

3. pathologically confirmed positive axillary lymph nodes with a clinical stage of N1-3.

4. received a full course of NST (including neoadjuvant chemotherapy, neoadjuvant targeted therapy, neoadjuvant immunotherapy).

5. positive axillary lymph nodes successfully marked stained by carbon nanoparticle suspension.

6. all patients are required to undergo immunohistochemical staining (IHC) for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor2 (HER2), Ki-67 proliferation index, and further fluorescence in situ hybridization should be performed in HER2 2+ cases.

7. metastatic axillary lymph nodes downstaged to clinically negative after NST (ycN0) assessed by ultrasound and MRI.

8. ECOG score of 0–1.

9. patients voluntarily participated in this study and signed the informed consent form (see Supplementary File 1).

Exclusion criteria

Patients will be excluded on any of the following grounds:

1. bilateral breast cancer.

2. breast cancer during lactation or pregnancy.

3. presence of distant metastases confirmed by physical examination or imaging examination.

4. previous history of a malignant tumor.

5. history of previous surgery on the affected axilla, or history of surgery affecting the function of the upper extremity.

6. history of radiation therapy to the breast or chest.

7. positive surgical margins for breast-conserving surgery/mastectomy.

8. positive results of intraoperative frozen-section evaluation (including isolated tumor cells and micrometastases) for SrLNB (ypN+).

9. inability to complete the full course of adjuvant therapy as prescribed.

10. aspartate transaminase (AST) or alanine transaminase (ALT) ≥1.5 times the upper limit of normal, alkaline phosphatase (ALP) ≥2.5 times the upper limit of normal, total bile ≥ 1.5 times the upper limit of normal, serum creatinine ≥1.5 times the upper limit of normal; left ventricular ejection fractions (LVEF) <50% on cardiac ultrasound; severe coagulation dysfunction, serious systemic disease, or uncontrolled infection.

11. lack of personal freedom and independent civil capacity.

12. presence of mental disorders, addictions, and those deemed ineligible by the

investigator.

Sample size

This study is a single-arm objective performance criteria study, with the 3-year iDFS of ypN0 patients receiving standard treatment (ALND and RNI) as the target, which is expected to be 90% as described in previous studies[12-19 21-24]. A non-inferiority test will be used to evaluate the 3-year iDFS with the margin value of 10%, with a significance level of 0.05 and power of 0.8 . Assuming a 10% rate of loss to follow-up, the required sample size is 92 (calculated by PASS 15.0 software). In light of this, a 3-year iDFS >80% for enrolled patients who underwent SrLNB is considered non-inferior to ALND in terms of oncology safety, and SrLNB followed by RNI including the axilla is non-inferior to the standard of care.

Intervention

NST

All enrolled patients will receive NST, which will be determined by the molecular subtype as clarified by the status of ER, PR and HER2. ER and PR positive are both defined as ≥ 1% of the invasive tumor cells staining positive via IHC. HER2 positive is defined as HER2 3+ and HER2 2+ by IHC with the presence of HER2 amplification by FISH (ISH+). The chemotherapy regimen combines sequential paclitaxel and anthracycline, with trastuzumab and/or pertuzumab added for HER2-positive patients. The course of NST typically spans 6–8 cycles and will be

individually adjusted.

Breast surgery

Breast-conserving surgery or mastectomy is selected based on tumor characteristics and patient preference. Patients who undergo breast-conserving surgery will receive whole-breast radiotherapy (WBI) afterwards.

Axillary surgery

SrLNB procedure

Before the NST, the most abnormal lymph node will be selected for FNA. At the same time, carbon nanoparticle suspension (0.5 mL containing 25 mg; LUMMY®, Chongqing Lummy Pharmaceutical Co. Ltd, China) will be diluted (concentration: 0.05 mL per 0.15 mL saline) and injected into the cortex of the biopsied lymph node under ultrasound guidance to ensure that the biopsied lymph node is accurately stained and marked. Only the most abnormal node will be stained, unless multiple abnormal lymph nodes are distributed across a wide area, in which case the closest and most distal nodes within the affected region will be stained to delineate the surgical field boundaries. Lymph nodes will turn black after staining. During surgery, SrLNB involves the removal of the stained lymph node (or the stained region in cases of widely distributed multiple abnormal lymph nodes), along with any abnormally palpated lymph nodes near the stained node. Those with negative results of intraoperative pathology will be enrolled, while those with macro-metastases, micrometastases, or isolated tumor cells will be excluded from this study.

Axillary lymph node dissection

Enrolled patients will be exempted from ALND, which includes axillary lymph nodes in region I and II. However, patients with residual axillary disease will routinely undergo ALND.

Radiotherapy

All patients enrolled will undergo whole-breast radiotherapy (WBI) or postmast ectomy radiotherapy (PMRT) according to breast surgery, and regional lymph n ode radiotherapy (RNI) including the axilla, regardless of treatment response to systemic therapy. Part of patients will receive prophylactic irradiation of the i nternal mammary node (IMN) drainage area when necessary.

Those undergoing breast-conserving surgery will receive WBI, with a boost to the tumor bed delivered sequentially immediately after. The prescribed dose for conventional fractionated irradiation is 50 Gy in 25 fractions with a single do se of 2 Gy, administered 5 times a week. The dose to the tumor bed is 60 G y in 25 fractions with a single dose of 2.4 Gy. Alternatively, when a hypofrac tionated regimen is applied, the dose will be 43.5 Gy in 15 fractions of 2.9 G y over 3 weeks, and the dose to the tumor bed will be 52.5 Gy in 18 fractio ns of 2.9 Gy.

PMRT is defined as radiotherapy to the chest wall for those undergoing maste ctomy. The dose is 50 Gy/25 F (50 Gy in 25 fractions) for conventional fracti onated irradiation or 43.5 Gy/15 F for hypofractionated irradiation.

RNI including the axilla covers the supraclavicular region and the entire axillary lymphatic drainage area (region I, II, and III), with a dose of 50 Gy/25 F.

IMN is added if any of the following criteria are met: (1) ≥4 positive axillary lymph nodes; (2) primary tumor located centrally or in the medial quadrant of the breast, or posterior to the nipple; (3) imaging-diagnosed suspicious metastasis of IMN, or pathologically confirmed metastatic IMN.

Adjuvant systemic treatment

Systemic adjuvant therapies will be administered in accordance with local and international guidelines. These therapies include chemotherapy, targeted therapy, endocrine therapy, and immunotherapy. Luminal patients mainly receive epirubicin and cyclophosphamide followed by docetaxel (EC-T), combined with endocrine drugs. HER2+ patients mainly receive epirubicin and cyclophosphamide followed by docetaxel, trastuzumab and pertuzumab (EC-THP), or docetaxel, carboplatin, trastuzumab and pertuzumab (TCbHP). To TNBC, docetaxel and carboplatin, followed by epirubicin and cyclophosphamide, with or without pembrolizumab, is the main regimen. The regimen will be individually adjusted based on the actual situation.

Follow-up

Follow-up will commence after surgery, with a 3-year follow-up as the primary endpoint. The schedule for follow-up examinations is as follows:

1. Physical examination: every 6 months;

2. Imaging examination: bilateral breast and axillary ultrasound every 6 months ; mammography with or without MRI annually for patients who have undergone breast-conserving surgery; Chest X-ray or CT, abdominal ultrasound, bone scanning, and head MRI will be conducted annually when necessary;

3. Objective assessment of BCRL: Relative Volume Change (RVC) and single-frequency bioimpedance analysis (SFBIA) will be tested and calculated at 1 week, 1, 3, 6, 12, 24, and 36 months post-operation;

4. Patient-reported questionnaires: FACT-B and QuickDASH will be surveyed at 1 week, 1, 3, 6, 12, 24, and 36 months postoperatively.

Endpoints

Primary endpoint

The primary endpoint of the trial is invasive disease-free survival (iDFS), defined as the time interval from the surgery to invasive LRR, distant metastasis, contralateral invasive breast cancer, or death from any cause. iDFS will be calculated after a 3-year follow-up. Patients without an event will be censored at the date of the last available assessment.

Secondary endpoints

1. LRR

This includes both local and regional recurrence. Local recurrence is defined as recurrence in the ipsilateral breast, chest wall, skin, or surgical scar. Regional recurrence is defined as recurrence in the affected lymphatic drainage area, in

cluding the axilla, supraclavicular region, subclavicular region, and IMN area. LRR will be calculated after a 3-year follow-up.

2. Breast Cancer Related Lymphedema (BCRL)

In our study, two objective methods will be used to assess upper limb lymphedema. If either criterion is met, BCRL will be diagnosed. In the event of inconsistency, the former results will prevail.

(1) RVC >10% in the affected upper extremity.

The volume of the upper limb is calculated based on arm circumference, where 5 points were taken to measure at 10cm intervals from ulnar styloid to 40cm away. Five points divide upper limb into 4 truncated cones. For each truncated cones, arm circumference of both ends are recorded as C1 and C2, the volume formula is[25]: $V = h(C_1^2+C_2^2+C_1C_2)/12\pi$ (h is the length, which was 10cm in the study). The volume of the upper limb is the sum of four truncated cones. The baseline volume of both upper limbs is measured and calculated preoperatively, with the volume of the affected arm recorded as A1 and the unaffected arm as U1. At the time of follow-up, the arm volume is calculated in the same way, with the affected arm recorded as A2 and the unaffected arm as U2. Then, the RVC can be calculated as follows: $RVC = (A_2/U_2)(A_1/U_1) - 1$. When RVC > 10%, BCRL will be diagnosed[26]. Follow-up of RVC will be conducted at one week and 1, 3, 6, 12, 18, 24, 36 months after the operation.

(2) Bioelectrical Impedance Analysis (BIA)

The SFBIA ratio is defined as the ratio of the impedance of the unaffected upper limb

to the affected one. BCRL is diagnosed when the impedance ratio exceeds the threshold value of the mean ± 2SD (standard deviation) of healthy controls. The impedance of both arms is measured by Inbody device at 1 kHz and 5 kHz, and the ratio is calculated. Referring to the study by Liu et al.[27], a diagnosis will be made when any one of the following criteria is met: when the affected arm is the dominant hand, its impedance ratio >1.067 at 1 kHz or >1.068 at 5 kHz; when the affected arm is the non-dominant hand, the ratio > 1.043 at 1 kHz or >1.044 at 5 kHz suggests edema.

3. QoL

PRO will be reported using the Chinese version of FACT-B (V4.0). These scal es will be used to subjectively assess QoL. They will be surveyed before surg ery as a baseline measurement, and follow-up will be conducted at one week and 1, 3, 6, 12, 24, and 36 months postoperative.

4. Patient-reported arm morbidity

Patient-reported arm morbidity will be reported using the Chinese version of Quick Disability of the Arm, Shoulder, and Hand (QuickDASH) questionnaires, They will be surveyed before surgery as a baseline measurement, and follow-up will be conducted at one week and 1, 3, 6, 12, 24, and 36 months postoperative.

Data collection and management

An electronic case report form (eCRF) is used for data management and recording. The principal investigator oversees all projects in this trial. Investigators perform data

collection, entry, correction, and revision. To ensure data accuracy, two data managers will independently perform double entry and proofreading.

Clinical supervisors audit data for completeness, consistency, and standardization. They will challenge any data that is nonsensical or non-standardized. Researchers are expected to respond to and address these challenges promptly, recording the reasons for any modifications. If a participant withdraws consent, no further data will be collected, but existing data will remain anonymized and retained for three years. At the study's conclusion, the principal investigator will submit a final report for review and written approval by the Ethics Committee.

Statistical analysis

Baseline characteristics, including demographics, pathology features, treatment re gimen, BCRL rate, and scores of QoL scales, will be described. For continuou s variables, data will be presented as mean, standard deviation, or median and interquartile range. For categorical variables, data will be analyzed using the ch i-square test or Fisher's exact test, and presented as totals, percentiles, and freq uencies. Continuous variables will be analyzed using the Student's t test or the Mann–Whitney U test.

Kaplan–Meier curves will be established to analyze iDFS and LRR, Hazard ratios (HRs) and 95% confidence intervals (CIs) are calculated. For iDFS with a single-group target value, a non-inferiority test will be performed: the target value of iDFS for the experimental group is set at 90%, with a non-inferiority cut-off value of

10%, α = 0.05, β = 0.2, and an estimated 10% loss rate of follow-up. Univariate and multivariate Cox regression models will be used to analyze impact factors on iDFS and LRR. If the LRR events are few, they could be reported as a crude rate. Differences are considered statistically significant with $p < 0.05$.

Data monitoring

An Independent Data Monitoring Committee (IDMC) will be established for this study and will undergo review every six months. Any SAEs that occur during the trial will be documented following the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0). Investigators are required to report all adverse reactions, suspected adverse reactions, unexpected adverse reactions, and unanticipated events during the trial, in writing to the sponsor and the Ethics Committee within 24 hour of occurrence. Clinical data administrators generate a review report post data cleansing. This report will be discussed at a data review meeting by the principal investigator, statistician, project manager, medical staff, and data management staff, and the IDMC will finalize the statistical analysis population.

Monitoring of Adverse Events

An adverse event (AE) is defined as any unfavorable experience that participants encounter during the study period, regardless of its relation to the treatment. Predefined AEs specific to the axilla include postoperative bleeding or infection, lymphedema of the upper limb or chest wall, neuralgia, sensory abnormalities,

decreased mobility, muscle weakness, or pain in the arm and shoulder. The severity of AEs is categorized as mild, moderate, or severe, based on the CTCAE v5.0 criteria. A serious adverse event (SAE) is defined as an adverse medical occurrence or effect related to surgical treatment that results in hospitalization, prolonged hospital stay, or death.

In this study, the procedural risk is low, and the likelihood of an SAE is minimal. In the event of BCRL and radiotherapy-related adverse effects, symptomatic treatment is typically sufficient. The principal investigators of this study are responsible for evaluating SAE, which should be reported within 15 calendar days to the ethics committee. For fatal or life-threatening cases, there is a maximum of 7 days for an initial report and 8 days for a completed report. All SAEs should be followed up until they have subsided or reached a steady state. Follow-up may necessitate additional tests or medical procedures and/or referral to a general practitioner or medical specialist, depending on the specific event.

Ethics and dissemination

The study will be conducted in strict compliance with the principles of the Declaration of Helsinki, and data are anonymized by removing identifiable information and using unique study codes. The study has received approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Ethical review number: 2023-SR-169). Protocol amendments require prior ethics committee approval and trial registry notification from the Ethics Committee of the

First Affiliated Hospital of Nanjing Medical University, and these changes will be notified to the trial registry. The findings from the study will be disseminated through academic conferences and published in peer-reviewed journals.

DISCUSSION

Although de-escalation procedures have replaced ALND as the standard surgical approach for the axilla in ypN0 patients, certain techniques such as TLNB still lack sufficient data on oncological outcomes, limiting their clinical application. To date, prognostic data[13-17] on recurrence or survival outcomes are mainly available for patients who underwent SLNB or TAD, while safety data on TLNB requires further validation.

Ongoing trials and our SrLNB trial are expected to provide safety evidence for axillary de-escalation surgery and expedite its clinical application. The ATNEC (NCT04109079) study enrolls patients with cN1 and performs SLNB without ALND in apCR patients. The MINIMAX[28] trial includes cN1-3 patients and axillary de-escalation surgery will be employed according to local protocol. The AXSANA[29] study utilizes a variety of marking procedures with the goal of identifying the optimal procedure. Another ongoing trial, NSABP B51/RTOG 1304[30], evaluates whether adding regional nodal irradiation (RNI) improves survival outcomes. These trials will provide further evidence on optimal locoregional treatment strategies for ypN0 disease regarding long-term prognosis, aiming to prevent both overtreatment and undertreatment. These trials will provide furthe

r evidence on optimal locoregional treatment strategies for ypN0 disease. Compared to these studies, our SrLNB study offers several advantages: First, SrLNB has the benefits of using carbon tattooing, including eliminating the need for a second localization prior to the surgery, avoiding the use of radioactive particles, and providing a long, identifiable period of black stained lymph nodes by carbon nanoparticles that lasts up to half a year. Secondly, our preliminary trial[11] has confirmed a high detection rate and a FNR below the threshold value (10%). More importantly, our study places greater emphasis on axillary morbidity and QoL. BCRL rate and PRO will be reported using multiple objective and subjective measurements. This study also has limitations. It is a a single-center study with a limited sample size. We hope to expand the experimental center and increase the number of enrollments after achieving preliminary results.

Trial status and Time plan

The protocol version number and date are V3.0 and 16 May 2023, respectively. The study protocol was developed in 2022. The first patient was enrolled on 11 September 2023, and the study is currently in the patient recruitment phase. Enrollment is projected to be completed by August 2025, with the 3-year follow-up expected to be preliminary completed by October 2026.

Acknowledgement

We thank all participants who volunteered to take part in this study and all investigators who have contributed to this study.

Contributors

All authors are from The First Affiliated Hospital with Nanjing Medical University. Jue Wang and Xiaoming Zha were the principal investigators of this study, they proposes the preliminary conception and conduct a tentative design, financial support is also provided. Lingjun Ma and Xuan Li developed the detailed protocol, Rui Chen and Mingyu Wang helped to register the trial and obtain ethical approval. Lexin Wang and Ran Zheng coordinated patient recruitment; Jingjing Ding and Hao Yao managed data collection; Xingye Sheng supervised imaging assessments. Lingjun Ma drafted the initial manuscript. Jue Wang is responsible for the overall content as guarantor. All authors critically revised the manuscript and approved the final study protocol.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Written consent obtained directly from patients.

Data availability statement

Data sharing is currently not applicable to this article as the study is still open for inclusion of patients. However, we are willing to consider requests for data sharing on a case-by-case basis. Researchers interested in accessing the data should contact the corresponding author with a detailed proposal outlining the purpose of their request.

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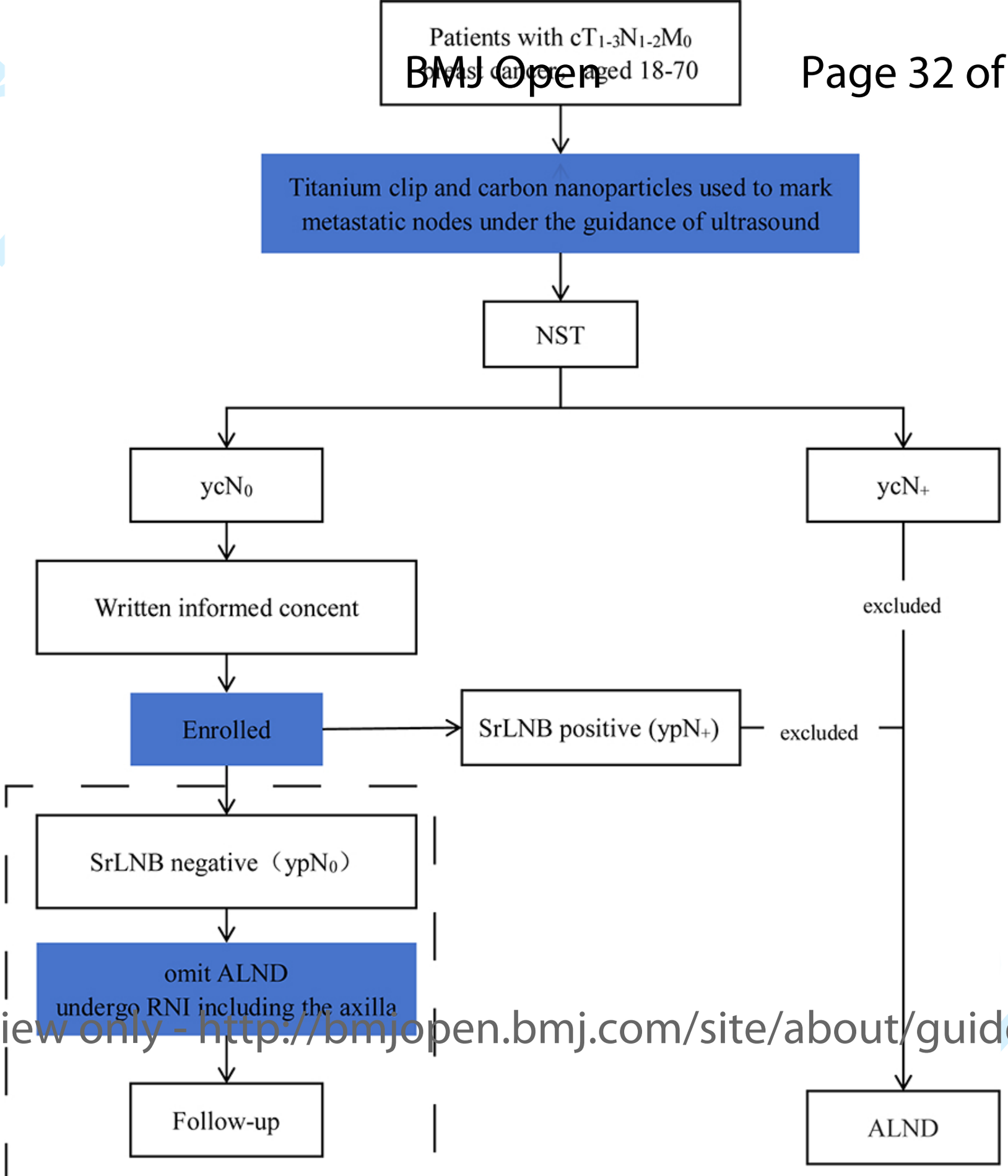
Figure Legends

Figure 1. Flow chart of SrLNB trial. cTNM, clinical classification of TNM; NST, neoadjuvant systemic therapy; SrLNB, Stained Region Lymph Node Biopsy; ypN0, pathologically assessed lymph node negative after NST; ALND, axillary lymph node dissection; RNI, regional lymph node radiotherapy.



Supplementary File 1:

Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

Informed Consent Form

Version:V2.0

Version date: June 5, 2023

Patient initials:

Patient study number:

Patient contact number:

Patient address:

Information Disclosure Page

Participant Information Sheet

Dear Patient,

We invite you to participate in the phase II clinical trial to exempt from axillary lymph node dissection (ALND) for breast cancer patients achieving axillary complete response (apCR) after neoadjuvant therapy. This study is initiated and led by Director Jue Wang and Xiaoming Zha, and will be conducted in the Department of Breast Diseases, Jiangsu Province Hospital. 92 subjects is expected to participate voluntarily. This study has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital). Ethical Review number is 2023-SR-169.

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

1. Why is this study conducted?

Up to 40% of patients with positive axillary lymph node breast cancer may convert to negative (ypN0) after NST. However, the current clinical guidelines still recommend ALND, which will bring about more complications, such as lymphedema, dysfunction, pain and numbness in affectedl upper limb and the axilla, to ypN0 patients. Actually, it is feasible to downgrade axillary surgery in such patients, with high requirements to technical and equipment, such as: (1) using dual tracers (dye and radionuclide) for Sentinel Lymph Node Biopsy (SLNB); (2) $\geqslant$3 axillary lymph nodes must be removed; (3) titanium clips must be used before neoadjuvant therapy to

localize metastatic axillary lymph nodes and ensure that the localized lymph nodes will be removed at the time of surgery. However, radioactive particles are under strict supervision in most countries and regions around the world (including our country), which prevents the vast majority of countries and regions from performing axillary downstaging procedures.

Team of Director Xiaoming Zha has been conducting clinical research since 2019, and successfully developed Stained Region Lymph Node Biopsy (SrLNB) to downstage axillary surgery. In SrLNB procedure, titanium clips is used to mark positive lymph node, and carbon nanoparticle suspension will also be injected under the guidance of ultrasound for staining at the time of diagnosis. When surgery after completion of NST, clipped and the stained lymph nodes will be biopsied. Data from the our previous study showed that the false-negative rate of stained-area lymph node biopsy was 5.3%, much lower than the generally recognized threshold of 10%, suggesting that SrLNB is safe and feasible.

In this study, team of Dr. Zha intends to enroll patients with positive axillary lymph nodes at diagnosis and receiving NST to undergo SrLNB. When SrLNB turns out to be negative, ALND is exempted, and then undergo axillary area radiotherapy after surgery. The physician team will further evaluate the efficacy and long-term safety of the procedure by following up and monitoring data such as local recurrence and complication rates.

2. Who is eligible (or not) to participate in the study?

The main inclusion criteria includes: (1) female breast cancer patients aged 18-70 years; (2) pathologically confirmed metastatic axillary lymph nodes at the time of diagnosis, with stage of cN1-3; (3) successful injection of carbon nanoparticle suspension to mark positive lymph nodes.

The main exclusion criteria includes: (1) bilateral breast cancer or during lactating/pregnant period; (2) clinically confirmed distant metastases; (3) unable to complete the subsequent adjuvant therapy in full course as prescribed for various reasons; (4) not eligible for enrollment as judged by the investigator.

3. What need to do if participate in the study?

If you are willing to participate in this study, the surgeon will perform SrLNB. When

intraoperative rapid pathology shows positive results (with micrometastases and isolated tumor cells), then you will be excluded from this study and proceed with ALND, other adjuvant treatments including chemotherapy, endocrine therapy and targeted therapy, will be performed according to guidelines. When intraoperative rapid pathology shows negative results, ALND will be omitted, postoperative regional lymph node radiotherapy (RNI) including axilla will be required, and other adjuvant treatments will be performed as prescribed. When the intraoperative pathology unable to clarify the presence of metastasis, the subsequent axillary will be suspended, and whether to add ALND will be depends on the routine pathology.

We will perform a follow-up within 3 years after surgery, which mainly includes: (1) breast and whole body imaging; (2) upper extremity circumference and bioelectrical impedance examination; (3) quality of life questionnaire.

4. What's the benefit to participate?

Patients who only undergo SrLNB are likely to experience less postoperative complications such as upper limb lymphedema, dysfunction, numbness and pain, and have improved quality of life,compared to ALND patients. In addition, our team will provide several examinations for free (see details in"Do I need to pay for participation in the study?") and closely monitor the results of subsequent visit to provide effective treatment and guidance timely.

5. What's the risk to participate?

SrLNB is the original and latest axillary downstaging procedure developed by team od Dr. Zha, which has been found with good operability and safety in our previous study. However, same as sentinel lymph node biopsy, there is possibility of false-negative results due to missed biopsy to metastatic lymph node, which may increase the possibility of recurrence in the axillary or even distant metastasis. Thus, all patients exempted from ALND in this study will undergo axillary radiotherapy to further eliminate tumor cell that may have been missed, reducing the risk of local recurrence and distant metastasis. Even with the boost of radiotherapy to the axilla and other subsequent systemic treatments, there may be an increased risk of local recurrence and distant metastasis. Our team will closely monitor recurrence and metastasis through regular follow-up, so that timely treatment measures (including complementary axillary surgery, etc.) can be taken.

In case of any discomfort and adverse reactions, please promptly contact the study physician. All agents of chemotherapy, targeted therapy, endocrine therapy, and radiation used in this study are part of the routine clinical treatment of breast cancer. Even if you do not participate in this study, these side effects/adverse reactions may occur as long as you receive these treatments. In addition, any treatment may failed to take effect, as well as the disease continue to progress due to ineffective treatment or other coexisting diseases.

6. Do I need to pay for participation in the study?

To compensate for any inconvenience this study may cause, bioelectrical impedance analysis, follow-up of quality of life and monitoring instructions will be provided free of charge during this study. The cost of medications for routine breast cancer treatment and follow-up examinations will not be covered. The cost of treatments and tests required for other medical conditions, or switching to other treatments if the treatment failed to take effect, will not be covered as well. Transportation, lost wages, and nutritional expenses are not covered by this study. In case of trial-related damages, appropriate treatment and compensation will be provided in accordance with relevant national regulations.

7. Is personal information confidential?

Your medical records will be kept at the hospital, and only the investigator, research authorities, and ethics committee are allowed to access your medical records. Any public reporting of the results of this study will not disclose your personal identity. Every effort will be made to protect the privacy of your personal medical information to the extent permitted by law.

8. What other treatments are available if not participating in this study?

If not participate in this study, you will undergo ALND, and the incidence and severity of arm mobidity may be higher. However, the subsequent adjuvant treatment will be performed according to clinical guidelines without change.

If you participate in the study but drop out halfway through the follow-up, you will still receive the most standardized and regular treatment.

9. Do I have to participate in the study?

Participation in this study is completely voluntary, and you may refuse to participate or withdraw at any time during the study, which will not affect your treatment. If you decide to withdraw, please contact the doctor, you may be asked to undergo related tests and complementary procedures (e.g., axillary lymph node dissection, etc.) that may be beneficial to protect your health.

If you have any questions related to your personal rights and interests, you can contact the Ethics Committee of our hospital at 025-68306360.

Informed Consent

Consent Signature Page

SUBJECT DECLARATION: I have read the above description of this study and fully understand the possible risks and benefits of participating in this study. I volunteer to participate in this study. I understand that I will be given a copy of this informed consent form.

I **agree□ or decline□** to other research utilizing my medical records and clinical specimens related to this study.

Patient Signature: _____Date of Signature: _____

Contact phone number: _____

Or

(To be used only if subject is incapacitated)

Signature of Legal Representative: _____

Date of Signature: _____

Relationship to the patient: _____

Contact phone number: _____

INVESTIGATOR DECLARATION: I confirm that I have explained the details of this study, particularly the possible risks and benefits of participating in this study, and answered all relevant questions from the subject, who has voluntarily agreed to participate in this study. This informed consent form is in duplicate, with one signed copy retained by the investigator and one by the subject.

Signature of Investigator: _____ Date of Signature: _____

Contact phone number: _____

知情同意书

尊敬的女士：

我们邀请您参加南京医科大学第一附属医院（江苏省人民医院）批准开展的“新辅助治疗后腋窝淋巴结阳性转阴性的乳腺癌患者豁免腋窝淋巴结清扫的 II 期临床研究”。本研究由乳腺病科查小明主任发起和负责，将在江苏省人民医院乳腺病科开展，预计将有 92 名受试者自愿参加。本研究已经得到南京医科大学第一附属医院（江苏省人民医院）伦理委员会的审查和批准，伦理审查编号：2023-SR-169。

为什么要开展本项研究？

腋窝淋巴结阳性的乳腺癌患者经新辅助治疗（Neoadjuvant Systemic Therapy，NST）后可有高达 40%的比例转为阴性（ypN0）。**然而即使淋巴结转为阴性，目前的临床指南仍然首推腋窝淋巴结清扫术（axillary lymph nodes dissection，ALND）**，而这也会带来更多的后遗症，如上肢淋巴水肿、上肢活动障碍、上臂内侧和腋窝疼痛麻木等。**实际上对此类患者进行腋窝淋巴结手术的降级是可行的，但是技术和设备的要求很高**，比如：(1) 使用双示踪剂（染料和放射性核素）进行前哨淋巴结活检（Sentinel Lymph Node Biopsy，SLNB）；(2) 必须切检≥3 枚腋窝淋巴结；(3) 必须在新辅助治疗前用钛夹定位转移的腋窝淋巴结，并且在前哨淋巴结手术时要确保取到钛夹定位的淋巴结。**由于放射性核素在全世界大多数国家和地区（包括我国）都要受到严格的监管，因此仅此一个要求就使绝大多数国家和地区无法开展腋窝的降级手术**。

查小明主任团队从 2019 年就开始开展临床研究，成功原创了染色区淋巴结活检术（Stained region Lymph Node Biopsy，SrLNB），即在初诊时经超声引导将纳米炭混悬注射液（卡纳琳）注射到腋窝阳性淋巴结的皮质中进行染色标记，在新辅助治疗完成后进行手术时活检染色区域的淋巴结。**前期临床研究数据初步显示染色区淋巴结活检术的假阴性率为 5.5%，远低于国际公认的 10%的阈值，初步证明染色区淋巴结活检术是安全可行的**。

在本研究中，查小明医生团队拟入组初诊腋窝淋巴结阳性且接受新辅助治疗的患者，行染色区淋巴结活检术，如为阴性则豁免腋窝淋巴结清扫手术，术后行腋窝区放疗。医生团队会通过随访和监测局部复发情况、并发症率等数据，**进一步评估染色区淋巴结活检手术的有效性和长期安全性**。

哪些人适（不）宜参加研究？

入选标准主要有：(1) 18-70 周岁的女性乳腺癌患者；(2) 初诊时经病理证实腋窝淋巴结

为阳性，N 分级为 cN1-3；(3) 阳性淋巴结经纳米炭混悬注射液成功注射标记。

排除标准主要有：(1) 双侧乳腺癌/哺乳期/孕期乳腺癌；(2) 临床或影像学证实存在远处转移；(3) 各种原因导致无法按医嘱足疗程完成后续辅助治疗者；(4) 经研究者判断不符合入组者。

如果参加研究，需要做什么？

如果您愿意参加本项研究，医生会在术中行染色区淋巴结活检术。如果术中快速病理显示腋窝淋巴结阳性（含微转移和孤立肿瘤细胞）则排除出本研究，并继续行腋窝淋巴结清扫手术，术后的其他综合治疗（如化疗、内分泌治疗、靶向治疗等）按照指南进行。**如果术中快速病理显示腋窝淋巴结阴性则不做腋窝淋巴结清扫术，术后需要接受包含腋窝的区域淋巴结放疗（RNI）**，术后的其他综合治疗也按照指南进行。**如果术中快速病理无法明确有无转移，则会暂停后续的腋窝淋巴结清扫术，等常规病理出来后再决定是否加做腋窝淋巴结清扫术**。

我们会在手术治疗结束的 3 年内对您进行随访，随访内容主要包括：(1) 乳腺及全身影像学检查；(2)上肢周径和生物电阻抗检查；(3) 生活质量量表问卷调查等。

参加研究有哪些好处？

只接受染色区淋巴结活检术的患者，术后发生淋巴水肿、上肢功能障碍、麻木疼痛等后遗症的概率及其严重程度大概率会明显降低，生活质量也可能提高。另外，本团队也会提供部分免费检查（见“参加研究需要支付有关费用吗”），并密切关注复查结果，以便及时提供有效的治疗和指导。

参加研究有哪些风险？

染色区淋巴结活检术是查小明医生团队原创的、最新的腋窝淋巴结活检手术，前期研究发现其具有较好的操作性和安全性。**但正如前哨淋巴结活检术一样，染色区淋巴结活检术同样会存在漏检导致假阴性结果的可能。因漏检而残留在腋窝的转移淋巴结有可能会增加腋窝区的复发率甚至远处转移率，因此本研究中所有豁免腋窝淋巴结清扫手术的患者都需要接受腋窝放疗，以进一步消灭可能漏检的阳性淋巴结，从而减少局部复发和远处转移的风险**。当然，**即使增加了放疗和后续其他全身治疗，也可能会出现局部复发和远处转移的风险增高，医生团队将会通过定期密切随访，及时监测疾病复发、转移等情况，以便及时采取治疗措施（包括补充腋窝手术等）**。

如果出现任何不适和不良反应，请及时与研究医生联系。本研究方案中使用的所有化疗

药、靶向药、内分泌药物以及放疗等都属于临床治疗乳腺癌的常规方式，即使您不参加本临床研究，只要接受该治疗方法，就有可能发生这些副作用/不良反应。此外，任何治疗都可能出现无效的情况，以及因治疗无效或者因合并其他疾病等原因而导致病情继续发展。

参加研究需要支付有关费用吗？

为了补偿本研究可能给您带来的不便，本研究期间将免费提供生物电阻抗检测，免费进行生活质量随访和随访监测指导。乳腺癌常规的治疗药物以及随访检查费用不在免费范围之内。如果您同时合并其他疾病所需的治疗和检查，以及因治疗无效而改用其他治疗的费用，也不在免费范围之内。本研究不提供交通费、误工费、营养费等。如果出现试验相关的损害，将依据国家有关规定提供相应的治疗与补偿。

个人信息是保密的吗？

您的医疗记录将保存在医院，研究者、研究主管部门、伦理委员会将被允许查阅您的医疗记录。任何有关本项研究结果的公开报告将不会披露您的个人身份。我们将在法律允许的范围内，尽一切努力保护您个人医疗资料的隐私。

如果不参与本研究还有哪些治疗方法？

如果您不参加本项研究，则接受标准的腋窝淋巴结清扫手术，腋窝后遗症的发生率和严重程度可能会较高。但后续的综合治疗会按照临床指南的要求进行，不会有任何改变。

如果您参加了研究，但后续中途退出了，您仍会得到最标准正规的治疗。

我必须参加研究吗？

参加本项研究是完全自愿的，您可以拒绝参加研究，或在研究过程中的任何时间退出本研究，这都不会影响医生对您的治疗。如果您决定退出本研究，请与您的医生联系，您可能被要求进行相关检查和补充手术（如腋窝淋巴结清扫术等），这对保护您的健康是有利的。

如您有涉及个人权益方面的问题可与本院伦理委员会联系，联系电话：025-68306360。

受试者声明：我已经阅读了上述有关本研究的介绍，对参加本研究可能产生的风险和受益充分了解。我自愿参加本研究。我将获得一份签署姓名和日期的本知情同意书副本。

我同意□ 或拒绝□ 其他研究利用我的与本研究相关的医疗记录和临床标本。

受试者签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

受试者的联系电话：_____ 手机号：_____

（适用时）法定监护人/见证人签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

法定监护人/见证人联系电话：_____ 手机号：_____

研究者声明：我确认已向受试者解释了本研究的详细情况，特别是参加本研究可能产生的风险和受益，并回答了受试者的所有有关问题，受试者是出于自愿同意参加本研究。此知情同意书一式两份，研究者与受试者各留一份已签字的知情同意书。

研究医生签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

研究医生的工作电话：025-68308172 手机号：_____