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ORIGINAL ARTICLE – BREAST ONCOLOGY

Pretreatment Tattoo Marking of Suspicious Axillary Lymph Nodes: Reliability and Correlation with Sentinel Lymph Node

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ABSTRACT

Background. Tattooing is an alternative method for marking biopsied axillary lymph nodes (ALNs) before initiation of treatments for newly diagnosed breast cancer. Detection of black ink-stained nodes is performed under direct visualization at surgery and is combined with sentinel node (SLN) mapping procedures.

Methods. Women with newly diagnosed breast cancer who underwent fine or core-needle biopsy of suspicious ALNs were recruited. The nodal cortex and perinodal soft tissue was injected with 0.1–1.0 ml of Spot™ (GI Supply) black ink under ultrasound guidance. Intraoperatively, black stained nodes were removed along with SLNs, noting concordance between the two.

Results. Sixty-six evaluable patients were enrolled (2013–2017). Nineteen received surgery first (Group 1) and 47 neoadjuvant therapy (NAT, Group 2). The average number of nodes tattooed was 1.16 for Group 1 and 1.04 for Group 2. The average interval from tattoo to surgery was 21 days (range 1–62) for Group 1 and 148 days (range 71–257) for Group 2. The tattooed node(s) were visually identified at surgery and corresponded to the sentinel lymph node(s) in 98.5% of cases (18/19 in Group 1 and 47/47 in Group 2). Of the 14 patients in Group 2 whose nodes remained positive following NAT, the tattooed node was the SLN associated with carcinoma.

Conclusions. Tattooing is an alternative method for marking biopsied ALNs. Tattooed nodes coincided with SLNs in 98.5% of cases. This technique is advantageous, because it allows for fewer procedures and lower costs compared with other methods.

Staging and tumor molecular markers are the key factors that guide clinical management of newly diagnosed breast cancer and the use of neoadjuvant chemotherapy or endocrine therapy (NAT).[1,2] Pretreatment sampling of palpable or sonographically abnormal axillary lymph nodes is becoming more routine. Determining the presence of nodal involvement before initiation of NAT is critical to evaluate responsiveness to treatment and accurately assess downstaging of disease.[3–5]

Clinical examination of regional nodes has long been recognized as inaccurate and unreliable.[6] Axillary ultrasound evolved as a noninvasive technique to detect abnormal nodes. This approach has become part of diagnostic imaging practices in conjunction with fine-needle aspiration or core-needle biopsies. Presurgical nodal sampling gained momentum in the era of sentinel lymph node biopsy as a means of bypassing this step and proceeding to axillary node dissection (ALND) in cases of positive nodes.[7–9] It also eliminated the need for radiotracer and/or blue dye injection, lymphoscintigraphy, and reduced the number of delayed ALNDs in the cases of false-negative intraoperative sentinel lymph node (SLN) evaluations.[10,11]

Sentinel node mapping after neoadjuvant therapy has gained acceptance in recent years.[5,12] Removal of pretreatment marked positive nodes has been shown to decrease false-negative rates at the time of postneoadjuvant sentinel node mapping.[13] However, concordance between

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these biopsied nodes and sentinel nodes may be discordant in up to 23% of cases, as reported by Caudle et al.[14] In addition to marker displacement, similar to that observed with biopsied and tagged breast lesions, cancer treatment causes changes in the tissue and may exacerbate the occurrence of displacement.[15] Therefore, it is possible that some techniques may be superior for tagging nodes subjected to neoadjuvant treatments. Typically, biopsied nodes are marked with a metallic clip placed within or adjacent to the lymph node at the time of biopsy. Other types of markers have emerged as alternatives to clips, namely radioactive and magnetic seeds, biopolymer, or optical reflectors.[14,16–18] Each of these methods can require different levels of complexity with respect to storing, insertion, and detection at surgery.[19,20] Their costs vary, and some newer devices are substantially more expensive than traditional techniques.

Our group has proposed black ink tattooing as an alternative method for marking biopsied axillary lymph nodes.[21] A major advantage of this method is that no image guidance or special equipment is necessary at the time of surgery, because the black staining is visible to the surgeon during surgical exploration of the axilla. The technique is simple, and the material is inexpensive.

The study was designed to determine whether tattooing of biopsied axillary nodes with sterile black ink allows the surgeon visually to identify them intraoperatively. We hypothesized that tattooed nodes could be reliably identified, and there would be high concordance between the marked and sentinel nodes. This report represents our extended experience, including the previously published 28 cases.[21]

METHODS

The study was approved by the Scientific Review Committee of the Stanford Cancer Institute and the Administrative Panel on Human Subjects in Medical Research at Stanford University. Women older than age 18 years who were diagnosed with a clinical T0–T4 invasive breast cancer and a palpable or sonographically suspicious ALN were deemed eligible. Breast cancer cases presenting as axillary nodal metastases, without a known primary or large palpable DCIS lesions also were included. One of four breast surgeons or one of four dedicated breast imagers recruited participants for this study. Subjects signed written, informed consent following a clinical examination or ultrasound evaluation of the axilla. The criteria for tissue sampling included firm or enlarged axillary nodes on physical examination or sonographically detected abnormal-appearing nodes characterized by a thickened cortex and loss of fatty hilum. Sixteen women who had previously been biopsied, and for whom we were able to reliably identify the same abnormal node, also were included.

For this procedure, the patients were positioned either supine or in lateral decubitus, with or without a wedge behind the back, on an examining table with the ipsilateral arm outstretched 90°–165°. Suspicious nodes were visualized by ultrasound and tattooed immediately following tissue sampling by either fine-needle aspiration or core-needle biopsy. The injections targeted the antero-lateral cortex and perinodal fat of the biopsied node utilizing a 25-gauge needle. The volume of tattoo ink injected was adjusted slightly during the course of the study as the investigators gained experience. Initially smaller volumes of 0.1–0.2 ml of Spot™ (GI Supply) sterile black ink were injected, but volumes were increased to facilitate the recognition of tattoo at surgery. On average patients were injected with 0.3 ml, although five patients received between 0.6 and 1 ml based on depth, location, or larger node size. The majority of patients had only one node tattooed, but six patients had two separate nodes injected.

SLN mapping was performed in all patients with peritumoral injection of 4–5 ml of isosulfan blue dye and/or 1 or 2 mCi of periareolar Tc-sulfur colloid the same day or day before surgery, respectively. Palpable, blue, or radioactive ("hot") nodes were designated as sentinel nodes. After the clavipectoral fascia was incised, the axilla was inspected for the presence of black ink. The black, tattooed, perinodal fat guided the surgeon to the targeted tattooed node. The identified black nodes were then examined to determine whether the node was blue, "hot," or firm on palpation. Discerning blue nodes was challenging and was masked by the black ink in some instances (Fig. 1). However, careful inspection and the identification of a blue leading lymphatic served to confirm the tattooed node as a sentinel node. All black nodes were excised and

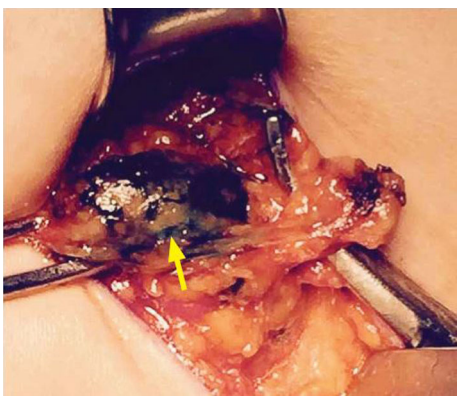


FIG. 1 Tattooed lymph node. Intraoperative evaluation of black tattooed node coinciding with sentinel node as evidence by blue leading lymphatics (arrow)

carefully examined to determine whether they fulfilled criteria of being an SLN, namely whether palpably suspicious, "hot," or blue. All black nodes and sentinel nodes were excised and submitted for intraoperative touch preparation or frozen-section evaluation. The axilla was visually examined further to determine the presence of any nonsentinel black nodes. Completion axillary dissection was performed for node positivity after NAT. However, Z0011 criteria were used for those receiving surgery first. Definitive pathological examination included determination of the presence of tumor as well as histological features associated with treatment effect in nodes previously involved with tumor. Microscopic evidence of tumor treatment effect, for example fibrosis or necrosis, was noted specifically in the designated black nodes (Fig. 2).

RESULTS

Between January 2013 and December 2017, seventy-six women with suspicious or biopsy-proven involved axillary lymph nodes were enrolled in the study and underwent tattoo marking of targeted nodes. Nineteen women (Group 1) underwent surgery first while the remaining 57 women (Group 2) received NAT before surgery. Of the latter group, 10 were excluded, because their surgery took place at an outside institution ($n = 4$), experienced progression of disease ($n = 5$), or withdrew from the study ($n = 1$). Pathologic nodal involvement was documented among 11 (57.9%) of the 19 women in Group 1 and 37 (78.7%) of the 47 women in Group 2 at pretreatment biopsy.

The mean time interval between tattooing and operation was 21 (range 1–62) days for Group 1 and 148 (range 71–257) days for Group 2. The average number of SLNs evaluated was 3.0 (range 1–5) for Group 1 and 3.1 (range 1–7) for Group 2 with an average of 1.0 (range 0–4) positive SLNs in Group 1 and 0.4 (range 0–3) in Group 2. The average number of nodes tattooed was 1.16 (range 1–2) for Group 1 and 1.04 (range 1–2) for Group 2. Black tattoo pigment staining the node or perinodal fat was visually identified intraoperatively in all Group 2 cases and all but one case in Group 1 (94.7%). The one patient in Group 1 without an identifiable pigmented node at surgery was injected with only 0.1 ml but had microscopic evidence of pigment within the positive palpable SLN. The average number of black sentinel nodes was 1.7 (range 0–3) for Group 1 and 1.7 (range 1–3) for Group 2 (Table 1). Although in most instances only one node was tattooed, additional black-stained sentinel nodes were found in 10 (52.6%) women in Group 1 and 26 (55.3%) women in Group 2, likely resulting from their proximity to the tattooed node. SLN involvement was demonstrated in 13 of 19 (68.4%) patients in Group 1 at surgery compared with 11 of 19 (57.9%) on preoperative diagnostic core biopsy. In other words, a false-negative pretreatment node biopsy was encountered in two patients (13.3%) of Group 1 who underwent ALND. All node-positive cases received ALND with the exception of two women who were treated by lumpectomy and adjuvant whole breast irradiation in accordance to Z011 criteria. Black pigment was microscopically detected in the perinodal fat of three ALNDs (Fig. 3).

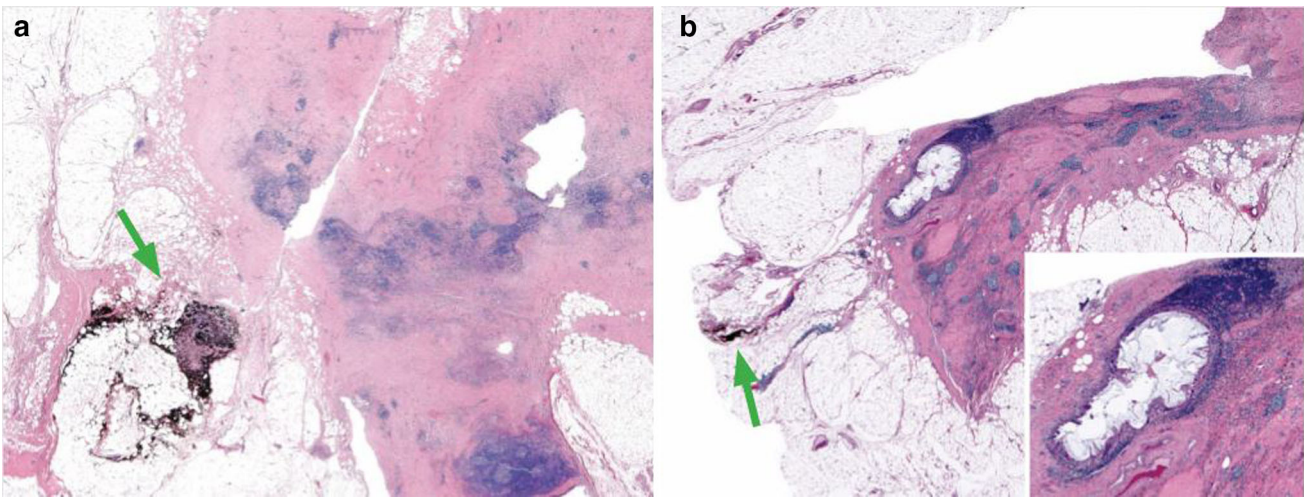
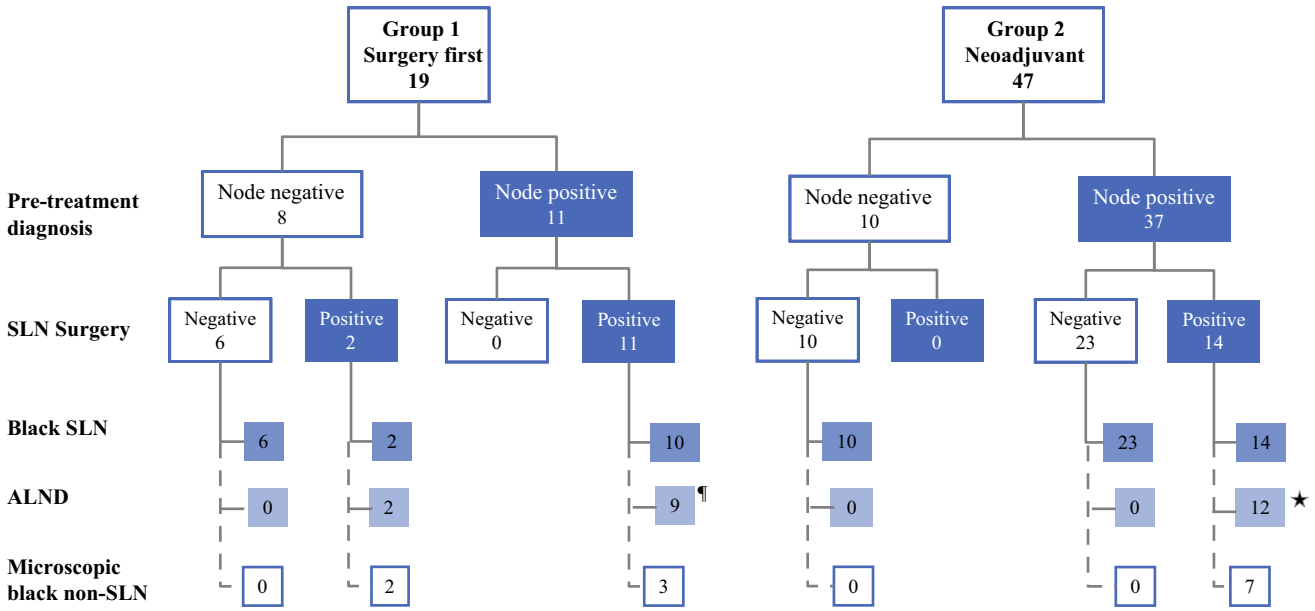


FIG. 2 Histologic findings after neoadjuvant chemotherapy. Low magnification H&E photomicrograph of **a** perinodal black ink tattoo (arrow) adjacent to a node demonstrating fibrosis as an indicator of treatment effect (pink); **b** perinodal tattoo (arrow) adjacent to biopsy site (inset)

TABLE 1 Patient demographics

	Group 1 (*N* = 19)	Group 2 (*N* = 47)
Age (year)	54 (29–71)	53 (33–76)
Clinical tumor size (*N*)		
T0	0	4
Tis	1*	0
T1	5	7
T2	11	22
T3	2	12
T4	0	2
Clinical nodal status (*N*)		
N0	6	6
N1	13	32
N2	0	7
N3	0	2
Receptor status (*N*)		
HR+, HER2−	12	14
HR−, HER2+	2	8
HR+, HER2+	0	11
HR−, HER2−	5	14
Injected by (*N*)		
Surgeon	9	27
Radiologist	10	20
Number nodes tattooed	1.16 (1–2)	1.07 (1–2)
Days from tattoo to surgery	21 (1–62)	148 (71–257)
No. of sentinel nodes	3.0	3.1
No. black nodes identified	1.7 (0–3)	1.7 (1–3)
Tattooed node also as sentinel node (*N*)	18 (94.7%)	47 (100%)

*Large palpable mass



¶ No ALND for 2 node positive cases

★ 2 cases with isolated tumor cells, no ALND performed

FIG. 3 Concordance of black node with sentinel lymph node. Presurgery or pretreatment biopsy findings and subsequent pathological evaluation of sentinel lymph nodes (SLN) are compared with black SLN identified

There were no false-negative pretreatment biopsies among the women in Group 2 (no case designated as negative before NAT had a positive node at definitive surgery). Tattooed SLNs were identified in all patients. Of the 37 (78.7%) node-positive cases in Group 2, twenty-three (62.1%) had a complete pathologic response after NAT (Fig. 2a). Fourteen (29.9%) had persistent lymph node involvement in the tattooed node after receipt of NAT. Intraoperative evaluation confirming nodal positivity in a first sentinel node allowed surgeons to proceed to ALND without removing additional sentinel lymph nodes. Altogether, 12 underwent completion ALND. Additional involved lymph nodes were found in 8 (67%) of these cases on final pathology. However, two cases found to have isolated tumor cells did not undergo completion ALND. Among these Group 2 cases, two were noted to have black pigment in association with a non-SLN located close to the biopsied and tattooed lymph node (Fig. 2b).

DISCUSSION

The current report represents an update of our experience with tattooing of biopsied suspicious axillary nodes before surgery or NAT. Our results validate this approach as an alternative technique for marking and identifying biopsied nodes. Unlike metallic clips, radioactive, or magnetic seeds and newer reflector markers, sterile black ink tattoos do not require any preoperative localization or specialized intraoperative equipment.[19,22,23] These other modalities invariably imply increased cost, patient discomfort, and surgical delays.

The practice of sampling and marking suspicious axillary nodes at the time of breast cancer diagnosis has become more common. Initially, it was introduced to circumvent the need for sentinel node mapping and intraoperative biopsy in order to proceed directly to axillary node dissection. While these diagnostic axillary node biopsies may be helpful in many cases, it engendered some controversy for those with early-stage disease who undergo surgery first and are eligible to avoid ALND consistent with Z0011 criteria.[24–26] Specifically, it has caused some confusion in the management of patients with positive nodes with seemingly low burden of axillary node disease who should in our opinion undergo sentinel node evaluation.[27–29]

The utility of marking biopsied lymph nodes before neoadjuvant chemotherapy was recognized in the ACOSOG Z1071 sentinel node biopsy prospective single-arm trial.[5] Among the 170 patients who had a clip placed in the biopsied positive node, the SLN biopsy false-negative rate (FNR) was 7.2%. Interestingly, the clipped node was not identified in the SLN resection in 24.1% of cases.[13] Caudle et al. expanded on the findings of the Z1071 trial and determined that removing the clipped node in addition to the SLNs decreased the FNR from 10.1 to 1.4%.[14] Similarly, Donker et al. reported a FNR of 7% among 100 patients who underwent ^{125}I radioactive seed marking of biopsied positive axillary lymph nodes.[16] However, radioactive seed placement is not approved for the neoadjuvant setting in the United States.[30,31]

In contrast, our study showed a reliably high correlation of 98.5% between tattooed nodes and SLNs. This may be attributed to improved technical consistency, because all cases were conducted at a single institution with the vast majority of procedures performed by a single senior surgeon. Our definition of SLNs are based on the guidelines used in the NSABP B-32 SLN prospective, randomized trial, which defined a SLN as a palpably suspicious, radioactive, blue node, or blue leading lymphatic.[7] We speculated that easy visibility of the black-stained node allowed us to identify the tattooed node first and then, secondarily, to determine whether it was firm, blue, and/or "hot." Additionally, dual-agent sentinel node mapping was utilized in 80% of cases compared with 55% of cases reviewed by Caudle et al., which also may explain our higher concordance.

Tattoo marking of biopsied lymph node also has been reported by a group at Yonsei Cancer Center in Korea. They used a charcoal suspension before NAT in 20 patients and were able to identify all of those nodes intraoperatively up to 197 days later.[32] In this study, 75% of tattooed nodes were concordant with hot or blue nodes. By comparison, our concordance of 98.5% between tattooed and SLNs is an indicator of the high accuracy and reliability of our approach. One reason may be the inclusion of palpably suspicious nodes in our series in addition to hot and/or blue nodes.

The volume of black ink utilized and the injection technique were modified as we gained experience with the procedure. To facilitate visualization of the black pigment intraoperatively, we found that optimal injection should target the antero-lateral surface of the node and the perinodal fat. While inking the perinodal fat may result in tattooing of an adjacent node, it ensures the likelihood of detection of the biopsied node. Tattoo pigment may extend beyond the site of injection, as was seen in some of our cases with multiple black nodes. This also may be explained by migration of pigment via lymphatics or phagocytosis as has been described for charcoal particles by tumoral and peritumoral macrophages.[33–35] Discerning blue dye within a tattooed node can be challenging if there is much black pigment. However, the color ambiguity often can be resolved by identifying a blue leading lymphatic as well as reliance on radiotracer signaling. It is important to point out that the black pigment has not interfered with the histopathological evaluation of the nodes in either group.

Tattooing of suspicious axillary lymph nodes at the time of biopsy before treatment can be a useful adjunct to clinical lymph node staging surgery, particularly in the neoadjuvant setting. Tattooing facilitates visual localization of biopsy-proven involved nodes and is less likely to be displaced or lost as in the case of clips or seeds. Our analysis showed that in 65 of 66 cases the tattooed nodes coincided with one of the sentinel nodes. Overall, pretreatment tattoo marking of biopsies axillary lymph nodes is less expensive and requires no specialized equipment, making it an attractive technique and one that is readily exportable to low resource environments.

REFERENCES

1. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*. 2014;384(9938):164–72. https://doi.org/10.1016/s0140-6736(13)62422-8.
2. Spring LM, Gupta A, Reynolds KL, et al. Neoadjuvant endocrine therapy for estrogen receptor-positive breast cancer: a systematic review and meta-analysis. *JAMA Oncol*. 2016;2(11):1477–86. https://doi.org/10.1001/jamaoncol.2016.1897.
3. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol*. 1997;15(7):2483–93.
4. Bear HD, Anderson S, Smith RE, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol*. 2006;24(13):2019–27. https://doi.org/10.1200/jco.2005.04.1665.
5. Boughey JC, Suman VJ, Mittendorf EA, et al. Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. *JAMA*. 2013;310(14):1455–61. https://doi.org/10.1001/jama.2013.278932.
6. Krishnamurthy S, Sneige N, Bedi DG, et al. Role of ultrasound-guided fine-needle aspiration of indeterminate and suspicious axillary lymph nodes in the initial staging of breast carcinoma. *Cancer*. 2002;95(5):982–8. https://doi.org/10.1002/cncr.10786.
7. Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. *Lancet Oncol*. 2010;11(10):927–33. https://doi.org/10.1016/s1470-2045(10)70207-2.
8. Giuliano AE, Chung AP. Long-term follow-up confirms the oncologic safety of sentinel node biopsy without axillary dissection in node-negative breast cancer patients. *Ann Surg*. 2010;251(4):601–3. https://doi.org/10.1097/sla.0b013e3181d6115f.
9. Krag DN, Ashikaga T, Harlow SP, et al. Surgeon training, protocol compliance, and technical outcomes from breast cancer sentinel lymph node randomized trial. *J Natl Cancer Inst*. 2009;101(19):1356–62. https://doi.org/10.1093/jnci/djp281.
10. van Rijk MC, Nieweg OE, Valdés Olmos RA, Rutgers EJT, Hoefnagel CA, Kroon BBR. Non-axillary breast cancer recurrences after sentinel node biopsy. *J Surg Oncol*. 2005;92(4):292–8. https://doi.org/10.1002/jso.20407
11. Holwitt DM, Swatske ME, Gillanders WE, et al. Scientific Presentation Award: the combination of axillary ultrasound and ultrasound-guided biopsy is an accurate predictor of axillary stage in clinically node-negative breast cancer patients. *Am J Surg*. 2008;196(4):477–82. https://doi.org/10.1016/j.amjsurg.2008.06.006.
12. Boileau J-F, Poirier B, Basik M, et al. Sentinel node biopsy after neoadjuvant chemotherapy in biopsy-proven node-positive breast cancer: the SN FNAC study. *J Clin Oncol*. 2015;33(3):258–64. https://doi.org/10.1200/jco.2014.55.7827.
13. Boughey JC, Ballman KV, Le-Petross HT, et al. Identification and resection of clipped node decreases the false-negative rate of sentinel lymph node surgery in patients presenting with node-positive breast cancer (T0–T4, N1–N2) who receive neoadjuvant chemotherapy: results from ACOSOG Z1071 (Alliance). *Ann Surg*. 2016;263(4):802–7. https://doi.org/10.1097/sla.0000000000001375.
14. Caudle AS, Yang WT, Krishnamurthy S, et al. Improved axillary evaluation following neoadjuvant therapy for patients with node-positive breast cancer using selective evaluation of clipped nodes: implementation of targeted axillary dissection. *J Clin Oncol*. 2016;34(10):1072–8. https://doi.org/10.1200/jco.2015.64.0094.
15. Pinkney DM, Mychajlowycz M, Shah BA. A prospective comparative study to evaluate the displacement of four commercially available breast biopsy markers. *Br J Radiol*. 2016;89(1065):20160149. https://doi.org/10.1259/bjr.20160149.
16. Donker M, Straver ME, Wesseling J, et al. Marking axillary lymph nodes with radioactive iodine seeds for axillary staging after neoadjuvant systemic treatment in breast cancer patients: the MARI procedure. *Ann Surg*. 2015;261(2):378–82. https://doi.org/10.1097/sla.0000000000000558.
17. Dashevsky BZ, Altman A, Abe H, et al. Lymph node wire localization post-chemotherapy: towards improving the false negative sentinel lymph node biopsy rate in breast cancer patients. *Clin Imaging*. 2017;48:69–73. https://doi.org/10.1016/j.clinimag.2017.10.003.
18. Nguyen TT, Hieken TJ, Glazebrook KN, Boughey JC. Localizing the clipped node in patients with node-positive breast cancer treated with neoadjuvant chemotherapy: early learning experience and challenges. *Ann Surg Oncol*. 2017;24(10):3011–6. https://doi.org/10.1245/s10434-017-6023-z.
19. Trinh L, Miyake KK, Dirbas FM, et al. CT-guided wire localization for involved axillary lymph nodes after neo-adjuvant chemotherapy in patients with initially node-positive breast cancer. *Breast J*. 2016;22(4):390–6. https://doi.org/10.1111/tbj.12597.
20. Mango VL, Wynn RT, Feldman S, et al. Beyond wires and seeds: reflector-guided breast lesion localization and excision. *Radiology*. 2017;284(2):365–71. https://doi.org/10.1148/radiol.2017161661.
21. Choy N, Lipson J, Porter C, et al. Initial results with preoperative tattooing of biopsied axillary lymph nodes and correlation to sentinel lymph nodes in breast cancer patients. *Ann Surg Oncol*. 2014:1–6. https://doi.org/10.1245/s10434-014-4034-6.
22. Falcon S, Weinfurtner RJ, Mooney B, Niell BL. SAVI SCOUT® localization of breast lesions as a practical alternative to wires: outcomes and suggestions for trouble-shooting. *Clin Imaging*. 2018;52:280–6. https://doi.org/10.1016/j.clinimag.2018.07.008.
23. Siso C, de Torres J, Esgueva-Colmenarejo A, et al. Intraoperative ultrasound-guided excision of axillary clip in patients with node-positive breast cancer treated with neoadjuvant therapy (ILINA Trial): a new tool to guide the excision of the clipped node after neoadjuvant treatment. *Ann Surg Oncol*. 2018;25(3):784–91. https://doi.org/10.1245/s10434-017-6270-z.
24. Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA*. 2011;305(6):569–75. https://doi.org/10.1001/jama.2011.90.

25. Giuliano AE, Ballman KV, McCall L, et al. Effect of axillary dissection vs no axillary dissection on 10-year overall survival among women with invasive breast cancer and sentinel node metastasis: the ACOSOG Z0011 (Alliance) randomized clinical trial. *JAMA*. 2017;318(10):918–26. https://doi.org/10.1001/jama.2017.11470.
26. Wellington J, Sanders T, Mylander C, et al. Routine axillary ultrasound for patients with T1–T2 breast cancer does not increase the rate of axillary lymph node dissection based on predictive modeling. *Ann Surg Oncol*. 2018;25(8):2271–8. https://doi.org/10.1245/s10434-018-6545-z.
27. Pilewskie M, Jochelson M, Gooch JC, Patil S, Stempel M, Morrow M. Is preoperative axillary imaging beneficial in identifying clinically node-negative patients requiring axillary lymph node dissection? *J Am Coll Surg*. 2016;222(2):138–45. https://doi.org/10.1016/j.jamcollsurg.2015.11.013.
28. Ahmed M, Jozsa F, Baker R, Rubio IT, Benson J, Douek M. Meta-analysis of tumour burden in pre-operative axillary ultrasound positive and negative breast cancer patients. *Breast Cancer Res Treat*. 2017;166(2):329–36. https://doi.org/10.1007/s10549-017-4405-3.
29. Wallis MG, Kilburn-Toppin F, Taylor-Phillips S. Does preoperative axillary staging lead to overtreatment of women with screen-detected breast cancer? *Clin Radiol*. 2018;73(5):467–72. https://doi.org/10.1016/j.crad.2017.11.023.
30. Straver ME, Loo CE, Alderliesten T, Rutgers EJT, Vrancken Peeters MTFD. Marking the axilla with radioactive iodine seeds (MARI procedure) may reduce the need for axillary dissection after neoadjuvant chemotherapy for breast cancer. *Br J Surg*. 2010;97(8):1226–31. https://doi.org/10.1002/bjs.7073.
31. van Nijnatten TJA, Simons JM, Smidt ML, et al. A novel less-invasive approach for axillary staging after neoadjuvant chemotherapy in patients with axillary node-positive breast cancer by combining radioactive iodine seed localization in the axilla with the sentinel node procedure (RISAS): a Dutch prospective multicenter validation study. *Clin Breast Cancer*. 2017;17(5):399–402. https://doi.org/10.1016/j.clbc.2017.04.006.
32. Park S, Koo JS, Kim GM, et al. Feasibility of charcoal tattooing of cytology-proven metastatic axillary lymph node at diagnosis and sentinel lymph node biopsy after neoadjuvant chemotherapy in breast cancer patients. *Cancer Res Treat*. 2018;50(3):801–12. https://doi.org/10.4143/crt.2017.210.
33. Langlois SL, Carter ML. Carbon localisation of impalpable mammographic abnormalities. *Australas Radiol*. 1991;35(3):237–41.
34. Bonhomme-Faivre L, Depraetere P, Savelli MP, et al. Charcoal suspension for tumor labelling modifies macrophage activity in mice. *Life Sci*. 2000;66(9):817–27.
35. Kang TW, Shin JH, Han B-K, et al. Preoperative ultrasound-guided tattooing localization of recurrences after thyroidectomy: safety and effectiveness. *Ann Surg Oncol*. 2009;16(6):1655–9. https://doi.org/10.1245/s10434-009-0431-7.