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Improving breast cancer outcome by preoperative systemic therapy and image-guided surgery

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Chapter 1

General introduction and outline of thesis

Breast cancer is the most common cancer in women and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of cancer deaths.[1] In the Netherlands, 12.000 women are diagnosed with the disease annually and the lifetime risk of developing breast cancer is 12-13%. The incidence of breast cancer is still increasing, which likely results from changes in reproductive factors (including the increased use of postmenopausal hormone therapy) as well as an increased screening intensity.[2] Breast cancer is strongly related to age. Only 5% of all breast cancers occur in women under 40 years old.[3] The age distribution of breast cancer shows a bimodal characterization and early- and late-onset modes are observed near ages 50 and 70 years, respectively (Figure 1).[4] Over the last decades, mortality trends for breast cancer are declining. Currently, 90% of breast cancer patients are expected to survive at least five years. The increase in breast cancer survival seen since the mid-1970s has been attributed to improved systemic treatment. Nonetheless, surgery is still the cornerstone of the curative treatment of breast cancer.

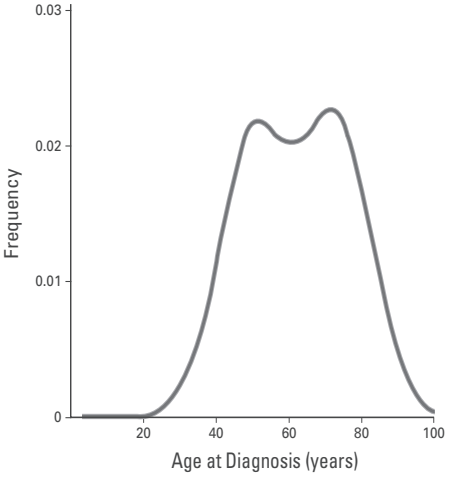

Figure 1. Bimodal age distribution at diagnosis for invasive female breast cancer cases (n = 94,813) in the National Cancer Institute's Surveillance, Epidemiology, and End Results Program during the years 1994 through 1997.[4]

HISTORY OF BREAST CANCER SURGERY

The local treatment of breast cancer has undergone a dramatic paradigm shift during the last century, characterized by a more conservative surgical approach. The radical mastectomy, published in a landmark paper by Dr. William Halsted in 1894, was regarded as standard of care for every breast cancer patient regardless of any tumor characteristic or status of the axilla for several decades. As this operation included an *en bloc* excision of the breast gland, both pectoral muscles and all relevant lymph nodes, it was associated with a high morbidity.[5] In an attempt to decrease morbidity, the modified radical mastectomy was introduced, in which both pectoral muscles were spared and a less extensive axillary dissection was performed. The efficacy of both

operations was equal, while the morbidity was markedly decreased.[6,7] During the 1970s, the breast-conserving therapy was introduced, which comprised of a lumpectomy in combination with radiotherapy to the breast. While the survival rates were equal for mastectomy and lumpectomy,[8] the surgical morbidity and patients' self-image owing to better cosmetic outcome were ameliorated.[9, 10] In the 1990s, the sentinel lymph node procedure was introduced to further reduce surgical extent.[11] The sentinel lymph node procedure prevents the morbidity of an axillary lymph node dissection in lymph node negative patients. In line with this changing surgical approach of breast cancer, preoperative or neoadjuvant chemotherapy was introduced in early breast cancer to down size breast tumors in order to improve surgical possibilities and increase the rate of breast-conserving surgery.

PART I: NEOADJUVANT SYSTEMIC THERAPY

Neoadjuvant chemotherapy in breast cancer treatment defines the use of cytotoxic chemotherapy before any local treatment, either surgery or radiotherapy. Although other terms such as 'primary', 'preoperative', 'induction', 'upfront' or 'initial' are perhaps more accurate descriptions, it was decided during the 2003 Consensus Conference to retain the more commonly used term 'neoadjuvant'.[12]

The use of neoadjuvant chemotherapy in breast cancer was introduced almost simultaneously with the establishment of adjuvant chemotherapy in the 1970s in patients with locally advanced disease in order to convert inoperable tumors into operable tumors. At present, neoadjuvant chemotherapy is the standard of care for patients with locally advanced and inflammatory breast cancer. Soon after reaching positive results in locally advanced breast cancer, randomized controlled trials were conducted to evaluate neoadjuvant chemotherapy in earlier, operable stages. A major benefit of neoadjuvant chemotherapy is the increase in breast conservation rate, which is associated with less morbidity and improved body image compared with complete breast removal. However, concerns exist on local control after down staging of the tumor and the delay of surgery in patients with tumors resistant to chemotherapy.

Besides an increase in breast conservation, an increase in overall survival was also anticipated with the use of neoadjuvant chemotherapy in early stage breast cancer. The rationale for this survival benefit was derived from several experimental studies, in which an increase in the proliferation index of residual tumor was shown after removal of the primary tumor, which resulted in acceleration of tumor growth.[13, 14] This increase in tumor growth was repressed by preoperative chemotherapy, which diminished the release of circulating growth factors and prolonged survival.[15] A more theoretical advantage is the Goldie–Coldman hypothesis, which proposes that, as a tumor cell population increases, an ever-expanding number of drug-resistant phenotypic variants arise that are more difficult to eradicate with chemotherapy.[16]

Moreover, early introduction of systemic therapy in the biological life of the tumor could tackle micrometastatic tumor cells several months earlier than in the adjuvant setting. Upon these considerations, it was reasoned that neoadjuvant chemotherapy might improve overall survival in early stage breast cancer patients.

Since the mid-1980s, several trials have been conducted to evaluate the efficiency of neoadjuvant chemotherapy compared with adjuvant chemotherapy in early stage breast cancer. In **Chapter 2**, a meta-analysis of these trials is performed in order to assess the overall effectiveness of neoadjuvant chemotherapy on clinical outcome.

Prediction of tumor response to therapy: towards personalized treatment

Neoadjuvant chemotherapy facilitates the *in vivo* monitoring of changes in tumor volume during systemic treatment. The achievement of complete eradication of local disease by systemic neoadjuvant therapy is strongly associated with a favorable long-term prognosis.[17] So, a pathological complete response during neoadjuvant therapy reflects chemosensitivity of distant micrometastasic disease. These findings have led to the use of pathological complete response as a surrogate marker for prognosis of survival and its use in clinical trials provides an early indication of drug activity. Moreover, the assessment of tumor response during neoadjuvant therapy is an excellent study model to identify predictive factors. A predictive factor is any clinical or biological characteristic associated with a response or lack of a response to a specific treatment. Identification of predictive factors may lead to more personalized treatment strategies.

In **Chapter 3**, predictive factors are identified that are capable of predicting pathological complete and overall clinical tumor response to preoperative anthracycline-based chemotherapy. For this, the pre-treatment core biopsies of women with operable breast cancer who enrolled in the European Organization for Research and Treatment of Cancer (EORTC) trial 10902 were used.

One of the most important predictive factors of tumor response to chemotherapy is the estrogen receptor (ER) status of the tumor. Several neoadjuvant chemotherapy studies have demonstrated that patients with ER negative tumors are more likely to achieve a pathological complete response than those with ER positive tumors.[18-20] Moreover, these studies found that, when patients with ER negative tumors achieved a pathological complete response, their survival was comparable to that of ER positive patients. Translating these results to the adjuvant setting, some authors have argued that chemotherapy should not be administrated to patients with node negative, ER positive breast cancer, but, instead, should be treated with hormonal treatment alone.

Young age (< 40 years) at the time of diagnosis of breast cancer is an independent factor of poor prognosis and current consensus guidelines have included young age as an absolute indication for adjuvant systemic chemotherapy irrespective of other tumor characteristics, such as stage, grade, or ER status.[21] However, young patients with hormone receptor positive breast cancer might receive limited benefit from

chemotherapy alone. Due to the fact that breast cancer at a young age is a relatively rare event and accounts for 5-7.5% of all cases, limited data on predictive and prognostic factors are available for this patient group. Therefore, concerns exist on the overtreatment with chemotherapy of these young patients. In particular the long-term toxicity of chemotherapy and the implications of possible fertility impairment and premature menopause are of concern in young women.[22] More refined knowledge of predictive and prognostic factors in young breast cancer patients will be of use in guiding systemic therapy in these women.

In **Chapter 4**, the effect of chemotherapy is studied in young patients with breast cancer in relation to hormone receptor status. In this study, the paraffin-embedded tumor material was used from a large cohort early stage breast cancer patients younger than 40 years who participated in one of four EORTC trials.

In **Chapter 5**, prognostic factors are identified in the above-described cohort and in the node negative subpopulation of which most patients did not receive chemotherapy.

Resistance to therapy and breast cancer stem cells

Despite recent advances in systemic therapy and radiotherapy, a significant proportion of early stage breast cancer patients still develop loco regional recurrences and distant metastases. Often, these recurrences occur after a considerable follow-up period. This phenomenon, referred as tumor dormancy, is a particular clinical problem in breast cancer, where disease recurrences are witnessed 20 years after initial curative treatment.[23] Recent biological research has provided evidence that the cancer stem cell theory might explain these treatment failures.

Cancer stem cells, defined as a small subset of tumor cells with stem cell-like features, including epithelial-to-mesenchymal transition, have the capacity of self-renewal and differentiation; giving rise to heterogeneous tumor cell population.[24] Various studies have shown that cancer stem cells have the ability to survive drugs and radiotherapy by a number of properties including high expression of ABC drug transporters, higher levels of DNA repair, and more anti-apoptotic traits.[25-27] Selective survival of cancer stem cells might provide opportunities for understanding treatment resistance and tumor dormancy. Several cancer stem cell markers have been suggested for breast cancer. However, expression of the detoxifying enzyme aldehyde dehydrogenase-1 (ALDH1) has shown the most promise as a clinically relevant prognostic cancer stem cell marker in breast cancer.[28-30]

Breast cancer stem cells could also provide a biological explanation for the well-known age-specific difference in breast cancer survival (Figure 1). Young age (< 65 years) is associated with more aggressive tumors with a relatively high risk of distant metastasis and loco-regional recurrence,[3] whereas old age is associated with more

indolent tumors.[31, 32] However, it is unknown whether the expression of ALDH1 is associated with age and has an influence on clinical outcome.

In **Chapter 6**, the age distribution of ALDH1 expression and its prognostic role in young and elderly patients was analyzed using the long-term follow-up data of a large cohort of breast cancer patients primarily treated with surgery at the Leiden University Medical Center.

To further elucidate the biological pathways involved in the formation and growth of cancer stem cells, the role of the putative coagulation protein tissue factor (TF) has been suggested. A large number of tumor types show tumoral expression of TF and the complex of TF and activated factor VII (TF:FVII) have been implicated in tumor growth and metastasis. TF exhibits its effect through a protease activated receptor-2 (PAR2)-dependent pathway, which results in proliferation, increased oncogene expression and cell migration. TF expression and the TF:FVII/PAR2 axis has been linked to cancer stem cells. However, the role of TF and its alternatively spliced isoform (asTF), which exhibit its role through an integrin-related pathway, have not been tested in breast cancer.

In **Chapter 7**, the expression of TF and asTF in early breast cancer was assessed as well as the association with clinicopathological characteristics, patient outcome and ALDH1 expression in the above-described cohort.

PART II: IMAGE-GUIDED SURGERY

Intraoperative tumor visualization

The main challenge in the surgical treatment of breast cancer is the complete removal of tumor tissue taking into account an adequate tumor-free margin and an acceptable cosmetic result. During breast-conserving surgery, the surgeon has to rely on palpation and visual inspection to discriminate tumor tissue from normal tissue. The distinction between tumor and normal tissue is often not evident, resulting in irradical resections in 5 to 40% of patients undergoing breast-conserving surgery, which requires additional resection or intensified radiotherapy regimens.[33-35] Particularly, after neoadjuvant chemotherapy the assessment of remnant cells may be difficult and tumor response can be either under- or overestimated owing to fibrosis, weakening of the tumor margins and resolution of edema. Local recurrence rates following breast-conservative therapy of 6.7 to 11% are reported,[36] which can be explained by remnant tumor tissue that is not identified during surgery. Loco regional recurrences are associated with a decrease in overall survival.[36] Therefore, there is a need for a diagnostic tool that can discriminate tumor tissue from normal tissue in real-time during surgery.

Optical imaging using near-infrared (NIR) fluorescence is a new technique that can be used to visualise structures in real-time during surgery. Advantages of

NIR fluorescent light (700-900nm) include high tissue penetration (millimetres to centimetres deep) and low autofluorescence, thereby providing sufficient contrast.[37] Because the human eye is insensitive to NIR wavelengths, the use of NIR light does not alter the surgical field. Several imaging systems have recently become available that are capable of visualizing NIR fluorescence in real-time (reviewed in [38]). Besides these imaging systems, tumor-targeted NIR fluorescent contrast agents ("probes") are necessary to visualize cancer cells. Various mechanisms are available for probes to target tumor cells: they can target increased metabolism, upregulated enzymes, or specific cell surface markers. Therefore, the use of NIR fluorescence imaging can be of great value in the intraoperative detection of critical anatomical structures and oncologic targets. The ultimate goal of NIR fluorescence imaging is a real-time visualization of cancer cells during surgery in order to achieve an increase of the radical resection rate and thereby an improvement in breast cancer outcome.

In **Chapter 8**, a novel, hand-held, intraoperative NIR fluorescence imaging system is tested. The minimal detection limits, resolving power and intraoperative utility are addressed in primary breast cancer and metastatic colorectal cancer in two syngeneic rat models.

In **Chapter 9**, the technique of NIR fluorescence imaging is assessed in a syngeneic breast cancer rat model using a protease-activated NIR probe and the accuracy is determined of intraoperative tumor detection to obtain an adequate tumor-free resection margin.

In **Chapter 10**, the technique of NIR fluorescence imaging is assessed in a syngeneic breast cancer rat model using monoclonal antibodies conjugated to a NIR fluorescence dye and its utility for image-guided resection is tested.

Sentinel lymph node mapping

The sentinel lymph node (SLN) procedure, as introduced in the treatment of breast cancer by Giuliano *et al*,[11] is currently regarded as standard of care in staging of the axilla. The SLN is the first lymph node that receives lymphatic drainage from a tumor, and identification of the SLN and analysis for tumor involvement should predict the status of the remaining lymph nodes.

Despite widespread acceptance of SLN procedure, the current technique can be improved. The SLN procedure utilizes a gamma ray-emitting radiotracer or a blue dye or a combination. Radiocolloids require involvement of a nuclear medicine physician, can be difficult to localize with a handheld gamma probe and there is some exposure to ionizing radiation. Moreover, the time-window for SLN identification after injection of the radiocolloid is limited. Blue dyes cannot be seen easily through skin and fat and allow limited visualization of afferent lymphatic vessels and the SLN. Surgical time needed to complete the SLN procedure may take up to 30-45 minutes, in particular when identification is difficult, requiring extensive axillary exploration.

NIR fluorescence imaging using the NIR fluorescence agent indocyanine green has the potential to provide an alternative for, or an addition to, conventional techniques used for SLN mapping.[39] Indocyanine green (ICG) is currently the only clinically available NIR fluorophore that can be used for SLN mapping. Preclinically, ICG adsorbed to human serum albumin (ICG:HSA) improves its performance as a lymphatic tracer. The benefit of ICG:HSA for SLN mapping of breast cancer has not yet been assessed in a clinical trial.

In **Chapter 11**, the development of a miniaturized version of the fluorescence-assisted resection and exploration (FLARE) imaging system is described. Using this Mini-FLARE, preclinical and clinical optimization of the lymphatic tracer ICG:HSA was performed, followed by a more refined optimization in a phase II clinical trial. During this dose-escalating trial, the use of NIR fluorescence was directly compared with the combination of radioactive colloid and blue dye in breast cancer patients undergoing SLN mapping.

In **Chapter 12**, a double-blind, randomized clinical study is performed to determine if ICG:HSA has advantages over ICG alone in the SLN mapping in breast cancer.

Finally, **Chapter 13** includes a summary of this thesis as well as a general discussion. **Chapter 14** provides a summary in Dutch.

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