Advancing Surgical Strategies for Breast Cancer in the Era of Neoadjuvant Chemotherapy

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To my wife, our children and family

"The times they are a-changin"

— Bob Dylan (1964)

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ABSTRACT

This thesis aimed to explore strategies to enhance surgical outcomes in patients receiving neoadjuvant chemotherapy (NACT). **Paper I** evaluated the impact of implementing surgical care bundles (SCB) on postoperative surgical site infections (SSI) in a retrospective cohort (n=958). SCB reduced the incidence of SSI by 2.9%, with a 37% reduction in the odds of infection. **Paper II** investigated the association between tumor-infiltrating lymphocytes (TILs) and tumor response and survival after NACT in a retrospective cohort (n=220). Higher TILs levels were predictive of axillary tumor response following NACT but showed no statistically significant correlation with survival. **Paper III** presented the interim results of a multicenter randomized trial of NACT patients (n=44), comparing a novel one-stage tumor localization using a paramagnetic marker (Magseed), with the conventional two-stage clip and guidewire approach. The new method demonstrated feasibility, safety, and potential improved surgical precision in terms of resection volume ratio. **Paper IV** assessed a novel approach for pre-marking axillary lymph nodes using superparamagnetic iron oxide nanoparticles (SPIO) injected *before* NACT (up to five months prior to surgery), compared with the standard technique of radioactive tracer injection *after* NACT (one day prior to surgery), in a prospective cohort (n=80). SPIO showed superior sentinel lymph node detection, with nearly half of the identified nodes differing from those detected by the radioactive tracer. **In conclusion**, collectively, these studies identify key opportunities to advance surgical practice by minimizing patient harm, personalizing axillary management based on predicted tumor response, and achieving greater precision in surgery after NACT.

Keywords: breast cancer, neoadjuvant chemotherapy, surgical site infection, tumor-infiltrating lymphocytes, sentinel lymph node biopsy, superparamagnetic iron oxides nanoparticles, and Magseed.

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SAMMANFATTNING PÅ SVENSKA

Vid bröstcancer, möjliggör neoadjuvant kemoterapi (NACT), som ges före kirurgi, monitorering av tumörrespons in vivo, vilket kan bidra till en mer effektiv och individualiserad behandling. Dessutom kan NACT krympa tumörens storlek, vilket i sin tur möjliggör mindre omfattande kirurgiska ingrepp och underlättar bröstbevarande kirurgi. Införandet av NACT har emellertid medfört nya utmaningar för kirurger, eftersom tumörsresponsen på behandlingen kan variera mycket. I vissa fall kan tumören till och med bli radiologiskt osynlig efter behandling, vilket kan försvåra kirurgin och påverka det slutliga kirurgiska utfallet. Dessutom kan NACT påverka patientens immunförsvar och potentiellt ökar risken för postoperativa infektioner. Syftet med denna avhandling var därför att utvärdera strategier för att optimera de kirurgiska resultaten hos patienter som behandlas med NACT.

Artikel I utvärderade effekten av ett förebyggande åtgärdspaket, så kallat surgical care bundle (SCB) på postoperativa sårinfektioner i en retrospektiv kohort (n=958). Införandet av SCB visade sig minska incidensen av sårinfektioner med 2,9 %, motsvarande en oddsreduktion på 37 %.

Artikel II undersökte sambandet mellan tumörinfiltrerande lymfocyter (TIL) och tumörrespons samt överlevnad efter NACT i en retrospektiv kohort (n=220). Högre nivåer av TIL i tumören var associerade med förbättrad axillär tumörrespons efter NACT, men ingen signifikant korrelation med överlevnad kunde påvisas.

Artikel III redovisade resultaten från en randomiserad studie (n=44) av patienter med bröstcancer som genomgick NACT, där en ny paramagnetisk markör för tumörlokalisering (Magseed) jämfördes med den standardmetoden som använder markör av metall och ståltråd. Studien visade att den nya metoden var genomförbar, säker och bättre precision avseende, frekvensen av tumör resektionsradikalitet samt resektionsvolym.

Artikel IV utvärderade en ny metod för utmärkning av axillära lymfkörtlar genom att injicera superparamagnetiska järnoxidpartiklar (SPIO) i bröstet före start av NACT (upp till fem månader före operation), i jämförelse med standardmetoden där ett radioaktivt spårämne injiceras efter avslutad NACT (en dag före operation). I en prospektiv kohort (n=80) visade resultaten att SPIO-metoden var överlägsen när det gällde identifiering av portvaktkörtlar i

axillen. Nästan hälften av de detekterade körtlarna av SPIO inte överlappade med dem som identifierades av det radioaktiva spårämnet.

Sammanfattningsvis visar dessa studier på möjligheter att optimera den kirurgiska behandlingen efter NACT genom att minska risken för komplikationer, möjliggöra mer individualiserad axillkirurgi och öka precisionen vid kirurgiska ingrepp.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Chin K, Wärnberg F, Kovács A and Olofsson Bagge R.

Impact of Surgical Care Bundle on Surgical Site Infection after Non-Reconstructive Breast Cancer Surgery: A Single-Centre Retrospective Comparative Cohort Study.

Cancers (Basel). 2023 Feb 1;15(3):919

II. Chin K, Landén A, Kovács A, Wärnberg F, Ekholm M, Karlsson P, and Olofsson Bagge R.

Tumor-infiltrating lymphocytes as a predictor of axillary and primary tumor pathological response after neoadjuvant chemotherapy in patients with breast cancer: a retrospective cohort study.

Breast Cancer Res Treat. 2024 Aug; 207(1):49-63.

III. Chin K, Kovac P, Henriksson R, Kovács A, Ekholm M, Dussán Luberth C, Vikhe-Patil E, Olofsson Bagge R, Eriksson S, Karakatsanis A, and Wärnberg F.

Paramagnetic seed versus standard marker plus wire-guided tumor localization in patients with breast cancer undergoing neoadjuvant chemotherapy: interim results of a multicenter randomized trial (Maglocal)

In manuscript form.

IV. Chin K, Olofsson Bagge R, Mirzaei N, Kovács A, Leonhardt H, Zaar P, Karakatsanis A, Pantiora E, Eriksson S, Ekholm M, Thompson A, Barry P, Boland M, Man V, Kwong A, and Wärnberg F.

Superparamagnetic tracer and paramagnetic seed for marking of sentinel lymph nodes and index metastatic nodes before neoadjuvant chemotherapy to facilitate subsequent sentinel lymph node biopsy and targeted axillary dissection in breast cancer patients: A feasibility study.

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ABBREVIATIONS AND DEFINITIONS

Actual-RV	Actual resection volume
ALND	Axillary Lymph Node Dissection
BCFI	Breast cancer-free interval
BCS	Breast-conserving surgery
CI	Confidence interval
cN+	Clinically proven axillary lymph node metastasis
FNR	False negative rate
Optimal-RV	Optimal resection volume
Index-met	Index metastatic node
Marker-WGL	Two-stage marker plus wire-guided localization
MDT	Multidisciplinary Team
MPG	Miller Payne five-point grading system
MRI	Magnetic Resonance Imaging
NACT	Neoadjuvant chemotherapy. This is defined as systemic therapy that include chemotherapy with or without anti-HER2 therapy and immunotherapy administered before surgery
NKBC	National Quality Register for Breast Cancer (Sweden)
OR	Odds ratio
OS	Overall survival
Post-TILs	Post-treatment (NACT) TILs in surgical specimens
Pre-TILs	Pre-treatment (NACT) TILs in diagnostic specimens
RV-ratio	Resection volume ratio (Actual RV : Optimal-RV)
SCB	Surgical care bundle
SLN	Sentinel lymph nodes
SLNB	Sentinel lymph node biopsy
SPIO	Superparamagnetic iron oxide nanoparticle (Magtrace)
SSI	Surgical site infection
TAD	Targeted axillary dissection
Tc^{99m}	Technitium 99 metastable
TILs	Tumor-infiltrating lymphocytes
ΔTILs	Change of TILs after NACT
TNBC	Triple-negative breast cancer

1 THESIS BACKGROUND

More than a century after Halsted Mastectomy defined the surgical approach to breast cancer treatment [1], we are now witnessing a paradigm shift. The advent of neoadjuvant chemotherapy (NACT) has significantly altered the landscape of breast cancer surgery. As systemic therapy becomes increasingly guided by individual tumor biology, the traditional algorithm of upfront surgery as the primary treatment is no longer the universal gold standard.

Historically, NACT was reserved for patients with inoperable or locally advanced tumors. Nowadays, it is also employed in early-stage breast cancers with biologically high-risk profile, reflecting the principles of personalized medicine. Neoadjuvant chemotherapy not only facilitates tumor downstaging to enable breast- and axillary-conserving surgery but also provides a unique opportunity to assess in vivo tumor response. The achievement of a pathological complete tumor response (pCR) following NACT has emerged as an important surrogate marker of improved long-term outcomes, particularly in aggressive subtypes such as triple-negative (TNBC) and human epidermal growth factor receptor-2 (HER2) positive breast cancers. The ability to monitor treatment response in real time informs prognosis and guides postoperative escalation or de-escalation of systemic therapy based on residual disease. Likewise, the rising rate of pCR challenges the need for surgery to remain extensive, emphasizing the potential for surgical de-escalation.

In the current era of modern oncology, surgery needs to adapt to become a more tailored and biologically driven component of multidisciplinary care for breast cancer. Surgical decision-making now extends beyond the technical procedure itself and needs to include a multidisciplinary approach. This thesis provides an overview of contemporary understanding of breast cancer pathophysiology and advances in disease management, with a specific research focus on optimizing surgical strategies for patients undergoing NACT.

2 INTRODUCTION

2.1 EPIDEMIOLOGY

Breast cancer is the most common malignancy among women, with 2.3 million new cases diagnosed globally in 2020, representing nearly one in four female cancers [2]. Incidence varies geographically, with the highest rates in high-income regions such as Western Europe, North America, and Australia, and lower but rising rates in many low- and middle-income countries [2-4]. Risk factors are broadly classified as non-modifiable—age (>50 years), female sex, genetic predisposition, family history, ethnicity, and reproductive history—and modifiable, including obesity, physical inactivity, diet, hormone replacement therapy, smoking, and prior radiation exposure. Geographic differences are largely due to variations in these factors and public health measures [3, 4]. In Sweden, 9,898 new cases were reported in 2024, with 5- and 10-year breast cancer–specific survival averaging 93% and 89%, respectively [5], reflecting advances in early detection, multimodal treatment, and access to high-quality care.

2.2 PUBLIC HEALTH PERSPECTIVES

Patient outcomes in breast cancer are shaped not only by clinical factors but also by socioeconomic, educational, and psychosocial factors, which influence the timing of diagnosis, access to care, surgical decisions, and postoperative recovery. Socioeconomic disparities, in particular, affect surgical choice and survival. For example, women from higher socioeconomic backgrounds are more likely to undergo breast-conserving surgery (BCS) or immediate reconstruction and generally experience better outcomes, due to earlier presentation and greater participation in screening [6].

Meanwhile reluctance to seek healthcare early, lead to delayed diagnosis among lower socioeconomic groups with poorer survival. Limited health literacy further compounds these inequities by delaying presentation, affect treatment compliance, and limiting engagement in preventive strategies. All of these factors impact negatively on the outcome of breast cancer [7]. Moreover, improved survival has brought prolonged exposure to psychosocial challenges. Disease stigma can cause chronic stress, constant fear of cancer recurrence, body image concerns, and social exclusion [8, 9]. These sequelae adversely affect mental health, treatment compliance and recovery. Addressing these

socioeconomic and psychosocial determinants alongside biological and therapeutic considerations is therefore essential for achieving equitable and optimal outcomes in breast cancer care.

3 BREAST CANCER PATHOGENESIS

The pathogenesis of breast cancer exhibits heterogeneity across individuals and molecular subtypes. There are distinct underlying biological pathways that influence tumor behavior, therapeutic response, and prognosis in different disease subtypes. Cancer development is a multifactorial process, influenced by genetic, epigenetic, hormonal, lifestyle, and host immune-related factors, which collectively drive tumor initiation, progression, and metastasis. Understanding these mechanisms has been a major focus of research, aiming to identify molecular targets for therapy.

3.1 CARCINOGENESIS

Carcinogenesis refers to the cellular process by which normal breast epithelial cells transform into malignant cells. This multistep progression typically begins with initiation, where genetic or epigenetic alterations arise in ductal or lobular epithelial cells. These altered cells may proliferate abnormally, leading to hyperplasia with or without atypia, which, although non-malignant, confers increased future cancer risk [10]. Abnormal cells may clonally expand leading to the development of in-situ carcinoma, which is confined within ducts or lobules. Invasion occurs once the basal membrane is breached, marking the development of invasive breast cancer with the potential for regional lymph node and distant metastasis [11]. This cancerous progression aligns with the Hallmarks of Cancer framework described by Hanahan and Weinberg, which outlines the key biological capabilities acquired during tumor evolution [12]. Key factors influencing breast carcinogenesis include:

3.1.1 GENETIC PREDISPOSITIONS

Germline mutations in BRCA1, BRCA2, CHEK2, PALB2, and TP53 increase susceptibility to breast cancer. Typically, one allele of a tumor suppressor gene is mutated in carriers, and loss of the second allele initiates the carcinogenic process. Carriers of high-risk mutations in BRCA1 and BRCA2 have an estimated cumulative breast cancer risk by age 80 of approximately 72% and 69%, respectively [13]. Genetic testing is recommended for patients with high-risk breast cancer subtypes (e.g., HER2-positive and TNBC) or significant family history without germline mutations in order to guide both treatment and surgical planning, particularly in the NACT setting [14].

In addition, epigenetic factors also play a crucial role in breast carcinogenesis. These factors typically cause changes in the regulation of gene expression without altering the underlying DNA sequence . For example, abnormal DNA methylation can influence hormone receptor expression in breast cancer thereby, affecting, the efficacy for endocrine therapy [15].

3.1.2 HORMONAL FACTORS

Prolonged exposure to endogenous or exogenous hormones contributes to breast cancer risk. Hormone replacement therapy, particularly combined estrogen–progestogen therapy, significantly increases risk [16]. Early menarche, late menopause, and nulliparity extend hormonal exposure, while breastfeeding and multiple childbirths are protective [17, 18].

3.1.3 LIFESTYLE FACTORS

Modifiable lifestyle factors such as obesity, alcohol consumption, smoking, high-fat diet, and low physical activity are linked to breast cancer risk [19]. Diets rich in fiber and adherence to a Mediterranean diet are protective [20]. Physical activity improves quality of life and survival, although most evidence comes from non-NACT settings. The Swedish Neo-NACT study will provide further insights on the impact of structured exercise during NACT, with results expected in 2026 [21].

3.1.4 HOST IMMUNE SYSTEM

The immune system plays a central role in both defending against and shaping the development of breast cancer. Innate immunity, mediated by macrophages, natural killer cells, dendritic cells, and granulocytes, provides rapid but non-specific protection, while adaptive immunity, driven by antigen-specific T and B lymphocytes, generates lasting immunological memory. The link between immunity and cancer was first proposed by Paul Ehrlich [22] in the early 20th century and later formalized by Burnet and Thomas [23] through the concept of immunosurveillance—the idea that the immune system detects and eliminates emerging tumor cells. Subsequent research revealed that the immune system not only suppresses tumor growth but also shapes tumor evolution, leading to the concept of **cancer immunoediting** [24]. This immune process was described to encompass three phases. In the **elimination** phase , immune effector cells destroy transformed cells, as evidenced by the increased cancer incidence in immunosuppressed individuals [25]. During **equilibrium** phase, residual tumor cells persist under immune pressure, selecting for less immunogenic variants that may remain dormant for years, as seen in late

relapses of luminal breast cancer [26]. In the **escape** phase, tumors evade immunity through antigen loss, immune checkpoint activation (PD-1/PD-L1 interactions), and immunosuppressive signaling [27, 28]. The discovery of checkpoint molecules such as CTLA-4 and PD-1 transformed the understanding of the interplay between immune system and cancer development, enabling the development of immune checkpoint inhibitors that restore T-cell activity and achieve durable responses in several malignancies, including triple-negative breast cancer.

3.2 TUMOR MICROENVIRONMENT

The connection between inflammation and cancer was first proposed by Virchow in 1863, who observed leukocyte infiltration in tumors, suggesting a role for chronic inflammation in tumorigenesis [29]. Paget's 1889 "seed and soil" hypothesis highlighted that tumor growth and metastasis depend not only on malignant cells but also on a supportive host environment [30], pre-dating the modern concept of the tumor microenvironment (TME). Now recognized as an active driver of cancer, the TME—comprising stromal fibroblasts, immune cells, endothelial cells, and extracellular matrix—promotes tumor progression, immune evasion, angiogenesis, and metastasis [31]. Landmark studies, including Folkman's demonstration of angiogenesis as essential for tumor growth, have informed targeted therapies such as bevacizumab (an anti-angiogenic monoclonal antibody), while cancer-associated fibroblasts remodel the extracellular matrix to facilitate malignancy [32]. These discoveries underscore the important interplay between tumor cells and their microenvironment, including tumor-infiltrating lymphocytes (TILs), which hold significant clinical and therapeutic potentials in breast cancer management.

3.2.1 TUMOR-INFILTRATING LYMPHOCYTES

Tumor-infiltrating lymphocytes are immune cells, primarily lymphocytes, that migrate into and reside within the breast cancer, reflecting the host immune response to the tumor. The density and composition of TILs vary widely across breast cancer subtypes, reflecting differences in intrinsic immunogenicity. Generally, TNBC and HER2-positive tumors exhibit higher TIL densities. In TNBC, this stems from a higher mutational load and increased neoantigen expression that enhance cytotoxic T-cell recognition [33], whereas HER2-positive tumors are less immunogenic, driven primarily by proliferative signaling rather than immune processes, resulting in fewer TILs [34]. Luminal

cancers (hormone receptor–positive, HER2-negative) are the least immunogenic, with low mutation rates and estrogen receptor dependence contributing to weak immune activation and sparse lymphocytic infiltration [35].

The prognostic significance of lymphocytic infiltration was first recognized in melanoma and later confirmed in breast cancer, where $CD8^+$ cytotoxic T-cell–rich tumors are associated with improved outcomes, while enrichment of $CD4^+$ regulatory T-cells predicts poorer prognosis [36, 37]. Additionally, $CD4^+$ helper T-cells further enhance antitumor immunity by secreting interferon-gamma, indicating the complex interplay within the TIL population [38]. Clinically, the strongest evidence supporting TILs as biomarkers in breast cancer arises from the NACT setting: Denkert et al. demonstrated that TILs independently predict pCR, and Loi et al. reported survival benefits associated with higher TIL densities, particularly among node-positive patients receiving adjuvant chemotherapy [39, 40]. Emerging data suggest TILs may also predict therapeutic response in axillary metastases, though larger, long-term studies are needed for validation [41]. Despite published evidence, TIL assessment has not yet entered routine clinical use due to the absence of standardized scoring, interobserver variability, and unclear clinical thresholds. Further prospective trials are required to confirm the utility of TIL-guided strategies and clarify their potential in refining patient selection for immunotherapy and checkpoint blockade—an evolving yet unresolved area [42].

3.2.2 ASSESSING TUMOR-INFILTRATING LYMPHOCYTES

In precision oncology, reliable biomarkers are essential for guiding diagnosis, prognosis, and treatment decisions. In breast cancer, early biomarker adoption—such as estrogen receptor assessment and later Ki-67—was initially limited by variability in testing methods, highlighting the need for standardized evaluation [43, 44]. Building on these lessons, the International TILs Working Group issued consensus guidelines in 2014 for scoring TILs on routine hematoxylin and eosin-stained, formalin-fixed paraffin-embedded sections [45]. The recommendation is to quantify the proportion of stromal area within the invasive tumor boundary occupied by mononuclear immune cells, including lymphocytes and plasma cells, while excluding polymorphonuclear cells. Although TILs are inherently a continuous variable, semi-decimal categories are used as visual estimation cannot yield an exact count when assessing large number of tumor cells. Studies often group TILs into

approximate categories such as low (<10%), intermediate (10–59%), and high (≥60%), with the latter referred to as lymphocyte-predominant breast cancer [46]. This standardized approach has improved reproducibility and enables more consistent incorporation of TILs into clinical and translational research (Figure 1).

However, despite standardized scoring criteria, TIL assessment is not yet routine in clinical practice, largely due to interobserver variability, tumor heterogeneity, and the absence of validated automated tools. Emerging digital pathology and machine learning approaches show promise in improving reproducibility, but their performance varies across algorithms and datasets [47, 48]. To address this, large multi-center benchmarking efforts, such as the TIGER Challenge (https://tiger.grand-challenge.org), are evaluating automated TIL quantification in HER2-positive and TNBC [49]. These initiatives aim to establish robust, standardized workflows that could support reliable clinical implementation and help refine patient selection for immunotherapy [42].

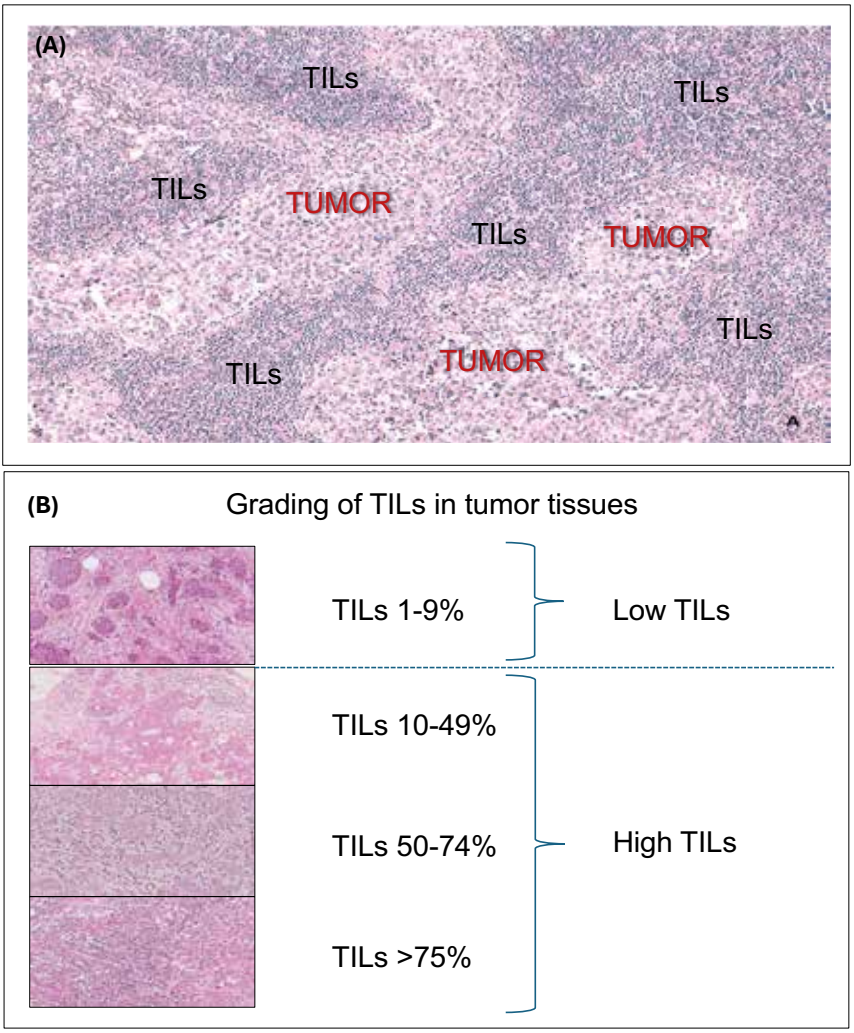


Figure 1. A) Tumor-infiltrating lymphocytes (TILs) surrounding tumor cells (Tumor). **B**) The proportion of stromal TILs within the tumor was evaluated, with categorical percentage cut-offs defined by our local histopathology department protocol in accordance with the International TILs Working Group guidelines. [45]. The cut-offs for low and high TILs for paper II were chosen pragmatically, based on thresholds commonly reported in published studies. No artificial intelligence was used for the assessment of TILs. Pictures: courtesy of Dr Anikó Kovács, Consultant Histopathologist, Sahlgrenska University Hospital, Göteborg, Sweden

4 IMAGING MODALITIES

Breast imaging is central to breast cancer management, evolving from X-ray discovery in by Röntgen 1895 [50] and Albert Salomon's early correlation of radiographic calcifications with pathology [51], to a sophisticated tool for diagnosis, surgical planning, treatment decisions, and surveillance. The shift to digital mammography further improved image resolution, diagnostic accuracy, and workflow [52], while artificial intelligence now offers automated lesion detection, risk modeling, and clinical optimization [53]. This section highlights modern advances in breast imaging and their impact on surgical management in breast cancer care.

4.1 MAMMOGRAPHY

Mammography remains an important first-line imaging tool for breast cancer diagnostic, using low-dose X-rays in craniocaudal and mediolateral oblique views. However, its effectiveness can be limited in women with dense breasts due to tissue overlap. Innovations such as digital breast tomosynthesis create three-dimensional reconstructions that improve cancer detection and reduce recall rates, with only a modest increase in radiation [54]. Contrast-enhanced spectral mammography (CESM) provides functional imaging by highlighting abnormal vascularity after iodinated contrast administration. It offers MRI-like information more quickly and cost-effectively particularly for dense breasts, indeterminate lesions, and tumor response assessment [55, 56].

Emerging technologies further enhance mammography: radiomics links imaging patterns to molecular features for subtype prediction [57], photon-counting mammography improves detection of microcalcifications [58], and artificial intelligence-assisted screening, as shown in the Swedish MASAI trial, can increase early detection while reducing radiologist workload without raising overdiagnosis [53].

4.2 ULTRASOUND

Breast ultrasound, first explored for medical use in the early 1950s [59], has evolved into an essential adjunct to mammography. Improvements in high-frequency transducers allow clear visualization of small, clinically relevant lesions, and the development of compact probes enabled ultrasound-guided biopsy and marker placement with high precision [60]. Ultrasound is a

particularly valuable imaging tool in women with dense breasts, although it is not cost-effective as a primary screening modality.

More recent advances like automated whole-breast ultrasound for standardized volumetric imaging, and contrast-enhanced ultrasound for assessing preoperative and neoadjuvant therapy response—have expanded its role beyond lesion detection toward functional and treatment-monitoring applications [61, 62]. Overall, ultrasound now serves as a key component of multimodal breast imaging, complementing mammography, tomosynthesis, and MRI in both diagnostic evaluation and surgical planning.

4.3 MAGNETIC RESONANCE IMAGING

The potential of magnetic nuclear resonance for tumor detection was first proposed by Damadian in 1971 [63]. The first breast MRI images were reported by Peter Mansfield in 1979 [64], finally leading to clinical adoption in the 1980s [65]. Later, the introduction of gadolinium-based contrast agents marked a major advance by visualizing neoangiogenesis, a hallmark of malignancy. The malignant tissue exhibits leaky vasculature allowing rapid contrast uptake and washout, distinguishing benign from malignant lesions [66]. Today, MRI is used for high-risk screening (e.g., BRCA mutation carriers), evaluating disease extent in invasive lobular carcinoma and assessing tumor response to NACT. However, its high sensitivity can lead to overdiagnosis, highlighting the importance of careful patient selection [67, 68].

Overall, though not used universally, MRI is more accurate than mammography for assessing tumor response during neoadjuvant chemotherapy, as demonstrated in prospective trials and meta-analyses [69, 70]. It is also noteworthy that there is emerging data that demonstrate CESM may be an acceptable alternative tool to monitor tumor response during NACT [71]. Furthermore, there is currently no robust randomized evidence demonstrating that MRI-guided changes in therapy or surgery lead to improved long-term outcomes in breast cancer.

5 BREAST CANCER TREATMENTS

Breast cancer treatment is essentially based on three main approaches: surgery, radiotherapy, and systemic therapy. Surgery remains the primary method of achieving local control by removing the tumor, while radiotherapy reduces the chance of recurrence in the breast and regional lymph nodes [72]. Systemic therapy, which includes endocrine therapy, chemotherapy, and targeted treatments, is designed to address disease that may have spread beyond the breast, thereby lowering the risk of distant relapse and improving survival. Additionally, systemic therapy has also been increasingly used in the upfront setting to down-stage and down-size tumors in order to facilitate BCS. In modern practice, these treatments are rarely used in isolation. Instead, they are combined and tailored to the characteristics of both the disease and the patient. This has shifted breast cancer management toward a more individualized and multidisciplinary approach.

5.1 SURGERY: A CENTURY OF DE-ESCALATION AND EVOLUTION

It is largely accepted that surgery was first described as treatment for breast cancer in ancient Egypt. Although various other remedies were also used, surgery was regarded as the main treatment for a long time, until the discovery and clinical adoption of endocrine- and radiotherapy in the 1900s. Here, we discussed the evolution of surgical approaches to breast cancer (Figure 2).

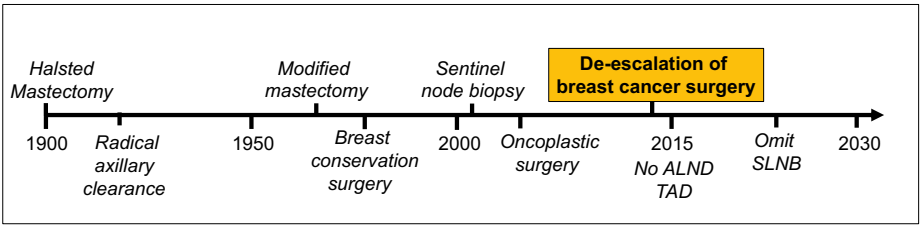


Figure 2. This shows the evolution timeline of breast cancer surgery. Illustrated by Kian Chin. Data source from Zurrida et al, The Breast journal, 2015, Milestones in breast cancer treatment [73]. Abbreviations: ALND: axillary lymph node dissection, SLNB: sentinel lymph node biopsy, and TAD: targeted axillary dissection.

5.1.1 BREAST SURGERY

5.1.1.1 FROM MASTECTOMY TO BREAST CONSERVATION

The earliest known references to breast tumor surgery appear in the Edwin Smith Papyrus, dating back to ancient Egypt [74]. For centuries thereafter, treatment consisted largely of radical resections involving removal of the breast, chest wall musculature, and regional lymph nodes—techniques described by physicians across both Western and Eastern traditions [75]. However, the outcomes of these extensive operations were not systematically evaluated.

A turning point came in the late 19th century when William Halsted introduced the radical en-bloc mastectomy, meticulously described in his 1894 report on 50 patients. The procedure achieved low rates of local recurrence and became the gold standard for the ensuing decades, reinforcing the belief that wider resections translated into better oncological control [1]. This philosophy of extensive surgery dominated until the 1970s, when pivotal randomized trials by Veronesi et al. in Milan and Fisher et al. in the United States challenged the Halstedian paradigm. Their landmark studies demonstrated that BCS followed by radiotherapy provided equivalent long-term survival to radical mastectomy, even after 20 years of follow-up [72, 76]. These findings marked a watershed moment in breast cancer surgery, leading to an era of surgical de-escalation focused on oncological safety with improved quality of life.

5.1.1.2 ONCOPLASTIC BREAST CONSERVATION

Over time, BCS became the preferred approach for appropriately selected patients instead of mastectomy. In Sweden, the proportion of women undergoing BCS for early cancer increased from 53% in 2008 to 77% by 2024, with rates approaching 90% for invasive tumors smaller than 30 mm [5]. This trend was facilitated by national screening programs, which enable earlier detection of smaller, more operable cancers [77, 78].

The evolution of standard BCS occurred with oncoplastic surgery first proposed in the early 1990s [79, 80]. The concept of combining tumor removal with aesthetic breast reshaping led to advances that now wider resection of cancers while preserving cosmetic outcomes and achieving clear margins, thereby reducing the need for mastectomy [81, 82]. These innovations have broadened indications for BCS and contributed to another shift in surgical management.

Beyond aesthetic benefits, BCS offers lower morbidity, shorter recovery, and reduced hospital resource utilization compared to mastectomy [83-85]. Emerging evidence even suggests that BCS may confer superior survival in a study cohort adjusted for co-morbidity and socioeconomic status [86], challenging older assumptions that survival is equivalent between BCS with radiotherapy and mastectomy. However, despite this newer evidence based on retrospective data, mastectomy remains essential for selected patients, including those for whom BCS would produce unacceptable cosmetic outcomes despite oncoplastic techniques, individuals with hereditary breast cancer syndromes, patients with inflammatory breast cancer, those ineligible for postoperative radiotherapy, or those who elect mastectomy after informed discussion.

5.1.2 DE-ESCALATION OF AXILLARY SURGERY

Axillary lymph node dissection was a major part of the Halstedian radical approach to breast cancer surgery, but it carried significant morbidity, including chronic lymphedema, pain, and reduced arm mobility, factors that profoundly affected long-term quality of life [87]. These complications led to increased focus in re-evaluating axillary management, with the aim of safely reducing extent of surgery without compromising oncological outcomes.

Over the past two decades, research evidence has culminated in a significant shift towards conservatism in axillary surgery. This transition reflects a deeper understanding of tumor biology and lymphatic spread, associated with the availability of effective systemic and locoregional therapies. As a result, many patients can now avoid the arm morbidities historically linked to radical axillary clearance, achieving excellent oncological outcomes with less invasive approaches.

5.1.2.1 ADVENT OF SENTINEL LYMPH NODE BIOPSY

The presence of breast cancer spread via lymphatic was first described by Jean Louis Petit and Le Dran between the 18th and 19th centuries, who noted that tumor recurrences were often associated with nearby axillary lymph nodes [74, 88]. The clinical significance of this observation was formalized in 1894 when William Halsted incorporated axillary lymph node removal into radical mastectomy, based on the belief that breast cancer spread sequentially—from the primary tumor to regional nodes and then systemically [1]. For nearly a century, this approach dominated surgical practice.

The concept of the sentinel lymph node (SLN), the first node to receive drainage from a tumor, emerged in the late 1970s. Cabanas used lymphangiography in penile cancer to show that if the primary draining nodes were metastasis-free, further nodal dissection could often be avoided [89]. This principle laid the foundation for SLNB with the MSLT-1 trial in melanoma providing key evidence of procedural accuracy and oncological safety compared to complete lymph node dissection [90].

Subsequent randomized clinical trials confirmed the safety and effectiveness of SLNB used in breast cancer. The NSABP B-04 showed that leaving occult axillary metastases unresected did not compromise overall survival (OS) [72]. Later, NSABP B-32 demonstrated that SLNB, with ALND performed only if the SLN was positive, yielded equivalent overall and disease-free survival [91]. Similarly, the UK ALMANAC [92] and Milan clinical trials [93] reported significantly lower arm morbidity with SLNB without increasing locoregional recurrence. These findings established SLNB as a reliable axillary staging method in clinically node-negative breast cancer, paving the first stage to axillary de-escalation.

5.1.2.2 OMITTING AXILLARY LYMPH NODE DISSECTION

Despite the established safety of SLNB for clinically node-negative breast cancer, ALND continued to be the standard for patients with sentinel node metastases. By early 2010s, evidence began to emerge that less invasive surgery to the axilla, that contained metastases in the SLN, could achieve comparable outcomes. The pivotal ACOSOG Z0011 trial (USA) randomized patients with 1–2 SLN macrometastases to either ALND or no further axillary surgery. The study enrolled patients with T1–T2 invasive breast cancer who were clinically node-negative, underwent BCS, SLNB, and whole-breast irradiation. At both 5- and 10-year follow-up, there were no significant differences between the two groups in OS or regional recurrence [94]. Despite its landmark findings, the trial was associated with controversies: early closure due to slow recruitment and lower-than-expected event rates may have limited statistical power, and inadvertent irradiation of the lower axilla could have influenced outcomes [95-97]. These factors have raised questions about the reliability of drawing definitive conclusions regarding ALND omission.

However, subsequent randomized studies have validated the oncologic safety of omitting ALND in the setting of limited SLN metastases. The AMAROS trial compared ALND to axillary radiotherapy in patients with positive SLN, demonstrating equivalent axillary control but significantly lower morbidity

with radiotherapy. Ten-year follow-up confirmed non-inferior locoregional control and no difference in OS [98, 99]. The IBCSG 23-01 trial addressed SLN micrometastases, comparing ALND to no further axillary surgery. Both 5- and 10-year results showed no significant difference in disease-free survival [100, 101]. More recently, the SENOMAC trials compared ALND to no surgery if SLNB showed two or less lymph nodes with macrometases but clinically node-negative. The results also confirmed the safety in omitting ALND at 5-year followed up non-inferior recurrence-free survival [102].

Collectively, these studies supported safe omission of ALND in patients with minimal to moderate nodal tumor burden and underpin current standards of care.

5.1.2.3 TARGETED APPROACH IN AXILLARY STAGING

The shift toward axillary de-escalation laid the groundwork for targeted axillary dissection (TAD), a strategy developed for patients receiving NACT with initially biopsy-proven axillary metastases at diagnosis. Many clinicians began to observe high rates of axillary pCR after NACT, especially among those patients with HER2-positive and TNBC subtypes and questioned the necessity to still perform ALND [103]. Meanwhile, trials exploring post-NACT SLNB, including ACOSOG Z1071 [104], SENTINA [105]), SN FNAC [106], and the Swedish Multicentre Trial [107], revealed unacceptably high false-negative rates (12.6–14.4%) and lower SLN detection compared with the adjuvant setting [108].

To address this high false-negative rates, the concept of TAD was developed. This technique involves pre-marking the previously biopsied metastatic node (index node) and retrieving it during surgery alongside SLNB using a lymphatic tracer. Caudle et al. demonstrated that combining SLNB with retrieval of the index node reduced the false-negative rate from 10.1% to 2% [109]. Multiple subsequent studies have now confirmed TAD as a reliable and effective approach for de-escalating axillary surgery after NACT, with consistently low rates of nodal recurrence [110-112].

The reliability of the TAD approach has also prompted further research in evaluating upfront surgery in patients with low axillary metastatic burden, randomizing them to TAD compared to ALND, TADPOLE trial (UK) [113]. In Sweden, TAD is currently only recommended for patients showing a clinically good or complete axillary response after NACT.

5.1.3 PARAMAGNETIC DEVICES IN SURGERY

Accurate localization of breast tumors and axillary lymph nodes is essential for safe surgical de-escalation, ensuring complete tumor removal and precise nodal staging. Traditionally, wire-guided localization is used to mark non-palpable lesions, while lymphatic tracers like blue dye and Tc^{99m} guide SLNB. However, guide wires insertion can cause discomfort, risk wire displacement, and pose logistical challenges in planning for surgery. Blue dye may induce allergic reactions and skin staining, while Tc^{99m} requires specialized handling due to its radioactivity. Although new devices have become available for localizations [114], the advent of paramagnetic technology offers an effective alternative to overcome these clinical and logistical limitations [115] (Figure 3).

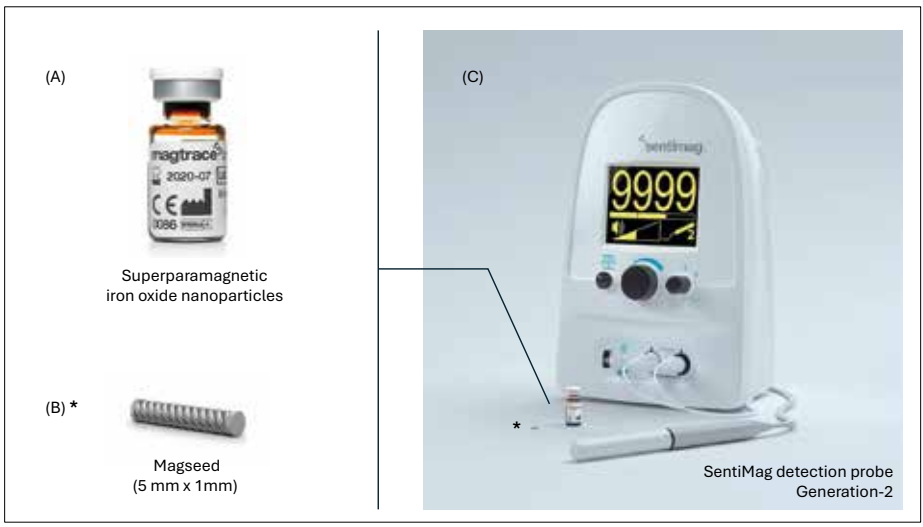


Figure 3. This shows **A**) an ampoule of Magtrace containing superparamagnetic iron oxides nanoparticles, **B**)* paramagnetic marker, Magseed, and **C**) SentiMag detection probe (magnetometer) – Generation 2

5.1.3.1 IRON OXIDE NANOPARTICLES AS LYMPHATIC TRACER

Superparamagnetic iron oxide nanoparticles are widely used in biomedical applications due to their biocompatibility, biodegradability, low allergenicity, affordability, and ease of synthesis [116]. They are typically produced by co-precipitating ferrous (Fe^{2+}) and ferric (Fe^{3+}) salts in alkaline solutions, yielding high-purity particles with controlled sizes [117]. Particles smaller than 20 nm exhibit superparamagnetism, becoming magnetic only under an external field and returning to a non-magnetic state afterward, making them safe for clinical use.

Superparamagnetic lymphatic tracers such as Magtrace® (SPIO) (Endomag, Hologic Inc.) have a 3.5–10 nm iron oxide core coated with carboxydextran, producing an overall size of 45–60 nm [118]. For clarity and consistency, the term SPIO is used synonymously with Magtrace throughout this thesis. They generate a transient magnetic signal detectable intraoperatively with the SentiMag®, a handheld magnetometer, allowing SLN identification. Unlike blue dye or Tc^{99m}, SPIO typically persist in lymph nodes for a longer period (up to months), enabling flexible surgical planning. However, SPIO is CE-marked for clinical use with an approved injection-to-surgery interval of up to 30 days. However, SPIO can also create MRI artifacts, limiting its use in patients requiring MRI-based assessment of breasts.

5.1.3.2 PARAMAGNETIC SEED FOR TUMOR LOCALIZATION

Magseed is a 5 × 1 mm biocompatible stainless-steel marker designed for tumor and lymph node localization (Endomag, Hologic Inc.). Unlike SPIO, it is a macroscopic iron alloy. Its paramagnetic properties allow temporary magnetization in a magnetic field, facilitating intraoperative detection with a handheld probe. Magseed can remain in situ until surgery (no CE-marked time limitation) and is delivered via an 18-gauge needle under mammographic or ultrasound guidance, though it is not compatible with MRI-guided placement (Figure 4 and 5).

5.1.3.3 CLINICAL UTILITIES

Tracer like SPIO have demonstrated accuracy in guiding SLNB compared to Tc^{99m} [119-122]. Similarly, Magseed has demonstrated non-inferior performance compared to wire localization, with high patient and clinician satisfaction [123, 124]. However, most of these studies focused on upfront

surgery; the role of Magseed for tumor marking after NACT is less well-defined, therefore, will be explored further in this thesis.

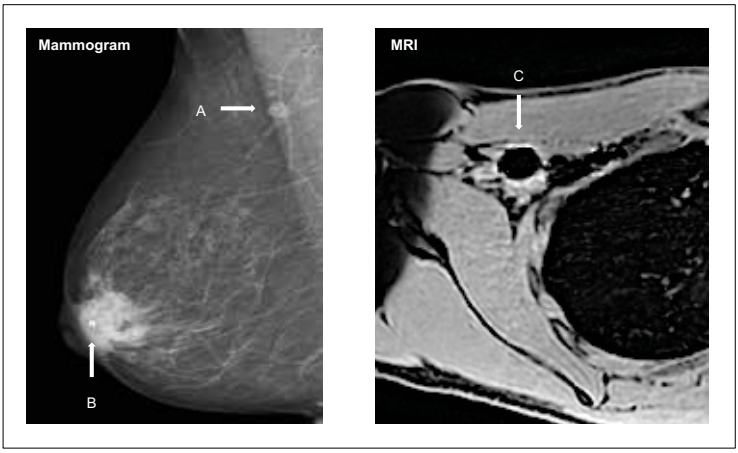


Figure 4. A) Magseed inserted to mark the index node with metastasis seen on mammogram. **B**) a standard marker used to mark the tumor. **C**) T1-weighted magnetic resonance (MR) image showing a sentinel node with signal void, "black hole", appearance (arrow) in the right axilla, due to an artefact caused by the magnetic property of superparamagnetic iron oxide (SPIO) nanoparticles that interfere with the magnetic field generated by the MR camera, where accumulation of SPIO results in loss of signal.

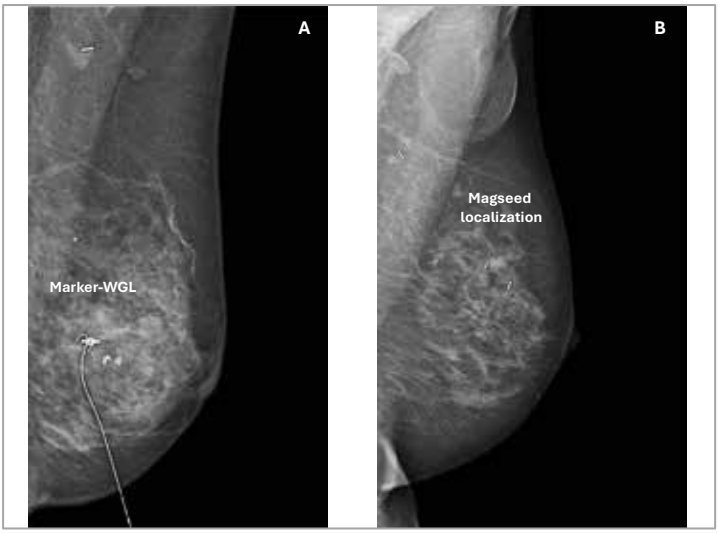


Figure 5. Mammography showing two different methods in marking the tumor in the breast in patients undergoing neoadjuvant chemotherapy. **A**: Marker-WGL standard approach for tumor localization was used. A Magseed can been inserted into the index lymph node. **B**: Bracketing tumor localization with double Magseeds in the breast and a Magseed seen in the axilla

5.2 SYSTEMIC THERAPY

Historically, general local or systemic remedies containing poultices and balms have been documented, though not specifically, for use in cancer treatment,[125]. However, as breast cancer treatment was mainly dominated by surgery during ancient times, systemic therapy was seldom used due to its limited clinical impact and its mechanisms were not well understood. By 1955, George Crile challenged the surgical dominance in his article 'Cancer and Common Sense', advocating for more conservative approaches and recognizing that breast cancer often involved micrometastases at diagnosis [126]. This early view of breast cancer as a systemic disease laid the foundation for the development of systemic therapies (Figure 6), based on modern research evidence. This chapter provide an overview of the historical evolution as summarized in Figure 7. Some of the key advancements, and emerging directions in systemic breast cancer treatment will be discussed.

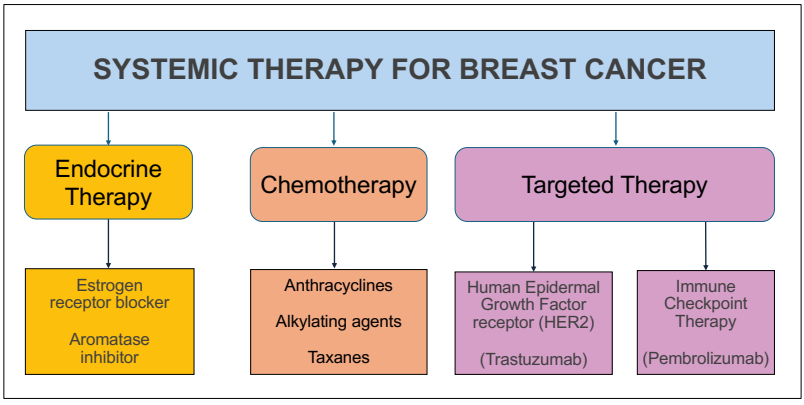


Figure 6. Diagram showing different classes of systemic therapy for breast cancer

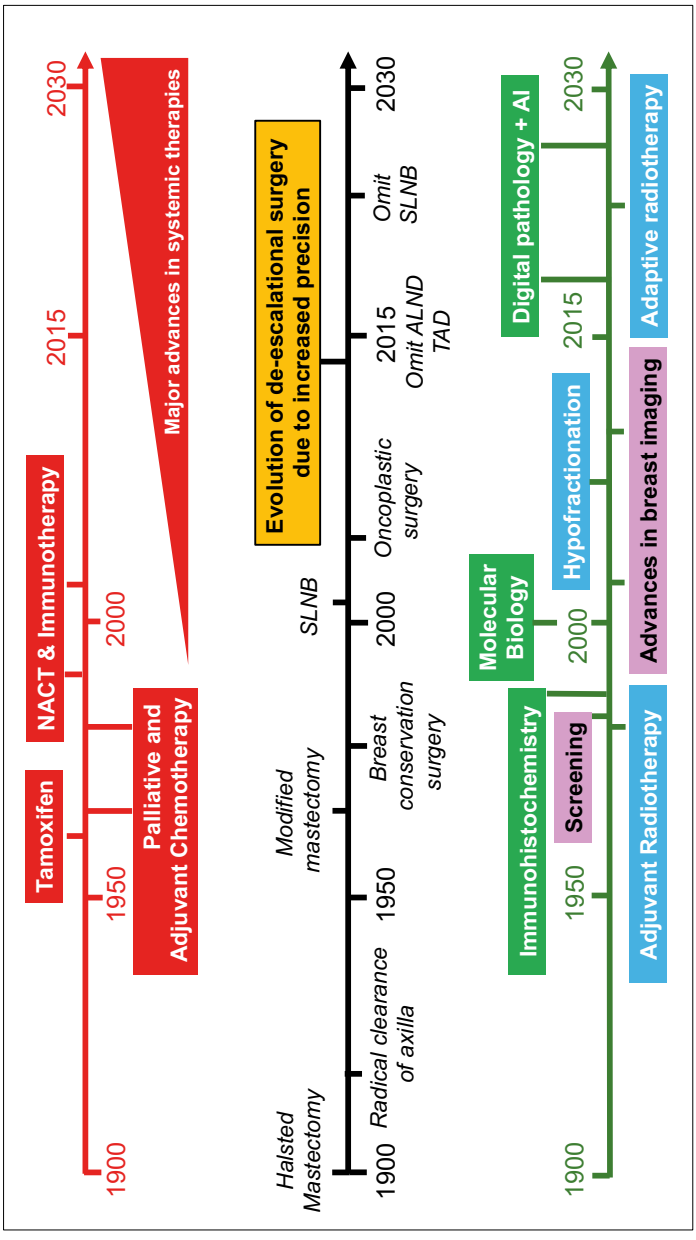


Figure 7. Diagram indicating the evolutional timeline of systemic therapy, radiotherapy, imaging, and histopathology, in relation to surgical de-escalation Specifically, the advances in non-surgical domains of breast cancer managements have clearly paralleled the advent of surgical conservatism. Illustration by Kian Chin. Data source from Zurrida et al, The Breast journal, 2015, milestones in breast cancer treatment [73]. Abbreviations: ALND: axillary lymph node dissection, NACT: neoadjuvant chemotherapy, SLNB: sentinel lymph node biopsy, and TAD: targeted axillary dissection

5.2.1 ENDOCRINE THERAPY

Endocrine therapy represents the first targeted systemic treatment for breast cancer, originating from George Beatson's 1896 observation that ovarian ablation induced tumor regression in premenopausal women. The biological basis for this effect became clear following the discovery of estrogen [127] and its receptor (ER) in the mid-20th century [128]. Later on, Elwood Jensen demonstrated estrogen's specific receptor-mediated action in human breast tissue [129]. The identification of antiestrogenic activity of tamoxifen in the 1970s [130, 131] and ER-α as a driver of cell proliferation [132] established the clinical foundation of hormone therapy.

Tamoxifen, the first selective estrogen receptor modulator (SERM), acts as an estrogen antagonist in breast tissue but retains agonist effects in the endometrium and bone [133]. For decades, Tamoxifen became the cornerstone of adjuvant systemic therapy after trials such as NSABP B-14 demonstrated improved outcomes [134]. Aromatase inhibitors that block estrogen synthesis—anastrozole, letrozole, and exemestane—later supplanted tamoxifen in postmenopausal women. Major trials (ATAC [135] , BIG 1-98 [136], IES [137]) confirmed their efficacy led to improved survival outcome. For premenopausal patients, the SOFT [138] and TEXT [139] trials showed that ovarian suppression with tamoxifen or an aromatase inhibitor further improved survival. Fulvestrant, the first selective estrogen receptor degrader (SERD), expanded treatment options by inducing receptor degradation and inhibiting estrogen signaling, as validated in the FALCON [140], PALOMA-3 [141], and CONFIRM [142] trials. More recently, combination therapies have advanced endocrine treatment: Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors (palbociclib, ribociclib, abemaciclib) that disrupt cell growth cycle could significantly prolong survival in advanced hormone-positive disease (PALOMA-3 [141] , MONALEESA-7 [143], MONARCH-E [144], NATALEE [145]). Meanwhile, inhibition of the mammalian target of rapamycin (mTOR) pathway with everolimus plus exemestane improves outcomes in resistant cases (BOLERO-2) [146].

Neoadjuvant endocrine therapy (NET), initially tested with tamoxifen and later with aromatase inhibitors, can achieve overall clinical response rates up to 55% [147]. However, NET remains underutilized due to longer treatment durations to achieve tumor response and uncertain management of partial responders. Tools such as the Preoperative Endocrine Prognostic Index (PEPI) may help stratify post-NET risk, though further validation is needed [148, 149].

5.2.2 CHEMOTHERAPY

Chemotherapy is the systemic administration of cytotoxic drugs that interfere cell division and DNA replication, resulting in cell death or growth arrest of malignant cells. Nitrogen mustard, originally developed as a chemical warfare agent, was the first alkylating compound shown to induce tumor regression in lymphoma, leading to the FDA approval of mechlorethamine as the first chemotherapeutic drug in 1949 [150]. Subsequent landmark trials by Bonadonna [151] and Kjellgren [152] demonstrated that adjuvant chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) significantly improved survival in node-positive and later node-negative breast cancer [153]. These findings established the efficacy of chemotherapy as a standard adjunct to surgery and spurred the development of more effective agents (Table 1).

Doxorubicin, an anthracycline antibiotic derived from Streptomyces peucetius, subsequently replaced CMF as the backbone of therapy after meta-analyses confirmed its superiority in reducing recurrence and mortality [154-156]. Further advances included the discovery of paclitaxel (Taxol®) from the pacific yew tree [157], which, when combined with anthracycline-based regimens, improved outcomes in early-stage breast cancer [156]. The introduction of platinum-based agents, particularly carboplatin, added another major advance, showing marked efficacy in triple-negative and BRCA1/2-mutated breast cancers, with trials such as TNT [158] and subsequent neoadjuvant studies demonstrating higher response and pCR rates [159-161].

Together, these breakthroughs transformed breast cancer chemotherapy from rudimentary alkylating therapy into a refined, evidence-based, and subtype-directed component of modern systemic treatment.

Table 1. Mechanisms of actions for various types of systemic therapies

Table 1	Category	Mechanism of action	Examples
Conventional Chemotherapy	Anthracylines	Intercalate with DNA – DNA breaks	Doxorubin, Epirubicin
	Taxanes	Stabilize microtubules - stop mitosis	Paclitaxel, Docetaxel
	Alkylating agents	Crosslink DNA – impaired replication	Cyclophoshamide
	Antimetabolites	Interfere with nucleotide metabolism – inhibit DNA synthesis	5-FU, Capecitabine, Methotrexate
	Platinum agents	Form DNA adducts – Crosslinks - apoptosis	Cisplatin, Carboplatin
Targeted therapy	HER2-directed	Monoclonal Antibodies block HER2 signalling	Trastuzumab
	CDK 4/6 inhibitors	Inhibit cyclin-dependent kinases 4/6 – cell cycle arrest	Palbociclib, Abemaciclib, Ribociclib
	PARP inhibitors	Stop PARP-mediated DNA repair – death of BRCA mutated cells	Olaparib, Tazoparib
	PI3K/mTOR inhibitors	Block PI3K/mTOR signalling – inhibit proliferation	Alpelisib, Everolimus
	Immune checkpoint inhibitors	Block PD-1 / PD-L1 interaction - restore T cell immunity	Pembrolizumab, Atezolizumab
	Antibody-drug conjugates	Monoclonal antibody delivers cytotoxic payload directly to tumor cells	Sacituzumab govitecan Trastuzumab deruxtecan

Abbreviations: CDK 4/6: cyclin dependent kinase, DNA: Deoxyribose nucleic acids, 5-FU: fluorouracil, HER2: human epidermal growth factor receptor 2, mTOR: mammalian Target of Rapamycin, PARP: poly (ADP-Ribose) polymerase, PD-1: programmed cell death protein 1; PD-L1: programmed cell death protein-ligand 1; PI3K: phosphoinositide 3-kinase

5.3 TARGETED SYSTEMIC THERAPY

5.3.1 HER2 TARGETED THERAPY

The development of targeted monoclonal antibody therapy in breast cancer was driven by the discovery of HER2 receptor, overexpressed in 15–20% of breast cancers [162]. Overexpression HER2 activates proliferative signaling pathways and is linked to poor prognosis. The pivotal finding that a monoclonal antibody could inhibit HER2-driven tumor growth [163] led to the advent of trastuzumab. Trastuzumab is a monoclonal antibody that binds to the extracellular domain of the HER2 receptor, blocking downstream signaling and simultaneously recruiting immune effector cells through antibody-dependent cellular cytotoxicity [164]. The efficacy of trastuzumab transformed outcomes for patients with HER2-positive breast cancer, with landmark trials demonstrating the drug was associated with significant survival benefits [165, 166]. In the neoadjuvant setting, trastuzumab markedly increased rates of pCR [167, 168] (Table 1).

5.3.2 IMMUNE CHECKPOINT THERAPY

Advances in immunotherapy were enhanced by the discovery of immune checkpoints—transmembrane receptors on immune cells that regulate cytotoxic activity. Among the most clinically significant are programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), both of which suppress immune responses when engaged by tumor ligands, allowing cancer cells to evade detection.

Tasuku Honjo and colleagues identified PD-1 as a receptor upregulated on T cells during activation [27], later shown to mediate immune suppression when bound by tumor PD-L1, leading to $CD8^+$ T-cell exhaustion and tumor survival [169]. Whereas, CTLA-4, discovered earlier in 1987 [170], was later characterized by James Allison's group as an inhibitory receptor whose blockade could restore antitumor immunity [28]. Expressed mainly on $CD4^+$ T cells and regulatory T cells (Tregs), CTLA-4 dampens cytotoxic responses when interacting with antigen-presenting cells. Tumors exploit this pathway by recruiting Tregs to the tumor microenvironment via chemokines release, creating local immunosuppression that facilitates immune escape [171] (Figure 8). The knowledge that blocking these immune checkpoints can inhibit tumor growth has been pivotal to the development of modern targeted cancer therapy (Table 1).

In breast cancer, the PD-1 inhibitor pembrolizumab has significant benefit against triple-negative disease, improving pCR and survival in both early and advanced TNBC [172, 173]. In contrast, the CTLA-4–targeting antibody ipilimumab remains under investigation for breast cancer but has demonstrated efficacy in other malignancies such as melanoma.

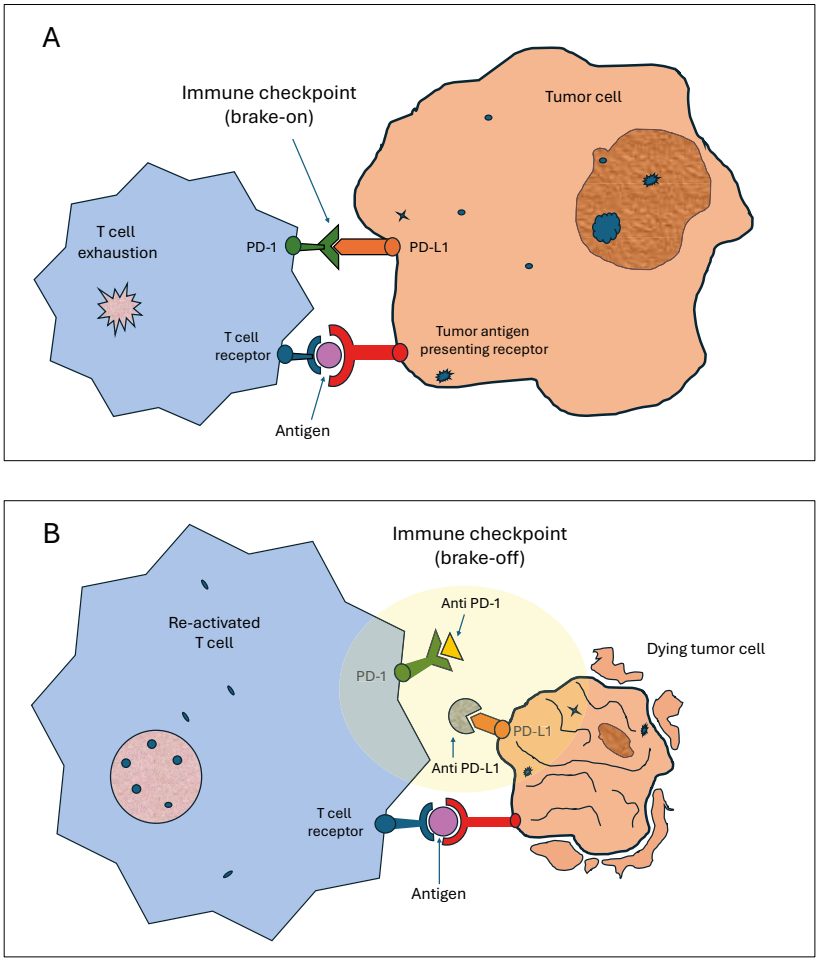


Figure 8. Schematic demonstration of interactions between T cell and tumor cell through the immune checkpoint receptors. **A**) Binding of PD-1 / PD-L1, renders the T cell exhausted and decrease in cytotoxic activity. **B**) when PD-1 and PD-L1 inhibitors are used, the binding of the immune checkpoint (PD-1 / PD-L1) are no longer intact, leaving the T cell becomes activated for cytotoxicity, killing the tumor cell. (Illustrated by Kian Chin).

5.3.3 ANTIBODY-DRUG CONJUGATES

Another significant advance for targeted monoclonal antibody therapy in breast cancer is the advent of antibody-drug conjugates (ADCs). These conjugates are constructed by fusing an antibody that binds specifically to tumor cells with a cytotoxic “payload” drug. Consequently, ADCs are more selective and precise than traditional chemotherapy, due to their targeted delivery to cancer cells. Simultaneously, they are associated with fewer side effects. Randomized controlled trials using ADCs such as trastuzumab emtansine and trastuzumab deruxtecan have expanded treatment options for HER2-positive metastatic breast cancer [174], while sacituzumab govitecan has shown efficacy in patients with previously treated metastatic TNBC [175].

Collectively, these advances in targeted drugs, given alongside cytotoxic chemotherapy, represent a significant move toward precision medicine.

5.4 NEOADJUVANT CHEMOTHERAPY

Neoadjuvant chemotherapy was first introduced in the late 1970s for patients with locally advanced or inflammatory breast cancer to enable surgical resection. By 1997, the landmark NSABP B-18 trial, which compared NACT to adjuvant chemotherapy in early breast cancer, had demonstrated the efficacy of NACT in significant tumor shrinkage and increased rates of breast-conserving surgery [176]. Over the ensuing years, the concept of NACT has the focus of extensive research, developments, and clinical adoptions. Between 2012 and 2023 in Sweden, adjuvant chemotherapy use declined from 40% to 30%, while NACT increased from 3.5% to 15%, reflecting a major shift in treatment practice, particularly for HER2-positive and triple-negative breast cancers [5] (Figure 9).

Despite increasing use, early neoadjuvant chemotherapy (NACT) trials did not demonstrate improvements in overall or disease-free survival compared with adjuvant therapy [177]. These studies had several limitations, including the absence of direct comparisons with adjuvant therapy [178], uncertainty regarding whether pCR reliably reflects systemic disease control [179] , and inconsistent definitions of pCR that limited its utility as a surrogate endpoint [180]. Moreover, most early trials predated molecular profiling and did not stratify patients by breast cancer subtype. A subsequent pooled analysis by Cortazar et al. [181] demonstrated a strong association between pCR and improved prognosis, particularly in HER2-positive and triple-negative breast cancers, which has contributed to NACT becoming routine in these biologically aggressive subtypes. However, patients who achieve pCR generally have tumors with favorable chemosensitivity, and they likely would have experienced similarly good outcomes if the same chemotherapy had been administered postoperatively. In contrast, the role of NACT in hormone receptor–positive, HER2-negative breast cancer remains limited, as pCR rates are low and no survival advantage over adjuvant chemotherapy has been observed [182].

Beyond improved survival with pCR, NACT also allows real-time assessment of tumor response and guides post-neoadjuvant therapy. Landmark trials such as CREATE-X (capecitabine versus no further chemotherapy) and KATHERINE (adjuvant a versus continuation of trastuzumab) also demonstrated significant benefit for patients with residual disease, who additional systemic treatment [183, 184]. In summary, NACT does not inherently confer a survival advantage over adjuvant chemotherapy. Notably,

while pCR is a robust prognostic marker, many patients who achieve pCR would likely have experienced similar outcomes with postoperative therapy, without the need for in vivo response monitoring. Therefore, for patients likely to be exceptional responders, delaying systemic therapy until after surgery may represent a safe and individualized treatment approach.

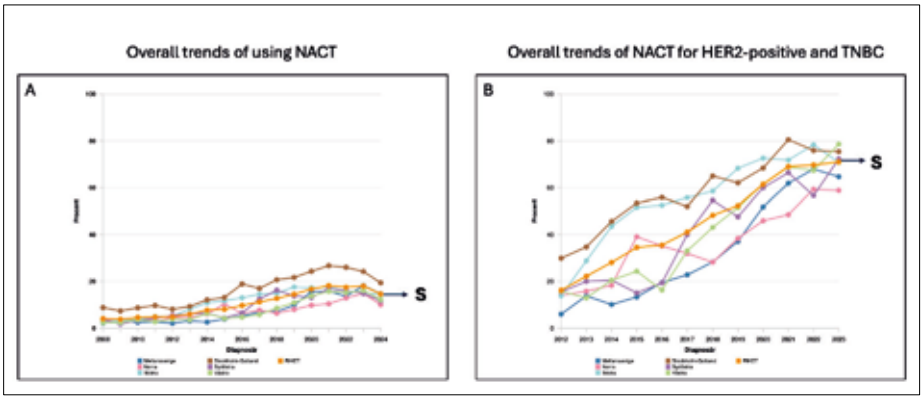


Figure 9. Trends of neoadjuvant chemotherapy (preoperative systemic therapy) between 2008 and 2024 in Sweden. A) Overall trends between regions for all subtypes of breast cancers. B) Overall trends between regions for HER2-positve and triple negative breast cancers. Interpretation: Yellow-colored line (S) represents the whole country while other lines represent different regions in Sweden. Source: Swedish National Quality Register for Breast Cancer (online public access)

5.4.1 HISTOPATHOLOGICAL ASSESSMENT OF TUMOR RESPONSE

Assessing tumor response and defining pCR after NACT can pose significant challenges in breast cancer pathology. Past grading systems such as Chevallier [185] and Miller–Payne [186] differ in how they measure residual tumor cellularity, in situ components, and nodal involvement, resulting in variable interpretations across centers (Figure 10). Differences in specimen handling, tissue sampling, and the inherently subjective nature of estimating residual disease further contribute to inconsistency between pathologists [187, 188].

The definition of pCR itself also lacks uniformity, with some trials considering only the absence of invasive disease in the breast (ypT0/is), while others require no invasive or in situ tumor in both breast and axilla (ypT0 ypN0) [189]. Such variation makes it difficult to compare outcomes between studies or establish consistent prognostic indicators. However, the introduction of

Residual Cancer Burden system (RCB) [190] has led to more homogenous standard of histopathological reporting [191].

Moving toward standardized assessment protocols, supported by digital pathology and artificial intelligence-assisted quantification, may offer a path to greater reproducibility and clinical relevance in evaluating treatment response [192].

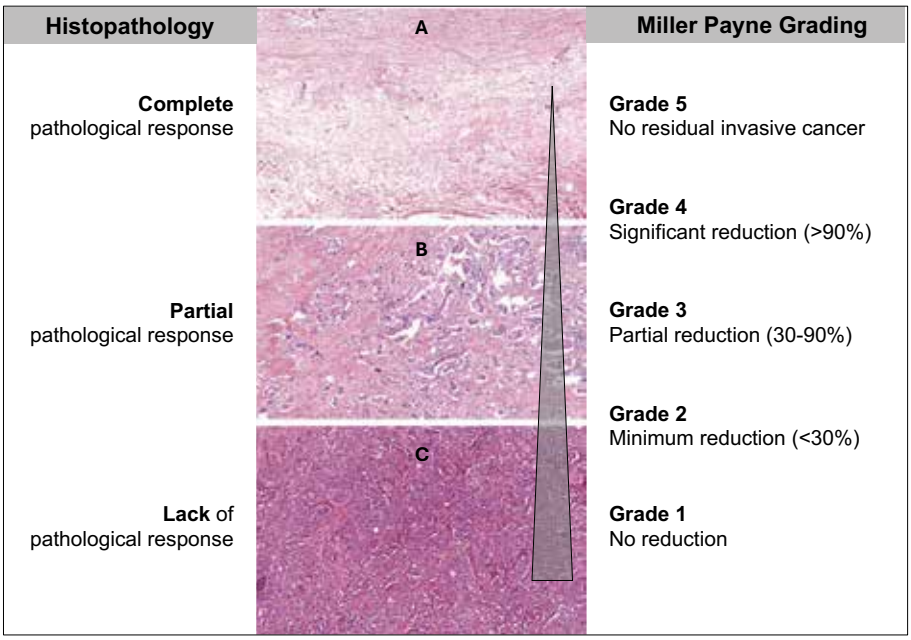


Figure 10. On the left: Shows the histopathological description of tumor responses corresponding to the hemotoxylin and eosin staining of tissues **A**) Complete, **B**) Partial, and **C**) Lack of response. On the right: Miller Payne 5-point grading of tumor response after neoadjuvantt chemotherapy [186] corresponding to the histopathology. This grading system was used in paper II as the residual cancer burden tool was not adopted as clinical routine during the study period. Pictures source: Sejben et al, Pathology and Oncology research, 2020 [193]. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).

5.4.2 FUTURE ADVANCEMENTS OF NEOADJUVANT CHEMOTHERAPY

The concept of NACT provides unique opportunities for research in personalized and precision medicine. Treating tumors in vivo allows real-time assessment of response and facilitates the testing of novel therapies. Traditionally, usage of NACT has been guided by fixed clinical and pathological tumor subtypes. Future progress lies in moving away from the static, one-size-fits-all treatment decisions and towards a more dynamic selection or exclusion protocols based on biologically informed approaches. For example, emerging biomarkers such as TILs and circulating tumor DNA have shown promise in predicting NACT tumor response [45, 194, 195].

Real-time clinical- and image-based monitoring of tumor response has also enabled the rise of adaptive trial designs. These methodologies allow treatment modifications according to pre-specified rules, tailoring therapy to patient response during NACT. Beyond individualization, adaptive designs offer broader benefits: adjusting cohort size to achieve statistical power, discontinuing ineffective treatments, and accelerating the identification of active agents [196-198].

Alongside biomarkers, imaging has become central to assessing and predicting NACT outcomes. Conventional tools such as mammography and ultrasound are widely available but show variable accuracy, particularly in dense or multifocal disease [199, 200]. Doppler ultrasound can help distinguish tumor tissue and predict pCR [201]. Magnetic resonance imaging is currently the most reliable imaging method and has been extensively used in adaptive NACT trials [168, 202, 203]. Yet, despite its sensitivity, MRI may overestimate disease extent, potentially leading to unnecessary mastectomy [204]. Additionally, contrast enhanced spectral mammography has shown promising results to assess tumor response and requires less resources [56].

These advances in biomarkers, adaptive trial designs, and imaging innovations are driving more individualized and effective breast cancer care in the NACT era.

6 SURGICAL CHALLENGES IN NACT

Neoadjuvant chemotherapy is now an established treatment option for a range of indications at diagnosis and is no longer reserved for large or inoperable tumors. Its use has steadily increased worldwide, particularly for HER2-positive and TNBC subtypes, though adoption varies by region. In Sweden, for example, NACT now accounts for only 15% of systemic therapies in operable early breast cancers [5] but yet it contribute substantially to surgical workload due to the added complexities of preoperative planning. Specifically, planning for surgery can have direct impact on patient well-being during prolonged treatment, radiological surveillance, tumor localization, and decisions regarding breast and axillary surgery.

6.1 PATIENT-RELATED OUTCOMES

The impact of NACT on patient well-being is unclear. Some studies suggest that living with cancer during chemotherapy may cause psychological distress [205], while others indicate that patients undergoing upfront surgery may experience higher stress levels [206]. Overall, patient-reported outcome measures have not demonstrated any consistent psychological advantage for either treatment sequence, whilst smaller studies suggested comparable quality of life regardless of treatment timing [207, 208]. Therefore, in the absence of robust prospective evidence, the psychological impact of NACT deserves further evaluation.

6.2 SURGICAL MORBIDITY - INFECTION

Patients undergoing NACT are often immunocompromised, raising concerns about postoperative surgical site infections (SSI). While some studies report no increased SSI risk [209], infections can delay adjuvant therapies such as radiotherapy, which may worsen outcomes [210-212]. In contrast, Adwall et al. analyzed over 80,000 patients and found that postoperative SSIs did not increase the risk of systemic or locoregional recurrence, though OS was reduced [213]. These findings highlight the need to minimize postoperative complications, particularly in immunocompromised NACT patients. Surgical care bundles, including perioperative antibiotics, risk-factor optimization, and normothermia, have successfully reduced SSI rates in other surgical fields

[214], but their role in breast cancer surgery post-NACT remains underexplored and warrants further study.

6.3 ASSESSMENT OF TUMOR RESPONSE

In-vivo assessment of tumor response is a key advantage of NACT, yet current imaging modalities cannot reliably predict pCR. This uncertainty complicates surgical planning for both the breast and axilla. Although MRI is more accurate than mammography or ultrasound for detecting residual disease, false positives from fibrosis and inflammation limit its predictive value [215]. However, studies have shown that CESM provides equal specificity and greater sensitivity compared to MRI, making it a viable alternative, especially in settings where MRI is contraindicated or unavailable. Additionally, CESM offers advantages in terms of cost-effectiveness and accessibility, which can be particularly beneficial in resource-limited environments [56].

Image-guided vacuum-assisted biopsy of the tumor site after NACT has been proposed to enhance accuracy [216]. Early data combining imaging and histopathology suggest that surgery may be safely omitted in so-called exceptional responders [217, 218]. However, until more mature randomized trial data are available, surgical decisions remain complex and require multidisciplinary, evidence-based evaluation.

6.4 TUMOR LOCALIZATION

Accurate tumor localization after NACT is crucial due to changes in tumor size and morphology. While many tumors shrink concentrically (52–64%), some exhibit diffuse patterns, complicating identification of the tumor bed [219, 220] (Table 2) (Figure 11). Traditional clip and wire methods are increasingly supplemented by one-step techniques, including paramagnetic seeds [221], radioactive iodine-125 seeds [222], radar reflectors [223], and radiofrequency identification devices [224]. These innovations may enhance patient experience and workflow, but their clinical effectiveness, cost-efficiency, and long-term outcomes remain under investigation. Tumor localization is particularly challenging in HER2-positive and TNBC, where high pCR rates often leave no detectable lesion, complicating surgical planning [225].

Prospective studies such as MELODY are expected to provide more robust evidence on these technologies [226].

Table 2. Comparing tumor subtypes with incidence of (**A**) compete pathological tumor response, and (**B**) patterns of tumor shrinkage

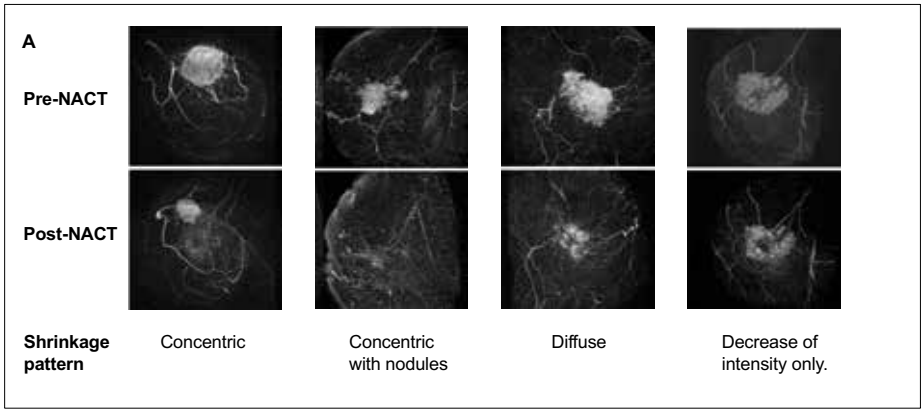
A

	Incidence	pCR
Luminal A	50-70%	< 10% (2-5%)
Luminal B	15-20%	10-20%
HER2-positive	15-20%	Up to 70%
TNBC	10-15%	50-60%

B

Shrinkage patterns	Concentric	Diffuse	Decrease intensity only	Stable disease
Luminal / HER2-negative	52%	19%	2%	27%
HER2-positive	54%	33%	3%	10%
TNBC	64%	18%	0%	18%

Abbreviations: HER2: human epidermal growth factor 2, TNBC: triple negative breast cancer: Source: A) Google scholar, april 2025, B) Wang et al Breast Cancer Research, 2024 [227]



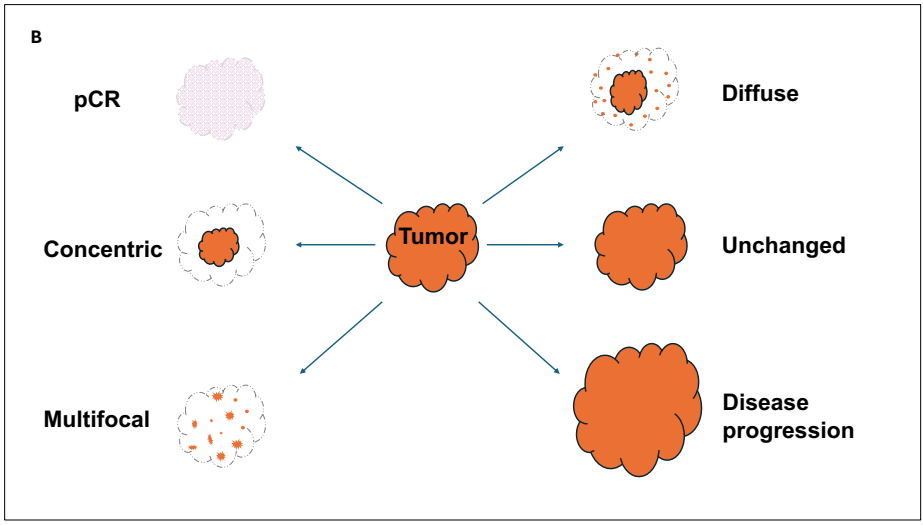


Figure 11. Showing different patterns of tumor shrinkage following neoadjuvant chemotherapy. **A**) Magnetic Resonance Images of tumor shrinkage in luminal cancers. Kind permission from Dr Ippei Fukada, Department of Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan [220]. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). **B**) Schematic illustration of possible patterns of tumor shrinkage after NACT. Illustrated by Kian Chin with reference to source; Huang et al, Frontiers in Bioengineering and Biotechnology, 2021 [219]

6.5 AXILLARY SURGERY

Downstaging of nodal disease after NACT presents opportunities to de-escalate axillary surgery. However, methods for reliably predicting axillary pCR are not yet well established. Furthermore, SLNB after NACT for initially cN+ patients carries a false-negative rate of over 50% [105], raising concerns about inaccurate staging and potential undertreatment. Furthermore, chemotherapy-induced fibrosis of lymphatic channels can cause hindrance to flow of injected lymphatic tracers, contributing to lower detection and increased false negative rates in axillary staging after NACT [228]. The use of lymphatic tracers such as SPIO and Magseed shows promise in improving axillary staging accuracy and facilitating TAD. However, in a large prospective AXSANA study, this technology was used in only 8% of cases [229], with the majority of axillary staging performed using the conventional two-step wire and coil method to mark the target or index metastatic node. Given the trend toward increasing axillary conservatism and higher nodal pCR rates after NACT, further research is needed to guide evidence-based management of the axilla.

In summary, NACT presents several unique surgical challenges: (1) risks of surgical and psychological morbidity that may delay subsequent therapy; (2) uncertainties in assessing treatment response; (3) difficulties in tumor localization, particularly in complete responders; and (4) dilemmas in axillary staging and de-escalation; These challenges underscore the need for robust evidence to optimize surgical strategies, forming the basis of research described in this thesis.

7 AIMS

Neoadjuvant chemotherapy induces changes in both the tumor micro- and macroenvironment, as well as in the patient's overall clinical condition, including the immune system, all of which challenge conventional approaches to breast cancer surgery. The overarching aim of this thesis is to explore different strategies to optimize surgical management for these patients. The specific objectives are as follows:

I. **Primary objective**: To evaluate the impact of implementing surgical care bundle on surgical site infection among patients, including those following NACT, who had non-reconstructive breast-conserving surgery and mastectomy with or without sentinel lymph node biopsy or axillary lymph node dissection. **Secondary objective**: To evaluate adherence to surgical care bundle and any adverse effects associated with it.

II. **Primary objective**: To investigate the relationship between TILs in the primary breast tumor and the pathological response in both the axilla and breast following NACT. **Secondary objective** was to evaluate TILs as a prognostic factor for breast cancer-free interval and overall survival.

III. **Primary objective**: To evaluate the feasibility of Magseed in tumor localization compared to conventional marker plus guidewire localization in patients undergoing NACT. **Secondary objective:** To explore the feasibility using SPIO and Magseed simultaneously in axillary staging.

IV. **Primary objective**: To evaluate the feasibility of pre-marking axillary lymph nodes before NACT using a combined paramagnetic approach with SPIO for sentinel lymph nodes and Magseed for index metastatic lymph nodes. **Secondary objective:** To evaluate whether if a lymph node tracer injected before NACT identify the same sentinel lymph nodes as those identified by a tracer injected after NACT.

8 PATIENTS AND METHODS

8.1 SUMMARY OF INCLUDED STUDIES

Table 3. Papers included in this thesis with respective designs

Paper	Aim	Design *(Study period)*	Population	N	Endpoints
I	To evaluate the impact of sugical care bundle (SCB) on surgical site infection (SSI).	Retrospective comparative cohort study *(Period: 2016-2020)*	All patients who underwent non-reconstructive breast cancer surgery	958	- SSI rate - Adherence of SCB - Adverse effects of SCB
II	To investigate the relationship between TILs and tumor response in the axilla and breast *after* NACT	Retrospective cohort study *(Period: 2013-2020)*	NACT patients	220	- pCR - Breast Cancer Free Interval - Overall survival
III	To evaluate the feasibility of pre-marking tumor in the breast with Magseed *before* NACT	Prospective feasibility randomized trial *(Period. 2024-2025)*	NACT patients	44	- Resection volume ratio - Tumor resection margin - Detection of sentinel lymph nodes
IV	To investigate the feasibility of pre-marking axillary lymph nodes with SPIO and Magseed *before* NACT	Prospective feasibility comparative cohort study *(Period: 2021-2023)*	NACT patients	80	- Detection of sentinel lymph nodes - Concordance of tracers in lymph nodes based on SPIO and Tc^{99m}

Abbreviations: NACT: Neoadjuvant chemotherapy, pCR: pathological complete response, SPIO: Superparamagnetic iron oxides nanoparticles, and Tc^{99m}: Technitium-99 metastable.

8.2 PAPER I

8.2.1 STUDY DESIGN

This is a single center retrospective comparative cohort study before and after the implementation of surgical care bundle (SCB) to reduce surgical site infections (SSI) after surgery. The subjects were unmatched. The SCB routine was implemented in October 2018 at the Department of Breast Surgery, Sahlgrenska University Hospital. It consisted of the eight peri- and intraoperative clinical action points: (1) preoperative wash using soap and water instead of chlorhexidine, (2) prophylactic antibiotics (intravenous 2 g cloxacillin or 600 mg clindamycin) to be given from 2 hour up to 30 min before skin incision, (3) wound irrigation with normal saline, (4) use of monofilament triclosan coated sutures, and (5) wound dressing with surgical tapes only, avoiding the need for regular changes of dressing, which can itself be resource-intensive and a potential risk factor for SSI. Other incorporated non-WHO

measures were (6) routine local anesthetic infiltration to minimize pain in order to facilitate ambulatory surgery whenever possible and (7) avoidance of routine use of low molecular weight heparin (LMWH) as thromboprophylaxis since postoperative hematoma was considered a risk factor for SSI. The routine use of (8) surgical drains was considered a low-evidence-based practice that hindered day-case surgery and was therefore not recommended.

8.2.2 SELECTION AND INCLUSION

Between 2016 and 2020, patients who underwent BCS and mastectomy (non-reconstructive) with an axillary procedure were identified from hospital registry based on International Classification of Diseases (ICD) surgery codes. Subsequently, patients were included in separate consecutive steps: 1) first step –all NACT patients, 2) second step - all non-NACT patients with breast surgery and ALND, and 3) third step - random selection of non-NACT patients with breast surgery and SLNB. The third step was performed as a randomized procedure because

8.2.3 DATA COLLECTION

All relevant clinical information was registered retrospectively, including age, body mass index, smoking status, comorbidity (diabetes), types of surgery, SCB measures, SSI, microbiological cultures, chemo-radiotherapy, tumor biology, reoperations, postoperative thromboembolic events, seroma aspirations, length of stay and time to start of adjuvant treatments,.

8.2.4 ENDPOINTS

The **primary endpoint** was postoperative 30-day SSI. Infection was diagnosed according to the Centre for Disease Control and Prevention criteria: erythema, localized swelling pain, purulent discharge with or without fever or positive bacterial culture, as well as if diagnosis was made by a qualified physician. The retrospective assessment on SSI was also based on photographic documentations in the patient record system. **Secondary endpoints** were adherence rate and adverse events of SCB defined at 30-day postoperatively.

8.2.5 STATISTICAL ANALYSIS

This was a retrospective unmatched comparative study. No prior sample size or post hoc power analysis were performed. Data was analyzed using SPSS (Statistical Package for the Social Sciences) version 28.0.1.0. The study cohort

was divided into two groups relating to time periods: before and after SCB implementation. The immediate first two months following SCB implementation were considered an introductory phase and therefore excluded from the analysis. Primary endpoint was dichotomized, with SSI categorized as either 'present' or 'absent'. The secondary endpoint of adherence to SCB categorized as either 'yes' or 'no'. Other variables were dichotomized where appropriate. Binomial distribution of data was assumed. Adequate adherence was deemed present if a patient received at least six of the eight measures described in the bundle protocol. Descriptive analyses were performed for all variables and are presented as absolute numbers with corresponding percentages. Comparisons of proportions were assessed using the chi-squared test. Risk factors for SSI—including SCB implementation, age, BMI, smoking, diabetes, type of surgery, NACT, and seroma aspirations—were assessed using univariable and multivariable binary logistic regression analyses. Statistical significance was defined as $p<0.05$.

8.3 PAPER II

8.3.1 STUDY DESIGN

A retrospective cohort study aimed to primarily investigate the relationship between TILs and tumor response, both in the axilla and breast among patients who underwent NACT between 2013 and 2020. Secondarily, to assess TILs as a prognostic factor for survival outcome.

8.3.2 SELECTION AND INCLUSION OF PATIENTS

All patients who underwent NACT were identified from the hospital database. Of these NACT patients, those diagnosed with cN+ disease but without distant metastases were included.

8.3.3 DATA COLLECTION AND DEFINITIONS

Data collections were based on a set of pre-defined outcome measures. Assessment of TILs was performed in preoperative core biopsies before NACT (pre-TILs) and surgical specimens after NACT (post-TILs) and defined as the percentage of stromal areas in the breast cancer occupied by mononuclear inflammatory cells [230]. A cutoff of 10% was used to classify TILs as either low (< 10%) or high (≥ 10%) [231]. Any increase or decrease in TILs after NACT was denoted as delta-TILs (ΔTILs). Definitions of tumor biology is available in attached published manuscript (Paper II).

8.3.4 ENDPOINTS

The **primary endpoint** was TILs in relation to histopathological tumor response observed within the metastatic axillary lymph nodes and breast cancer after NACT. Tumor response in the breast was graded using Miller-Payne five-point grading system (MPG) [186]. **Secondary endpoints** were BCFI and OS, both defined as per the Standardized Definitions for Efficacy End Points in Adjuvant Breast Cancer Clinical Trials [232].

8.3.5 STATISTICAL ANALYSIS

As this was a retrospective analysis, sample size calculation for formal hypothesis testing was not performed. Primary endpoint of tumor response was dichotomized as 'pCR' or 'no pCR'. Specifically, pCR rates were divided into three separate categories: axillary, breast, and combined axilla/breast pCR. Comparison of proportions of patients with pCR were analyzed using the chi-squared test. Univariable and multivariable binary logistic regression analyses were used to compare TILs of three different pCR categories to other clinic-histopathological parameters. Results from the regression analyses were reported as odd ratios (OR) and their 95% confidence intervals (CI). Secondary endpoints of BCFI and OS were defined as the time from the diagnosis to an event of locoregional, distant recurrences or death, respectively. To evaluate BCFI and OS in relation to pCR, Kaplan-Meier curves were plotted and compared by using the log-rank test. To adjust for multiple covariates (pre-TILs, age, tumor subtypes and NACT completion), Cox proportional regression analyses were performed and hazard ratio (HR) with 95% CI were reported. The performance characteristics for TILs as a predictor for pCR was assessed by calculating the sensitivity, specificity, positive- (PPV) and negative (NPV) predictive values. The level of statistical significance was defined as $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics version 29.0.0.0 (241), licensed in 2022.

8.4 PAPER III

8.4.1 STUDY DESIGN

This was an open label, multicenter randomized trial of using Magseed to localize breast cancers in patients prior to start of NACT compared to that of conventional two-stage Marker-WGL approach

8.4.2 SELECTION AND INCLUSION

Between March 2024 and July 2025, patients scheduled for NACT were offered to participate in the study. Eligible patients were adults (>18 years) with breast cancer planned for NACT and preliminarily considered for BCS with SLNB, TAD or ALND. After providing informed consent, patients were randomized in 1:1 ratio using block randomization (block of 8) generated in Microsoft Excel (version 16.93.1). All assignments were sealed in opaque envelopes individually and opened sequentially at inclusion. Randomization sequence-generation and envelope-preparation were performed by separate team members blinded to each other's tasks. The assigned tumor-localizing device was inserted into the breast before the start of NACT. In the presence of axillary metastases irrespective of assigned localizing method for breast tumor, a Magseed was used to mark the most prominent metastatic axillary lymph node prior to NACT. Subsequently, 1 mL of SPIO was injected peritumorally after NACT to facilitate SLNB and TAD.

8.4.3 DATA COLLECTIONS

Data were registered prospectively. Specifically, outcome parameters like patient and tumor characteristics, operative details, tumor localization, and weight of resected tumor specimens were collected.

8.4.4 ENDPOINTS

The **primary endpoint** was the resection volume ratio (RV-ratio), which reflects the accuracy of the surgical resection in relation to the optimal target volume. The RV-ratio was defined as the actual resected volume (Actual-RV) divided by the optimal resection volume (Optimal-RV). The Actual-RV was derived from the intraoperative specimen weight (in grams), based on the established correlation between breast tissue weight and volume, assuming a tissue density of 0.958 g/cm³ [233]. The Optimal-RV was calculated from the post-NACT residual tumor dimensions using the formula for the volume of a sphere, $4/3\pi r^3$, where r represents the largest tumor radius measured on imaging, with an additional centimeter added to account for circumferential resection margin clearance (Figure 12). **Secondary endpoints** were: 1) negative resection margin rate, 2) detection of SLN based on SPIO and Magseed-marked index metastatic lymph nodes, 3) time interval from marker/Magseed localization to surgery, 4) operation time, and 5) time interval for completion of NACT.

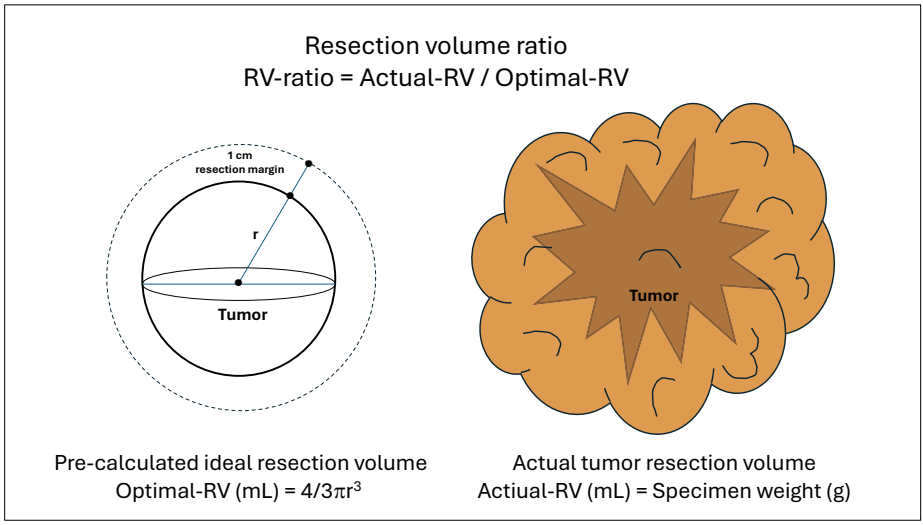


Figure 12. Illustration of how the resection volume ratio (RV-ratio) is derived by comparing the actual volume of resection of tumor (right) to the pre-calculated Optimal resection volume with an added one-centimeter surgical margin for resection (left)

8.4.5 STATISTICAL ANALYSIS

This randomized pilot study was not powered for formal hypothesis testing. Instead, the study was designed to estimate the between-group mean difference in the primary endpoint (RV-ratio) with pre-specified precision (95% confidence), in order to inform the design of a future definitive trial. Accordingly, the sample size (n) was determined by precision-based calculations as below, where 95% CI for $Z_{1-\alpha/2}$ is 1.96, σ is standard deviation, and ME is margin of error (half-width of CI).

$$n = 2\left[\frac{Z_{1-\alpha/2}\,\sigma}{ME}\right]^2$$

Based on an internal audit of 16 patients (by correspondence), the mean specimen volume was 185 mL with a standard deviation of 16 mL. Targeting a 95% CI with a ±15 mL margin of error for the between-group mean difference, the required sample size was calculated as 18 participants (9 per group). Allowing for a 10–15% drop-out rate, this was conservatively

increased to 24 participants (12 per group). Additionally, in line with recommendations that feasibility studies should include 24 to 50 participants [234], we further expanded the sample size to a total of 44 participants (22 per group) to enable exploration of the designated secondary endpoints.

The primary analysis was conducted according to the intention-to-treat principle, with patients analyzed in the groups to which they were randomized. All data was reported with descriptive statistics. A secondary per-protocol analysis, taking into consideration of crossovers was also performed to assess robustness of the findings. However, due to skewed distribution of data after data collection, non-parametric median with interquartile range was used. In line with the concept of a feasibility study, comparative statistical analyses were performed for exploratory reasons only and not for hypothesis testing. For continuous variables, medians were compared with Mann-Whitney test. Categorical variables were summarized as percentage or proportions and compared with Chi-square or Fisher's exact test. Level of statistical significance was defined as $p<0.05$. All analyses were performed using SPSS Version 29.0.2.0 (20) (IBM)

8.5 PAPER IV

8.5.1 STUDY DESIGN

This was a non-randomized single-arm prospective study. The aim was to investigate the feasibility of pre-marking relevant axillary lymph nodes with SPIO (1 mL injected peritumorally for SLN detection) and Magseed (inserted in index metastatic node) before NACT. Outcomes were compared with those axillary lymph nodes obtained using Tc^{99m} as the reference tracer, injected after NACT in the same patients. Thus, each patient served as their own control, allowing comparison of lymph node markings with two different tracers administered at two different time points. Blue dye was injected perioperatively if signals from SPIO and Tc^{99m} were deemed clinically suboptimal before the start of surgery.

8.5.2 SELECTION AND INCLUSION

All patients over 18 years old, who were planned for NACT and eligible for breast cancer surgery subsequently were offered to participate. Patients were not eligible if the Multidisciplinary Team recommended MRI for monitoring

tumor response during NACT. Mammography and ultrasound are the routine imaging modalities used to assess tumor response during NACT.

8.5.3 DATA COLLECTIONS

All data were prospectively collected. Paramagnetic and radioisotope signals were measured and registered for each removed lymph node. Only at the first postoperative hospital visit, skin staining due to SPIO was documented

8.5.4 ENDPOINTS

The **primary endpoint** was detection of SLNs and index metastatic node (Index-met) per patient. Detection was defined as the proportion (expressed as a percentage) of patients in whom at least one lymph node was successfully localized by the respective tracer (or Magseed), relative to the total number of patients who underwent SLNB (or TAD) based on the same tracer. **Secondary endpoints** were concordance and reversed concordance between tracers per node. ***Concordance*** was defined as the proportion of nodes identified by the reference tracer, Tc^{99m} that were also identified by the new tracer, SPIO. This is calculated as the number of nodes containing both SPIO and Tc^{99m} divided by all nodes marked by Tc^{99m}, irrespective of presence of SPIO, expressed as a percentage:

$$\text{Concordance} = \frac{\text{SPIO} + Tc^{99m}}{Tc^{99m}}$$

Whereas ***Reversed concordance*** was defined by the proportion of nodes identified by the new tracer, SPIO that were also identified by the reference tracer, Tc^{99m}. This is calculated as the number of nodes containing both SPIO and Tc^{99m} divided by all nodes marked by SPIO, irrespective of presence of Tc^{99m}), expressed as percentage:

$$\text{Reversed Concordance} = \frac{\text{SPIO} + Tc^{99m}}{\text{SPIO}}$$

Therefore, concordance reflects how accurately SPIO mimic Tc^{99m} (the reference tracer) in identifying SLNs. High concordance indicates SPIO detects the same nodes as Tc^{99m}. In contrast, reversed concordance reflects how

precisely Tc^{99m} mimic SPIO (the reference tracer) in identifying SLNs, with high reversed concordance indicating Tc^{99m} detected the same nodes as SPIO, and that SPIO does not identify other additional nodes (Figure 13).

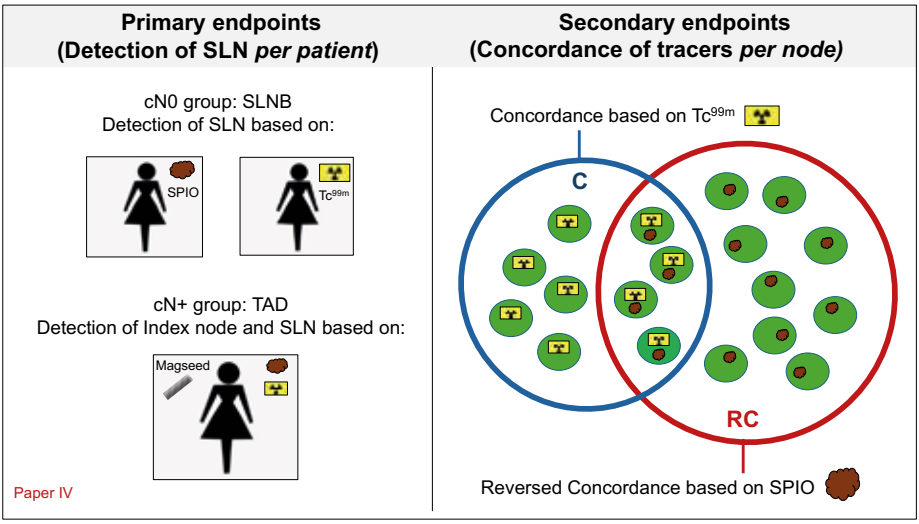


Figure 13. Illustrate the primary and secondary endpoints in paper IV. **Detection** of SLN per patient was defined as the ratio of proportion of patients with at least one lymph node successfully detected by a particular tracer (or Magseed) to the total number of patients that underwent SLNB (or TAD). **Concordance**, C, defined as: nodes containing Tc^{99m} and SPIO divided by all nodes detected by Tc^{99m} (blue circle). **Reversed concordance**, RC, defined as: nodes containing Tc^{99m} and SPIO divided by all nodes detected by SPIO (red circle).

8.5.5 STATISTICAL ANALYSIS

Outcome measures were divided into two groups, that with cN0 and cN+. Based on the principles of a feasibility study, the generally recommended sample size ranges from 24 to 50 study subjects [234]. As study patients acted as their own controls, preliminary calculations indicated that a sample of 40 patients per group would be sufficient to inform the design of a future larger trial. However, this sample size estimation was not strictly based upon the pre-defined primary endpoint. Descriptive statistics were applied. Differences in proportions for primary and secondary endpoints were assessed using chi-square and McNemar's test. For normally distributed data, independent t-test was used to compare means and 95 % CI. For skewed data, non-parametric tests were used to compare medians with interquartile range (IQR). Level of statistical significance was defined as $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics version 29.0.0.0 (241), licensed in 2022.

9 METHODOLOGICAL CONSIDERATIONS

Every research project is influenced and shaped by various methodological considerations. Options related to study design, participant selection, data collection, and analytical approach each is associated with inherent advantages and drawbacks. The validity of the results depends on the formulation of the scientific question and the null hypothesis. Therefore, recognizing these factors is essential for understanding the robustness of the results, the potential sources of bias, and the extent to which the results can be applied beyond the study setting.

9.1 PAPER I

In this study, several methodological considerations should be highlighted. First, the nature of a retrospective single-center design introduces inherent risks of selection bias. Patient inclusion was based on surgical codes, which are subject to coding errors and may result in under- or over-representation of SSI within the cohort. There is also reliance on the electronic patient record documentation by multiple healthcare personal, being optimal. Despite photographic records, mis-reporting cases of SSI and other risk factors like smoking and comorbidities may occur. Inclusion process that occurred in three separate consecutive stages could have also led to bias. Random sampling of non-NACT SLNB patients instead of including all patients, may introduce sampling bias, as those not sampled may differ. However, one possible mitigation is to compare the baseline characteristics between the sampled vs. non-sampled group though this analysis was not performed.

Second, the study compared outcomes before and after SCB implementation but patients were not matched. This may introduce confounding bias by temporal or organizational changes unrelated to SCB. Although multivariable logistic regression was performed to adjust for known risk factors (e.g., age, BMI, diabetes, smoking, type of surgery, and NACT), residual confounding bias cannot be excluded.

Third, the assumption of binomial distribution and use of parametric tests (such as chi-square and t-tests) may not fully account for presence of skewness or small subgroup sizes.

Fourth, as this was a retrospective study, there was no formal sample size calculation performed, which may limit the ability to detect small differences and increases the risk of type II error. Although post hoc power analysis could have been undertaken, it is generally considered unreliable and results are difficult to interpret, therefore not recommended.

Despite these limitations, the study also has strengths. It was based on a relatively large surgical cohort with standardized SSI criteria according to Centre for Disease Control and Prevention guidelines, strengthened by photographic documentation in the medical record. The study captured a complete consecutive series of breast cancer surgeries over a 5-year period with real-world data, thereby reducing the risk of selection bias.

9.2 PAPER II

As a retrospective cohort study, there is an inherent risk of bias. The study period (2013–2020) may be associated with information bias due to evolving practices in the documentation of clinical data. Furthermore, indications for NACT became increasingly guided by molecular subtyping in the later years, rather than the more heterogeneous patient selection seen earlier, which could lead to selection bias. As this is single center study, the cohort is small which constrains the robustness of the results.

There could also be potential confounding factors in a retrospective study. During the study period, NACT regimens also changed, which could influence tumor response rates, particularly pCR. Additionally, completion rate of NACT may also impact on pCR.

Although the International TILs Working Group has established standardized assessment methods, several potential confounding factors remain. Since this study focused on pre-treatment TILs in core biopsy specimens, sampling variability may limit the accuracy and representativeness of TIL evaluation. In addition, the use of categorical TIL classifications may oversimplify the assessment, whereas continuous TIL values could better reflect the true extent of immune cell infiltration within the tumor. These factors should be considered as important methodological considerations in the study design and interpretation of findings.

However, the strengths of this study included its comprehensive assessment of TILs, with evaluations conducted both pre- and post-NACT, as well as analysis

of the amount of TILs changes after NACT. This approach provided a more detailed understanding of TILs dynamics throughout NACT.

9.3 PAPER III

This study was designed as a randomized feasibility trial, and several methodological considerations should be noted when interpreting the results. First, clearly defined inclusion and exclusion criteria were applied to ensure that patients in both study arms had comparable characteristics. The randomization process further helped to maintain balanced treatment groups. Given the estimated small sample size, block randomization was used to keep the number of participants in each group balanced at regular intervals throughout the study. Despite the advantages of block randomization, it has certain limitations. In particular, if participants drop out after randomization—such as disease progression during NACT making them no longer eligible to the study—it may be difficult to replace them in a way that preserves the original balance within blocks. This can lead to temporary or permanent imbalances between study arms, potentially affecting the comparability of groups and the interpretation of results.

A statistically estimated sample size is important to ensure the final results can be interpreted with some degree of accuracy so that it could be extrapolated into clinical practice. However, as this was a feasibility study, the estimated sample size, albeit not formally powered, was deemed sufficient to provide preliminary data to guide the design of a larger, definitive trial.

As the study endpoints included histopathological measures such as resection volume, tumor response, and margin status, blinding of the pathologist may be necessary to reduce assessment bias. However, because tumor sites were marked with either a wire or Magseed device, blinding was not feasible. To mitigate this limitation, standardized protocols for specimen handling in the laboratory and reporting were followed to ensure consistent and objective assessment across both study groups.

The introduction of a new surgical device, such as the Magseed system, can be influenced by the operator's learning curve, which may affect operative efficiency, accuracy of intraoperative tumor localization, and therefore the endpoints in this randomized study. It is therefore essential to ensure surgeons in both study arms possess adequate experience and competence with the respective localization techniques. Standardized training and pre-trial

familiarization with the devices could minimize variability related to operator skill, thereby minimizing the bias in the results. In addition, like any introduction of a new surgical methods, any adverse events would need to be monitored and reported.

Finally, careful follow-up of the entire treatment process in both study groups is crucial. It not only helps make sure patients are safe throughout but also ensures that the study protocol is adhered to consistently. Monitoring of each step, from tumor marking to surgery and histopathological assessments, helps minimize any deviations early and allows for a balanced comparison between the two approaches.

9.4 PAPER IV

This was a non-randomized prospective study to evaluate the feasibility of using a new lymphatic tracer (SPIO) and marker (Magseed) compared to a standard tracer (Tc^{99m}) to perform axillary staging after NACT. Both new and standard devices were injected into the same patient – SPIO / Magseed was injected / inserted pre-NACT and Tc^{99m} injected post-NACT. Therefore, the patient serves as own control in the comparison analysis. In such a study, several methodological considerations should be considered.

First, as SPIO contain paramagnetic properties, it could lead to image artefacts in subsequent MRI investigation. Therefore, the inclusion criteria need to be clear to ensure patients who required any MRI follow up for tumor response were not included into the study. Second, as any study introducing a novel surgical technique, there should be assurance of adequate technical competence to minimize bias related to operator variability. Prior to study launch, participating surgeons had undergone pre-trial training with mentorship support to standardize technique in using the new devices.

Third, there are no strict rules in guiding the decision on sample size for a feasibility study. This is because the primary aim is to assess safety and practical aspects of an intervention and not to focus on efficacy. Typically, the sample used are small and determined pragmatically based on either clinical experience of the investigators or data from published literature. The outcome of a feasibility study should be adequately reliable to inform the design of larger trial with formal statistical sample size calculations. Therefore, it was important to choose study endpoints that reflect the safety and practical success in using the new SPIO and Magseed. The results should answer questions like

- can the new device be placed easily, safely and reliably, how both clinicians and patients experienced the procedure, and whether the pre-marked lymph nodes could be consistently detected and retrieved. Together with per-protocol follow-up of patients, the study endpoints were designed to answer the questions on feasibility of the novel device.

Last but not least, while formal blinding of the histopathologists was not implemented, they were inherently unaware of the intraoperative findings related to the use of SPIO or Magseed devices when evaluating the surgical specimens. On this basis, additional measures to enforce blinding were not deemed essential for the validity of the study.

10 RESULTS

10.1 SUMMARY OF RESULTS

Table 4. Results of respective papers I to IV

Paper	Study	Findings	Implications
I	Retrospective evaluation of the the impact of surgical care bundle(SCB) on surgical site infection (SSI)	Overall, 10.4% of patients developed SSI. Implementation of SCB led to SSI rate reduced from 11.8% to 8.9% (p=0.15). SCB is an independent factor in reducing SSI (OR 0.63, 95% CI 0.4-0.99, p=0.04	Introduction of SCB into breast surgery led to a non-statistically but clinically significant reduction of SSI rate. Further adjustment of the bundle elements can potentially lead to larger benefit in patient safety after surgery
II	Retrospective study on the relationship between TILs and tumor response in the axilla and breast *after* NACT	High pre-TILs in the tumor stroma was in independent predictor of axillary and breast pCR. However, TILs did not predict BCFI and OS	Pre-TILs could be considered in clinical practice to facilitate de-escalation of axillary surgery after NACT
III	Randomized trial to evaluate the feasibility of pre-marking tumor in the breast with Magseed *before* NACT	Magseed tumor localization in NACT was feasible, performed comparably to the two-stage Marker–WGL approach, and raised no safety concerns.	Magseed tumor localization can be considered in surgery after NACT. But larger trial would be needed to confirm its validity.
IV	Non-randomized prospective study to investigate the feasibility of pre-marking axillary lymph nodes with SPIO and Magseed *before* NACT	SPIO showed higher SLN detection than Tc^{99m}. While both tracers had high concordance, SPIO also detected additonal nodes	Early pre-NACT SPIO seemed to lead to detection of different group of lymh nodes marked by conventional Tc^{99m} injected after NACT

Abbreviations: NACT: Neoadjuvant chemotherapy, pre-TILs: pre-treatment TILs found in tumor stroma in core biopsy specimens, SPIO: Superparamagnetic iron oxides nanoparticles, Tc^{99m}: Technitium-99 metastable, and Marker-WGL: standard tumor non-magnetic marker followed by wire guided localization.

10.2 PAPER I

The study analyzed data from 958 patients who underwent surgery between 2016 and 2020. Overall, the SSI rate decreased from 11.8% to 8.9% post-SCB implementation, though this reduction was not statistically significant (p=0.15). Notably, patients undergoing breast conservation with SLNB experienced a significant decrease in SSI rate from 18.8% to 9.8% (p=0.01). Multivariable analysis revealed that the SCB was associated with a 37% reduction in odds for SSI (OR 0.63, 95% CI 0.40–0.99, p=0.04). Subgroup analysis showed SSI rates were reduced in NACT patients (from 10.9% to 6.5%, p=0.28) and non-NACT patients (from 11.9% to 9.8%, p=0.37) after SCB implementation though these reductions were not statistically significant. In this study, the adoption of a structured SCB decreased the risk of SSIs in breast cancer surgery, particularly in specific surgical subgroups.

10.3 PAPER II

Analysis of 220 patients showed that high pre-TILs were associated with a higher likelihood of achieving axillary pCR (OR 2.03, 95% CI 1.02–4.05; p=0.04), whereas elevated post-TIL levels and increased in TILs (ΔTILs) were associated with lower likelihood of axillary pCR (i.e. only partial response). The performance of pre-TILs, post-TILs, and ΔTILs in predicting axillary pCR was as follows: **sensitivity**, 42%, 13%, and 34%; specificity, 78%, 73%, and 87%; **positive predictive value**, 43%, 17%, and 69%; and **negative predictive value**, 68%, 55%, and 69%, respectively.

For **secondary endpoints**, pre-TILs was an independent prognostic factor for BCFI (HR 0.46, 95% CI 0.22–0.96; p = 0.04) but not for OS (HR 0.73, 95% CI 0.35–1.55; p = 0.42). However, when pre-TILs was combined with axillary pCR as a single factor, it independently predicted OS (HR 0.09, 95% CI 0.12–0.72; p = 0.02).

10.4 PAPER III

Based on the **interim analysis**, this randomized trial demonstrated that it was technically feasible and safe to use Magseed for tumor localization in patients undergoing NACT. Specifically, the results showed Magseed performed comparably to the conventional two-stage Marker-WGL approach. The **primary endpoint** RV-ratio were similar between groups, with a slight, non-statistically significant trend favoring Magseed for more precise tissue excision: Marker-WGL 1.4 (IQR 0.8-1.6) *vs*. Magseed 1.2 (IQR 0.7-2.0), p=0.94.

For **secondary endpoints**, both groups achieved 100% success in achieving negative resection margins, as well as in detecting the SLN and index node. Timing from localization to surgery for Marker-WGL was 157 days (IQR 146.0-165-0) compared to Magseed 153.0 days (IQR 140.0-163.0), p=0.25. The median operating time was shorter with Magseed 78.5 minutes (IQR 58.3-112.5 vs. 92.5 minutes (IQR 62.8-121.3) with Marker-WGL, p=49. Time taken for completion of chemotherapy was 146.0 days (IQR 126.0-148.0) for Marker-WGL group versus 146.0 days (IQR 126.0-147.5) for Magseed group (p=0.95). No serious adverse events occurred.

10.5 PAPER IV

In this feasibility study of 80 patients undergoing NACT, the **primary endpoint** of SLN detection with pre-NACT SPIO was achieved in 86% of cases compared with 79% for post-NACT Tc^{99m} ($p = 0.01$), with the greatest advantage observed in cN+ patients (81% vs 62%, $p < 0.001$). Identification of Magseed-marked metastatic index node was successful in 97% of patients, significantly higher than Tc^{99m} detection at 56% ($p < 0.001$). For the **secondary endpoint**, overall concordance and reversed concordance were 79% versus 49% ($p < 0.001$), respectively. The median number of nodes retrieved with SPIO and Tc^{99m} was 2 (IQR 1–3) and 1 (IQR 1–2), respectively ($p < 0.001$). The false-negative rate of TAD using SPIO and Magseed was 0%. These results indicate that SPIO provides higher SLN detection than Tc^{99m}. Notably, pre-NACT SPIO injection allowed identification of different groups of axillary lymph nodes compared with post-NACT Tc^{99m}. The procedure was also associated with an excellent safety profile.

11 DISCUSSION

Neoadjuvant chemotherapy has significant impacts on both tumor biology and host physiology. These complexities challenge the established historical norm of surgical thinkings. The findings in this thesis further elucidate current understanding in **three** key areas: the prevention of postoperative infections, the role of TILs in predicting tumor response, and the development of novel surgical strategies incorporating paramagnetic technology for the treatment of breast cancer following NACT

11.1 PAPER I

Immunosuppression due to neutropenia is commonly reported during NACT. In some studies, about 30 to 60% rate of neutropenia was associated with those more aggressive treatment protocols [235, 236]. However, the extent to which patients remain neutropenic immediately prior to surgery is not well documented. Based on exclusion criteria in trials, one review study recommended a delay of up to three to four weeks after systemic therapy before surgery is performed [237]. It is therefore reasonable to assume that patients may undergo surgery with varying degrees of immunosuppression, potentially increasing susceptibility to postoperative infection.

In this paper, SCB implementation was associated with a clinically meaningful reduction in overall SSI. Similar trends were observed in both NACT and non-NACT subgroups, although they did not reach statistical significance—likely reflecting limited power to detect subgroup differences. Notably, multivariable regression analysis demonstrated a 37% reduction in the odds of SSI, underscoring the potential value of SCB particularly in patients receiving NACT.

Importantly, it should be recognized that the effectiveness of the SCB derives from the combined, synergistic application of its components as a bundled intervention, rather than from any individual infection-prevention measure alone.

Advancing surgical strategies: The implementation of a SCB demonstrates a practical approach to minimize postoperative infections, particularly in immunocompromised patients undergoing NACT.

11.2 PAPER II

Tumor-infiltrating lymphocytes reflect the host immune response against breast cancer and have been widely studied as biomarkers of primary tumor response to NACT. In contrast, their role in predicting response within metastatic axillary lymph nodes remains less well defined. Some studies have reported that higher levels of TILs—as well as $CD4^+$ and $CD8^+$ T cells and $CD56^+$ NK cells—in metastatic nodes are associated with axillary pCR to NACT [238]. Meanwhile, the development of secondary germinal centers following NACT in low-TIL TNBC has been linked to improved prognosis [239].

Unlike most studies that focus on post-NACT TILs, our findings showed that higher pre-treatment stromal TILs in the primary tumor were associated with an increased likelihood of axillary pCR. Additionally, a reduction in TILs from baseline to post-NACT correlated with axillary pCR, suggesting that the dynamic change in TILs during therapy carries clinical relevance. This highlights the potential value of evaluating TILs longitudinally throughout NACT, rather than relying solely on their assessment in the final surgical specimen.

Although this study did not demonstrate a statistically significant association, high pre-treatment TILs have been linked to improved BCFI and OS in previous reports. The absence of significance here was likely due to limited sample size and few recurrence or mortality events. Overall, these findings support the potential clinical utility of TIL assessment. Patients with high TILs may be more likely to achieve a favorable response to NACT and could be considered for earlier surgical intervention, whereas those with low TILs may benefit from additional therapeutic strategies to enhance chemosensitivity before surgery [240].

Advancing surgical strategies: Findings from paper II suggest that pre-treatment TIL assessment could inform individualized axillary management. Patients with high pre-TILs, who are more likely to achieve axillary pCR, may be suitable for less extensive axillary surgery or earlier operative intervention. In contrast, patients with low TILs may require intensified or alternative systemic therapy to improve nodal response before surgery. Incorporating dynamic TIL evaluation during NACT may further refine the timing and extent of axillary surgery, supporting a more personalized approach.

11.3 PAPER III

In paper III, we evaluated the feasibility of using Magseed to localize tumor in advance of NACT, to facilitate subsequent surgery afterwards. The **interim results** showed that the paramagnetic technique is feasible for tumor localization and lymph nodes detection. Specifically, there was no statistically significant difference in RV-ratios though there was a noticeable clinical indication that favors Magseed to have better precision in resecting the optimal volume of tumor compared to Marker-WGL. There was high rate of clear margin resections in both conventional Marker-WGL and Magseed methods. Sentinel lymph nodes pre-marked by SPIO and index metastatic lymph nodes by Magseed were all detected and removed. The safety profile for using paramagnetic devices was excellent.

However, it is important to acknowledge a potential confounding bias, as NACT is often selected for TNBC and HER2-positive subtypes; their higher pCR rates may contribute to increased negative-margin rates compared to other subtypes that tend to have poorer tumor responses. Performance of Magseed in localizing tumors in these poor responders will need to be further evaluated.

Neoadjuvant chemotherapy facilitates BCS through tumor downstaging but can also complicate resection. Sparse or dispersed residual disease increases the challenge of achieving clear margins, as imaging may underestimate tumor extent and satellite lesions can lie beyond the visible main mass. Although few studies have examined margin outcomes in relation to shrinkage patterns, imaging–pathology correlation studies indicate that non-concentric patterns such as mixed, nodular, or diffuse shrinkage—are more likely to harbor scattered residual disease, resulting in lower rates of clear margins [241]. Therefore, performance of Magseed in localizing tumors with various types of shrinkage patterns will need to be further evaluated. Conversely, some reports suggest that post-NACT margin widths of ≤1 mm, >1 mm, or unknown due to pCR do not affect local recurrence-free survival, BCFI, or OS over nearly 90 months of follow-up [242].

Although not formally evaluated, a one-stage approach with Magseed would conceivably be more preferable to a two-stage Marker-WGL, both for clinicians and patients. The results of this study suggest that the use of Magseed for tumor localization in the neoadjuvant setting is feasible and may offer a superior utility profile.

Advancing surgical strategies: Paper III demonstrated that Magseed localization is not only feasible but also streamlines workflow by allowing timely seed insertion independent of radiology scheduling. Magseed reduces patient discomfort as a one-step procedure whilst maintaining accuracy in localization until surgery after NACT. There is potential for overall efficiency gains in workflow, which supports its adoption as a flexible, patient-centered alternative to wire localization. Despite a higher upfront device cost, findings from this paper warrant further evaluation to confirm Magseed's clinical and cost-benefit efficacy.

11.4 PAPER IV

Neoadjuvant chemotherapy can induce nodal tumor response as well as fibrosis of lymphatic channels, reducing the accuracy of conventional techniques used for axillary staging. Specifically, SLN detection is often lower when conventional lymphatic tracers are injected only post-NACT. In Paper IV, we assessed the feasibility of pre-marking axillary lymph nodes by injecting SPIO before NACT and compared outcomes with Tc99m. The results demonstrated that SLNs could be reliably identified even when SPIO was injected up to 5 months before surgery, with a higher detection rate than Tc99m. Interestingly, the reversed concordance of lymph node tracers was only 50%, suggesting that SPIO detected a different group of lymph nodes than Tc99m. The study also showed the efficacy of Magseed in marking index metastatic lymph nodes for subsequent re-identification and surgery. These findings suggest that the timing of SPIO injection relative to NACT is critical for accurate SLN identification after NACT.

In Sweden, current guidelines recommend dual-tracer axillary staging after NACT, typically combining Tc99m with blue dye. Considering the logistical challenges, radioactive risks of Tc99m, and potential allergic reactions to blue dye, pre-NACT SPIO represents a promising alternative, particularly for mitigating the effects of lymphatic fibrosis.

An ongoing larger follow-up multicenter study based at Sahlgrenska University Hospital, Gothenburg, Sweden, will further evaluate the efficacy of pre-marking lymph nodes with paramagnetic devices and the potential negative impact of lymphatic fibrosis on axillary staging with tracers injected only after NACT (SentiNeo 2.0; n=453; Ethics approval: Dnr 2025-03967-01).

Advancing surgical strategies: Paper IV demonstrated pre-NACT SPIO injection enables reliable, radiation-free sentinel and targeted axillary node detection with full scheduling flexibility. It simplifies logistics, reduces costs associated with radioisotopes, and improves patient convenience. Injecting SPIO in advance of NACT offers a safe, efficient, and re-defined approach to modern axillary staging. These results deserve further evaluation in the concept of using dual tracer technique for axillary staging after NACT, namely injecting tracer both before and after NACT.

12 ETHICAL CONSIDERATIONS

All studies were conducted in accordance with the Declaration of Helsinki.

Papers I and II were retrospective cohort studies utilizing anonymized data analyses to evaluate surgical outcomes following NACT. These studies involved neither direct patient contact nor the introduction of new treatment interventions. Patients consent was waived due to the retrospective nature of the study. Temporary access to patient identifiers was required solely for the purpose of accurately extracting relevant clinical data from hospital records. All procedures related to data handling adhered strictly to Good Clinical Practice guidelines to ensure patient confidentiality and data integrity. Once data collection was completed, all identifiers were removed, and analyses were performed using anonymized datasets. Approvals for both studies were obtained rom the Regional Ethical Board in Gothenburg prior to commencement (Dnr: 479-18 2018-Jun-27).

Papers III and IV were prospective studies that involved direct patient interaction and the introduction of new interventions in addition to standard treatments. These additional procedures were deemed ethically acceptable within the research context, as they posed no more than minimal discomfort compared to the standard care patients would have received outside the study. Therefore, both studies were assessed to carry no significant additional clinical risks to patient safety. Nevertheless, discussing research participation with patients presented challenges, as many were emotionally affected by their cancer diagnoses and often felt overwhelmed when making decisions about study involvement. Therefore, careful consideration was given to eligibility criteria and patient wellbeing throughout the enrollment process. All participants were provided adequate time to reflect on their participation and were encouraged to ask questions before giving informed consent. To ensure scientific integrity, the study protocols detailed methodological approaches, data management procedures, and transparency in reporting. Participants were also offered the option to receive information about the study results upon completion. In particular, all participants in paper IV were formally informed of the study results via post upon the publication of the scientific manuscript. Prior approvals were obtained from the Regional Ethical Board in Gothenburg and the Swedish Medical Product Agency (Paper I: Dnr: 2023-03412-01) (Paper II: Dnr: 2021-04285).

13 CONCLUSION

The findings of research in this thesis have led to deeper understanding of the challenges that surgery encounter when performed after NACT. The results provided guidance for further advancement in various aspects of surgery. In **Paper I**, implementation of a structured SCB significantly reduced postoperative infections, underscoring the importance of standardized perioperative care in all patients undergoing breast surgery and not just those after NACT. In **Paper II**, the association between high pre-TILs and axillary pCR supported the potential use of immune biomarkers to guide surgical de-escalation. **Paper III** demonstrated the use of Magseed for tumor localization was both feasible and safe, offering workflow and potentially precision advantages over the conventional wire-guided approach. Lastly in **Paper IV**, pre-NACT SPIO injection for lymphatic tracing achieved better SLN detection compared to post-NACT Tc^{99m}, addressing the challenges of lymphatic fibrosis and possibly improving staging accuracy by finding the true relevant SLNs.

In conclusion, this thesis highlighted the need for adaptive surgical strategies. The results emphasized the importance of a personalized, multidisciplinary approach to breast cancer surgery aimed at de-escalation whilst ensuring oncological safety in an era of continuously evolving systemic therapies.

14 FUTURE PERSPECTIVES

Through the work presented in this thesis, I have come to appreciate how multidisciplinary collaboration, translational research, and thoughtful surgical innovation can together transform breast cancer care.

Studying the impact of the surgical care bundle taught me the importance of pragmatic, evidence-based interventions and how even simple procedural refinements can meaningfully improve patient outcomes. I have also learned that progress often lies not in singular breakthroughs, but in the cumulative refinement of each component of care.

My research in tumor-infiltrating lymphocytes deepened my understanding of the immune system's role in treatment response, sparking my interest in integrating immunological and molecular biomarkers into surgical decision-making. I look forward to exploring how digital pathology and dynamic immune profiling could personalize surgical strategies following NACT.

Likewise, evaluating paramagnetic localization techniques revealed how technology can be adopted to enhance precision, workflow, and patient experience while reducing reliance on radioactivity—a shift that aligns with a vision for more accessible and patient-centered breast surgery.

The feasibility of integrating SPIO and Magseed localization points toward a streamlined, fully paramagnetic approach that could redefine how we plan and perform surgery after systemic therapy. To this aspect, my immediate future plans include consolidating the results from this thesis via larger studies with statistical power.

Looking ahead, I aim to build on these new insights by focusing my research on response-adapted and biologically guided surgery after NACT—combining immunological, imaging, and technological innovation to make breast cancer surgery more precise, less invasive, and ultimately more tailored to each individual patient.

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