Supplementary File 1:

Omission of Axillary Lymph Node Dissection in Breast Cancer Patients With Axillary Pathological Complete Response Confirmed by Stained Region Lymph Node Biopsy After Neoadjuvant Systemic Therapy (SrLNB Study): Study Protocol for a Single-Arm, Single-Center, Phase-II Trial

Informed Consent Form

Version:V2.0

Version date: June 5, 2023

Patient initials:

Patient study number:

Patient contact number:

Patient address:

Information Disclosure Page

Participant Information Sheet

Dear Patient,

We invite you to participate in the phase II clinical trial to exempt from axillary lymph node dissection (ALND) for breast cancer patients achieving axillary complete response (apCR) after neoadjuvant therapy. This study is initiated and led by Director Jue Wang and Xiaoming Zha, and will be conducted in the Department of Breast Diseases, Jiangsu Province Hospital. 92 subjects is expected to participate voluntarily. This study has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital). Ethical Review number is 2023-SR-169.

Before making a decision, it is essential that you read and fully acknowledge this informed consent document. This document details the study's objectives, methodologies, potential benefits, and associated risks. It also provides an overview of your responsibilities and recommended precautions. If you decide to participate in this study, you can consult any questions with your research doctor. Upon reaching a clear understanding, both you and your research doctor will sign this document. You will be provided with a copy.

1. Why is this study conducted?

Up to 40% of patients with positive axillary lymph node breast cancer may convert to negative (ypN0) after NST. However, the current clinical guidelines still recommend ALND, which will bring about more complications, such as lymphedema, dysfunction, pain and numbness in affectedl upper limb and the axilla, to ypN0 patients. Actually, it is feasible to downgrade axillary surgery in such patients, with high requirements to technical and equipment, such as: (1) using dual tracers (dye and radionuclide) for Sentinel Lymph Node Biopsy (SLNB); (2) $\geqslant$3 axillary lymph nodes must be removed; (3) titanium clips must be used before neoadjuvant therapy to

localize metastatic axillary lymph nodes and ensure that the localized lymph nodes will be removed at the time of surgery. However, radioactive particles are under strict supervision in most countries and regions around the world (including our country), which prevents the vast majority of countries and regions from performing axillary downstaging procedures.

Team of Director Xiaoming Zha has been conducting clinical research since 2019, and successfully developed Stained Region Lymph Node Biopsy (SrLNB) to downstage axillary surgery. In SrLNB procedure, titanium clips is used to mark positive lymph node, and carbon nanoparticle suspension will also be injected under the guidance of ultrasound for staining at the time of diagnosis. When surgery after completion of NST, clipped and the stained lymph nodes will be biopsied. Data from the our previous study showed that the false-negative rate of stained-area lymph node biopsy was 5.3%, much lower than the generally recognized threshold of 10%, suggesting that SrLNB is safe and feasible.

In this study, team of Dr. Zha intends to enroll patients with positive axillary lymph nodes at diagnosis and receiving NST to undergo SrLNB. When SrLNB turns out to be negative, ALND is exempted, and then undergo axillary area radiotherapy after surgery. The physician team will further evaluate the efficacy and long-term safety of the procedure by following up and monitoring data such as local recurrence and complication rates.

2. Who is eligible (or not) to participate in the study?

The main inclusion criteria includes: (1) female breast cancer patients aged 18-70 years; (2) pathologically confirmed metastatic axillary lymph nodes at the time of diagnosis, with stage of cN1-3; (3) successful injection of carbon nanoparticle suspension to mark positive lymph nodes.

The main exclusion criteria includes: (1) bilateral breast cancer or during lactating/pregnant period; (2) clinically confirmed distant metastases; (3) unable to complete the subsequent adjuvant therapy in full course as prescribed for various reasons; (4) not eligible for enrollment as judged by the investigator.

3. What need to do if participate in the study?

If you are willing to participate in this study, the surgeon will perform SrLNB. When

intraoperative rapid pathology shows positive results (with micrometastases and isolated tumor cells), then you will be excluded from this study and proceed with ALND, other adjuvant treatments including chemotherapy, endocrine therapy and targeted therapy, will be performed according to guidelines. When intraoperative rapid pathology shows negative results, ALND will be omitted, postoperative regional lymph node radiotherapy (RNI) including axilla will be required, and other adjuvant treatments will be performed as prescribed. When the intraoperative pathology unable to clarify the presence of metastasis, the subsequent axillary will be suspended, and whether to add ALND will be depends on the routine pathology.

We will perform a follow-up within 3 years after surgery, which mainly includes: (1) breast and whole body imaging; (2) upper extremity circumference and bioelectrical impedance examination; (3) quality of life questionnaire.

4. What's the benefit to participate?

Patients who only undergo SrLNB are likely to experience less postoperative complications such as upper limb lymphedema, dysfunction, numbness and pain, and have improved quality of life,compared to ALND patients. In addition, our team will provide several examinations for free (see details in"Do I need to pay for participation in the study?") and closely monitor the results of subsequent visit to provide effective treatment and guidance timely.

5. What's the risk to participate?

SrLNB is the original and latest axillary downstaging procedure developed by team od Dr. Zha, which has been found with good operability and safety in our previous study. However, same as sentinel lymph node biopsy, there is possibility of false-negative results due to missed biopsy to metastatic lymph node, which may increase the possibility of recurrence in the axillary or even distant metastasis. Thus, all patients exempted from ALND in this study will undergo axillary radiotherapy to further eliminate tumor cell that may have been missed, reducing the risk of local recurrence and distant metastasis. Even with the boost of radiotherapy to the axilla and other subsequent systemic treatments, there may be an increased risk of local recurrence and distant metastasis. Our team will closely monitor recurrence and metastasis through regular follow-up, so that timely treatment measures (including complementary axillary surgery, etc.) can be taken.

In case of any discomfort and adverse reactions, please promptly contact the study physician. All agents of chemotherapy, targeted therapy, endocrine therapy, and radiation used in this study are part of the routine clinical treatment of breast cancer. Even if you do not participate in this study, these side effects/adverse reactions may occur as long as you receive these treatments. In addition, any treatment may failed to take effect, as well as the disease continue to progress due to ineffective treatment or other coexisting diseases.

6. Do I need to pay for participation in the study?

To compensate for any inconvenience this study may cause, bioelectrical impedance analysis, follow-up of quality of life and monitoring instructions will be provided free of charge during this study. The cost of medications for routine breast cancer treatment and follow-up examinations will not be covered. The cost of treatments and tests required for other medical conditions, or switching to other treatments if the treatment failed to take effect, will not be covered as well. Transportation, lost wages, and nutritional expenses are not covered by this study. In case of trial-related damages, appropriate treatment and compensation will be provided in accordance with relevant national regulations.

7. Is personal information confidential?

Your medical records will be kept at the hospital, and only the investigator, research authorities, and ethics committee are allowed to access your medical records. Any public reporting of the results of this study will not disclose your personal identity. Every effort will be made to protect the privacy of your personal medical information to the extent permitted by law.

8. What other treatments are available if not participating in this study?

If not participate in this study, you will undergo ALND, and the incidence and severity of arm mobidity may be higher. However, the subsequent adjuvant treatment will be performed according to clinical guidelines without change.

If you participate in the study but drop out halfway through the follow-up, you will still receive the most standardized and regular treatment.

9. Do I have to participate in the study?

Participation in this study is completely voluntary, and you may refuse to participate or withdraw at any time during the study, which will not affect your treatment. If you decide to withdraw, please contact the doctor, you may be asked to undergo related tests and complementary procedures (e.g., axillary lymph node dissection, etc.) that may be beneficial to protect your health.

If you have any questions related to your personal rights and interests, you can contact the Ethics Committee of our hospital at 025-68306360.

Informed Consent

Consent Signature Page

SUBJECT DECLARATION: I have read the above description of this study and fully understand the possible risks and benefits of participating in this study. I volunteer to participate in this study. I understand that I will be given a copy of this informed consent form.

I **agree□ or decline□** to other research utilizing my medical records and clinical specimens related to this study.

Patient Signature: _____Date of Signature: _____

Contact phone number: _____

Or

(To be used only if subject is incapacitated)

Signature of Legal Representative: _____

Date of Signature: _____

Relationship to the patient: _____

Contact phone number: _____

INVESTIGATOR DECLARATION: I confirm that I have explained the details of this study, particularly the possible risks and benefits of participating in this study, and answered all relevant questions from the subject, who has voluntarily agreed to participate in this study. This informed consent form is in duplicate, with one signed copy retained by the investigator and one by the subject.

Signature of Investigator: _____ Date of Signature: _____

Contact phone number: _____

知情同意书

尊敬的女士：

我们邀请您参加南京医科大学第一附属医院（江苏省人民医院）批准开展的“新辅助治疗后腋窝淋巴结阳性转阴性的乳腺癌患者豁免腋窝淋巴结清扫的 II 期临床研究”。本研究由乳腺病科查小明主任发起和负责，将在江苏省人民医院乳腺病科开展，预计将有 92 名受试者自愿参加。本研究已经得到南京医科大学第一附属医院（江苏省人民医院）伦理委员会的审查和批准，伦理审查编号：2023-SR-169。

为什么要开展本项研究？

腋窝淋巴结阳性的乳腺癌患者经新辅助治疗（Neoadjuvant Systemic Therapy，NST）后可有高达 40%的比例转为阴性（ypN0）。**然而即使淋巴结转为阴性，目前的临床指南仍然首推腋窝淋巴结清扫术（axillary lymph nodes dissection，ALND）**，而这也会带来更多的后遗症，如上肢淋巴水肿、上肢活动障碍、上臂内侧和腋窝疼痛麻木等。**实际上对此类患者进行腋窝淋巴结手术的降级是可行的，但是技术和设备的要求很高**，比如：(1) 使用双示踪剂（染料和放射性核素）进行前哨淋巴结活检（Sentinel Lymph Node Biopsy，SLNB）；(2) 必须切检≥3 枚腋窝淋巴结；(3) 必须在新辅助治疗前用钛夹定位转移的腋窝淋巴结，并且在前哨淋巴结手术时要确保取到钛夹定位的淋巴结。**由于放射性核素在全世界大多数国家和地区（包括我国）都要受到严格的监管，因此仅此一个要求就使绝大多数国家和地区无法开展腋窝的降级手术**。

查小明主任团队从 2019 年就开始开展临床研究，成功原创了染色区淋巴结活检术（Stained region Lymph Node Biopsy，SrLNB），即在初诊时经超声引导将纳米炭混悬注射液（卡纳琳）注射到腋窝阳性淋巴结的皮质中进行染色标记，在新辅助治疗完成后进行手术时活检染色区域的淋巴结。**前期临床研究数据初步显示染色区淋巴结活检术的假阴性率为 5.5%，远低于国际公认的 10%的阈值，初步证明染色区淋巴结活检术是安全可行的**。

在本研究中，查小明医生团队拟入组初诊腋窝淋巴结阳性且接受新辅助治疗的患者，行染色区淋巴结活检术，如为阴性则豁免腋窝淋巴结清扫手术，术后行腋窝区放疗。医生团队会通过随访和监测局部复发情况、并发症率等数据，**进一步评估染色区淋巴结活检手术的有效性和长期安全性**。

哪些人适（不）宜参加研究?

入选标准主要有：(1) 18-70 周岁的女性乳腺癌患者；(2) 初诊时经病理证实腋窝淋巴结为阳性，N 分级为 cN1-3；(3) 阳性淋巴结经纳米炭混悬注射液成功注射标记。

排除标准主要有：(1) 双侧乳腺癌/哺乳期/孕期乳腺癌；(2) 临床或影像学证实存在远处转移；(3) 各种原因导致无法按医嘱足疗程完成后续辅助治疗者；(4) 经研究者判断不符合入组者。

如果参加研究，需要做什么？

如果您愿意参加本项研究，医生会在术中行染色区淋巴结活检术。如果术中快速病理显示腋窝淋巴结阳性（含微转移和孤立肿瘤细胞）则排除出本研究，并继续行腋窝淋巴结清扫手术，术后的其他综合治疗（如化疗、内分泌治疗、靶向治疗等）按照指南进行。**如果术中快速病理显示腋窝淋巴结阴性则不做腋窝淋巴结清扫术，术后需要接受包含腋窝的区域淋巴结放疗（RNI）**，术后的其他综合治疗也按照指南进行。**如果术中快速病理无法明确有无转移，则会暂停后续的腋窝淋巴结清扫术，等常规病理出来后再决定是否加做腋窝淋巴结清扫术**。

我们会在手术治疗结束的 3 年内对您进行随访，随访内容主要包括：(1) 乳腺及全身影像学检查；(2)上肢周径和生物电阻抗检查；(3) 生活质量量表问卷调查等。

参加研究有哪些好处？

只接受染色区淋巴结活检术的患者，术后发生淋巴水肿、上肢功能障碍、麻木疼痛等后遗症的概率及其严重程度大概率会明显降低，生活质量也可能提高。另外，本团队也会提供部分免费检查（见“**参加研究需要支付有关费用吗**”），并密切关注复查结果，以便及时提供有效的治疗和指导。

参加研究有哪些风险？

染色区淋巴结活检术是查小明医生团队原创的、最新的腋窝淋巴结活检手术，前期研究发现其具有较好的操作性和安全性。**但正如前哨淋巴结活检术一样，染色区淋巴结活检术同样会存在漏检导致假阴性结果的可能。因漏检而残留在腋窝的转移淋巴结有可能会增加腋窝区的复发率甚至远处转移率，因此本研究中所有豁免腋窝淋巴结清扫手术的患者都需要接受腋窝放疗，以进一步消灭可能漏检的阳性淋巴结，从而减少局部复发和远处转移的风险**。当然，**即使增加了放疗和后续其他全身治疗，也可能会出现局部复发和远处转移的**

风险增高，医生团队将会通过定期密切随访，及时监测疾病复发、转移等情况，以便及时采取治疗措施（包括补充腋窝手术等）。

如果出现任何不适和不良反应，请及时与研究医生联系。本研究方案中使用的所有化疗药、靶向药、内分泌药物以及放疗等都属于临床治疗乳腺癌的常规方式，即使您不参加本临床研究，只要接受该治疗方法，就有可能发生这些副作用/不良反应。此外，任何治疗都可能出现无效的情况，以及因治疗无效或者因合并其他疾病等原因而导致病情继续发展。

参加研究需要支付有关费用吗？

为了补偿本研究可能给您带来的不便，本研究期间将免费提供生物电阻抗检测，免费进行生活质量随访和随访监测指导。乳腺癌常规的治疗药物以及随访检查费用不在免费范围之内。如果您同时合并其他疾病所需的治疗和检查，以及因治疗无效而改用其他治疗的费用，也不在免费范围之内。本研究不提供交通费、误工费、营养费等。如果出现试验相关的损害，将依据国家有关规定提供相应的治疗与补偿。

个人信息是保密的吗？

您的医疗记录将保存在医院，研究者、研究主管部门、伦理委员会将被允许查阅您的医疗记录。任何有关本项研究结果的公开报告将不会披露您的个人身份。我们将在法律允许的范围内，尽一切努力保护您个人医疗资料的隐私。

如果不参与本研究还有哪些治疗方法？

如果您不参加本项研究，则接受标准的腋窝淋巴结清扫手术，腋窝后遗症的发生率和严重程度可能会较高。但后续的综合治疗会按照临床指南的要求进行，不会有任何改变。

如果您参加了研究，但后续中途退出了，您仍会得到最标准正规的治疗。

我必须参加研究吗？

参加本项研究是完全自愿的，您可以拒绝参加研究，或在研究过程中的任何时间退出本研究，这都不会影响医生对您的治疗。如果您决定退出本研究，请与您的医生联系，您可能被要求进行相关检查和补充手术（如腋窝淋巴结清扫术等），这对保护您的健康是有利的。

如您有涉及个人权益方面的问题可与本院伦理委员会联系，联系电话：025-68306360。

受试者声明：我已经阅读了上述有关本研究的介绍，对参加本研究可能产生的风险和受

益充分了解。我自愿参加本研究。我将获得一份签署姓名和日期的本知情同意书副本。

我同意□ 或拒绝□ 其他研究利用我的与本研究相关的医疗记录和临床标本。

受试者签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

受试者的联系电话：_____ 手机号：_____

（适用时）法定监护人/见证人签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

法定监护人/见证人联系电话：_____ 手机号：_____

研究者声明：我确认已向受试者解释了本研究的详细情况，特别是参加本研究可能产生的风险和受益，并回答了受试者的所有有关问题，受试者是出于自愿同意参加本研究。此知情同意书一式两份，研究者与受试者各留一份已签字的知情同意书。

研究医生签名：_____ 日期：__ __ __ __ 年 __ __ 月 __ __ 日

研究医生的工作电话： 025-68308172 手机号：_____