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

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Chapter 13

Summary and general discussion

Partly based on:
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This thesis consists of two parts. In part I, we have demonstrated that preoperatively administrated systemic (neoadjuvant) therapy is a feasible treatment strategy in early stage breast cancer to achieve improved surgical options and to assess tumor response. We also demonstrated that overexpression of the breast cancer stem cell marker aldehyde dehydrogenase-1 in early stage breast cancer patients is inversely associated with age and is of prognostic importance. In part II, we have demonstrated proof-of-principle of intraoperative tumor detection and image-guided tumor resection by using the novel technique of near-infrared fluorescence imaging. We have performed two clinical trials to optimize the use of indocyanine green as a near-infrared fluorescence lymphatic tracer for the sentinel lymph node procedure in breast cancer patients.

PART I: NEOADJUVANT SYSTEMIC THERAPY

The meta-analysis described in **Chapter 2** demonstrated that neoadjuvant chemotherapy results in equivalent overall and disease-free survival rates and permits more breast-conserving therapies, while not significantly hampering loco regional control. An international expert panel recently recommended that all patients with a clear indication for adjuvant chemotherapy can be offered chemotherapy preoperatively.[1]

However, neoadjuvant therapy is still underutilized in many countries. Also, in the Netherlands, an apparent barrier exists for the routine administration of neoadjuvant strategies in the treatment of early stage breast cancer patients that might benefit from this approach. This is in spite of the obvious advantages associated with the use of neoadjuvant therapy, while little disadvantages exits (Table 1). Moreover, the compliance of patients to neoadjuvant therapy is high, thanks to the motivating decrease in tumor size that breast cancer patients can assess themselves by palpation. Even more, the indications for neoadjuvant strategies have broadened due to an increase in the availability of more tailored regimens, e.g. hormonal, chemo- and targeted therapies.

Table 1. Advantages and disadvantages of neoadjuvant chemotherapy

Advantages	Disadvantages
Tumor down staging: more breast conserving surgery	Delay of adequate surgery in the case of tumor resistance
Nodal down staging: less axillary lymph node dissection	Loss of information on pretreatment nodal status
In vivo assessment of tumor resistance or sensitivity	
Tumor response as surrogate prognostic marker	
Rapid screening of efficiency of new drugs	

Although the routine use of neoadjuvant therapy could be extended, an encouraging increase in the number of included patients in Dutch neoadjuvant studies is witnessed over the last years, as demonstrated in the recently closed INTENS study (BOOG 2007-02) and the lately started NEO-ZOTAC study (BOOG 2010-01). However, another national study, the TEAM-IIA trial (BOOG-2006-04a), in which the duration of neoadjuvant hormonal therapy is investigated, suffers from slow inclusion. Therefore, further research and propagation on the use of neoadjuvant systemic therapy as a powerful tool in the treatment of early stage breast cancer patients is still warranted.

Neoadjuvant hormonal therapy is an attractive alternative to neoadjuvant chemotherapy in treating women with estrogen receptor (ER) positive breast cancer because of the low toxicity of hormonal therapy and the low response rate in ER positive breast cancer (especially lobular cancer) treated with neoadjuvant chemotherapy. Neoadjuvant hormonal therapy was directly compared with neoadjuvant chemotherapy in one study demonstrating equal response rates (64%) and lower adverse events in the hormonal group.[2] Currently, the randomized NEOCENT study is underway to compare 6 cycles of neoadjuvant chemotherapy with 18-23 weeks of the aromatase inhibitor letrozole. Three randomized trials have compared 3-4 months of neoadjuvant tamoxifen with aromatase inhibitors and showed superiority of aromatase inhibitors in clinical response rate and breast conservation rate (RR, 1.36; 95% CI, 1.16- 1.59).[3] The tumor response to neoadjuvant hormonal therapy is slow, but sustained. To address the issue of the optimum duration of neoadjuvant hormonal therapy, several phase II studies have investigated the prolonged use of neoadjuvant hormonal therapy beyond 3-4 months and demonstrated response rates up to 80%. Even more, reductions in tumor volume were achieved up to 2 years of treatment.[4] For elderly women with a limited life expectancy, neoadjuvant hormonal therapy without surgery provided long-term disease control. So, these data suggest that a further reduction in tumor size can be achieved with prolonged treatment and that even surgery can be withheld for elderly women on continuing hormonal treatment. However, the optimum duration of neoadjuvant hormonal therapy remains to be established.

Several surgical aspects are associated with the use of neoadjuvant therapy. **Chapter 2** demonstrated that the major benefit of neoadjuvant chemotherapy is the down sizing effect on the primary tumor allowing for a breast conservative approach in selected patients. In addition, the total volume of excised breast tissue is significantly decreased in patients with tumors already suitable for breast-conserving surgery.[5] However, defining the optimal selection criteria for breast conservation remains a clinically relevant area of research, as breast tumors respond in a heterogeneous fashion to neoadjuvant chemo- and hormonal therapy and distinct types of regression can be recognized (Figure 1). After concentric shrinkage, the residual tumor can easily be

completely resected. However, tumors that regressed in a patchy fashion leaving scattered microscopic disease over the original tumor bed volume may predispose to a higher breast cancer recurrence rate when surgery is directed only at the 'center' of the residual tumor.[6]

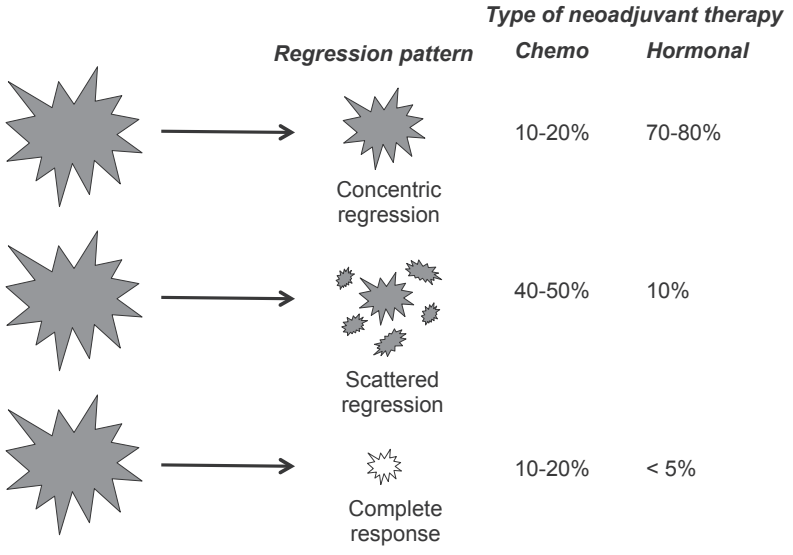

Figure 1. Regression patterns to neoadjuvant therapy. Adapted from [7, 8].

Conventional imaging modalities have demonstrated poor correlation between the radiological and the pathological tumor size after neoadjuvant chemotherapy.[9] **Chapter 2** demonstrated that the local recurrence rate in the neoadjuvant arm is greatly reduced after excluding studies in which patients received exclusive radiotherapy and no surgery after clinical complete tumor regression. These data emphasize the importance of incorporating surgery in the loco regional treatment after neoadjuvant chemotherapy. Otherwise stated, the current clinical assessment of tumor response is insufficiently sensitive to safely withhold surgery. Therefore, surgical planning and execution should take into account the size of the original tumor and the response to neoadjuvant chemotherapy.[1] Placement of radiopaque clips within breast lumps at the time of biopsy provides localization for subsequent surgical removal has been associated with better local control.[10] In order to improve risk assessment of a loco regional recurrence following breast-conserving surgery after neoadjuvant chemotherapy, a prognostic index has been developed, which includes four factors: clinical N2-3 disease, residual pathologic tumor size larger than 2 cm, a multifocal pattern of residual disease, and lymphovascular space invasion.[11] The index was able to predict loco regional recurrence in a retrospective study,[12] and may be useful in selecting type of surgical treatment for patients treated with neoadjuvant chemotherapy; however, confirmation in prospective patient series is warranted.

Sentinel lymph node (SLN) mapping provides the possibility of assessing the axillary lymph node status after neoadjuvant therapy. If the SLN becomes negative following neoadjuvant therapy in patients with proven nodal metastases prior to treatment, an axillary dissection could be prevented. Though, concerns exist on the accuracy of SLN mapping after neoadjuvant chemotherapy because the proposed alteration of the lymphatic network. However, scintigraphy data from patients before and after neoadjuvant chemotherapy demonstrated that the location of the SLN was the same in 99.2% of patients.[13] Moreover, a meta-analysis on SLN mapping after neoadjuvant chemotherapy reported a SLN identification rate of 91% and a false negative rate of 11%;[14] these rates are comparable in patients naïve to chemotherapy.[15] In patients with proven axillary metastases, the SLN becomes negative in approximately one-third of the cases and the SLN identification rate and false negative rate are 85% and 11%, respectively.[13] These results suggest that SLN mapping after neoadjuvant chemotherapy is feasible in node-positive patients with complete clinical response in order to avoid an axillary dissection. Currently, the prospective, multicenter SENTINA trial is accruing patients to evaluate the accuracy of the SLN procedure in both clinically node-negative and node-positive patients.

In **Chapter 3**, data are presented from the neoadjuvant chemotherapy trial EORTC 10902 that suggest that expression of the tumor suppression protein p53 is of predictive significance in anthracycline-containing chemotherapy regimens. To assess the value of p53 to predict sensitivity of anthracycline and taxane containing neoadjuvant chemotherapy regimens, the phase III randomized EORTC 10994/BIG 00-01 trial has been conducted using a functional yeast assay to detect biologically important mutations of p53. The final results of this trial were recently published and demonstrated that although p53 was highly prognostic, p53 status was not a predictive factor of sensitivity or resistance to taxanes or anthracyclines.[16]

In **Chapter 4**, the effect of chemotherapy in relation to ER status was studied in very young breast cancer patients. Patients with ER positive tumors had an improved overall survival compared to their ER negative counterparts. However, in the subgroup of patients treated with chemotherapy, no difference in overall survival was shown. These data suggest that young patients with ER positive tumors benefit less from chemotherapy than patients with ER negative tumors. Moreover, these results suggest that chemotherapy alone cannot be considered optimal adjuvant systemic treatment in very young breast cancer patients with hormone receptor positive tumors.

In **Chapter 5**, the impact of established prognostic factors was tested in very young breast cancer patients. Tumor size, nodal status and molecular subtype were independent prognostic factors. In the node negative patient group, molecular subtype was the sole prognostic factor for overall survival. These data support the use of established prognostic factors and in particular molecular subtype to plan systemic treatment strategies in these very young patients.

In **Chapter 6**, the prognostic effect of the breast cancer stem cell marker aldehyde dehydrogenase-1 (ALDH1) has been analyzed. ALDH1 expression was inversely associated with age. In elderly patients, ALDH1 status was not associated with clinical outcome. Alternatively, in young patients, ALDH1 expression was associated with poor outcome. These results support the hypothesis that breast cancer biology is different in elderly patients compared to their younger counterparts, which may be explained by differences in cellular pathways involved in cancer stem cell activity.

In **Chapter 7**, the association was studied between ALDH1 expression and the coagulation protein tissue factor (TF) and its alternatively spliced (asTF) isoform. Expression of both TF isoforms was very common in human breast cancer cells and was associated with poor histological grade. Expression of TF was associated with ALDH1 expression, whereas expression of asTF was not. Moreover, co-expression of TF and ALDH1 was strongly correlated with poor relapse-free period in patients younger than 65 years, whereas in older patients the inverse effect was demonstrated. These results provide new insights on the interaction between the tissue factor/protease activated receptor (TF/PAR) pathway and the growth potential of the cancer stem cell subset and suggest that the TF/PAR pathway is important for the cancer stem cell population in early breast cancer.[17, 18] Therefore, modulation of the activity of this pathway might interfere with survival of the cancer stem cell subset, which may subsequently improve patient survival.

Selective survival of cancer stem cells might be responsible for treatment failure witnessed in early stage breast cancer. Regrowth of tumors from this intrinsically chemotherapy-resistant subpopulation has been termed the 'dandelion hypothesis'.[19] Neoadjuvant systemic therapy offers an excellent model to test the dandelion hypothesis in breast cancer patients. By comparing the cancer stem cell content of the pretreatment specimens with the posttreatment specimens, an increase in the relative proportion of cancer stem cells is expected after neoadjuvant systemic therapy. Targeting with novel therapies of the residual cells with stem cell self-renewal properties may provide a specific approach to prevent cancer recurrence and improve long-term survival.[20]

PART II: IMAGE-GUIDED SURGERY

In **Chapter 8**, a novel near-infrared (NIR) fluorescence imaging system, the Fluobeam®, was tested both *in vitro* and *in vivo*. This study demonstrated the technical performance of this new imaging system and its intraoperative utility in guiding resection of tumors using a protease-activatable NIR fluorescent probe. Also, this study has provided several methods for the validated testing of new NIR fluorescence imaging system.

In **Chapter 9**, the accuracy was determined of real-time NIR fluorescence imaging in obtaining tumor-free resection margins in a rat model of primary breast cancer using a protease-activatable NIR fluorescence probe. Real-time NIR fluorescence guidance

of tumor resection resulted in a complete resection of all tumors with minimal excision of normal healthy tissue (mean minimum and a mean maximum tumor-free margin of 0.2 ± 0.2 mm and 1.3 ± 0.6 mm, respectively). Histological analysis revealed that the NIR fluorescence signal was highest at the invasive tumor border and in the stromal compartment of the tumor, which correlates with the mechanism of action of protease activity.

In **Chapter 10**, the performance was assessed of antibody-based detection of breast tumor margins in the same breast cancer rat model as described in Chapter 9. The monoclonal antibody MG1, directed against epithelial rat tumor cells, was conjugated to a NIR fluorophore. All breast tumors were identified and under direct image-guidance, all tumors could be resected with a clear margin. The fluorescence signal corresponded with histological tumor demarcation. Interestingly, the control experiment with a non-targeted antibody demonstrated equal fluorescent intensity and signal-to-background ratio. This observation can be explained by the 'enhanced permeability and retention' effect, by which the conjugated antibodies passively accumulate in the tumor due to the 'leaky vessels'; the characteristic large pores seen in neovascularization. This observation may provide a simple and effective targeting strategy for NIR imaging of solid tumors.

Chapter 8, **Chapter 9**, and **Chapter 10** demonstrated that NIR fluorescence detection of breast tumor margins was successful in a rat model. These studies suggest that clinical introduction of intraoperative NIR fluorescence imaging has the potential to increase the number of complete tumor resections in breast cancer patients undergoing breast-conserving surgery.

In **Chapter 11**, the clinical translation of a new, portable NIR fluorescence imaging system, the Mini-FLARE™, is described in a dose optimization study of SLN mapping in breast cancer using indocyanine green (ICG) absorbed to human serum albumin (ICG:HSA) as a lymphatic tracer. The imaging system, which was developed for capturing color video and two channels of NIR fluorescence (700 nm and 800 nm), enabled visualization of lymphatic channels and SLNs in all patients. Mean number of identified SLNs was 1.45. All SLNs were NIR fluorescent. Optimal injection dose of ICG:HSA ranged between 400 and 800 μM.

In **Chapter 12**, the effect of premixing ICG to HSA for SLN mapping in breast cancer was determined in a double-blind, randomized study. No significant differences were found in signal brightness and number of detected SLNs between the ICG:HSA group and the ICG alone group. This study has demonstrated that there is no direct benefit of premixing ICG with HSA prior to injection for SLN mapping in breast cancer patients, thereby reducing the cost and complexity of the procedure.

Chapter 11 and **Chapter 12** describe the optimization of NIR fluorescence SLN mapping using ICG in breast cancer and have paved the way for larger trials that can determine patient benefit of this technique.

The recent introduction of NIR fluorescence image guidance has provided new opportunities in cancer surgery. Several NIR fluorescence imaging systems have been designed for intraoperative clinical use.[21] Two of these imaging systems have been described in **Chapter 8** and **Chapter 11**, respectively. Although differing in their technical specifications, all of these systems provide the surgeon with an image of the NIR fluorescence signal that would otherwise be invisible to the human eye (Table 2). To permit precise localization of the NIR fluorescent signals in the context of surgical anatomy, several imaging systems acquire color video and NIR fluorescence emission simultaneously and provide overlay images.[22-24] The development of new imaging systems will further improve NIR fluorescence image-guided surgery. NIR fluorescence laparoscopic imaging systems are currently being developed, and a commercial system is already available, which will allow NIR fluorescence guided-surgery in a minimally invasive setting.[25, 26] Various techniques are being evaluated to correct photon scattering and thereby enhance the depth at which NIR fluorescent signal can be detected.[27-29]

Table 2. Clinically available NIR fluorescence imaging systems

Imaging system	Excitation source	Working distance	Field of view	White light illumination of surgical field	NIR-color overlay
PDE	LED 805 nm, power NS	15 - 25 cm	NS	No	No
SPY	Laser 806 nm, 2.0 W	30 cm	56 cm^2	No	No
Fluobeam®	Laser 780 nm, 10mW/cm^2	22 cm	80 cm^2	Yes	No
HyperEye	LED 760 nm, power NS	30 - 50 cm	78.5 cm^2	Yes	Yes
FLARE™	LED 745-779 nm, 14 mW/cm^2	45 cm	3.7 cm^2 - 169.5 cm^2	Yes	Yes
Mini-FLARE™	LED 760 nm, 8.6 mW/cm^2	30 cm	100 cm^2	Yes	Yes
FDPM imager	Laser 785 ± 10 nm, < 1.9 mW/cm^2	<76.2 cm	Max 900 cm^2	No	No
Munich prototype	Laser 750 nm, 300 mW	21 cm	1,5 cm^2 - 107 cm^2	Yes	Yes

PDE, Photodynamic Eye; LED, Light emitting diode; NS, not specified; FLARE, Fluorescence-Assisted Resection and Exploration; FDPM, Frequency Domain Photon Migration

Besides NIR fluorescence imaging systems, exogenous NIR fluorescent contrast agents are necessary to visualize specific tissues. The only NIR fluorescent contrast agents currently registered by the FDA and EMA for clinical applications are indocyanine green (ICG; peak emission ≈ 820 nm) and methylene blue (peak emission ≈ 700 nm).[30] ICG has preferable fluorescent characteristics because it provides a higher signal-to-background ratio as the autofluorescence is lower and tissue penetration is increased at 820 nm wavelength compared to 700 nm and ICG has a higher "brightness" (quantum yield).

ICG is a negatively charged, amphiphilic, water-soluble, tricarbocyanine with a molecular mass of 776 Da. ICG has been registered for several decades to determine cardiac output, hepatic function, and ophthalmic perfusion. ICG has a very favorable safety profile, as the number of allergic reactions, the most important side-effect, is very low (1: 10,000, as reported by manufacturer).[31] ICG is currently utilized in NIR fluorescence cancer surgery for four indications: sentinel lymph node (SLN) mapping, endoscopic marking of colorectal tumors,[32] intraoperative identification of certain solid tumors,[33] and angiography during reconstructive breast surgery.[34]

Sentinel lymph node mapping with NIR fluorescence imaging using ICG as described in **Chapter 11** and **Chapter 12** has demonstrated accuracy and percutaneous visualization of the lymphatic channels. Thereby, it can reduce time of surgery and improve localisation of the SLN so that surgical exploration can be minimized, while maintaining a high identification rate. It should be noted, however, that NIR fluorescence detection is currently in the millimetre to centimetre range, far less than radioactive tracers, which requires caution when interrogating thick tissues, for instance in patients with high body mass index. In **Chapter 11** and **Chapter 12**, it was demonstrated that NIR fluorescence using ICG could consistently be visualized before the blue dye could be observed. Therefore, NIR fluorescence imaging has the potential to replace blue dyes in SLN mapping of breast cancer patients. Whether NIR fluorescence could also replace radiocolloids is subject of an ongoing clinical trial, which is conducted by our group (NTR2674).

Intraoperative NIR fluorescence imaging tumor detection in cancer patients is currently limited because both ICG and methylene blue are so-called non-targeted probes as they cannot be conjugated to tumor-specific targets. Novel, non-toxic NIR fluorophores have been developed, such as IRDye CW800 (LI-COR Biosciences, Lincoln, USA), VivoTag family (PerkinElmer, Waltham, USA) and the 800 nm zwitterionic heptamethine indocyanine ZW-800-1 (John V. Frangioni, Center for Molecular Imaging, Boston, USA), with improved fluorescence properties for higher tissue penetration and with the ability to be conjugated to tumor-targeting ligands. Several academic and industry groups are translating these fluorophores to the clinic and the first toxicity results have been reported.[35] Once these fluorophores have obtained regulatory approval, the logical next step would be the conjugation of these fluorophores to already clinically available monoclonal antibodies, such as trastuzumab (directed to the HER2/neu receptor, which is overexpressed in 20% of breast cancer patients), cetuximab, or bevacizumab. Thereafter, novel tumor-targeting approaches should be exploited. Various mechanisms are available for tumor targeting, including the conjugation of NIR fluorophores to tumor-specific antibody, to glucose derivates in order to visualize elevated metabolic rate, and autoquenched fluorophores that can be activated by tumor-specific enzymatic cleavage in order to become fluorescent. An

important strategy is the conjugation of fluorophores to a tumor-specific antibody or ligand directed to markers overexpresed by tumors, such as the follicle-stimulating hormone receptor, which is selectively expressed by endothelial cells at the periphery in a layer of approximately one cm of a wide range of tumors.[36] Multiple targeted probes have already been developed, some of which are commercially available, and have successfully been tested in animal studies and this thesis also has provided examples of this in **Chapter 9** and **Chapter 10**. Translating these preclinical results to the clinical setting in order to improve breast cancer outcome remains a major challenge for the next decades.

NIR optical spectroscopy is a technique to non-invasively monitor tumor response to neoadjuvant therapy. The technique utilizes the differences in absorption and scattering spectra of endogenous compounds. Therefore, no contrast agents are necessary. For the breast, the major absorption chromophores are oxyhemoglobin, deoxyhemoglobin, lipids and water, which are altered by angiogenesis, tumor cell proliferation and hypoxia.[37, 38] The Softscan® (ART, Montreal, Canada) is an optical breast imaging device, which uses pulsed time domain optical spectroscopy to obtain 3D images of the breast.[39] Current imaging modalities for tumor response assessment (mammography, ultrasound, MRI) are mainly based on size measurements. However, it is expected from tumor biology data that physiological changes in the tumor can be noted long before any changes in size can be observed.[40] As the Softscan measurements are based on tissue physiology, it should theoretically be able to assess early changes in the tumor, which could indicate a good response to neoadjuvant therapy. Indeed, a small clinical trial has demonstrated that the Softscan can discriminate responders from non-responders after one cycle of neoadjuvant chemotherapy.[41] Our group is currently conducting a larger clinical trial with the Softscan to study the predictive value of early tumor response assessment to neoadjuvant therapy within the framework of the NEOZOTAC and TEAM-IIa studies. Another application of NIR imaging could be the *in vivo* monitoring of the response of axillary metastases, either by optical spectroscopy or by NIR fluorescence contrast agents either targeting apoptotic[42] or viable tumor cells. As discussed above, if axillary metastases are regressed after neoadjuvant therapy and this can be confirmed by fluorescent imaging, an axillary lymph node dissection can be prevented.

In summary, near-infrared fluorescence imaging has the potential to revolutionize human cancer surgery by providing sensitive, specific, and real-time intraoperative visualization of normal and malignant tissues, to optimize surgical removal of tumors and their (local or lymph node) metastases.

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