# Targeted axillary dissection using carbon marking for patients with node-positive breast cancer following neoadjuvant therapy (TADCOM): study protocol for a prospective, multicenter, randomized controlled trial

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# I. Background

Breast cancer is one of the most common cancers among women worldwide. Neoadjuvant chemotherapy (NAC) plays a pivotal role in the management of locally advanced cases, offering a pathway to pathological complete response (pCR) and reducing reliance on axillary lymph node dissection (ALND), which is often associated with complications like lymphedema and chronic pain. Approximately 21.1% of patients achieve pCR with NAC, with axillary pCR rates ranging from 23% to 74%. Such outcomes may allow some patients to avoid ALND, underscoring the clinical value of preserving axillary integrity.

For patients with confirmed axillary metastasis before NAC, the use of tissue markers enables precise localization and removal of these nodes during sentinel lymph node biopsy (SLNB), known as targeted axillary dissection (TAD). This technique, which has been validated in studies like ACOSOG Z1071 and SenTa, shows a false-negative rate (FNR) of 2% to 7%, reducing postoperative complications.

Despite its benefits, TAD using traditional tissue markers faces challenges like high costs and potential marker loss. Recent advancements include the use of carbon nanoparticle suspension injection (CNSI) for lymph node mapping, known as carbon marking. CNSI's unique properties allow for enhanced visibility and prolonged stability within lymph nodes, making it a promising alternative to traditional methods.

This research aims to compare various TAD techniques based on carbon marking against conventional methods, focusing on clinical feasibility, diagnostic accuracy, and the reduction of common issues such as marker loss. Additionally, the study explores the potential for CNSI in improving TAD outcomes in patients with pre-NAC cN2+ staging, seeking to develop a more effective, cost-efficient surgical approach.

# II. Objectives of the study

The TADCOM trial is designed as a prospective, multicenter, randomized controlled trial (RCT) utilizing a non-inferiority framework. This study aims to

compare the feasibility and efficacy of CNSI for TAD against conventional tissue marker clip-based methods in patients with breast cancer following NAC.

## III. Study endpoint

## Primary Endpoints

1. Marked lymph node retrieval rate: The proportion of successfully retrieved marked lymph nodes will be calculated and compared among the study groups to evaluate the effectiveness of each marking technique.
2. Number of sentinel and marked lymph nodes: The mean, median, and range of the number of sentinel and marked lymph nodes harvested during surgery will be recorded and compared to assess the efficacy of the marking techniques in identifying lymph nodes of interest.
3. Concordance between marked and sentinel lymph nodes: The consistency between marked lymph nodes and intraoperatively identified sentinel lymph nodes will be evaluated by calculating the percentage of marked nodes that are also sentinel nodes and vice versa to determine the accuracy of the marking techniques in identifying true sentinel lymph nodes.
4. Complication rate: All surgery-related complications, including but not limited to hemorrhage, lymphedema, infection, pain, tissue damage, clip displacement, clip loss, absence of CNSI staining, and excessive CNSI staining, will be recorded and analyzed. The overall complication rate and rates for specific types of complications will be reported. The severity of complications will be assessed using the Clavien-Dindo classification to provide a standardized evaluation of complication severity.

## Secondary Endpoints

1. Axillary and distant recurrence rates: During the 2-year and 5-year follow-up periods, the axillary and distant recurrence rates will be monitored and reported for

each study group to assess the long-term oncological outcomes of the different marking techniques.

2. Overall survival (OS), and disease-free survival (DFS): OS and DFS will be monitored and reported for each study group at 2 years, 5 years, and other relevant time points. Kaplan-Meier analysis and Cox proportional hazards models will be used to estimate these endpoints and compare outcomes among the study groups.

3. Surgical duration and learning curve: The total time from the start of the surgery to the removal of the last lymph node will be calculated to analyze the efficiency of different techniques in terms of surgical time. The learning curve associated with each marking technique will also be assessed to evaluate the impact on surgical duration and other outcomes over time.

4. Postoperative complications: Complications such as lymphedema, infection, and pain will be assessed at specific time points (e.g., 1 month, 6 months, and 1 year post-surgery) using validated tools or scales (e.g., Common Terminology Criteria for Adverse Events, Brief Pain Inventory) to determine the safety profile of each marking technique and its impact on patient morbidity.

5. Quality of life: The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) and the breast cancer-specific module (EORTC QLQ-BR23) will be used to evaluate patients' quality of life before treatment, at 6 months, 1 year, and 2 years after treatment to assess the impact of the different marking techniques on patient-reported outcomes.

6. Positive tumor margin rate and re-excision surgery: For patients undergoing breast-conserving surgery, the proportion of positive tumor margins, defined as tumor cells at the inked margin or within 1 mm from the margin, will be evaluated postoperatively. The rate of re-excision surgery due to positive margins will also be assessed to evaluate the impact of the marking techniques on the accuracy of surgical resection and the need for additional interventions.

## IV. Study subjects (national multi-center study in China)

## 4.1 Study population

Patients with cT1-4N1-2aM0 breast cancer undergoing neoadjuvant chemotherapy and surgery (N = 126)

## 4.2 Criteria for inclusion and exclusion

**Inclusion criteria:**

Participants in the study must meet the following criteria:

1. Female patients aged 18 to 75 years are eligible.
2. Participants must have a histologically confirmed diagnosis of breast cancer, classified as clinical stage T1-4N1-2aM0 according to the TNM classification system.
3. Eastern Cooperative Oncology Group (ECOG) performance status must be 0 or 1.
4. Clinical re-staging must indicate an axillary node status of ycN0 following NAC.
5. Participants must provide written informed consent to partake in the trial, acknowledging understanding and agreement to the procedures and risks involved.

**Exclusion Criteria:**

Patients who met any of the following exclusion criteria were excluded from the study:

1. Patients with metastatic breast cancer (Stage IV).
2. Diagnosed with inflammatory breast cancer or bilateral breast cancer.
3. History of axillary surgical procedures.
4. Any medical, psychological, or social conditions that would prevent adherence to the study protocol or completion of the treatment or follow-up.
5. Known allergy to carbon nanoparticles or presence of severe comorbid conditions or other serious underlying medical issues.
6. Current or prior participation in another clinical trial that could interfere with the outcome of this study or affect the safety and well-being of the participants.

## 4.3 Criteria for Termination

1. If a participant exhibits local tumor progression (including the ipsilateral breast, chest wall, axillary, and supraclavicular or infraclavicular lymph nodes) or develop

distant metastases during NAC, the NAC will be discontinued, resulting in the termination of the participant's involvement.

2. Participants may choose to withdraw from the study voluntarily at any point. Such withdrawal will have no adverse effects on the participant's future medical care or access to alternative treatment options.

3. If continued participation is deemed to compromise the safety of the participant, as determined by the investigating team, the participant's involvement will be terminated.

4. Any serious breach of the study protocol by a participant, as identified by the investigators, will result in immediate termination of their participation.

5. If a participant loses contact during the study and is unable to continue participating, they will be considered lost to follow-up and terminated from the study.

## 4.4 Criteria for ultrasound diagnosis of suspicious lymph nodes

**Ultrasound Scoring Criteria for Suspicious Axillary Lymph Nodes**

| Ultrasound Characteristics | Scoring Criteria | Points |
|---|---|---|
| Shape | Fusiform or elongated (ratio of long to short axis ≥2) | 0 |
| | Round or oval (ratio of long to short axis <2) | 1 |
| Border | Smooth, regular | 0 |
| | Irregular, lobulated | 1 |
| Hilum | Intact, central | 0 |
| | Absent or eccentric | 2 |
| Cortical thickness | Uniform, ≤3mm | 0 |
| | Focal thickening (>3mm) or diffuse thickening | 2 |

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| Calcification | No calcification | 0 |
| | Presence of microcalcifications | 1 |
| Necrosis | No necrotic area | 0 |
| | Presence of necrotic area | 1 |
| Vascular pattern | Predominantly central hilar vascularity, no significant peripheral vascularity | 0 |
| | Predominantly chaotic, irregular peripheral vascularity | 1 |

*A total score of ≥3 points indicates a suspicious lymph node, and a fine-needle aspiration (FNA) or core-needle biopsy (CNB) is recommended to obtain a pathological diagnosis

# V. Study Methodology

## 5.1 Subjects included

A prospective, multicenter, randomized controlled trial (RCT) utilizing a non-inferiority framework was conducted in 126 breast cancer patients who met the criteria of inclusion and exclusion. The relevant data were recorded according to the CRF form issued by the applicants.

## 5.2 Interventions

**Group 1: Clip-Guided Targeted Axillary Dissection (CG-TAD)**

Participants will undergo an ultrasound-guided placement of tissue marker clips into clinically suspicious axillary lymph nodes before NAC. Following the completion of NAC, methylene blue dye will be used to facilitate the mapping of sentinel lymph nodes. Subsequently, TAD will be performed to excise both the sentinel and previously clipped lymph nodes. This group serves as the control arm, employing standard

methodologies consistent with those reported in existing TAD literature (25).

**Group 2: CNSI Lymph Node Marking (CN-LNM)**

participants will receive injections of carbon nanoparticle suspension injection (CNSI) under ultrasound guidance to mark clinically suspicious axillary lymph nodes before NAC. Post-NAC, methylene blue dye will also be used to map the sentinel lymph nodes. The TAD procedure will then be conducted to remove both the sentinel lymph nodes and the lymph nodes marked with CNSI.

**Group 3: Peritumoral CNSI Mapping (PCN-MAP)**

Participants will be administered CNSI injections around the primary breast tumor under ultrasound guidance before NAC, allowing for natural lymphatic drainage to transport the carbon nanoparticles to both sentinel and potentially involved axillary lymph nodes. If a lymph node is confirmed as metastatic through pathology, an additional tissue marker clip will be placed under ultrasound guidance before NAC. After NAC, the sentinel lymph nodes will be mapped using methylene blue, followed by TAD to excise the sentinel lymph nodes, CNSI-marked nodes, and any additional clipped nodes.

## 5.3 Neoadjuvant chemotherapy

NAC regimens for breast cancer will be customized based on the patient's ECOG performance status, molecular subtype, and relevant biomarkers such as HER2 overexpression. Treatment durations range from 3 to 6 months, following the latest evidence-based guidelines. Anti-HER2 therapies are added for cases with HER2 overexpression to enhance efficacy. Throughout NAC, patient response is closely monitored via ultrasound, mammography, and MRI before and after chemotherapy cycles, adjusting treatments based on individual tolerance and effectiveness. This personalized approach ensures optimal safety and outcomes, incorporating patient and clinical input in decision-making.

## 5.4 Procedures for labeling axillary lymph nodes

**For CNSI:**

Using sterile technique, prepare the CNSI for injection.
Under continuous ultrasound guidance, inject a small volume of CNSI (specific volume as per protocol, e.g., 0.1 mL) directly into the identified suspicious lymph nodes.
Confirm the dispersion of CNSI within the lymph node visually on ultrasound.

**For Tissue Clips:**

Prepare the clip applier loaded with a sterile tissue marker clip.
Under ultrasound guidance, deploy the clip directly into or adjacent to the lymph node, ensuring the clip is securely placed within the node without displacement.

## 5.5 Post-Labeling Monitoring

1) Perform an immediate post-procedure ultrasound to ensure the integrity of the lymph node labeling.
2) Document any immediate adverse reactions or complications.
3) Schedule a follow-up ultrasound within a week to check the stability of the markers and the condition of the lymph nodes.

## 5.6 Pathological evaluation of lymph nodes

Following TAD, lymph nodes will undergo histological examination to determine the presence of disease. If the histological findings are positive, an extensive dissection of levels I-II axillary lymph nodes will be required. For enhanced accuracy in detecting axillary lymph node metastases, all specimens are processed using hematoxylin and eosin (H&E) staining, complemented by immunohistochemical (IHC) staining techniques. Following NAC, the detection of isolated tumor cells (ITCs) should be categorized as ypN0(i+), indicating non-invasive residual disease, while the identification of micro-metastases should be recorded as ypN1mi, denoting minimal residual disease. Considering the risk of residual disease, it is recommended that all

patients receive postoperative axillary regional nodal irradiation (RNI) to minimize the likelihood of local recurrence and enhance long-term disease management.

# VI. Record of patient information

## 6.1 Basic patient information

**Demographic and Physical Data:**

- **Age:** Documented in years.
- **Weight:** Measured in kilograms.
- **Body Mass Index (BMI):** Calculated using the formula (weight in kg)/(height in $m^2$).

**Clinical Assessment:**

- **Clinical Staging:** Includes documentation of tumor size, site, and lymph node status according to the TNM classification.
- **Neoadjuvant Therapy:** Details of any chemotherapy, hormone therapy, or radiation therapy administered prior to surgery.

**Surgical Intervention:**

- **Operation Mode:** Specified as either breast-conserving surgery or radical mastectomy, based on the clinical judgment and patient consent.

**Postoperative Care:**

- **Endocrine Therapy and Radiotherapy:** Specifics of any hormonal treatments or radiation therapy administered post-surgery.

**Histopathological Evaluation:**

- **Histopathology:** Classification of the tumor as invasive carcinoma or carcinoma in situ.
- **Histologic Grading:** Grading of the tumor based on cellular differentiation.
- **Hormone Receptor Status:** Results for estrogen and progesterone receptors.
- **Ki-67:** Proliferation index measured as a percentage.
- **HER-2 Status:** Determined via immunohistochemistry or fluorescence in situ

hybridization (FISH).

- **Lymph Node Status:** Documented findings from the pathological examination of excised lymph nodes.

**Additional Clinical Data:**

- **Menopausal State:** Classified as premenopausal, perimenopausal, or postmenopausal.
- **Family History:** Detailed record of any familial incidence of breast cancer or related cancers.

## 6.2 Placement information

Brand and Model: Each CNSI clip used in the study is meticulously documented, noting the specific brand and model to ensure consistency and traceability in the research findings.

Placement Position: The exact anatomical location where each clip is placed within the axillary region is recorded. This includes specifying the depth and proximity to critical structures to facilitate accurate replication and assessment of the clip's effectiveness in marking the lymph nodes.

Puncture Details: The condition of the puncture site (e.g., any immediate complications such as bleeding or infection) and the precise time of puncture are recorded to provide a comprehensive timeline and context for subsequent analyses.

Operator Information: The identity of the operator performing the clip placement is logged to assess the impact of operator skill and experience on the success of the procedure.

Follow-Up: Follow-up data on each clip are collected, focusing on whether any displacement or loss of the clip occurred post-placement. This is essential for evaluating the reliability and stability of the CNSI clips used in marking axillary lymph nodes.

## 6.3 Surgical pathology information

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| **SLNB/TAD region** | | | | | |
| **Marked** | X<br>X | Positive | X | pCR | X |
| **Unmarked** | X<br>X | Positive | X | | |
| **ALND does not contain the SLNB/TAD region** | | | | | |
| **Marked** | X<br>X | Positive | X | pCR | X |
| **Unmarked** | X<br>X | Positive | X | | |

* reporting concerns about post-treatment responses

## 6.4 Long-term Follow-up Information

Survival/Death: The survival status of each participant will be tracked, recording the time from study entry until death or the last follow-up, whichever comes first.

Recurrence: The occurrence and location of any cancer recurrence will be documented, along with the timing of such events relative to the initial treatment.

Follow-up Treatment: Any subsequent treatments administered post-recurrence or as part of ongoing care will be systematically recorded, noting the type and timing of treatment.

# VII. Data Management

**1. Patient Identification**:

- Upon enrollment, each participant will be assigned a unique identification number (ID). This ID will be used to maintain participant confidentiality and ensure that all collected data can be accurately linked to the corresponding participant without revealing personal information.

**2. Data Updating Protocol**:

- All study data will be updated on a quarterly basis to ensure that information is current and reflects any new developments or changes in participant status. This regular updating is crucial for maintaining the integrity of the data set and ensuring that analyses are based on the most recent information.

**3. Annual Data Analysis and Reporting**:

- Comprehensive data analysis will be conducted annually. The findings from these analyses will be compiled into progress reports that will be disseminated to all participating units. These reports will provide insights into the study's progress, preliminary outcomes, and any significant findings or deviations from expected trends.

## VIII. Quality Control of clinical trials

**1. Ethics and Protocol Approval**:

- Prior to implementation, the pilot program of each multicenter clinical trial must be reviewed and approved by the Ethics Committee responsible for the respective unit. This ensures that all ethical guidelines and regulatory requirements are met.

**2. Participant and Researcher Consistency**:

- To maintain consistency in participant management across all centers, clinical trial participants should be relatively fixed. Key researchers must conduct technical training sessions before the trial commences. These sessions will focus on a thorough review of the clinical trial protocols and technical procedures to ensure uniformity in technology application, equipment use, data judgment standards, and documentation methods.

**3. Data Handling and CRF Compliance**:

- Researchers are required to fill out the Case Report Form (CRF) accurately, promptly, and in detail according to the instructions provided. Data should be uploaded in real time to maintain data integrity and facilitate timely analysis.

**4. Verification of Observations and Findings**:

- All observations and findings from the clinical trials must be rigorously verified.

This verification process ensures the reliability of the data and confirms that the trial conclusions are directly derived from the original data collected.

**5. Mid-Term Reviews and Data Verification**:

- Each responsible unit must organize regular mid-term seminars to discuss the ongoing progress of the trial, address any issues encountered, and verify the data collected from each center. These discussions are critical for assessing interim outcomes and making necessary adjustments to the trial protocol.

**6. Participant Retention Strategies**:

- Researchers must implement proactive strategies, such as regular notifications and follow-up appointments, to maintain a dropout rate below 30%. These measures are essential for minimizing bias and ensuring the statistical power of the trial results.

**7. Quality Control Systems**:

- Each test center must establish a stringent quality control system to oversee and ensure the quality of the trial execution. This system should include regular audits and reviews to monitor compliance with the trial protocols and maintain high standards of research integrity.