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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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Abstract

Background Neoadjuvant chemotherapy (NAC) for breast cancer enables pathological complete response (pCR) in patients initially diagnosed with axillary lymph node metastases, potentially obviating the need for axillary lymph node dissection (ALND). Current targeted axillary dissection (TAD) techniques, guided by traditional tissue markers placed prior to NAC, face challenges such as marker loss and high costs. Carbon nanoparticle suspension injection (CNSI) offers a stable and reliable alternative for marking, which could enhance the TAD procedure. This study aims to evaluate the feasibility and accuracy of different TAD strategies using CNSIs and to explore their clinical utility in locally advanced breast cancer.

Methods This prospective, multicenter, randomized controlled trial will enroll 126 biopsy-proven breast cancer patients with suspicious axillary lymph node metastases (cN1-2a) who achieve ycN0 status following NAC. Participants will be randomized in a 1:1:1 ratio to undergo TAD guided by: [1] conventional tissue clips (CG-TAD); [2] CNSI lymph node marking (CN-LNM); or [3] peritumoral CNSI mapping (PCN-MAP). Primary endpoints include retrieval rate of marked lymph nodes, number of sentinel and marked lymph nodes, concordance rates, and complication rates. Secondary endpoints encompass regional and distant recurrence rates, survival outcomes, surgical duration, postoperative complications, quality of life scores, and margin status in breast-conserving surgery. Statistical analyses will adhere strictly to the CONSORT guidelines.

Discussion This study aims to evaluate the feasibility and accuracy of CNSI for targeted axillary dissection in breast cancer patients following neoadjuvant chemotherapy and to explore its clinical significance in reducing surgical complications and costs, as well as improving surgical precision.

Trial registration Clinicaltrials.gov, NCT04744506, Registered 27 December 2020, Updated 24 September 2024. Protocol Version Ver 1.2, 17/9/2024.

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Keywords Breast Cancer, Neoadjuvant Chemotherapy, Targeted Axillary Dissection (TAD), Carbon Nanoparticle suspension injection (CNSI), Randomized Controlled Trial

Background

Breast cancer is one of the most common malignancies among women globally [1]. Neoadjuvant chemotherapy (NAC) plays a crucial role in managing locally advanced breast cancer, offering the potential for pathological complete response (pCR) and reducing reliance on axillary lymph node dissection (ALND) [2]. ALND, while effective for staging, is associated with significant morbidities such as lymphedema and chronic pain. NAC has enabled a shift toward more conservative surgical approaches, which aim to reduce these complications. NAC demonstrates variable effectiveness across breast cancer subtypes. Overall pCR rates range from 10.1 to 74.2%, with an average of approximately 21.1% [3, 4]. The efficacy of NAC is particularly pronounced in specific subtypes: triple-negative breast cancer (TNBC) exhibits pCR rates of approximately 40–50% [5], while human epidermal growth factor receptor 2 (HER2)-positive breast cancer, when treated with targeted therapies such as trastuzumab, could achieve even higher pCR rates of 50–70% [6]. Notably, axillary pCR rates show similar variability, spanning from 23 to 74% [7, 8]. The achievement of axillary pCR is clinically significant as it may allow patients to avoid ALND, thus preserving axillary integrity post-NAC.

For patients with axillary metastasis confirmed before NAC, placement of tissue markers facilitates the localization of these nodes post-NAC, enabling their complete removal during sentinel lymph node biopsy (SLNB), a technique known as targeted axillary dissection (TAD) [9, 10]. This approach allows for a more precise and less invasive management of the axilla. Studies such as ACOSOG Z1071, SenTa, and ILINA have demonstrated that TAD after NAC accurately assesses the nodal status with a false-negative rate (FNR) of 2–7%, significantly reducing the incidence of postoperative upper limb lymphedema and pain [3, 9, 11–14]. Current guidelines and expert consensus generally recommend that for patients with clinically node-positive (cN1) axillary lymph nodes who convert to clinically node-negative (ycN0) after NAC, TAD combined with axillary regional nodal irradiation (RNI) may serve as an alternative to ALND [15]. However, the appropriateness of using TAD combined with axillary RNI as an alternative to ALND for patients initially presenting with more advanced nodal disease (cN2+) who convert to ycN0 remains to be further explored. Additionally, current TAD techniques, which rely on tissue marker clips, face challenges such as high costs, intraoperative localization difficulties, and potential marker loss. Addressing these issues by refining lymph node marking methods could potentially enhance the effectiveness of TAD and represents a critical area for clinical research.

In recent years, the use of carbon nanoparticle suspension injection (CNSI) for lymph node mapping in breast cancer, known as carbon marking, has been extensively explored. CNSI consists of stable suspensions of carbon nanoparticles with a diameter of 150 nm. These particles are smaller than the intercellular gaps of lymphatic endothelial cells (120–500 nm) but larger than those of capillary endothelial cells (30–50 nm), which prevents their entry into the bloodstream post-injection around the tumor site [16]. Instead, they are rapidly phagocytized by macrophages and accumulate in the lymph nodes [17]. Therefore, CNSI's strong black pigmentation enhances the visibility of lymph nodes during surgical procedures [18]. One of the notable characteristics of CNSI is its slow metabolic rate, allowing it to remain stable and safely within tissues for several months [19–22]. This property ensures that CNSI remains effective throughout the entire course of NAC for breast cancer. Studies from South Korea have confirmed the efficacy and feasibility of carbon marking in tracking axillary lymph nodes in breast cancer, highlighting its advantages over traditional methods [23, 24]. Furthermore, carbon marking has demonstrated superior detection rates in surgical settings, allowing direct visual identification without the need for specialized intraoperative imaging equipment [25]. While CNSI demonstrates advantages in lymph node marking, such as stability and high visibility, studies on SLNB have revealed that carbon nanoparticles can migrate between axillary lymph nodes, potentially expanding the area of lymph node identification [26, 27].

Recent studies have explored the combination of CNSI with established dual-tracer methods (using both radioactive isotopes and methylene blue dye) for SLNB following NAC. These studies demonstrated superior stability, lower FNR, and enhanced accuracy, particularly in patients with pre-NAC clinical staging of cN2-3 [28]. Given the stable deposition of CNSI within lymph nodes [22], the potential for pre-NAC injection of CNSI near the primary tumor to effectively visualize sentinel lymph nodes via natural lymphatic drainage

has emerged. This approach not only marks the primary tumor but also the draining lymph nodes, suggesting a dual benefit that merits further investigation. Moreover, considering that most medical institutions in mainland China utilize methylene blue dye alone for marking sentinel lymph nodes [29, 30], the reliability of TAD procedures facilitated by carbon marking in the context of this single-dye method also requires additional study.

Thus, this research is conducted to evaluate the clinical feasibility and diagnostic accuracy of various TAD methodologies based on carbon marking compared to traditional approaches using tissue marker clips. This study aims to address common issues such as marker loss and enhance surgical precision. Additionally, the applicability of carbon marking-based TAD in patients with pre-NAC cN2+staging will be assessed, with the goal of developing an optimized, viable, and cost-effective surgical technique.

Methods

Study Design

The TADCOM trial is designed as a prospective, multicenter, open label, 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.

Study setting

The trial is coordinated by the Department of Breast Surgery at the Second Affiliated Hospital of Zhejiang University School of Medicine. It encompasses three distinct sites within Zhejiang province (Second Affiliated Hospital, Zhejiang University School of Medicine; Lanxi People's Hospital; Taizhou Municipal Hospital), including both academic research hospitals and rural medical centers. This diverse site selection ensures a broad demographic representation and enhances the generalizability of the findings. Details of the trial structure are provided in Table 1 and illustrated in Fig. 1.

Eligibility criteria

Inclusion criteria.

1. Female patients aged 18 to 85 years are eligible.
2. Participants must have a histologically confirmed diagnosis of breast cancer, classified as cT1-4N1-2aM0 according to the 8th edition of the AJCC (American Joint Committee on Cancer) 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.

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.

Recruitment, randomization and allocation

Recruitment will occur during routine outpatient visits at participating hospitals. Primary care physicians will conduct an initial screening to identify patients who may be interested in participating in the trial. Potential participants will then be referred to a dedicated research team for a comprehensive assessment to verify eligibility based on the pre-defined inclusion and exclusion criteria. Detailed information about the trial, including its objectives, procedures, potential risks, and benefits, will be provided to potential participants and their families.

Informed consent will be obtained using forms that have been reviewed and approved by the institutional ethics committee (Supplementary File-2). Participants will be clearly informed of their rights, including the ability to withdraw from the study at any point without any repercussions.

Participants will be randomly assigned to treatment groups using a computer-generated sequence, ensuring that the allocation is both unbiased and reproducible. This randomization will be managed centrally to eliminate any potential allocation bias. Given the nature of the interventions being compared, blinding will not be implemented for participants and investigators. To address potential participant withdrawals, contingency plans have been established to enroll additional subjects as needed. This strategy is designed to preserve the statistical power required to meet the trial's objectives.

Table 1 WHO trial registration data set for TADCOM trial

Data Category	Information
Primary Registry & Trial ID	NCT04744506
Date of Registration	March 1st, 2024
Secondary ID	N/A
Trial Protocol Version	Version 1.1
Source of Support	Second Affiliated Hospital, Zhejiang University School of Medicine, Natural Science Foundation of Zhejiang Province, China's National Key R&D Program
Primary Sponsor	Second Affiliated Hospital, Zhejiang University School of Medicine
Secondary Sponsor	Natural Science Foundation of Zhejiang Province, China's National Key R&D Program
Contact for Public Queries	WZ Chen, chenwuzhen@zju.edu.cn
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Public Title	TADCOM TRIAL: Precision Targeted Axillary Dissection for Breast Cancer
Scientific Title	Targeted axillary dissection using carbon marking for patients with node-positive breast cancer following neoadjuvant therapy (TADCOM): a prospective, multicenter, randomized controlled trial
Countries of Recruitment	China
Health Condition Studied	Breast Cancer, Axillary Lymph Node Metastasis, Neoadjuvant Chemotherapy, Targeted Axillary Dissection
Interventions	**Group 1: CG-TAD Group (Control)** US-guided clip insertion into suspicious ALNs pre-NAC Post-NAC, TAD removing SLNs and clipped LNs **Group 2: CN-LNM Group** US-guided CNSI injection to tattoo suspicious ALNs pre-NAC Post-NAC, TAD removing SLNs and carbon-marked LNs **Group 3: PCN-MAP Group** US-guided CNSI injection around primary tumor pre-NAC, additional US-guided clip placement for metastatic LN Post-NAC, TAD removing SLNs, carbon-marked LNs, and clipped LNs
Key Inclusion/Exclusion Criteria	Ages: 18-75 years Sexes: Female Health Volunteers: No Inclusion Criteria: See "Eligibility Criteria" section Exclusion Criteria: See "Eligibility Criteria" section
Study Type	Investigator-initiated, prospective, multicenter, non-inferiority, randomized controlled, clinical trial Allocation: Centrally randomization Intervention Model: Parallel assignment Masking: Open-label Primary Purpose: Improving Accuracy and Safety in Breast Lymph Node Localization Phase: Phase III
Date of First Enrolment	November, 2024
Target Sample Size	126
Recruitment Status	Ready to Recruit
Primary Outcomes	1. Marked lymph node retrieval rate 2. Number of sentinel and marked lymph nodes 3. Concordance between marked and sentinel lymph nodes 4. Complication rate
Secondary Outcomes	1. Axillary and distant recurrence rates 2. Survival status (OS and DFS) 3. Surgical duration 4. Postoperative complications 5. Quality of life 6. Positive tumor margin rate and re-excision surgery

**ALN* Axillary lymph node, *CNSI* Carbon nanoparticle suspension injection, *DFS* Disease-free survival, *LN* Lymph node, *MB* Methylene blue, *NAC* Neoadjuvant chemotherapy, *OS* Overall survival, *SLN* Sentinel lymph node, *TAD* Targeted axillary dissection, *US* Ultrasound

Sample size calculation

To ensure sufficient statistical power for detecting the predefined non-inferiority margin in this study, we performed sample size calculations. The non-inferiority margin was set at 10%, which represents the maximum allowable difference in effect size between the carbon marking and the conventional tissue marker clip methods. Calculations were based on achieving a Type I error rate (α) of 0.05 and a statistical power (1-β) of 80%. Initial calculations indicated a need for 112 participants to meet

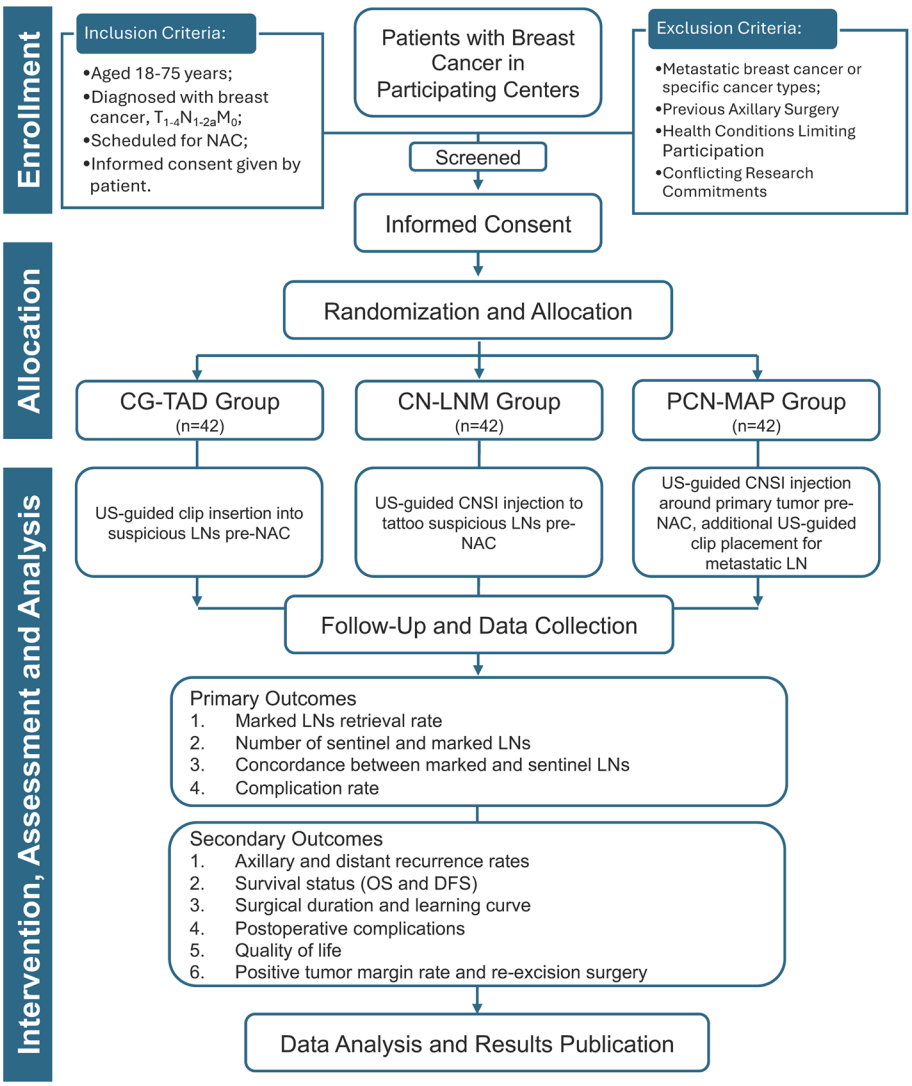

Fig. 1 TADCOM trial flow diagram. *CG-TAD: Clip-Guided Targeted Axillary Dissection; CNSI: Carbon Nanoparticle Suspension Injection; CN-LNM: CNSI Lymph Node Marking; DFS: Disease-Free Survival; LN: Lymph Node; NAC: Neoadjuvant Chemotherapy; OS: Overall Survival; PCN-MAP: Peritumoral CNSI Mapping; US: Ultrasound

these criteria. Considering an anticipated dropout rate of 10%, we adjusted the sample size to 126 participants to maintain the necessary statistical power throughout the study. This adjustment results in approximately 42 participants per treatment arm. These calculations were conducted using Python (version 3.8.5) with the SciPy stats module (version 1.5.2).

Neoadjuvant chemotherapy regimens

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. During 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.

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 clipped lymph nodes. This group serves as the control arm, employing standard methodologies consistent with those reported in existing TAD literature [31].

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

participants will receive CNSI injections 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 carbon-marked lymph nodes.

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 sentinel and 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, clipped lymph nodes, and carbon-marked lymph nodes.

Assessment Criteria for suspicious Axillary Lymph Nodes

To standardize ultrasound assessments of axillary lymph nodes, we established rigorous evaluation criteria outlined in Table 2, focusing on sonographic morphological and vascular characteristics to quantify lymph node suspicion. Nodes scoring three or more points are deemed suspicious, necessitating further diagnostic confirmation through fine-needle aspiration (FNA) or core-needle biopsy (CNB), depending on clinical judgment. This standardized scoring system ensures consistent evaluation across different clinicians and enhances the reliability of breast cancer staging and treatment planning.

Breast Cancer Primary Tumor Surgical Strategies

Surgical management of primary breast tumors in patients is meticulously customized according to individual clinicopathological characteristics and patient preferences. The medical team conducts a thorough assessment of the tumor, evaluating factors such as its location, size, and multicentricity, alongside the patient's physical health and aesthetic considerations. This assessment forms the basis for in-depth discussions with the patient about the most suitable surgical options available. The surgical approaches considered include mastectomy, breast-conserving surgery (BCS), and options for breast reconstruction. Each strategy is selected to optimize oncological outcomes while addressing the patient's personal treatment goals and quality of life considerations.

Pathological evaluation and Post-TAD Management of Axillary 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.

Table 2 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
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

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.

Termination criteria

The following circumstances will lead to the termination of participation in the study:

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.

Endpoints

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. This will help us understand potential changes in lymphatic drainage patterns following NAC.
4. Complication rate: All surgery-related complications, including but not limited to hemorrhage, lymphedema, infection, pain, tissue damage, clip displacement, clip loss, absence of carbon staining, and excessive carbon 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: 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.
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 Organization for Research and Treatment of Cancer Quality of Life Questionnaire - Breast Cancer Module 23 (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.

Data Collection and Follow-Up

To enhance the standardization and consistency within the TADCOM clinical collaboration, a comprehensive set of Standard Operating Procedures (SOPs) has been established. These SOPs cover all aspects of the trial process, including recruitment, grouping, intervention, data collection, and sample management. All research personnel, comprising physicians, evaluators, and trial assistants at each participating center, are mandated to undergo rigorous training on these SOPs and adhere strictly to the protocols outlined in the SOP manual.

Adequate hospitalization time will be maintained at all participating centers to meet the trial's requirements, ensuring that all enrolled patients can complete the necessary diagnostic and evaluation procedures. For patients discharged early, evaluators will implement robust follow-up strategies to prevent the loss of critical data points. Follow-up assessments, scheduled at 2-year and 5-year intervals, may involve contacting patients by phone, inviting them to hospital visits for further evaluations, or conducting home visits to their residences or healthcare facilities when necessary. Details of these follow-up activities are delineated in Table 3.

Monitoring and safety

Data Monitoring Committee (DMC) The DMC, an independent body of experts in oncology, biostatistics, and ethics, oversees participant safety and data integrity. The DMC reviews safety and efficacy data periodically, ensures adherence to ethical guidelines, and decides on the trial's continuation, modification, or termination based on critical data analysis.

Trial Conduct auditing Quarterly audits are conducted by an independent firm to ensure compliance with the clinical trial protocol and Good Clinical Practice (GCP) standards. These audits involve checking the accuracy of data collection, storage, and analysis; verifying informed consent documentation; and ensuring site adherence to trial regulations. Audit findings are promptly reported to the DMC and the trial sponsors to rectify any deviations from the standard procedures.

Table 3 TADCOM trial follow-up schedule and assessments

Time Point	Clinical Assessment	Imaging	Interventions	QoL and Survival
Baseline	Demographics Medical history Physical exam ECOG PS Blood tests	Mammography Ultrasound Breast MRI	Biopsy US-guided clip insertion or CNSI injection	-
Neoadjuvant Therapy (Every 2 cycles)	Treatment regimen Physical exam Adverse events	Response evaluation Mammography Ultrasound Breast MRI	-	-
Surgery	Operative details Intraoperative findings Complications	-	TAD Surgery	-
1 Month Post-Surgery	Physical exam Adverse events	-	-	-
6 Months Post-Surgery	Physical exam Adverse events	Mammography Ultrasound	-	EORTC QLQ-C30 / BR23
1 Year Post-Surgery	Physical exam Adverse events	Mammography Ultrasound Breast MRI	-	EORTC QLQ-C30 / BR23
2 Years Post-Surgery (Every 6 months)	Physical exam Adverse events	Mammography Ultrasound	-	EORTC QLQ-C30 / BR23 DFS / OS
3-5 Years Post-Surgery (Annually)	Physical exam Adverse events	Mammography Ultrasound	-	EORTC QLQ-C30 / BR23 DFS / OS

**ECOG PS* Eastern Cooperative Oncology Group Performance Status, *MRI* Magnetic resonance imaging, *QoL* Quality of life, *EORTC QLQ* European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire, *DFS* Disease-free survival, *OS* Overall survival

Management of adverse events Adverse events are immediately assessed and managed by the clinical team, with all events rigorously documented and reviewed. Serious adverse events trigger instant notifications to the DMC and regulatory bodies, following strict regulatory guidelines. Continuous monitoring and detailed risk-benefit analyses guide treatment adjustments or trial discontinuation decisions to prioritize participant safety at all times.

Statistical analysis

This study adheres to the Consolidated Standards of Reporting Trials (CONSORT) guidelines for the reporting and analysis of clinical trial data. Statistical analyses are conducted using R software (R Foundation for Statistical Computing, version 4.3.0). The study employs t-tests and Mann-Whitney U tests to assess statistical significance in baseline characteristics and treatment features between groups for continuous variables, and Pearson's chi-squared (χ^2) tests for categorical variables. The 95% confidence intervals (CI) for false-negative rates are calculated using the Clopper-Person exact method.

Survival data are analyzed using the Kaplan-Meier method and log-rank tests to evaluate overall survival (OS), and disease-free survival (DFS). Additionally, the Cox proportional hazards regression model is utilized to compute hazard ratios (HR) and their 95% CIs, incorporating a multivariable model that adjusts for potential confounders to identify clinically and treatment-related parameters that significantly influence treatment outcomes.

Stratified analyses are conducted based on the following factors: molecular subtype of breast cancer, preoperative clinical nodal status (cN1 vs. cN2+), postoperative pathological nodal status (ypN0 vs. ypN1mi), and whether marked lymph nodes are within the sentinel nodes. All tests are two-sided, with a significance level set at $p < 0.05$.

Patient and public involvement

In this study, patients and the public have been integral from the outset, shaping the study through initial discussions facilitated by patient advocacy groups and community forums. Their priorities and experiences directly influenced the design, helping to ensure that our research questions and methods align with their real-world needs. Patients contribute to steering committees, guide the selection of meaningful outcome measures, and help design participant recruitment strategies, making our approaches more patient-centered and accessible. This ongoing involvement ensures our research remains relevant, accessible, and impactful.

Study status

Recruitment for the study will commence in November 2024. We anticipate completing all study activities and concluding the trial by July 2028. This timeline allows for comprehensive data collection and analysis, adhering to the study's methodological framework.

Discussion

This prospective, multicenter, non-inferiority randomized controlled trial evaluates the efficacy and accuracy of innovative TAD techniques employing carbon marking combined with methylene blue dye following NAC. This research addresses critical challenges in traditional TAD approaches, particularly the issues with preoperative tissue marker clips such as displacement or loss that can compromise the accuracy of surgical interventions [32, 33]. Our study utilizes the unique properties of CNSI, specifically its tendency to accumulate in lymphatic vessels and create a persistent black stain, by injecting it into suspicious lymph nodes or around the primary tumor site. This approach aims to enhance the precision of lymph node identification and excision following NAC.

A significant concern in TAD procedures has been the high FNR, especially in patients initially diagnosed with cN2 + axillary lymph nodes who are re-staged to ycN0 following NAC. Current clinical guidelines recommend ALND for the cN2 + patients [34]. Our study explores an alternative approach by combining CNSI-traced TAD with axillary radiation, aiming to enhance lymph node detection and reduce the FNR. For patients with initial cN2 + staging, this combination has the potential to enhance the detection and retrieval rates of lymph nodes, positioning CNSI-enhanced TAD as a potentially preferred method for managing complex cases. The precision afforded by CNSI tracing facilitates more accurate localization of lymph nodes during surgery, thereby reducing the likelihood of missing affected nodes and addressing the critical concern of under-treatment in these high-risk patients.

Furthermore, subgroup analyses will be crucial in identifying patient groups that show significant lymph node downstaging in response to NAC. This stratification allows for tailored axillary management strategies that not only aim for the complete eradication of disease but also prioritize the preservation of quality of life by preventing unnecessary overtreatment. These targeted strategies are particularly crucial for managing patients with adverse prognostic features such as hormone receptor negativity, elevated Ki-67 expression, and high histological grades, enhancing the overall efficacy of breast cancer treatment protocols.

Traditional TAD methods that rely on tissue marker clips often require intraoperative imaging equipment such as mammography [35, 36], which are costly and have high requirements for equipment and protection, limiting their widespread adoption in primary healthcare settings., In contrast, carbon marking, utilizing CNSI, leverages the natural aggregative and staining properties of carbon nanoparticles in lymph nodes [37]. This approach enables visual identification by the surgeon without the need for additional intraoperative localization devices, enhancing its applicability across various healthcare settings. Economically, the cost of a single unit of CNSI ($165 for 0.5 mL) is significantly lower than that of a tissue marker clip ($344), offering a more cost-effective solution particularly in scenarios where multiple lymph nodes and tumor margins need to be marked. The per-use cost of CNSI can be further distributed as only 0.1 mL is required per marking, compared to the escalating costs when multiple clips are used.

The post-NAC setting poses unique challenges such as tumor shrinkage and fibrosis, which can impede the drainage pathways typically highlighted by conventional tracers [11]. CNSI's ability to mark relevant lymph nodes before treatment and maintain visibility for several months addresses these issues. While CNSI offers significant benefits for lymph node marking, studies have shown carbon nanoparticles can migrate from initially marked lymph nodes to others in the axilla [26, 27], potentially marking additional nodes beyond the original target. When combined with conventional tracers, this characteristic of CNSI may enhance lymphatic mapping, potentially increasing lymph node detection and reducing FNR. Previous study has shown instances where tissue marker clips identified positive lymph nodes that were neither blue-stained nor radioactive hot spots intraoperatively, resulting in a FNR of 19.0% (95% CI: 5.4-41.9%) [11]. The hypothesis is that changes in lymphatic pathways due to chemotherapy or tumor-related lymphatic disruption might contribute to these outcomes [38, 39]. Recent literature [40] supports the concept that the initially positive pre-NAC marked node represents the true sentinel node at diagnosis, and any post-NAC discordance with intraoperatively identified sentinel nodes likely indicates treatment-induced lymphatic drainage rerouting rather than a flaw in marking techniques. By injecting CNSI around the tumor pre-NAC, this study aims to improve the detection of potentially metastatic lymph nodes by utilizing natural lymphatic drainage, thereby circumventing these issues.

Furthermore, as breast cancer NAC protocols continue to evolve, the precise localization of the tumor bed post-NAC has become critically important. Retrospective studies indicate that placing tissue marker clips before NAC at the primary tumor aids in intraoperative identification, enhancing the surgical margin negativity rate and breast conservation rates [41–43]. Long-term follow-up shows that the local recurrence rate in the clip-marked group is lower than in the control group [41–43]. The use of CNSI for pre-NAC marking of the tumor could further improve these outcomes by providing a clearer demarcation of the tumor bed, enhancing breast conservation rates, and reducing positive margin rates.

Despite the potential benefits of carbon marking in TAD, concerns remain regarding its safety and long-term effects, especially on the lymphatic and reticuloendothelial systems [20, 44]. This study will monitor adverse reactions related to CNSI, ready to adjust or halt the trial to ensure patient safety. The use of CNSI requires advanced surgical skills due to its learning curve, which could influence treatment consistency. Moreover, existing research, focusing on short-term outcomes like lymph node retrieval and postoperative complications, lacks long-term follow-up data to confirm benefits in reducing recurrence and improving survival outcomes (DFS and OS).

This study aims to evaluate the efficacy and safety of various CNSI-based TAD techniques in the management of axillary lymph nodes in breast cancer patients following NAC. If results validate the unique advantages of CNSI tracing, it could offer a new paradigm for axillary management in cN + patients, potentially improving staging accuracy and complication prevention. Additionally, by marking originally positive lymph nodes and assessing their status post-treatment, CNSI could help identify patients who are less responsive to neoadjuvant treatments, suggesting the need for more aggressive treatments to improve prognostic outcomes. Future research should focus on integrating CNSI with other tracers in larger, long-term studies to refine its clinical application, promoting more personalized and precise axillary surgical approaches.

Abbreviations

ALND	Axillary Lymph Node Dissection
BCS	Breast-Conserving Surgery
CI	Confidence Interval
CG-TAD	Clip-Guided Targeted Axillary Dissection
CNSI	Carbon Nanoparticle Suspension Injection
CN-LNM	CNSI Lymph Node Marking
CNB	Core-Needle Biopsy
CONSORT	Consolidated Standards of Reporting Trials
DFS	Disease-Free Survival
EORTC	European Organization for Research and Treatment of Cancer Quality of Life
QLQ-BR23	Questionnaire Breast Cancer-Specific Module
EORTC	European Organization for Research and Treatment of Cancer Quality of Life
QLQ-C30	Questionnaire Core 30
ECOG	Eastern Cooperative Oncology Group
FNA	Fine-Needle Aspiration
FNR	False-Negative Rate

H&E	Hematoxylin and Eosin
HR	Hazard Ratio
IHC	Immunohistochemical
ITCs	Isolated Tumor Cells
NAC	Neoadjuvant Chemotherapy
OS	Overall Survival
pCR	Pathological Complete Response
PCN-MAP	Peritumoral CNSI Mapping
RCT	Peritumoral CNSI Mapping
RCT	Randomized Controlled Trial
RNI	Regional Nodal Irradiation
SLNB	Sentinel Lymph Node Biopsy
SOP	Standard Operating Procedure
TAD	Targeted Axillary Dissection

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Authors' contributions
W.Z.C. and J.H. conceptualized and designed the study. W.Z.C., L.W.P., H.L.C., X.Y.J., and J.H. were involved in preparing and drafting the study protocol. L.W.P. and W.Z.C. contributed to the protocol design and the writing of the manuscript. J.H. provided critical revisions to the protocol. L.W.P. and H.L.C. were responsible for preparing the standard operating procedures and training the medical staff. L.W.P. and W.Z.C. handled the design of the randomization process. L.W.P., H.L.C., X.Y.J., and the trial assistants from the study collaboration group were tasked with patient recruitment, data acquisition, protocol adherence, and trial coordination. W.Z.C. and J.H. managed data and quality control. L.W.P. was responsible for translating informed consent documents and developed the statistical analysis strategy, pivotal for interpreting the trial outcomes. All authors have critically reviewed and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This clinical trial has received ethical approval from the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (Approval No. IRB-2024-1199, dated March 1st, 2024, Supplementary File-1). Any significant modifications to the research protocol will only be made under the strict supervision and explicit authorization of the ethics committee. The conduct of all trial procedures will adhere strictly to the ethical standards mandated by institutional and national research committees, in alignment with the Declaration of Helsinki (version: October 2013, Fortaleza, Brazil) and its subsequent amendments, as well as other globally recognized ethical standards in clinical research. Informed consent will be obtained from all participants or their legal guardians before any protocol-related activities commence. This process will be voluntary and informed, with participants and their guardians retaining the right to revoke consent at any point without justification. Throughout all phases of the study - pre-operative, intra-operative, and post-operative - the provision of non-trial related treatments will conform to standard care protocols established at each participating site. Comprehensive documentation of primary and secondary outcomes will be included in the final clinical trial report, which will adhere to CONSORT guidelines to ensure high-level scientific communication. The results of the trial will be disseminated regardless of the outcome (positive, negative, or inconclusive) to maintain transparency and contribute to the broader scientific knowledge base. Findings will be submitted for publication in a peer-reviewed international journal and presented at national and international conferences to foster academic discussion and peer engagement.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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