

The THAI Journal of SURGERY

Official Publication of The Royal College of Surgeons of Thailand

Vol .47

July - September 2026

No. 3

The THAI Journal of SURGERY 2026;47(3):112-20.

Official Publication of The Royal College of Surgeons of Thailand

Original Article

Feasibility of Using Carbon Tattoo for Targeted Axillary Dissection in Node-Positive Breast Cancer Patients Treated with Neoadjuvant Systemic Therapy: Preliminary Results of a Prospective Study

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Abstract

Background: Neoadjuvant systemic therapy (NST) frequently achieves axillary downstaging in node-positive breast cancer. Targeted axillary dissection (TAD)—combining sentinel lymph node biopsy (SLNB) with removal of pre-NST marked metastatic nodes—provides accurate post-NST axillary restaging. While advanced wireless markers are standard in high-resource settings, carbon tattooing offers an economical, radiation-free visual alternative. This prospective study evaluates the feasibility, accuracy, and volume-optimized safety of carbon tattooing for TAD within Thailand's public health infrastructure.

Materials and Methods: Patients with biopsy-proven axillary metastasis underwent dual marking (metallic clip + cortical carbon tattoo injection) prior to NST. We evaluated a refined injection volume (0.1 mL post-pilot) to minimize background spillage. After NST, we visually identified carbon-tattooed nodes intraoperatively and confirmed clip retrieval via specimen radiography. We performed SLNB using dual mapping with indocyanine green (ICG) fluorescence and blue dye to assess optical clarity between tracers. Primary endpoints were identification rate, accuracy (tattooed matching clipped node), and concordance (tattooed matching SLN).

Results: Twelve patients completed TAD post-NST (mean tattoo-to-surgery interval: 156 days). We visually identified carbon-tattooed nodes in 11 of 12 patients (identification rate: 91.7%). Mean targeted node retrieval was 5.58 nodes. Clipped and tattooed nodes were identical in 11 cases (accuracy: 91.7%), whereas tattooed nodes matched SLNs in 6 cases (concordance: 50.0%). SLN detection was 100% with ICG fluorescence without carbon interference, and 83.3% with blue dye. Cutaneous staining occurred in one patient (8.3%) with no procedural complications.

Conclusions: Carbon tattooing for TAD is a highly accurate, safe, and economical strategy post-NST. By optimizing injection volume and using real-time visual localization, this technique eliminates the need for expensive secondary radiologic localization procedures, providing a scalable TAD solution tailored to resource-constrained surgical oncology settings.

Keywords: Targeted axillary dissection, Carbon tattooing, Node-positive breast cancer, Neoadjuvant systemic therapy, Sentinel lymph node biopsy, Low-resource setting.

Received for publication 28 November 2025; Revised 3 September 2025; Accepted 3 September 2026

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<https://doi.org/10.64387/tjs.2026.278682>

INTRODUCTION

Neoadjuvant systemic therapy (NST) frequently achieves axillary nodal downstaging in node-positive breast cancer, particularly within triple-negative and HER2-positive subtypes.¹ This downstaging allows surgical de-escalation from axillary lymph node dissection (ALND) to sentinel lymph node biopsy (SLNB), significantly reducing long-term upper extremity morbidity such as chronic lymphedema, pain, and shoulder dysfunction.² However, in patients presenting with initial biopsy-proven node-positive disease (cN1/N2) who convert to cN0 post-NST, performing SLNB alone yields an unacceptably high false-negative rate (FNR > 20%).³⁻⁷ Targeted axillary dissection (TAD)—which combines SLNB with selective retrieval of the pre-NST marked metastatic node—suppresses the FNR to below 5%.⁵⁻⁹

Although advanced localization technologies such as radioactive iodine seeds (¹²⁵I), magnetic seeds (Magseed®), and radar reflectors (SAVI SCOUT®) demonstrate high surgical retrieval rates, their widespread adoption remains heavily constrained in resource-limited public healthcare settings like Thailand. High hardware costs (e.g., ~100 USD or 3,500 THB per clip), regulatory burdens surrounding radioactive materials, and the need for secondary pre-operative radiologic re-localization make these devices financially and logistically inaccessible for many institutions.^{10,11} Carbon tattooing offers a compelling, low-cost, radiation-free alternative that enables real-time

visual localization during surgery without requiring additional wire/probe equipment or secondary pre-operative imaging.¹³⁻¹⁶ However, prior international trials utilizing larger carbon injection volumes (0.3–5.0 mL) frequently reported diffuse background tissue staining that compromised intraoperative target identification (reporting detection rates as low as 64%).^{25,27}

This prospective feasibility trial addresses these clinical gaps by evaluating a refined, volume-optimized (0.1 mL) carbon injection protocol designed to prevent background tissue spillage while maintaining high target node visibility. Furthermore, we assess whether carbon tattooing interferes with modern Indocyanine Green (ICG) fluorescence and blue dye dual-tracer mapping. Herein, we report the preliminary intraoperative detection rate, diagnostic accuracy, tracer concordance, and safety profile of carbon-guided TAD at the National Cancer Institute, Thailand.

MATERIALS AND METHODS

Study Design and Ethical Approval

This prospective, single-center, single-arm feasibility trial was conducted at the National Cancer Institute (NCI), Thailand, starting in January 2024 (Clinical Protocol: EC 66028). The trial followed the Declaration of Helsinki and Good Clinical Practice guidelines. We obtained written informed consent from all participants before enrollment.

Table 1 Overview and Diagnostic Metrics of Various Targeted Axillary Dissection (TAD) Localization Modalities¹⁰⁻¹¹

Modality	Placement Timing	Primary Advantages	Technical Limitations & Risks	FNR	IR
Clip + Guidewire	Day of surgery	- Low material cost - Avoids ionizing radiation	- Requires pre-op radiology - Clip migration - Pain - Hematoma - Adjacent tissue injury - Patient discomfort	6.8%	97%
Clip+I seed¹²⁵	5-7 days pre-op	- Minimal migration risk - Excellent patient comfort	- Involves radiation handling - Expensive - Mandates nuclear medicine workflow - Seed loss/migration	4.2%	96.7%
Clip+ Skin mark¹²	Day of surgery	- Non-invasive - Wireless - Radiation-free - Cost-effective	- Highly operator-dependent - Depth limitation	N/A	100%
Clip + Intraoperative US	At initial biopsy	- Cost-effective - Radiation-free - Permits long-term implantation	- Operator-dependent - Potential clip signal obscuration or migration	7%	95.7%
Carbon Suspension Tattoo¹³⁻¹⁶	At initial biopsy	- Highly economical - Stable long-term visual marker; - Requires no localization imaging	- Potential skin hyperpigmentation - Risk of localized dye migration	8.3%	95-100%
Magnetic seeds: (Magseed®)¹⁷⁻¹⁸	Up to 30 + days pre-op	- Highly accurate - Radiation-free - Flexible surgical scheduling	- High equipment costs - Creates MRI signal void artifacts - Depth limitations	1.4%	97-100%
Radar/Infrared (SAVI SCOUT®)¹⁹	At initial biopsy	- Real-time audio feedback - Good depth signal penetration	- High financial cost - Potential signal interference from nickel components	N/A	97%

Abbreviations: FNR, false-negative rate; IR, identification rate; N/A, not available; US, ultrasound.

Patient Selection Criteria

To minimize selection bias inherent to single-arm designs, we prospectively screened all consecutive female patients presenting to the Multidisciplinary Breast Cancer Clinic who met the eligibility criteria and offered participation without physician-directed selection. Certified breast radiologists and pathologists independently evaluated diagnostic imaging and histopathology, blinded to intraoperative trial parameters.

Inclusion and Exclusion Criteria

Patients eligible for inclusion in this study were female patients aged 18 years or older with cytologically (fine-needle aspiration) or histologically (core needle biopsy) confirmed primary invasive breast carcinoma and limited axillary nodal metastasis, defined as 1 to 2 suspicious nodes (clinical stage cN1) prior to treatment. All included patients were scheduled to receive neoadjuvant systemic therapy (NST) followed by surgery and achieved clinical and ultrasonographic conversion to node-negative status (cN0) after completing NST. Conversely, patients presenting with inflammatory breast carcinoma or extensive initial nodal involvement (clinical stage cN2 to cN3) were excluded, as axillary de-escalation via targeted axillary dissection is not oncologically indicated in these settings. Additional exclusion criteria comprised pathologically confirmed distant systemic metastasis (cM1), active pregnancy or lactation, and a documented history of severe hypersensitivity or systemic

allergic reaction to isosulfan blue dye, indocyanine green (ICG), or carbon dye particles.

Rationale for Nodal Selection (cN1) and Baseline Assessment

Initial axillary nodal staging was established using high-resolution bilateral axillary ultrasonography (US). Suspicious nodes were defined morphologically by ultrasound (cortical thickness > 3 mm, asymmetric cortical bulge, or fatty hilum effacement). The rationale for limiting enrollment to patients with limited (1-2 node) cN1 disease is based on international consensus guidelines (NCCN); patients with extensive nodal burden (cN2/N3) have significantly higher residual disease rates after NST, where complete ALND remains mandatory.

Tattoo Injection and Metallic Clip Localization

Under ultrasound guidance, the biopsy-confirmed metastatic node was targeted. A sterile carbon particle suspension (Spot Ex Endoscopic Tattoo, GI Supply, USA) in a volume of 0.1- 0.3 mL was injected directly into the nodal cortex. Simultaneously or immediately following pathological confirmation, a metallic marker clip (UltraClip II, 17G, BD Medical) was deployed into the cortex of the same metastatic node (**Figure 1**). Single-node tattooing and clipping were strictly performed across all participants to establish consistency for targeted retrieval. (**Figure 1**).

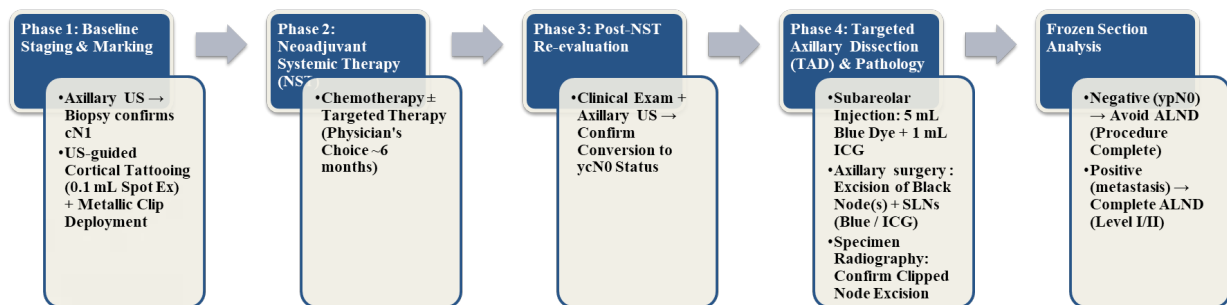


Figure 1 Scheme 1 Flow chart illustration process of this study

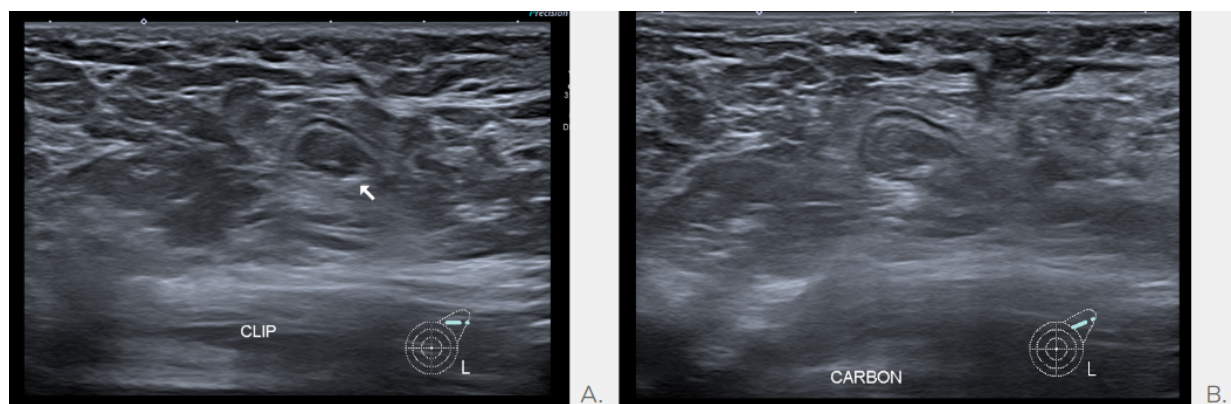


Figure 1 (A) Ultrasound shows metastatic lymph node after clip inserted. (B) Clipped node

Neoadjuvant Systemic Therapy (NST) Regimens

Multidisciplinary consensus determined NST indications based on tumor molecular subtype, clinical T-stage, and eligibility for breast-conserving surgery. Patients received Treatment of Physician's Choice (TPC) over a standard duration of approximately 6 months (24 weeks). Regimens consisted of standard anthracycline- and taxane-based chemotherapy for triple-negative and hormone receptor-positive disease, combined with dual HER2-targeted agents (trastuzumab and pertuzumab) for HER2-overexpressing subtype.

Post-NST Response Evaluation

Nodal response was rigorously evaluated within 2-4 weeks prior to surgery through combined clinical physical examination and repeat high-resolution axillary ultrasonography. Conversion to clinically node-negative status (ycN0) was strictly defined as the complete absence of palpable axillary lymphadenopathy and ultrasonographic resolution of nodal suspicion (restoration of fatty hilum and cortical thickness <3 mm). Patients demonstrating residual clinical or radiological node-positive disease (ycN1) were excluded from TAD and routed directly to completion ALND.

Surgical Technique: Targeted Axillary Dissection (TAD)

All patients underwent TAD using a dual-tracer mapping technique combined with targeted visual pigment retrieval. Intraoperatively, 5 mL of isosulfan blue dye and 1 mL of ICG were injected into the subareolar plexus. Visual inspection of the axilla was performed to identify carbon-tattooed (black) nodes (Figure 2). SLNB was performed simultaneously by tracking blue-stained lymphatic channels and real-time ICG fluorescence imaging using a near-infrared camera system (Figure 3). We respected visualized carbon-tattooed nodes and all identified SLNs. Excision of the clipped node was confirmed intraoperatively via specimen radiography. Resected nodes underwent intraoperative frozen section evaluation; completion Level I/II ALND was executed immediately if frozen section analysis demonstrated metastasis.

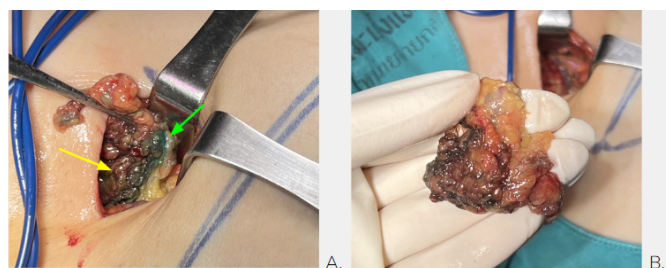


Figure 2 (A) Intraoperative detection of the tattooed perinodal tissue. Yellow arrow: black node. Green arrow: blue lymphatic vessels. (B) Tattooed node immediately after removal

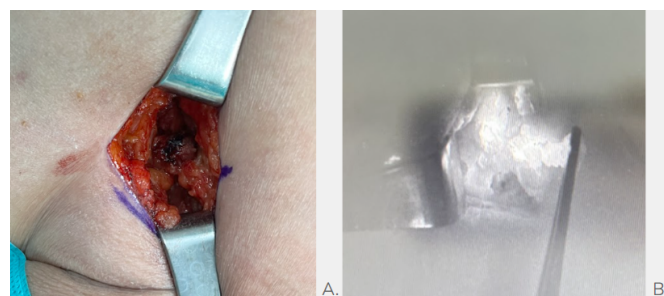


Figure 3 (A) Intraoperative detection of tattooed node(s). (B) Seeking sentinel nodes using fluorescent imaging; ICG-positive node (s) seen

Sample Size Justification and Study Workflow

The study target sample size ($N = 30$) was calculated based on feasibility guidelines for novel surgical localization trials, assuming an expected target node detection rate of 95%, a significance level of $\alpha = 0.05$, and a marginal error of 10%. Because of extended NST durations and patient accumulation timelines, this planned interim feasibility report presents data from the first 12 consecutive patients completing the protocol. Scheme 1 details the structured study workflow.

Statistical analysis

We used descriptive statistics to summarize patient and tumor characteristics. We defined the detection rate as the proportion of patients with black pigment (Black nodes) detected over the total number of patients. Accuracy was the proportion of patients in whom the intraoperatively detected tattooed node and clipped node were identical, out of the total number of patients. Concordance is the proportion of patients in whom tattooed nodes match SLNs. We did not calculate the false negative rate because ALND was not performed in cases with negative SLNs. We performed statistical analyses using SPSS.

RESULTS

A total of 12 consecutive breast cancer patients with biopsy-proven axillary nodal metastasis (cN1) who were scheduled for neoadjuvant systemic therapy (NST) were prospectively enrolled in this trial. All 12 patients underwent ultrasound-guided localization of a single metastatic node via carbon tattooing (0.1 mL Spot Ex) and metallic clip placement before initiating NST. After completing NST, all 12 patients achieved conversion to clinically and ultrasonographically node-negative status (ycN0) and underwent targeted axillary dissection (TAD) with dual-tracer mapping. The clinicopathological and treatment details are shown in Table 2. The mean age was 57 years (range 42-79). All tumors were invasive ductal carcinoma. There were 6 HR-positive cases and 7 HER2-positive cases, but only 3 received HER2-targeted therapy. Breast-conserving surgery was

Table 2 Baseline Clinicopathological Characteristics of Study Participants (N = 12)

Characteristic	Value
Age (years), Mean ($\pm$SD)	57 ($\pm$ 11)
Menopausal status, n (%)	
Premenopausal	4 (33.3)
Postmenopausal	8 (66.7)
Clinical T stage, n (%)	
cT1	2 (16.7)
cT2	9 (75)
cT3	0 (0)
cT4	1 (8.3)
Clinical N stage, n (%)	
cN1	12 (100)
cN2	0 (0)
Histologic grade, n (%)	
1	1 (8.3)
2	6 (50)
3	5 (41.7)
Histologic subtype, n (%)	
Luminal A	1 (8.3)
Luminal B / HER2 negative	3 (25)
Luminal B / HER2 positive	2 (16.7)
HER2 overexpression	5 (41.7)
Triple-negative (TNBC)	1 (8.3)
Tumor Histopathology, n (%)	
Invasive ductal carcinoma	12 (100)
Invasive lobular carcinoma	0 (0)
Others	0 (0)
Neoadjuvant Regimen, n (%)	
Anthracycline–Taxane sequential chemotherapy	9 (75)
Anthracycline–Taxane + Trastuzumab + Pertuzumab	3 (25)
Others	0 (0)
Surgical Procedure, n (%)	
Breast-conserving surgery	7 (58.3)
Mastectomy	5 (41.7)
Mastectomy with reconstruction	0 (0.0)

performed in 58.3%, and the rest underwent total mastectomy. In all cases, carbon injections targeted only 1 node.

Based on the surgical assessment presented in **Table 3**, the mean interval from carbon injection to the day of operation is 156 days (range 26–199). The mean total number of targeted nodes (TLNs = tattooed nodes and/or clipped nodes and/or SLNs) retrieved is 5 nodes (range 3–7). We visualized the tattooed nodes intraoperatively in 11 of 12 cases. The identification rate is 91.7%. The number of black nodes seen intraoperatively varies from 0 to 3. The mean total number of SLNs retrieved is 4 nodes, which are identified by blue dye in 83.3% and by ICG in 100%. Tattooed nodes matched SLNs in 6 of 12 cases. The concordance rate is 50%. Clipped nodes matched tattooed nodes in 11 cases. The accuracy rate is 91.7%. The frozen section of TLNs was positive in 7 cases (58.3%), so axillary dissection was performed in those cases. Pathological tumor response reveals

pCR/pTis in 5 of 12 cases. Pathological nodal response revealed pN0 in 5 out of 12 cases. Four cases appeared as ypT0/is N0; all were HER2-expressing subtypes, and 3 received neoadjuvant systemic chemotherapy combined with targeted therapy. The complications were observed after injecting carbon particles into the axillary nodes. Only one case showed skin staining. Some cases showed minimal spillage during surgery, resulting in retrieval of more than one black node, as shown in **Figure 2**.

Table 3 Intraoperative Localization Metrics and Pathological Responses (N = 12)

Variable	Value
Interval from tattoo to surgery (days), Mean ($\pm$SD)	156 ($\pm$ 52)
Total targeted nodes retrieved, Mean ($\pm$SD)	5.58 ($\pm$ 1.62)
Intraoperative carbon node visual identification, n (%)	
Yes	11 (91.7)
No	1 (8.3)
Number of carbon-pigmented nodes retrieved, n (%)	
0 node	1 (8.3)
1 node	8 (66.7)
2 nodes	2 (16.7)
3 nodes	1 (8.3)
Number of SLNs retrieved, n (%)	
1 node	0 (0)
2 nodes	3 (25)
3 nodes	0 (0)
4 nodes	3 (25)
> 4 nodes	6 (50)
Sentinel lymph node mapping technique, n (%)	
Isosulfan Blue Dye detection	10 (83.3)
Indocyanine Green (ICG) detection	12 (100)
Tattooed vs. SLN Concordance Rate, n (%)	6 (50)
Confirmed clip removal, n (%)	12 (100)
Clipped vs. Tattooed Node Accuracy Rate, n (%)	11 (91.7)
Frozen Section Result, n (%)	
Negative for metastasis	5 (41.7)
Positive for metastasis	7 (58.3)
Completion ALND Performed, n (%)	7 (58.3)
Pathologic response, n (%)	
pCR/pTis	5 (41.7)
ypT1	7 (58.3)
ypT2	0 (0.0)
ypT3	0 (0.0)
Pathologic nodal response, n (%)	
ypN0	5 (41.7)
ypN1	6 (50)
ypN2	1 (8.3)
ypN3	0 (0)
Tattoo-Related Morbidity, n (%)	
Minor dye spillage	3 (25)
Cutaneous hyperpigmentation	1 (8.3)
Allergic or adverse events	0 (0)
No symptom	8 (66.7)

DISCUSSION

Axillary status is important for planning systemic adjuvant treatment and RT. NCCN guideline²⁰ states that highly selected patients with biopsy-proven axillary metastases who then convert to clinically node-negative after preoperative systemic therapy may undergo SLNB with removal of the clipped lymph node. This is currently a category 2B recommendation, as the rate of false negatives is high when SLNB is performed after preoperative systemic therapy. Based on available data, the false-negative rate can be reduced by marking biopsied lymph nodes to document removal, using dual tracers, and removing 3 or more sentinel nodes (targeted ALND). When sentinel nodes are not successfully identified, level I and II axillary dissection is recommended for axillary staging. NCCN mentions clipping and marking techniques but does not specify which marking technique is best, so our study aimed to validate the feasibility of carbon tattooing, the optimal marking method in Thailand, for the reasons mentioned above. We used clip insertion as the standard technique to calculate accuracy. Choy et al.²¹ performed the first prospective pilot study using carbon marking. The study involved tattooing biopsied ALNs with a sterile black carbon suspension (SpotTM) 0.1–0.5 mL. The study divided 28 patients into two groups. Group I (16 patients) underwent surgery first, and group II (12 patients) underwent NST followed by surgery. The mean interval from tattooing to surgery was 22.9 days (group I) and 130 days (group II). Black tattoo was visualized intraoperatively in all cases, except in one case in which only microscopic black pigment was observed. This trial confirmed that tattooed nodes by SpotTM are visible intraoperatively, even months later. Further studies have been published since then. Two South Korean studies^{22–23} used CharcotraceTM (Charcotrace; Healthdirect, Haymarket, New South Wales, Australia) for tattooing. Park et al. injected 0.2–0.8 mL of the solution into the TLN of 20 patients. The detection rates for TLN and SLN were 100%. Kim et al. conducted a retrospective analysis of 45 patients in whom CharcotraceTM (1.0–3.0 mL) was injected after NST into axillary lymph nodes that remained suspicious on ultrasound. The tattooed lymph nodes were detected during operation in 44 patients (98%) and matched with the SLN in 57%. Neither trial reported complications, so our study aimed to observe complications associated with carbon suspension. In another retrospective trial, 24/75 patients were tattooed pre-NST with a sterile carbon suspension (Spot) 0.3–0.7 mL. Intraoperative identification of tattooed nodes was successful in 71 of 75 patients (94.6%). The concordance rate of SLN and tattooed lymph nodes was 75.7%. The authors reported that pathological examination revealed tattoo ink migration from one node to another, resulting in more stained nodes than initially tattooed. Accordingly, identifying black pigment on pathological

examination of SLNs is not always evidence of marked node retrieval. That is why, in our study, we used both clip and carbon tattoos to confirm pathologic nodes initially and may adjust to using only carbon tattoos in future studies.

This was our initial experience using carbon tattooing for axillary nodes at our institution. In our first case, the protocol specified injecting 0.3 mL of carbon particles into the cortex of the pathological axillary node. Intraoperative findings showed minimal spillage (**Figure 2A**), but the black and blue nodes were still distinguishable. After retrieving targeted lymph nodes (TLN) (**Figure 2B**), the nodes were dissected, and fluorescence imaging showed no ICG signal in the tattooed (clipped) node, in contrast to the remaining blue nodes, which were positive for ICG (**Figure 4B**). Clip removal was confirmed by mammograms of the TLN specimen, as shown in **Figure 4C**. In this case, the tattooed node and clipped node were identical but did not correspond to SLNs.

Multicentre prospective studies (25) have used Spot[®] or Black EyeTM (0.3–2.9 mL); however, only 22 patients received NST. The identification rate was lower at 64%, explained by the higher median volume of carbon solution (2.0 mL), which causes diffuse black staining in the axillary tissue. After observing minimal intraoperative spillage of carbon particles in our first three cases, we adjusted the protocol to reduce carbon injection to 0.1 cc from case four onward. Although localized background carbon spillage occurred in a minority of cases, it did not adversely affect intraoperative decision-making, nodal identification, or histopathological analysis. The volume-optimized (0.1 mL) injection protocol effectively prevented extensive dye diffusion. The distinct, localized black pigmentation of the target node remained easily distinguishable from surrounding soft tissues and did not obscure blue dye uptake or quench Indocyanine Green (ICG) fluorescence mapping. In a prospective study by Patel et al.²⁶, data from 47 patients who received primary systemic therapy were published. 0.1–1.0 mL of Spot[®] was injected, including 12 patients initially reported by Choy et al., and a detection rate of 100% was confirmed. The tattooed nodes corresponded to the sentinel lymph node in 98.5% of cases. The concordance of TLNs and SLNs varies from 50–98%.^{22–24,26} Similar to our study, the identification rate is 91.7%, but tattooed nodes match SLNs in half of patients; the concordance rate is only 50%. Beyond visual masking caused by dense black carbon pigment obscuring blue dye uptake in nearby lymphatic channels, the observed moderate concordance rate (50%) may reflect therapy-induced anatomical alterations within the axillary lymphatic system. Neoadjuvant systemic therapy (NST) frequently induces tumor regression, perilymphatic fibrosis, and microvascular obliteration. These structural changes can physically obstruct or reroute initial lymphatic pathways,

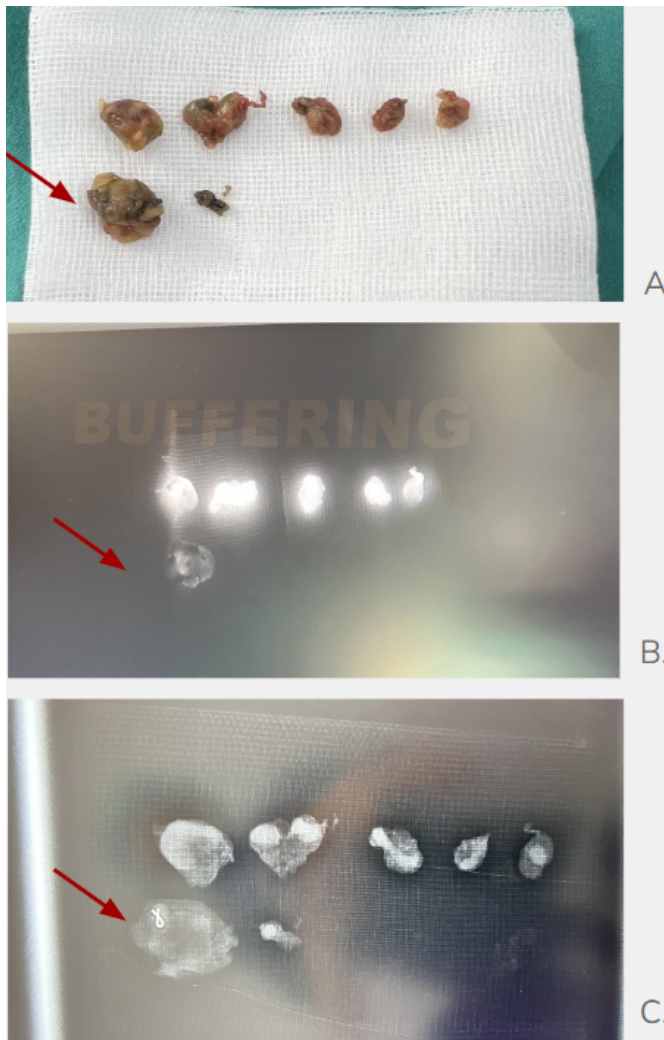


Figure 4 These are samples of all targeted lymph nodes that were retrieved. First row are sentinel nodes. The red arrow indicates the tattooed node.

- (A) Visualized in blue in the first row and in black in the second row. The arrow points to the black tattooed node.
- (B) Under fluorescence imaging: ICG was seen in all sentinel nodes in the upper row but not in the tattooed node.
- (C) Specimen radiograph confirms the clipped node is the tattooed node and confirms the clipped removal

resulting in aberrant lymphatic flow that directs intraoperatively injected tracers (e.g., isosulfan blue or ICG) away from the true index node and toward alternative non-target sentinel nodes. Furthermore, differential tumor response across multiple involvement sites can lead to asymmetric lymphatic drainage. While the pre-NST marked node undergoes fibrotic replacement or complete pathologic response, newly dominant, uninvolved lymphatic channels may preferentially take up blue dye during post-NST mapping. Consequently, the moderate concordance rate of 50% reflects a combination of optical interference from high-density carbon particle retention and true physiological

rerouting of lymphatic channels secondary to NST. These further underscores why dual-tracer mapping paired with targeted axillary dissection (TAD) is necessary to ensure retrieval of the clipped/tattooed node, regardless of post-treatment tracer migration. Fig. 5 demonstrates that the tattooed nodes were also blue and appeared with ICG. This may be due to the use of carbon, which adds darker pigmentation and makes it harder to distinguish blue from black, thereby contributing to higher concordance rates in some other studies. The largest prospective study of tattooing TLNs is the multicentre TATTOO study.²⁷ A total of 110 patients who received NST underwent Spot or CARBO-REP 0.3-5.0 ml injection. TLNs were detected at operation in 93.6%. Skin staining occurred in 4.5% of cases as an undesirable side effect. SLNs were identical to the TLNs in 60%. This TAD study's FNR was 9.1%, slightly higher than in previous studies because fewer patients completed ALND. In our study, the detection rate of tattooed nodes is 91.7%. The accuracy is 91.7%. We identified only one case where we couldn't identify clipped nodes by carbon, but they appeared as blue nodes instead. Pathological tumor response reveals pCR/pTis in 5 of 12 cases. Pathological nodal response reveals pN0 in 5 out of 12 cases. Following neoadjuvant systemic therapy, four patients (33.3%) achieved complete eradication of both invasive and in situ disease in the breast and axilla (ypT0, ypN0), representing strict pathological complete response (pCR). One additional patient (8.3%) demonstrated clearance of invasive carcinoma with residual ductal carcinoma in situ (ypTis, ypN0). While both outcomes represent complete local eradication of invasive breast cancer and carry comparable favorable long-term disease-free survival benefits, we report them separately to preserve precise histopathological distinctions. In our cohort, seven patients were identified as having HER2-positive disease; however, only three received neoadjuvant anti-HER2 targeted therapy (trastuzumab). This discrepancy is primarily due to financial constraints and healthcare reimbursement structures within Thailand's national public health framework. Consequently, four patients were unable to afford anti-HER2 therapy prior to surgery due to financial barriers and received standard neoadjuvant cytotoxic chemotherapy regimens alone. Although seven patients underwent completion ALND, this subgroup cannot be used to estimate the false-negative rate (FNR) of TAD due to verification bias. We performed completion ALND selectively in node-positive cases, while node-negative (ypN0) patients were spared dissection to avoid upper extremity morbidity. Calculating FNR requires routine ALND across all participants, including pathologically negative cases, to identify missed non-target node metastases, a practice no longer ethically justifiable in surgical de-escalation trials. Skin staining was found in 1 of 12 cases, but no other major complications from carbon particles.



Figure 5 (A) Tattooed node with a sentinel also identified by the technique, as shown by a blue-green arrow, and (B) sparkling with ICG. (C) MMG specimen confirms tattooed node concurrence with the node

While dual localization with a metallic clip remains the optimal reference standard for radiological confirmation, carbon tattooing alone represents a highly viable, cost-effective, and practical alternative in resource-limited settings where clips, wireless seeds, or wire-localization technologies are financially or logistically inaccessible. In our prospective cohort, single-node carbon tattooing demonstrated a high identification rate of 91.7%, underscoring its strong visual reliability for direct intraoperative guidance without requiring specialized detection probes or additional preoperative interventional procedures. In middle-income countries like Thailand—where healthcare cost constraints limit access to expensive localization devices—carbon tattooing alone offers surgeons high confidence in localized targeted node retrieval. When combined with standard post-NST dual-tracer sentinel lymph node biopsy (SLNB), carbon tattooing provides a scalable, low-cost strategy for safe axillary surgical de-escalation. Several methodological limitations of this trial must be acknowledged to appropriately contextualize our findings. First, the small sample size ($N = 12$) and single-center prospective design at a national tertiary cancer institution limit the immediate generalizability of our results to broader community healthcare settings. Second, because of the short postoperative follow-up, we could not evaluate long-term oncologic endpoints such as regional axillary recurrence rates and overall survival. Third, because we ethically omitted completion axillary lymph node dissection (ALND) in pathologically node-negative (ypN0) patients to minimize upper extremity surgical morbidity, we could not calculate the true False-Negative Rate (FNR) of this carbon-tattooed TAD protocol without introducing severe verification bias. Larger, multicenter prospective trials with extended long-term follow-up are warranted to confirm the oncologic safety and long-term efficacy of this approach.

CONCLUSION

This prospective evaluation demonstrates that single-node carbon tattooing combined with metallic clip placement is a highly feasible, safe, and cost-effective approach for targeted axillary dissection (TAD) following neoadjuvant systemic therapy. When evaluated against specimen radiography of clipped nodes as the reference standard, carbon tattooing achieved an identification accuracy of 91.7% without requiring additional pre-operative radiological wire localization or nuclear medicine procedures. Furthermore, in clinical scenarios or resource-limited settings where metallic clip deployment is financially or technically unfeasible, carbon tattooing alone provides a reliable, scalable, and radiation-free visual localization alternative to support safe surgical axillary de-escalation.

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