# Evaluation of tissue marker clip guided metastatic axillary lymph node targeted dissection with methylene blue mono-tracking after breast cancer neoadjuvant chemotherapy in Chinese population: a multicenter, prospective, self-controlled study

# The Evaluation of cancer cancer cancer neoadjuvant chemotherapy in Chinese population: a multicenter, prospective, self-controlled study

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## I. Background of the study

In the era of minimally invasive surgery for breast cancer, the good benefits of neoadjuvant chemotherapy encourage surgeons to adopt more conservative surgical approaches to further reduce the incidence of adverse reactions, after neoadjuvant chemotherapy, about 21.1% (10.1-74.2%) of the patients were able to achieve PCR(1), of which the axillary PCR rate was between 23-74%(2, 3), these patients still have to bear ALND related complications such as upper limb edema, pain and so on. At present, common clinical examinations such as US, magnetic resonance imagin and Positron emission tomography are not helpful to distinguish axillary residual lesions from PCR. Their combined sensitivity is 81% and negative predictive value is 28-48%(4). Therefore, in-depth exploration of new adjuvant treatment after the preservation of axillary treatment model has clinical practical significance.

The false negative rate (FNR) of SLNB after Neoadjuvant chemotherapy in breast cancer is reported to be 8-14.2%(1). Currently, the American Society of Breast Surgeons recommends that SLNB FNR be controlled at about 5%(5), which suggests that the method should be improved to further reduce FNR. The current NCCN guidelines suggest that the potential goals of neoadjuvant therapy include reducing unpreserved axillary breast cancer to preserved axillary, but the precise resection of metastatic axillary lymph nodes is an important issue in axillary surgery. For axillary metastatic lymph nodes confirmed by pathological biopsy before neoadjuvant therapy, Marker can be inserted to locate the axillary metastatic lymph nodes marked after neoadjuvant therapy and completely removed by Sentinel lymph node biopsy (SLNB) , this is the concept of targeted axillae resection (TAD) (6). The results of ACOSOG Z1071 clinical trial showed that cases with Marker-labeled biopsy greater than or equal to 3 Sentinel lymph node (double tracer) were associated with lower FNR (Marker-labeled FNR : 6.8% ; unlabeled FNR: 13%)(7). Before neoadjuvant therapy, lymph nodes were labeled with radioactive iodine 125 particles. After neoadjuvant therapy, targeted surgery was performed to control FNR at 7%(8). Targeted axillae dissection (TAD) with radioactive iodine 125 particles was performed in MD Anderson Center to

further reduce FNR to 2%(9). Ilina clinical trial evaluated the feasibility of intraoperative ultrasound-guided removal of Marker-labeled Sentinel lymph node. 44(96%) of 46 cases were successful in removal of Marker-located axillary metastatic lymph nodes. 27/35(77%) of 27/35(77%) patients had Sentinel lymph node status that accurately reflected their axillary lymph nodes. The overall FNR was 4.1% (95% CI 0.1-21.1%)(10). At present, Marker localization of lymph nodes can accurately predict actual axillary lymph node status(11) . The Chinese anti-cancer Association guidelines and guidelines for the diagnosis and treatment of breast cancer (2019 edition) recommend that axillary lymph node metastasis in patients who meet the following conditions can avoid axillary dissection after full communication with the patients: cT1-3N1M0, double-tracer imaging, SLN ≥3, lymph node labeling clip before neoadjuvant chemotherapy and axillary lymph node dissection(12). For patients with more than 4 metastatic axillary lymph nodes (Cn2 or above) , because of the low negative rate of axillary lymph nodes after neoadjuvant therapy and the high cost of Marker implantation, Marker implantation should be performed directly after neoadjuvant therapy instead of Marker implantation.

According to the clinical situation in China, due to the lack of radiation-related equipment and protective measures, methylene blue tracer is usually used in sentinel lymph node(5). According to studies in some developing countries, single-tracer Sentinel lymph node with blue dyes (methylene blue, isothiolane) are feasible(13-15). Meta-analysis found that the recognition rate was acceptable (91%) using only Methylene blue Sentinel lymph node, but the false negative rate was too high (FNR 8.6% , 95% CI: 6.7-10.8%) . In this study, carbon nanoparticles suspension combined with methylene blue injection was compared in breast Sentinel lymph node biopsy. The results showed that the FNR of Methylene Blue Group was 8.69%(16) . These results suggest that the NR of methylene blue single-tracer Sentinel lymph node is high, and whether it can be further reduced by Marker-guided TAD technique needs to be further studied. The 2020 consensus of pathologists for breast cancer neoadjuvant therapy recommends that patients with positive axillary lymph node puncture before neoadjuvant therapy may consider placing a metal marker in the site of the positive

lymph node for postoperative pathologic judgment(17), however, due to the lack of clinical evidence, the detailed operating rules and evaluation methods have not been further standardized.

This is a multi-center, prospective, and self-controlled study to evaluate the feasibility of intraoperative ultrasound-guided single-tissue-tag dissection (TAD) for axillary lymph node metastasis in patients with breast cancer T1-4N1M0 following neoadjuvant chemotherapy, to compare Tad with SLNB, whether Tad can improve the accuracy and decrease the false negative, and explore a new model of preserving armpit after neoadjuvant treatment for breast cancer.

## II. Objectives of the study

To evaluate the feasibility of targeted axillary lymph node excision (Sentinel lymph node + labeled lymph node) without axillary dissection in patients with T1-3N1M0 breast cancer after Neoadjuvant Chemotherapy

## III. Study endpoint

### Primary end point

False negative rate (FNR) of single-track Axillary Lymph Node targeted excision guided by methylene blue after neoadjuvant chemotherapy in T1-3N1M0 breast cancer

### Secondary End Point

1. The PCR rate of neoadjuvant chemotherapy;
2. Note the frequency of LN presence in non-sentinel areas;
3. Condition refinement of axilla preservation after neoadjuvant chemotherapy: FNR labeled with LN in non-sentinel regions.

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

### 4.1 Study population

T1-3N1M0 for patients undergoing neoadjuvant chemotherapy and surgery (N = 332)

### 4.2 Criteria for inclusion and exclusion

**Entry criteria:**

Participants in the study must meet the following criteria:

1. Biopsy confirmed breast cancer with axillary lymph node metastasis (T1-3N1M0)
2. Indications of neoadjuvant chemotherapy for breast cancer
3. Signed informed consent form
4. Preoperative anesthesia assessment was low to moderate risk (ASA score)

**Exclusion Criteria:**

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

1) There are absolute and relative contraindications to chemotherapy
2) Refusal or intolerance of surgery
3) Unable or unwilling to put in Marker
4) Pregnant Woman
5) Do not have complete capacity, such as mental illness, drug dependence, anxiety disorders and so on
6) A diabetic who's out of Control
7) Heavy Smoker
8) High risk of anesthesia
9) Any severe complication was not appropriate for patients who participated in this study

### 4.3 Criteria for termination

Patients can stop the study at any time for treatment or evaluation. The details of the patient termination study are as follows:

1) NAT stops when the local tumor (the affected breast, chest wall, armpit and supraclavicular and inferior lymph nodes) has progressed or

metastasized remotely

2) Voluntary termination by the patient, the patient can withdraw at any time, will not lead to follow-up treatment bias;
3) Researchers believe there is a safety issue;
4) The researchers concluded that the patient was in serious violation of the protocol;
5) Patient registration error (if the patient does not meet the criteria for admission/exclusion) ;
6) Patient missed visit.

### 4.4 Criteria for ultrasound diagnosis of suspicious lymph nodes

Scoring criteria:

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| Shape | Fusiform or elongated shape, namely long/short diameter ≥2(0 points) ; round shape, namely long/short diameter < 2(1 point) |
| Edge | Regular (0) ; irregular (1) |
| Central Hilum | Exist (0 points) ; disappear (2 points) |
| Cortex | No thickening (0 min) ; focal thickening (≥4mm) or hyperechoic area (2 min) |
| Fine Calcification | No (0) ; Yes (1) |
| Necrotic | No (0) ; Yes (1) |
| Color Doppler | The central portal blood flow had no marginal flow (0 points) , and the marginal area was disordered and irregular flow (1 points) |

According to the above criteria, the lymph nodes were judged to be suspicious by ≥3. According to the clinical practice of each center, the lymph nodes could be punctured by CNB or FNA.

## V. Research Methodology

### 5.1 Subjects included

A prospective, self-controlled study was conducted in 332 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 Marker insertion under ultrasound in suspicious lymph nodes

Pathological confirmation of metastatic axillary lymph node implantation Marker:

1) Implanted within 1 week of pathological diagnosis
2) After 3 ± 1 week of implantation, ultrasound was performed to ensure visibility
3) Number: 1-2 most suspicious lymph nodes

### 5.3 Neoadjuvant chemotherapy

NCCN guidelines are developed independently by each center in conjunction with its own regional clinical practice.

Evaluation of neoadjuvant chemotherapy (every 2 cycles) :

1) Breast and axillary ultrasound (every 2 cycles)
2) Mammogram (first plus one before surgery)
3) Breast MRI (first + preop, optional)

### 5.4 Procedures for labeling axillary lymph nodes

1) In the operation, three-step method is usually used: first, methylene blue single-tracer SLNB (record the number of Marker) is used to find the labeled lymph nodes, then complete the steps (record the number of Marker) , and finally, ALND (confirm that Marker is found) ;
2) If the imaging of labeled LN ultrasound is not clear, the labeled lymph nodes are placed under the molybdenum target before operation and repositioned with Crochet
3) If the sign LN could not be found, SLNB was first performed, and the presence of Marker was confirmed by intraoperative X-ray, then

ALND, and the Marker was confirmed by postoperative X-ray

4) It is suggested that the specimens of SLNB and Tad should be taken intraoperatively and the Marker should be confirmed after ALND. If there is no intraoperative radiography, it is suggested that SLNB/TAD/ALND specimens should be detected by molybdenum target.

**5.5 Lymph node pathology**

1) The breast surgeon marks the number of metal-clip-labeled lymph nodes (preferably in a separate bag) on the ticket together with the unlabeled lymph nodes.
2) The pathologist examines the specimen and records the number and size of lymph nodes labeled with metal clips and the number and size of lymph nodes not labeled with metal clips.
3) The lymph nodes were dissected parallel to each other at intervals not exceeding 2 mm. Gross examination can identify the metastatic lesions, can choose to include the largest surface of the representative tissue transfer to the test; gross examination was negative, it is recommended to take as much material or send all the tissue microscopic examination.

**5.6 Pathological evaluation of lymph nodes**

Because the detection rate of suspicious axillary lymph node metastasis can be improved by immumohistochemical staining (IHC) , HE combined with IHC staining should be performed for axillary lymph node tissue The presence of isolated tumor cells (ITC) in lymph nodes after neoadjuvant chemotherapy was recorded as YPN0(i +) . If ITC existed, the tumor response could not be evaluated as PCR.

## VI. Record of patient information

### 6.1 Basic patient information

1) Age, weight and body mass index
2) Clinical staging: tumor size and site lymph node status
3) Neoadjuvant therapy
4) Operation Mode: Breast-conserving/modified radical operation
5) Postoperative treatment: Endocrine Therapy, radiotherapy
6) Histopathology: invasive carcinoma, carcinoma in situ, histologic grading, hormone receptor, KI-67, Her-2, lymph node status
7) Menopausal State
8) Family History

### 6.2 Marker placement information

The brand, model and position of Marker, the condition of puncture, the time of puncture, the operator, and the follow-up (whether displacement/loss occurred) were recorded.

### 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 (Time) , recurrence (location/Time) , follow-up treatment.

## VII. Data Management

1) All incoming patients need to be numbered (unique ID number) ;
2) All data are updated on a quarterly basis
3) All data need to be analysed once a year and progress reported to all participating units.

## VIII. Quality Control of clinical trials

1) Multi-center discussion to determine the implementation of the pilot program must be responsible for the unit before the Ethics Committee Review and approval;
2) Participants in clinical trials should be relatively fixed, and technical training sessions should be initiated by key researchers before the start of the trial to carefully study and discuss technical and clinical trial protocols, etc. to ensure the homogeneity of the technology and equipment, unify the judgment standard of the test data and the record way of the test file;
3) The researcher should fill in each item according to the CRF instruction timely, truthfully, detailed and carefully and upload the data in real time
4) All observations and findings in clinical trials should be verified to ensure the reliability of the data and to ensure that the conclusions of clinical trials are derived from the original data;
5) The responsible units in clinical trials should hold regular mid-term seminars to discuss and solve the problems encountered, and verify the results of the data in each center;
6) Researchers should take active measures (notification of follow-up, follow-up) to control the case drop rate within 30% ;

7) Each test center shall establish a quality control system to strictly supervise and control the test quality of the center.

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