Impact of age, tumor characteristics, and treatment on local control and disease outcome in early stage breat cancer : an EORTC translational research project

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An EORTC translational research project

Jos Alexander van der Hage

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Part I

CHAPTER 1

General Introduction

Evolution of breast cancer treatment

Breast cancer is the most common female cancer, the second most common cause of cancer death in women (after lung cancer), and the main cause of death in women aged 45 to 55 years. In the Netherlands, the lifetime risk of developing breast cancer is 11% amongst women [1]. In the United States similar rates are reported. This translates into 11.500 new cases of breast cancer in the Netherlands and 211.240 new cases of breast cancer in the United States each year. In addition, 1 out of 20 to 25 women will die because of breast cancer [2].
Currently, overall survival rates for breast cancer are 80% after 5 years and 69% after 10 years of follow-up respectively [3].
Although mortality trends have been declining since 1992, the incidence of breast cancer in the Western World has risen since then, possibly due to the introduction of breast cancer screening programs. This means that although breast cancer treatment has improved over the past three decades, breast cancer still is a major subject of concern in terms of healthcare in our society [4].

Originally, treatment of breast cancer consisted of surgery alone and was aimed at aggressive locoregional eradication of tumor cells. In 1894 Halsted introduced the radical mastectomy in an era where breast cancer was normally treated by wide local excision alone which was associated with a high rate of locoregional recurrences [5]. The rationale of more aggressive locoregional therapy was based upon the hypothesis that more extensive resection would provide a better chance of disease control. The radical mastectomy implied “en bloc” removal of the breast, the overlying skin, both the pectoralis major and minor muscles, and the entire axillary contents (level I, II, and III nodes). The radical mastectomy resulted in a significant drop in local recurrence rates, and it became the standard of care for the treatment of breast cancer. However, despite the improvement in local control, the curative potential of this operation remained limited. In one series that followed 1438 women who had undergone radical mastectomy for 30 years, only 13 percent were free of disease, while 57 percent had died of breast cancer [6]. Therefore, in the 1970-ies, it was hypothesized that a less extensive operation, the modified radical mastectomy (MRM), could be performed without compromising survival. The term MRM implied complete removal of the breast tissue and the underlying fascia of the pectoralis major muscle, and removal of some but not all of the axillary nodes (levels I and II). Several prospective randomized trials documented equivalent survival rates with MRM as compared to radical mastectomy, with less morbidity [7-10]. These findings significantly changed the surgical approach to invasive breast cancer. More importantly, however, it supported the concept that breast cancer was not a local disease that spread contiguously, as Halsted proposed, but rather that systemic disease was ultimately the main determinant of survival. Variations in the treatment of local or regional disease were unlikely to affect survival.

While MRM was a less morbid procedure than radical mastectomy, it still required the loss of the breast. The question arose as to whether the breast could be preserved without compromising survival. Therefore breast-conserving therapy was introduced in the seventh and eighth decade of the twentieth century. Breast conserving therapy

refers to surgical removal of the tumor (with negative surgical margins) followed by radiotherapy to eradicate any residual disease. Six randomized clinical trials directly comparing breast conserving therapy with mastectomy and an overview of all completed trials have shown equivalent survival between the two treatment approaches although locoregional control rates after breast conserving therapy were significantly lower than after modified radical mastectomy [11-20].
In addition to changes in locoregional strategy of breast cancer treatment, the introduction of adjuvant polychemotherapy changed the concept of breast cancer treatment dramatically.
Over the past few decades, many randomized trials have been undertaken of various chemotherapy regiments for early breast cancer, and the data of these trials were included in quinquennial meta-analyses published by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG).

This meta-analysis summarized results of every randomized trial that began before 1990 and involved treatment groups that differed only with respect to the chemotherapy regimens that were being compared. In 47 trials comparing combination chemotherapy with no chemotherapy, a significant reduction in mortality occurred in patients receiving chemotherapy irrespective of nodal status (negative vs. positive), estrogen receptor (ER) status (ER-rich vs. ER-unknown, or ER-poor), and whether or not hormonal therapy was administered. The benefit of chemotherapy, however, did vary substantially according to patient age and menopausal status. For all women younger than 50 years at randomization, combination chemotherapy improved 10-year survival from 71% to 78% for those with node-negative disease (an absolute benefit of 7%), and from 42% to 53% for those with node-positive disease (an absolute benefit of 11%). For women between 50 and 69 years at randomization, combination chemotherapy improved 10-year survival from 67% to 69% for those with node-negative disease (an absolute gain of 2%), and from 46% to 49% for those with node-positive disease (an absolute gain of 3%) [21, 22].

European Organization for Research and Treatment of Cancer

All studies presented in this thesis are derived from trials originated and conducted by the EORTC Breast Cancer Group. The work of this thesis has to a significant extent been performed during a fellowship at the Data Center of the European Organization for Research and Treatment of Cancer (EORTC) in Brussels, Belgium.

This organization was founded in 1962 by oncologists working in the main cancer research institutes of the EU countries and Switzerland. It was named Groupe Europe´en de Chimiothe´rapie Anticance´reuse (GECA), and became the European Organization for Research and Treatment of Cancer (EORTC) in 1968.

In 2004, group members entered a total of 4508 new patients in EORTC trials. An additional 971 patients from other research groups were treated as part of the intergroup study scheme managed by the EORTC Data Center, and in 2005 no less than 85 studies are open for entry and are being conducted by the EORTC Data Center.

The EORTC Breast Cancer Group is a multidisciplinary group involving surgeons, radiation oncologists and medical oncologists, pathologists, radiologists, biologists, psychologists and research fellows. Currently, the Group includes 17 institutions with the status of active member and 75 institutions with the status of probationary member. The main activity of the Group has been to carry out large clinical studies covering a wide spectrum of breast cancer patients. Translational research evaluating correlations between clinical outcomes and biologic tumor characteristics has become a high priority as well.
Examples of such investigations include studies presented in this thesis. Current activities include the potential predictive value of P53 gene mutation in primary chemotherapy of locally advanced breast cancer (EORTC 10994) and detection of micrometastasis in sentinel lymph nodes by PCR (EORTC 10981) and the role of radiotherapy after sentinel node biopsy in axillary node positive patients (AMAROS). Recently a hereditary task force addressing several aspects of hereditary breast cancer has been installed. This group is performing a large retrospective study on archival tumor in paraffin selected from 8000 patients previously treated in randomized EORTC trials, comparing treatment outcomes from patients carrying a proven BRCA 1 or 2 mutation or non-carriers.
The Group has prepared and is continuously updating the Manual for Clinical Research in Breast Cancer, used as a reference for protocol elaboration, data collection and reporting of results (recently also online: www.bco.org breast cancer online). This manual summarizes the major points in assessment, staging, treatment and follow-up of breast cancer patients. It enhances the uniformity of definitions and procedures in the various breast cancer protocols.
Within the last three years the number of patients included in clinical studies is stable: 1008 in 2002, 1020 in 2003 and in 2004 it was 856. A total of 9 clinical trials were open for the accrual in 2004.
Additionally, thousands of patients included in previous studies have been under continuous follow up in order to obtain long term results.

Rationale and aims of this thesis

In this thesis, several questions regarding specific issues both in locoregional treatment and in systemic treatment are evaluated. Therefore, the thesis is divided into three parts. Part I addresses questions concerning systemic treatment. Part II studies several aspects of locoregional treatment and outcome, and finally part III discusses the question whether specific tumor characteristics can discriminate very young patients with early stage breast cancer with a good outcome in terms of survival from similar patients who have a poor outcome.

Part I
Concerning adjuvant systemic polychemotherapy, the aspect of timing of administration of chemotherapy is studied. Experimental studies using murine models in the seventies and the eighties suggested that the administration of chemotherapy before or immediately after removal of the primary breast tumor resulted in a significant decrease in tumor cell proliferation in metastases and a

decrease in the upregulation of growth factors due to surgery [23-27].
Therefore, we tested the hypothesis that adjuvant chemotherapy given before or immediately after surgery improves disease outcome in terms of survival and locoregional control. In this thesis, two prospective studies conducted by the EORTC Breast Cancer Group are presented in which neoadjuvant and perioperative chemotherapy are evaluated.

EORTC trial 10854 studied the question whether or not chemotherapy given directly after surgery would yield better results in terms of locoregional control, disease-free survival and overall survival. Perioperative chemotherapy consisted of one short intensive course of fluorouracil, doxorubixin, and cyclophosphamide, administered within 36 hours after surgery. The eleven-year follow up results are presented in this thesis.

EORTC trial 10902 was conducted to study whether or not the administration of neoadjuvant chemotherapy in early breast cancer patients would lead to improved treatment outcome as well. This thesis reports the 5-year follow up results of EORTC trial 10902. The study group received 4 courses of fluorouracil, epirubicin, and cylclophosphamide, administrated before surgery. The control group received the same chemotherapeutic regimen given postoperatively.

Part II
As described above, breast-conserving surgery is similar effective in terms of long term outcome as compared to modified radical mastectomy but is associated with a higher locoregional recurrence rate [28]. The rationale for this finding can be explained by the fact that breast cancer is a systemic disease rather than a loco-regional disease. On the other hand, women who experience a locoregional recurrence have unfavorable prognosis and not surprisingly, a locoregional recurrence is a strong independent prognostic factor associated with unfavorable survival rates. Nevertheless, the general assumption is therefore that more aggressive surgery does not lead to better survival.

In relative contradiction with these findings are better survival rates described with subsequent adjuvant radiotherapy after modified radical mastectomy compared to modified radical mastectomy alone [29-32].

Therefore, we hypothesized that any improvement in long term outcome due to more aggressive locoregional treatment should be accompanied by an improvement in locoregional control.
Next, we hypothesized that a subset of patients can be identified that might benefit from more aggressive locoregional therapy at time of diagnosis to prevent an isolated locoregional recurrence. This subset consists of patients that developed a locoregional recurrence after primary treatment, received therapy and eventually developed systemic disease, but only after being disease-free for a long follow-up period. These are patients in which locoregional recurrence is an instigator rather than an associative factor for subsequent metastatic disease.

Therefore, we studied the question whether it is possible to identify patients in which

the prevention or successful treatment of locoregional recurrences could lead to better disease outcome. In addition, we studied the association between tumor characteristics and locoregional recurrence.

Part III

In the last part of this thesis, tumor characteristics of breast cancer in the very young breast cancer patient are studied. The prognostic impact of young age at onset is well known. However, the underlying pathophysiological mechanisms remain uncertain. Since patient age is a well-established risk factor associated with poor local control as well as unfavorable outcome in terms of survival [33-38], we studied the possibility to divide the very young patient group into a good- and a bad prognosis cohort. Next, we tried to gain further insight in chemotherapy responsiveness in hormone receptor positive- and hormone receptor negative young breast cancer patients groups since the effect of adjuvant systemic chemotherapy in the former group has been subject of discussion due to alternative treatment strategies and impaired chemosensitivity in this patient group [39-43].

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CHAPTER 2

Improved survival after one course of perioperative chemotherapy in early breast cancer patients: long-term results from the European Organization for Research and Treatment of Cancer (EORTC) Trial 10854

J.A. VAN DER HAGE, C.J.H. VAN DE VELDE, J.-P. JULIEN, J.-L. FLOIRAS, T. DELOZIER, C. VANDERVELDEN, L. DUCHATEAU AND COOPERATING INVESTIGATORS

Abstract

The aim of this study was to examine whether one course of perioperative polychemotherapy yields better results in terms of survival, progression-free survival (PFS) and locoregional control than surgery alone in early stage breast cancer. From 1986 to 1991, 2795 patients with stage I/II breast cancer were randomised to receive either one perioperative course of an anthracycline containing chemotherapeutic regimen within 36 hrs after surgery or surgery alone. Patients were followed-up for overall survival, PFS and locoregional recurrence. The median follow-up period at time of the analysis was 11 years. PFS and locoregional control were significantly better (P=0.025 and P=0.004, respectively) in the perioperative chemotherapy arm. Node-negative patients seemed to benefit most from the perioperative FAC. Patients who received perioperative chemotherapy and locoregional therapy alone had significantly better overall survival rates than patients who received locoregional therapy alone (P=0.004). Patients who received additional systemic therapy did not seem to benefit from one course of perioperative chemotherapy (P=0.65). One course of perioperative polychemotherapy does improve PFS and locoregional control in early stage breast cancers. This effect is still present after 11 years of follow-up.

Introduction

Systemic adjuvant therapy has been shown to improve both disease-free survival and overall survival in breast cancer patients [1]. Over the past three decades, many investigators have studied the benefits of adjuvant chemotherapy in breast cancer. However, the significance of the timing of administration of chemotherapy in relation to locoregional treatment is still a matter of debate. Experimental studies, as well as mathematical hypotheses [2–6], have demonstrated that early timing of chemotherapy may be more effective than standard postoperative administration of chemotherapy.

Several randomised trials studying the effect of the administration of one dose of chemotherapy immediately after surgery with or without subsequent prolonged chemotherapy demonstrated better disease-free or relapse-free survival rates using this therapeutic regimen [7–9].

In the European Organization for Research and Treatment of Cancer (EORTC) trial 10854, of which preliminary results have been published previously, a similar effect was observed at a median follow-up time of 41months [10]. In this report, we will focus on the effect of perioperative chemotherapy after long-term follow-up.

Patients and methods

Patient characteristics

Eligibility requirements, randomisation procedures, surgical and radiation techniques used, characteristics of patients and tumours, and the distribution of patients among the treatment groups have been published previously [10]. In brief, eligible patients had primary operable breast cancer, T1-3, N0-2, M0, and had to be younger than 70 years of age at the time of randomisation.

	Perioperative chemotherapy $n=1398$	Control $n=1395$
	n (%)	*n* (%)
Age (years)		
⩽50	557 (40)	560 (40)
>50	841 (60)	835 (60)
Nodal status (pathological)		
Negative	741 (53)	728 (52)
Positive	644 (46)	661 (47)
Missing data	13 (1)	6 (0.5)
Tumour size (clinical)		
⩽2 cm	407 (29)	416 (30)
>2 cm	969 (69)	954 (68)
Missing data	22 (2)	25 (2)
ER status		
ER+	910 (65)	909 (65)
ER−	324 (23)	347 (25)
Missing data	164 (12)	139 (10)
Type of surgery		
BCS[a]	773 (55)	770 (55)
Mastectomy	616 (44)	618 (44)
Missing data	9 (1)	7 (1)
Radiotherapy		
Yes	1082 (77)	1063 (76)
No	316 (23)	332 (24)
Additional systemic therapy		
Yes	606 (43)	592 (42)
No	756 (54)	776 (56)
Missing data	36 (3)	27 (2)

[a] Breast-conserving surgery. ER, oestrogen receptor.

Table 1. Patient characteristics; all patients (N = 2793)

Exclusion criteria were bilateral breast cancer, previous treatment for breast cancer and previous systemic treatment for other cancers, distant metastases, and a poor World Health Organization (WHO) performance (>2). Patients were randomised to either receive one course of perioperative chemotherapy within 36 hrs after surgery or surgery alone. Patient characteristics are shown in Table 1. Patients who were younger than or equal to 50 years of age at the time of diagnosis were classified as premenopausal. Patients older than 50 years were considered postmenopausal. Tumour oestrogen receptor status (ER) was measured using a biochemical assay according to the best method locally available at every institution. A value of 510 fmol ER per mg protein was considered positive and a value of 0–9 fmol ER per mg protein was considered negative [11]. No information on the progesterone receptor status was collected. In the subgroup of node-negative premenopausal patients, ER status was also measured by immunohistochemistry in a central pathology review.

Treatment

Patients were treated with either (modified) radical mastectomy or breast-conserving surgery. Perioperative chemotherapy consisted of one single course of 50 mg/m^2 doxorubicin, 600 mg/m^2 5-fluorouracil, and 600 mg/m^2 cyclophosphamide (FAC), administered intravenously (i.v.) within 36 h after surgery. Axillary lymph node-positive premenopausal patients in the perioperative chemotherapy group were recommended to receive an extra five cycles of cyclophosphamide, methotrexate and 5-fluorouracil (CMF). Node-positive patients, younger than 50 years, who did not receive perioperative chemotherapy, were advised to have one conventional course of FAC followed by five cycles of CMF after surgery. Adjuvant hormonal therapy at the time was not routinely given in the management of breast cancer and the decision to give tamoxifen was therefore left to the discretion of the respective investigators. Radiotherapy was given in both arms. Postoperative radiation had to be started 6 weeks after surgery and was given in all cases in which surgery was considered not to be radical. A detailed description concerning the administration of radiotherapy was given previously [10].

Statistical considerations

The primary endpoint of the EORTC 10854 trial is overall survival. Secondary

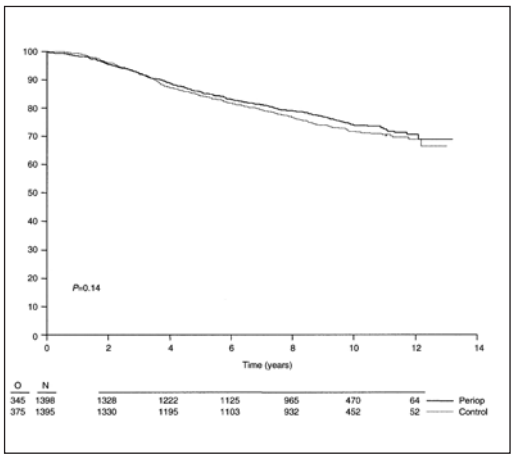

Figure 1. Overall survival

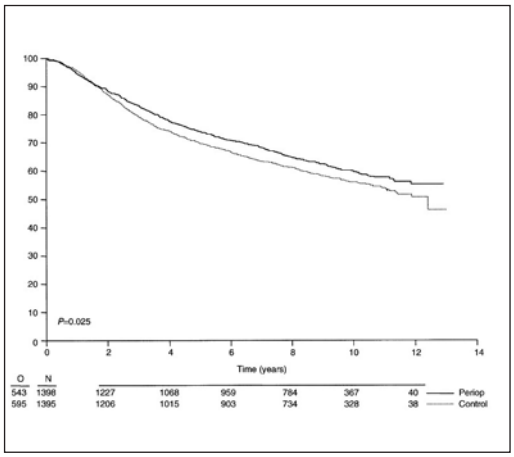

Figure 2. Progression-free survival

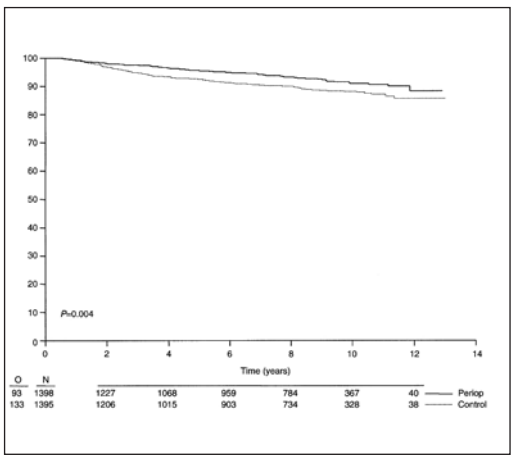

Figure 3. Locoregional recurrence as first event

endpoints are progression-free survival (PFS) and locoregional recurrence as the first event. PFS was defined as the time between the date of randomisation and the date of relapse (including secondary primary tumours and contralateral breast cancers) or death, whichever came first. A locoregional recurrence was defined as any recurrence in the breast or axilla. Only recurrences which occurred before the diagnosis of a distant metastasis and/or a new primary tumour were regarded as a locoregional recurrence as the first event and added to the analysis. Statistical calculations were performed using the 'intent-to-treat principle'. This means that all data are used in the statistical calculations, regardless of the fact whether a patient was eligible or not. PFS and overall survival curves, as well as locoregional recurrence rates, were estimated using the Kaplan–Meier method [12] and log-rank tests for the comparison of treatment effects were also used [13]. Cox proportional-hazard regression models [14] were used to estimate hazard ratios (HR) with their 95% confidence interval (CI). All tests were two-sided.

Results

Main analysis

From May 1986 to March 1991, 2795 patients were enrolled from 16 institutions from nine different countries onto this trial (Appendix). 41patien ts were ineligible. 2793 patients were included in the analysis. 2 patients, of whom information concerning randomisation was missing, were excluded from the analysis. After a median follow-up of 11 years, overall survival (71% versus 74%) was not significantly different between the two treatment groups (HR=0.9; 95% CI:

0.78–1.37; P = 0.14) (Fig. 1). However, PFS rates (53% versus 59%) are significantly different in favour of the perioperative chemotherapy group (HR=0.88; 95% CI: 0.78–0.98; P = 0.025) (Fig. 2). In line with the PFS results, locoregional control (86% versus 91%) was significantly different also in favour of the study-arm; (HR=0.69; 95% CI: 0.54–0.89; P = 0.004) (Fig. 3).

Subgroup analyses
To study the effect of perioperative chemotherapy in the specified groups of patients, subgroup analyses were carried out. However, one must interpret the outcome of these analyses with caution, as these were not preplanned analyses and are therefore only to be regarded as exploratory analyses.

Node-negative patients
1467 patients without axillary lymph node metastases were included in the trial. Node-negative patients in the study-arm did not have a significant better overall survival (HR=0.89; 95% CI: 0.70–1.13; P = 0.33) after perioperative FAC. However, a significant effect of perioperative FAC was observed on the PFS rate (HR=0.83; 95% CI: 0.70–0.99; P = 0.035). In addition, perioperative chemotherapy did have a profound effect on locoregional control in this subgroup, resulting in a significant difference in the locoregional control rates (HR=0.67; 95% CI: 0.48–0.93; P = 0.018) in favour of the study-arm.

Patients with T1 tumours
Patients with small tumours who received perioperative FAC did not benefit significantly in terms of overall survival (HR=0.86; 95% CI: 0.62–1.18; P = 0.34) and PFS (HR=0.92; 95% CI: 0.73–1.17; P = 0.50). However, perioperative chemotherapy had a marginally favourable effect on locoregional control (HR=0.64; 95% CI: 0.42–0.99; P = 0.047).

Premenopausal patients
Premenopausal patients have been established as the patients that benefit the most from adjuvant chemotherapy for breast cancer. Patients younger than or equal to 50 years of age at the time of diagnosis were deemed to be premenopausal patients in this study. Perioperative chemotherapy did not yield better overall survival rates (HR=0.91; 95% CI: 0.72–1.15; P = 0.43) or PFS rates (HR=0.87; 95% CI: 0.73–1.05; P = 0.15) in this subgroup. Moreover, the administration of one course of perioperative FAC did not result in better locoregional control rates (HR=0.75; 95% CI: 0.53–1.05; P = 0.096).

Timing of administration
We presumed that if timing influences treatment efficiency, this effect could only be demonstrated in patients who received extra adjuvant systemic therapy. To study the 'timing-effect' of one course of perioperative FAC, we therefore selected all patients who received prolonged adjuvant systemic treatment. In total, 1198 patients were included in the 'timing' analysis, but no effect of timing was found on overall survival (HR=0.65; 95% CI: 0.78–1.17; P = 0.65) or PFS (HR=0.94; 95% CI: 0.80–1.12; P = 0.50), respectively. In addition, no effect of timing was found on locoregional control (HR=0.88; 95% CI: 0.59–1.31; P = 0.52).

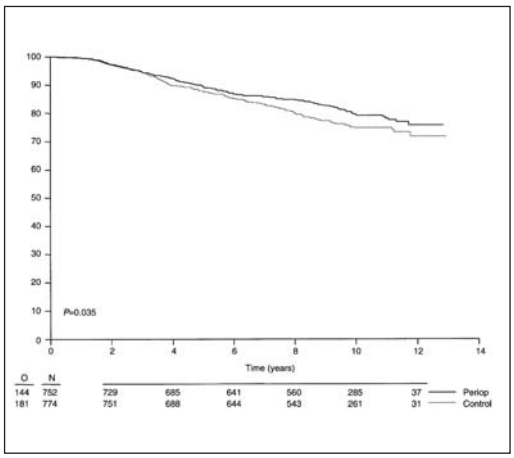

Figure 4. Overall survival in patients with 1 course of peri-operative FAC and no further systemic therapie versus patients treated with locoregional therapie alone.

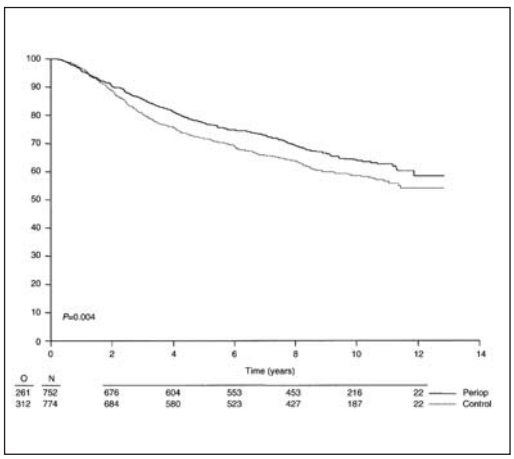

Figure 5. Progression-free survival in patients with 1 course of peri-operative FAC and no further systemic therapie versus patients treated with locoregional therapie alone.

Perioperative FAC as the sole systemic therapy

To test the absolute effect of one perioperative course of FAC, we compared the data of the patients in the control group who did not receive adjuvant systemic treatment with the patients who received perioperative chemotherapy alone in the study-arm. Patient characteristics are listed in Table 2. In this subset, which consisted of 1532 patients, a significant difference in favour of the perioperative chemotherapy group was shown in terms of overall survival (HR=0.80; 95% CI: 0.64–0.98; P = 0.035) and PFS (HR=0.79; 95% CI: 0.67–0.93; P = 0.004) (Figs. 4 and 5). Locoregional control was also significantly better in the study-arm (HR=0.60; 95% CI: 0.43–0.83; P = 0.0023) (Fig. 6).

Type of surgery

Perioperative chemotherapy did not have a significant effect on overall survival when patients were compared according to type of surgery (data not shown). Interestingly, perioperative chemotherapy has a significant impact on PFS (HR=0.84; 95% CI: 0.72–0.98; P = 0.031) and locoregional control (HR=0.71; 95% CI: 0.52–0.97; P = 0.029) in patients who underwent breast-conserving surgery, but not in patients who underwent mastectomy (HR=0.92; 95% CI: 0.78–1.08; P = 0.30 and HR=0.67; 95% CI: 0.43–1.04; P = 0.074, respectively).

ER-status

ER-status was known in 89% of the patients. 65% was ER-positive, 24% were ER-negative. In the ER-positive population, patients who received perioperative chemotherapy had a marginally significant better locoregional control rates (HR=0.71; 95% CI: 0.52–0.98; P = 0.04). Perioperative chemotherapy did not have a significant effect on overall survival and PFS in ER-positive patients. In ER-negative patients, locoregional control as well as PFS or overall survival rates were not significantly altered by perioperative chemotherapy.

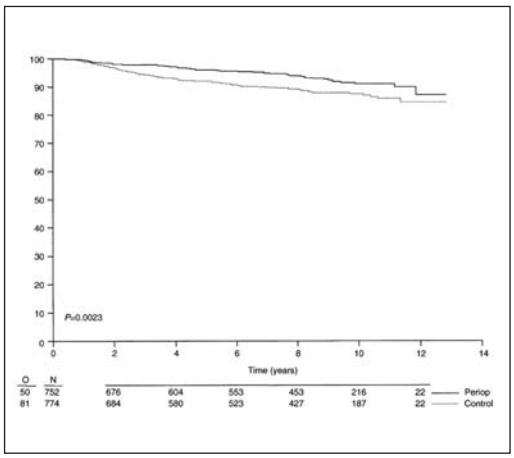

Figure 6. Locoregional recurrence in patients with 1 course of peri-operative FAC and no further systemic therapie versus patients treated with locoregional therapie alone

Discussion

This trial was set up primarily to study whether one course of chemotherapy given directly after surgery would yield better results in terms of prognosis than surgery alone in early stage breast cancer patients. As demonstrated in the main analyses, our results firmly demonstrate that perioperative chemotherapy after surgery leads to better locoregional control than surgery alone. We also showed that one course of perioperative FAC significantly improves progression-free survival rates.

Moreover, in a subset of patients who received locoregional treatment alone, one course perioperative FAC resulted in significant higher survival rates for those given perioperative chemotherapy. However, when patients who also received prolonged courses of chemotherapy or patients who received hormonal therapy were studied, no significant effect of perioperative administration of FAC could be shown.

Ever since chemotherapy became part of the therapeutic strategy against breast cancer, timing has been a matter of discussion. Several trials have studied early administration of (poly) chemotherapy after surgery [7–9,15–17]. These trials and their overall results are listed in Table 3. To date, however, no evidence of a significant effect of early timing of chemotherapy after primary tumour removal on treatment outcome has been demonstrated.

Since EORTC trial 10854 was initiated in 1986, the indication guidelines of adjuvant chemotherapy have shifted substantially. In the 1980s, chemotherapy was given on the basis of nodal- and menopausal status. At present, the decision to administrate chemotherapy is based on a combined evaluation of tumour stage, tumour grade and menopausal status in order to pursue a 10% disease-free survival gain after 10 years [19]. This shift has

	1× perioperative FAC $n=756$	Control $n=776$
	n (%)	*n* (%)
Age (years)		
⩽50	319 (42)	329 (42)
>50	437 (58)	447 (58)
Nodal status (pathological)		
Negative	666 (90)	651 (86)
Positive	71 (10)	104 (14)
Missing data	19 (3)	21 (3)
Tumour size (clinical)		
<2 cm	266 (36)	274 (36)
>2 cm	480 (64)	488 (64)
Missing data	10 (1.5)	14 (2)
ER status		
ER+	477 (63)	478 (62)
ER−	188 (25)	207 (27)
Missing data	91 (12)	91 (12)
Type of surgery		
BCS	494 (65)	490 (63)
Mastectomy	259 (34)	283 (36)
Missing data	3 (0.5)	3 (0.5)

BCS, breast conserving surgery; ER, oestrogen receptor; FAC, doxorubicin, 5-fluorouracil and cyclophosphamide.

Table 2. Patient characteristics; patients treated with 1 course of peri-operative FAC and no further systemic therapy versus patients treated with locoregional therapy alone

Study	*n* patients	Median follow-up (months)	TNM	Study design	RFS	OS
Nissen–Meyer The Scandinavian Adjuvant Chemotherapy Study 1 1965–1975	1026	205	T1-3, N0-2, M0 T1-2 80%	I: Mast→1×C→RT vs	I 52% (17 years FU)	I 36%
			N0 60%	II: Mast→RT	II 40% $P<0.001$	II 31% NS
Cancer Research Campaign 2 Trial [18] 1980–1985	2230	48	T1-2, N0-1, M0	I: Mast/BCT→1×C±RT & Tam	I ±67% (4 years FU)	NS
			T1 28% N0 49%	Vs. II: Mast/BCT→±RT & Tam	11 ±67%	NS
IBCSG (Trial V)[a] 1981–1985	2628	42	T1-3a, N0-1, M0	Ia: N0: Mast→1×CMFL (<36 h)	N0: Ia 77%	N0: Ia 90%
			N0 51%→(T1 41%)	Ib: N+: Mast→1×CMFL (<36 h) ±vs	(3.5 years FU) IIa 73% $P=0.04$	IIa 86% NS
				IIa: N0: Mast	N+: Ib 40%	N+: Ib 69%
				IIb: N+: 5×CMFLP±Tam	IIb 60% IIc 62%	IIb 74% IIc 80%
				IIc: N+: 6×CMFLP±Tam	$P<0.0001$	$P=0.011$
Sertoli 1985–1992	600	68	T1-3a, N0-2, M0	I: Mast/BCT→1×FEC (<72 h), if N+: 5×FEC & 6×CMF±RT & Tam	I 76%	I 88%
			T1 53% T2 39% N0 53%	vs	(5.7 years FU)	
				II: Mast/BCT→RT, if N+: 6× FEC and 6× CMF & Tam	II 70% $P=0.053$	II 84% NS
EORTC 10854 1986–1991	2795	132	T1-3, N0-1, M0	I: Mast/BCT→1×FAC±RT & Tam	I 65% (11 years FU)	I 74%
			T1 30% N0 53%	vs II: Mast/BCT→±RT & Tam	II 60% $P=0.025$	II 71% NS

Mast, mastectomy; C, cyclophosphamide; RT, radiotherapy; BCT, breast-conserving therapy; FEC, 5-fluorouracil epirubicin cyclophosphamide; CMF, cyclophosphamide methotrexate 5-fluorouracil; Tam, tamoxifen; CMFL, cyclophosphamide methothrexate 5-fluorouracil leucovorin; CMFLP, cyclophosphamide methothrexate 5-fluorouracil leucovorin prednisone; FAC, 5-fluorouracil doxorubicin cyclophosphamide; RFS, relapse-free survival; OS, overall survival; NS, not significant.

[a] The International (Ludwig) Breast Cancer Study Group (trial V).

Table 3. Perioperative chemotherapy trials

led to a higher fraction of early stage breast cancer patients receiving chemotherapy nowadays compared with two decades ago. If this trial was to be executed now, the subgroup of patients who would not receive additional systemic therapy would be much smaller. The question therefore is whether patients in which additional systemic therapy is not indicated nowadays (i.e. node-negative patients with small tumours and favourable histological parameters) would benefit from one course of chemotherapy.

Based upon our results, this question is difficult to answer. However, the presented results can be of use in designing future clinical trials.

However, the outcome that one course of chemotherapy as a sole systemic therapy is able to induce a modest, but significant increase in overall survival and better locoregional control rates in a subset of low-risk breast cancer patients regardless of the tumour stage and menopausal status, is an important finding. One could advocate on the basis of this finding that all patients with early stage breast cancer should at least receive some form of chemotherapy. Arguments against this policy have always been based on treatment-related toxicities and the long-term risks of

developing haematological malignancies after chemotherapy that would not be counterbalanced by the merits of systemic cytotoxic therapy in node-negative breast cancer patients. This group of patients, however, is known to have a 70–80% long-term survival rate after locoregional therapy alone, meaning that 20–30% of these patients will eventually develop distant metastases and subsequently die of breast cancer. The argument concerning toxicity may be real in a setting where adjuvant chemotherapy consists of prolonged schemes like in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-13 trial [20]. This trial investigated whether 12 cycles of methotrexate and 5-fluorouracil followed by leucovorin after surgery would yield better results than surgery alone in premenopausal, node-negative, ER-negative patients. In accordance with our results, this trial demonstrated a significant better disease-free survival rates and better locoregional control in favour of the adjuvant chemotherapy group. A comparable study conducted by Amadori and colleagues [21] using CMF showed similar data. In the EORTC trial 10854, only one course of an anthracycline-containing chemotherapeutic regimen was given. This type of adjuvant treatment induced a significant improvement in progression-free survival and locoregional control in the overall analysis, as well as overall survival in a large subset of patients without intolerable mortality and morbidity [22].
Therefore, one should be aware of these data when developing a treatment strategy for patients with early stage node-negative breast cancer.

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CHAPTER 3

Preoperative Chemotherapy in Primary Operable Breast Cancer: Results From the European Organization for Research and Treatment of Cancer Trial 10902

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Abstract

Purpose:
To evaluate whether preoperative neoadjuvant chemotherapy in patients with primary operable breast cancer results in better overall survival (OS) and relapse-free survival rates and whether preoperative chemotherapy permits more breast-conserving surgery procedures than postoperative chemotherapy.
Patients and Methods:
Six hundred ninety-eight breast cancer patients (T1c, T2, T3, T4b, N0 to 1, and
M0) were enrolled onto a randomized phase III trial that compared four cycles of fluorouracil, epirubicin, and cyclophosphamide administered preoperatively versus the same regimen administered postoperatively (the first cycle administered within 36 hours after surgery). Patients were followed up for OS, progression free survival (PFS), and locoregional recurrence (LRR).
Results:
At a median follow-up of 56 months, there was no significant difference in terms of OS (hazards ratio: 1.16; P = 0.38), PFS (hazards ratio: 1.15; P = 0.27), and time to LRR (hazards ratio: 1.13; P = 0.61). Fifty-seven patients (23%) were downstaged by the preoperative chemotherapy, whereas 14 patients (18%) underwent mastectomy and not the planned breast-conserving therapy.
Conclusion:
The use of preoperative chemotherapy yields similar results in terms of PFS, OS, and locoregional control compared with conventional postoperative chemotherapy. In addition, preoperative chemotherapy enables more patients to be treated with breast-conserving surgery. Because preoperative chemotherapy does not improve disease outcome compared with postoperative chemotherapy, future trials should involve
quality-of-life studies to investigate whether patients will benefit from this treatment modality.

Introduction

Trials that studied the role of adjuvant chemotherapy in the management of primary operable breast cancer conducted during the 1970s and 1980s showed significant improvements in progression-free and overall survival [1]. Conventionally, adjuvant systemic therapy is administered after local treatment in early breast cancer [2]. However, since the introduction of conservative treatment modalities, there has been considerable interest in the efficacy of preoperative chemotherapy to decrease tumor size. One of the potential benefits of preoperative chemotherapy is the more frequent usage of breast-conserving treatment modalities. Moreover, it has been hypothesized that preoperative chemotherapy has a more powerful effect on survival compared with postoperative chemotherapy. The rationale for these hypotheses comes from several biologic premises. Findings in various animal models [3-5] showed an increase of labeling index in residual tumor cells as well as an increase of circulating growth-stimulating factors after the removal of the primary tumor in murine models. In these models, adjuvant systemic therapy administered before the removal of the primary tumor impaired this increase in cell-kinetic mechanisms [6,7].
In addition, Goldie and Coldman [8] developed a hypothesis, which implies that as a tumor cell population increases, an ever-expanding number of drug-resistant

phenotypic variants arise as a result of spontaneous somatic mutations. Although the merits of preoperative chemotherapy in the treatment of locally advanced breast cancer are well established, the feasibility of preoperative chemotherapy in early breast cancer is still a matter of discussion.
The European Organization for Research and Treatment of Cancer (EORTC) Breast Cancer Cooperative Group started a randomized trial in 1991 to investigate the value of preoperative chemotherapy in early breast cancer, EORTC trial 10902. The primary objective of this trial was to test whether preoperative chemotherapy yields better results in terms of progression-free and overall survival than the same chemotherapeutic regimen administered postoperatively. Another objective was to study whether preoperative chemotherapy would permit more breast-conserving therapies by reducing primary tumor size. A third objective was to determine whether preoperative chemotherapy resulted in better locoregional control, especially after breast-conserving surgery.
An additional objective of the study was to evaluate the response of the primary tumor to preoperative chemotherapy and to correlate this response to disease-free and overall survival. This report compares the outcome of 698 women with early breast cancer randomized to receive either preoperative chemotherapy or the same regimen administered postoperatively.

Patients and methods

Patient Characteristics
Between April 1991 and May 1999, 698 women were enrolled onto the EORTC study 10902 in 17 institutions in 14 countries. Patients had primary operable breast cancer (T1c, T2, T3, T4b, N0 to 1, and M0). Breast cancer was preferably diagnosed by core needle biopsy (CNB) or by fine-needle aspiration cytology as part of triple diagnosis. For the diagnosis of T1c tumors, CNB was mandatory. CNB was also mandatory in case of doubt or suspicion of carcinoma-in-situ after fine-needle aspiration. Exclusion criteria consisted of age older than 70 years; bilateral breast cancer; previous treatment for breast cancer; presence of distant metastases; pregnancy or lactation at the time of diagnosis; previous or current other malignancies except adequately treated basal or squamous carcinoma of the skin or cervix uteri; World Health Organization performance status more than 2; active cardiac disease; and severe hematologic, renal, or hepatic abnormalities. All patients gave informed consent before entering onto the trial. Randomization was performed centrally by the EORTC Data Center. At randomization, patients were stratified for institution, age (50 years or 50 years), clinical tumor size, clinical nodal status (N or N), and planned type of surgery (mastectomy or breast-conserving surgery). Patients 50 years old or younger were considered to be premenopausal, and those older were deemed postmenopausal. Tumor estrogen receptor (ER) status was estimated by the ligand binding assay technique or by the immunohistochemistry technique. For the ligand binding assay, a concentration $\geq$ 10 fmol ER/mg protein was considered positive, and a value lower than 10 fmol ER/mg protein was considered negative. If the ER status was measured by immunohistochemistry, positivity or negativity was determined according to the scoring systems used by the individual institutions. There was no

standardization of the assay, so general cutoff points cannot be given for immunohistochemistry (EORTC Manual for Clinical Research in Breast Cancer) [9].

Treatment

Treatment consisted of surgery in combination with either preoperative or postoperative chemotherapy. Surgery consisted of either a modified radical mastectomy or breast-conserving surgery (wide local excision of the tumor or quadrantectomy plus axillary dissection and adjuvant radiotherapy). Before treatment, investigators had to report which type of surgery was indicated at the time of diagnosis. Subsequently, the planned type of surgery and performed type of surgery were compared to investigate whether preoperative chemotherapy induced a higher rate of breast-conserving surgery. Guidelines and selection criteria that concerned surgery were given in the study protocol, but patient selection for breast-conserving therapy was ultimately left up to the treating surgeon.

Chemotherapy consisted of four cycles of preoperative fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 (FEC) administered intravenously, at intervals of every 3 weeks. In the preoperative chemotherapy group, surgical therapy followed within 4 weeks of the fourth course of chemotherapy. In the postoperative chemotherapy group, the first cycle was administered within 36 hours after surgery, as has been advocated before [10,11]. Administration of FEC was delayed for a maximum of 2 weeks as a result of either hematologic, hepatic and renal, or gastrointestinal toxicities on day 1 of any cycle. Dose modifications were assessed according to the guidelines stipulated by the EORTC Breast Cancer Cooperative Group [9].

Adjuvant radiotherapy was administered after surgery in the preoperative chemotherapy group. In the postoperative chemotherapy group, it was decided to administer irradiation after the completion of chemotherapy. This way, radiotherapy did not interfere with the chemotherapeutic regimen, in which the first course was to be administered preoperatively. All patients who underwent breast-conserving therapy received irradiation of the whole breast. Other recommended guidelines for radiotherapy, as stipulated in the protocol, consisted of chest wall and parasternal irradiation in patients with an initial tumor of 5 cm or more in its largest dimension and irradiation of the infraclavicular and supraclavicular fossa in patients with a positive infraclavicular node after lymph node dissection. Radiotherapy was indicated in all cases where surgery was not considered to be radical. Specified dose at the target volume was 50 gray, administered in four or five weekly fractions in 5 weeks. For the parasternal/infrasupraclavicular fossa and chest wall, at least 45 Gy had to be administered in four or five weekly fractions in 4.5 to 5 weeks. However, some hospitals used their own radiation protocol. Patients $\geq$ 50 years of age also received tamoxifen 20 mg daily for at least 2 years, regardless of their ER and nodal status.

End Points

The primary end point of this study was overall survival. Survival time was defined as the time between randomization and death from any cause. Secondary end points were progression-free survival and locoregional recurrence. Progression-free survival was defined as the time between the date of randomization and the date of disease relapse (including distant metastases, locoregional recurrences, secondary primary

tumors, and contralateral breast cancers) or death, whichever came first. Locoregional recurrence was defined as a recurrence in the ipsilateral breast or in the ipsilateral regional lymph nodes, including supraclavicular nodes. Time to locoregional recurrence was defined as the time between date of randomization and locoregional recurrence, regardless of whether the locoregional recurrence was the first event or not. It is well known that the administration of adjuvant chemotherapy is a considerable burden to the patient in terms of quality of life. However, not many data are available that concern the effects of preoperative chemotherapy on quality of life compared with conventional postoperative chemotherapy. Therefore, a quality-of-life study program was set up that was, however, unsuccessful as a result of poor compliance.

Tumor Response

Clinical tumor size and nodal status were estimated before the start of chemotherapy as well as at the time of surgery by both palpation and mammography. The product of the two greatest perpendicular diameters was used to compare tumor size before and after chemotherapy, as defined by the International Union Against Cancer criteria [12]. A clinical complete response (cCR) was considered a complete disappearance of all clinically detectable malignant disease by palpation as well as mammography. Tumor specimens from patients who underwent preoperative chemotherapy were examined for the presence of microscopic residual tumor to correlate the clinical absence of tumor with pathologic evaluation. If no signs of residual malignant cells at the primary site and axillary lymph nodes were seen with histologic examination, this was scored as a pathologic complete response (pCR). Clinical tumor response to preoperative chemotherapy was assessed at the time of surgery. If the tumor had become undetectable before completion of the four cycles of preoperative chemotherapy, chemotherapy was continued as outlined in the protocol. Clinical partial response was defined as 50% decrease in total tumor size after four cycles of preoperative chemotherapy at the time of surgery. An increase of 25% in tumor size after a minimum of two courses of preoperative chemotherapy was considered to be progressive disease (PD).

In patients with clinically negative nodes at randomization, the development of palpable nodes during the administration of preoperative chemotherapy was considered evidence of PD. After a diagnosis of PD, patients immediately underwent surgery before completing the preoperative chemotherapy schedule. If the PD was not primary operable, the patient was declared to have experienced treatment failure, and subsequent treatment was left to the discretion of the responsible clinician. If patients did not meet one of the above-mentioned criteria after four cycles of chemotherapy, they were classified as having stable disease.

Follow-Up

All patients were followed up until death. In the first 2 years after surgery, patients were seen at least every 6 months and, in the following 3 years, every 6 to 12 months. Minimal requirements for follow-up were physical examination, locoregional evaluation, and performance scale assessment, with mammography, chest x-ray, and alkaline phosphatase and lactate dehydrogenase measurements every year postoperatively. One institute used CA 15-3 measurements instead of lactate dehydrogenase.

Characteristic	Total		Preoperative Chemotherapy		Postoperative Chemotherapy	
	No.	%	No.	%	No.	%
Total patients	698		350		348	
Age						
≤ 50 years	385	55.2	192	54.9	193	55.5
> 50 years	313	44.8	158	45.1	155	44.5
Axillary nodal status*						
Negative	333	47.7	171	48.8	162	46.6
Positive	355	50.9	176	50.3	179	51.4
Unknown	10	1.4	3	0.9	7	2.0
Tumor size†						
≤ 2 cm	96	13.8	49	14.0	47	13.5
> 2 cm	592	84.8	298	85.1	294	84.5
T4b	36	5.2	22	6.3	14	4.0
Unknown	10	1.4	3	0.9	7	2.0
ER status						
Negative	141	20.2	60	17.2	81	23.3
Positive	337	48.3	159	45.4	178	51.1
Unknown	220	31.5	131	37.4	89	25.6
Planned surgical procedure						
Mastectomy	536	76.8	268	76.6	268	77.0
Lumpectomy	151	21.6	77	22.0	74	21.3
No surgery	11	1.6	5	1.4	6	1.7
Additional radiotherapy						
Yes	452	64.8	237	67.7	215	61.8
No	246	35.2	113	32.3	133	38.2
Additional hormonal therapy						
Yes	273	60.9	139	39.7	134	38.5
No	425	39.1	211	60.3	214	61.5

*Clinical nodal status.
†Clinical tumor size.

Table 1. Baseline Patient Characteristics

Statistical Aspects

All analyses were based on the intent-to-treat principle. The two treatment arms were compared by the log-rank test for the time-to-event end points. The differences between the two treatment groups were graphically depicted by Kaplan and Meier curves. As it seemed from literature studies that both nodal and menopausal status could have a substantial impact on the treatment comparison, it was decided before the start of the analysis to perform subgroup analyses for these two variables. The nominal significance level for each subgroup analysis was adjusted by the Bonferroni method. Thus, in the cases of the four subgroup analyses for nodal and menopausal status, the nominal significance level for each of the subgroup analyses was set at .0125 (.05 divided by 4). The trial was designed to detect a 10% survival difference at 5 years (from 75% to 85%) with 80% power, for which 102 events were needed.

Results

Patients

Of the 698 patients, 350 patients were randomized to receive preoperative chemotherapy, and 348 patients were randomized to the postoperative chemotherapy group (first cycle of FEC administered within 36 hours after surgery). Tumor and patient characteristics were well distributed between the two treatment arms

Event	Preoperative Chemotherapy (n = 350)	Postoperative Chemotherapy (n = 348)
Cardiac function		
No dysfunction	297	289
Asymptomatic dysfunction	19	16
Symptomatic dysfunction, no therapy needed	1	5
Symptomatic dysfunction, responsive to therapy	1	3
Nausea/vomiting		
None	49	46
Nausea	89	85
Transient vomiting	119	125
Vomiting requiring therapy	69	62
Intractable vomiting	2	2
Diarrhea		
None	288	278
< 2 days	30	35
> 2 days	10	6
Requiring therapy	–	1
Drug fever		
None	299	283
< 38 °C	20	25
38-40 °C	8	9
> 40 °C	–	–
Fever with hypotension	–	1
Alopecia		
None	23	21
Minimal hair loss	65	37
Moderate patchy alopecia	68	68
Complete reversible alopecia	148	172
Drug allergy		
None	311	307
Edema	12	11
Bronchospasm, no therapy needed	1	1
Bronchospasm, therapy needed	1	–
Oral toxicity		
None	222	242
Erythema	59	45
Erythema/ulcers, normal dietary intake	31	22
Erythema/ulcers, requiring liquid diet	2	2
Thromboembolic complications		
None	314	305
Superficial phlebitis	1	1
Deep phlebitis	–	1
Embolism	–	–
No data	35	41

Table 2. Overall toxicity distribution

(Table 1), except for breast-conserving surgery rates, which were, as expected, higher in the preoperative chemotherapy group. Twenty-one patients were considered ineligible because of inadequate staging (n = 17), a World Health Organization performance status more than 2 (n = 3), or age greater than 70 years (n = 1).

Chemotherapy

Overall, 635 patients (91%) received the planned chemotherapy dose (321 patients [92%] in the study arm and 314 patients [90%] in the control arm). Sixty-three patients (8%) received less than 75% of the planned dose. Nineteen patients (3%) who began chemotherapy did not complete it (11 patients [3%] in the study arm and eight patients [2.5%] in the control group). Thirty-eight patients who were randomized to the preoperative chemotherapy group underwent treatment modification because of treatment-related febrile neutropenia versus 44 patients in the postoperative chemotherapy group. No treatment-related deaths were reported. An overview of chemotherapy-related toxicity is given in Table 2. Furthermore, four patients who were randomized to receive postoperative chemotherapy received preoperative chemotherapy, and three patients who were randomized to receive preoperative chemotherapy received postoperative chemotherapy. Forty patients did not receive chemotherapy. Of these patients, 16 patients were deemed ineligible. Eight patients refused chemotherapeutic treatment, two patients in the postoperative chemotherapy group did not receive chemotherapy as stipulated by the protocol because of postoperative complications, and seven patients did not receive chemotherapy for unknown reasons. From a further seven patients, no information that concerned treatment specifications or follow-up was received, even after repeated queries. Unfortunately, for approximately 30% of the patients, information that concerned ER status was missing. This was partly a result of the fact that this information was not mandatory and that tamoxifen was given irrespective of ER status in patients $\geq$ 50 years of age.

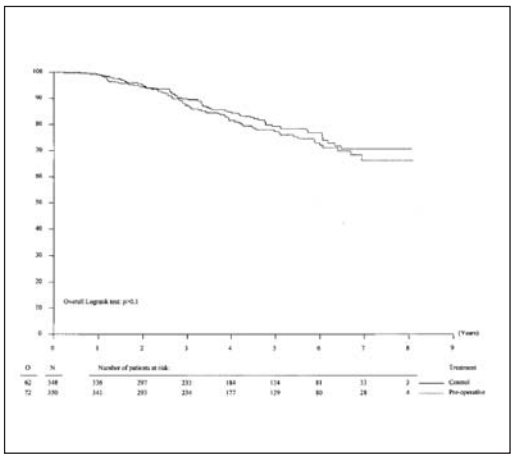

Figure 1. Overall survival

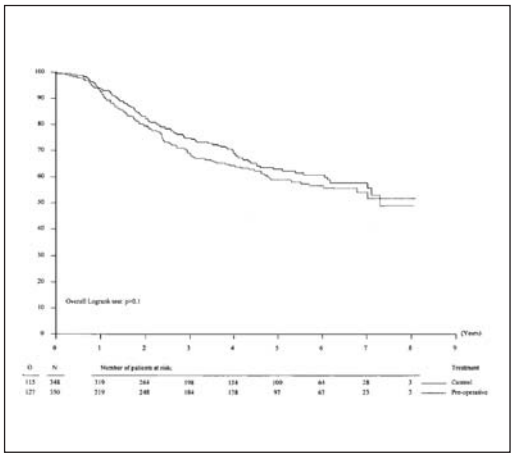

Figure 2. Time to disease progression or death

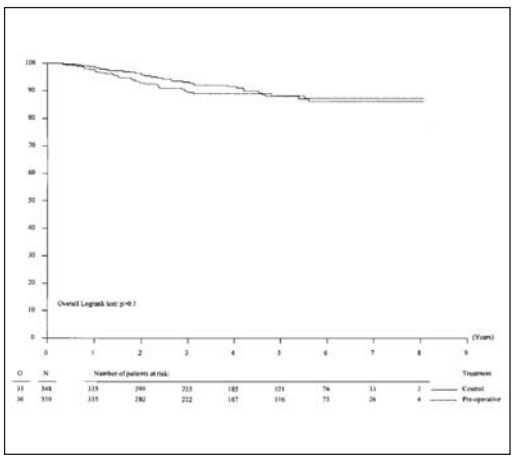

Figure 3. Locoregional recurrence rate

Surgery

Four hundred sixty-five patients underwent modified radical mastectomy, and 199 patients underwent breast-conserving surgery. Nineteen patients who underwent lumpectomy did not receive adjuvant radiotherapy. Thirty-eight percent of the patients on the study arm who were treated with mastectomy received radiotherapy, and 43% of the patients on the control arm who were treated with mastectomy received radiotherapy.

In the preoperative chemotherapy group, 20 patients did not receive the surgical treatment stipulated by the study protocol, versus 14 patients in the postoperative group. At the time of axillary clearance, at least six nodes had to be obtained for pathologic examination, and in 77% of the patients entered onto the study, 10 or more axillary nodes were examined. Surgical complications consisted of 16 grade 1/2 wound infections in the preoperative chemotherapy group, versus 25 grade 1/2 wound infections and two grade 3/4 wound infections in the postoperative chemotherapy group that required antibiotic treatment. Anticoagulant prophylaxis was administered on the basis of experience from previous trials that studied the efficacy of perioperative chemotherapy [13,14]. No severe postsurgical thromboembolic complications were observed in the postoperative chemotherapy group.

Overall Survival, Progression-Free Survival, and Locoregional Recurrence

Of the 698 randomized patients, 232 experienced relapse, from which 124 patients died. Furthermore, 10 patients died without experiencing a relapse. No significant differences between the two treatment arms were observed for progression-free and overall survival.

Tumor Response	Patients Randomized to Preoperative Chemotherapy	
	No.	%
CR	23	6.6
PR	148	42.3
No change	139	39.7
Progression of disease	5	1.4
Not assessable	16	4.6
No preoperative chemotherapy*	19	5.4

*Six ineligible patients; 10 patients refused treatment; three patients received postoperative chemotherapy.

Table 3. Clinical Tumor Response

	Patients Randomized to Preoperative Chemotherapy							
	T1 (n = 49)		T2 (n = 197)		T3/T4 (n = 101)		Unknown (n = 3)	Total (N = 350)
	No.	%	No.	%	No.	%	No.	No.
pT0/pTis	–	–	10	5	5	5	–	15
pT1	44	90	88	45	17	17	–	149
pT2	3	6	76	39	42	41	–	121
pT3/pT4	–	–	3	1	24	24	–	27
pTx	2	4	20	10	13	13	3	38

Table 4. Clinical tumor size vs. pathological tumor size after pre-operative chemotherapy

Overall survival after 4 years was 82% in the preoperative group and 84% in the postoperative group (hazards ratio [HR], 1.16; 95% confidence interval [CI], 0.83 to .63; *P* = 0.38) (Fig 1). Progression-free survival rates after 4 years for the preoperative and postoperative groups were 65% and 70%, respectively (HR, 1.15; 95% CI, 0.89 to 1.48; *P* = 0.27) (Fig 2). To date, 69 patients have experienced a locoregional recurrence, 36 in the preoperative chemotherapy group and 33 in the postoperative chemotherapy group. Time to locoregional recurrence was not significantly different between the two treatment arms (HR, 1.13; 95% CI, 0.70 to 1.81; *P* = 0.61) (Fig 3). Sixty-two of these patients experienced a locoregional recurrence as first event. Subgroup analyses were performed in an exploratory way for nodal status and menopausal status. The largest difference was found in clinical node-negative patients (n = 385) in terms of overall survival (HR, 1.77; 95% CI, 1.03 to 3.02; *P* = 0.04) and progression-free survival (HR, 1.53; 95% CI, 1.03 to 2.28; *P* = 0.03) in favor of the postoperative chemotherapy group. These *P* values failed to be significant compared with the adjusted nominal significance level of 0.0125.

Tumor Response

Table 3 shows the clinical tumor response after chemotherapy. An overall objective response was observed in 49% of the patients randomized to the preoperative chemotherapy group. Twenty-three patients (6.6%) experienced a cCR and progression of disease was seen in five patients (1.4%). Of the twenty-three patients who experienced a cCR, only six patients did not have any invasive tumor left. Apart from the six patients who experienced a pCR in accordance with the clinical assessment, seven other patients were microscopically free of tumor at the primary site and axilla after four cycles of preoperative chemotherapy but were not classified as having a cCR. The thirteen patients without evidence of residual malignancy do have a significant advantage in terms of overall survival (HR, 0.86; 95% CI, 0.77 to 0.96; *P* = 0.008) compared with patients who still had residual tumor cells left after preoperative chemotherapy.

Next, we tested the prognostic significance of clinical objective tumor response in terms of survival in a multivariate model together with clinical tumor size, clinical nodalstatus, and ER status. Clinical objective response, however, was not a significant prognostic factor. Table 4 shows the correlation between clinical tumor sizebefore preoperative chemotherapy and pathologic tumor size after preoperative chemotherapy. Both in the preoperative and postoperative arms, 14% of the patients had a clinical tumor size less than 2 cm at the time of diagnosis. After four courses of preoperative chemotherapy, 47% of the patients had a pathologic tumor size less than

	Patients Randomized to Preoperative Chemotherapy							
	N0 (n = 171)		N1 (n = 164)		N2 (n = 12)		Unknown (n = 3)	Total (N = 350)
	No.	%	No.	%	No.	%	No.	No.
pN0	95	56	36	22	2	17	–	133
pN1	64	37	121	74	8	66	–	193
pN2	–	–	–	–	2	17	–	2
pN unknown	12	7	7	4	–	–	3	22

Table 5. Clinical nodal status vs. pathological nodal status after pre-operative chemotherapy

	Preoperative Chemotherapy (n = 350)		Postoperative Chemotherapy (n = 348)	
Pathologic Tumor Size	No.	%	No.	%
T0	15	4	4	1
T1	149	43	88	25
T1 + T0	164	47	92	26
T2	121	35	173	50
T3	19	5	40	11
T4	8	2	12	3
Tx	38	11	31	10

Table 6. Differences in pathological tumor size between both study-arms

No. of Pathologic Positive Lymph Nodes	Preoperative Chemotherapy		Postoperative Chemotherapy	
	No.	%	No.	%
N0	134	38	111	32
N1-3	109	31	122	35
N4+	84	24	97	28
Other	23	7	18	5

Table 7. Differences in pathological nodal status between both study-arms

	Patients Randomized to Preoperative Chemotherapy				
	Performed MRM		Performed BCT		
	No.	%	No.	%	Total
Planned MRM	189	77	57	23	246
Planned BCT	14	18	63	82	77
Total	203	–	120	–	323

Abbreviations: BCT, breast-conserving therapy; MRM, modified radical mastectomy.

Table 8. Planned versus performed type of surgery

2 cm, whereas 26% of the patients in the postoperative arm had a pathologic tumor size less than 2 cm. Nodal status characteristics are listed in Table 5. At the time of diagnosis, 49% of the patients in the preoperative group and 47% of the patients in the postoperative group had clinical negative axillary lymph nodes. Pathologic examination after surgery showed a lower percentage of negative axillary lymph nodes in both the preoperative and the postoperative group 38% and 35%, respectively. Differences in pathologic tumor size and axillary nodal status between both study arms are listed in Table 6 and Table 7.

Downstaging

Before treatment, investigators had to report whether mastectomy or breast-conserving surgery was indicated. In the preoperative chemotherapy group, the rate of breast-conserving therapy was higher than in the postoperative chemotherapy group. In the preoperative chemotherapy group, 57 patients (23%) underwent breast-conserving surgery and not the planned mastectomy (Table 8), versus 14 patients (18%) who underwent mastectomy and not the planned breast-conserving surgery.

In the preoperative chemotherapy group, all patients who underwent breast-conserving surgery were compared according to their planned type of surgery. Patients who were planned for mastectomy but underwent breast-conserving therapy because of down-staging of the tumor did worse in terms of overall survival (HR, 2.53; 95% CI, 1.02 to 6.25) compared with patients who were initially planned to receive breast-conserving therapy and were treated accordingly (Fig 4), which suggested a relation between the outcome of locoregional treatment and tumor response. This, however, is not a randomized comparison. The observed difference in disease outcome might be a result of a selection bias as a result of different patient characteristics in the two groups. Therefore, we evaluated patient characteristics to detect potential differences. Of the patients who were downstaged, 35% were clinically node-negative before the start of chemotherapy versus 46% in the group in which breast-conserving therapy

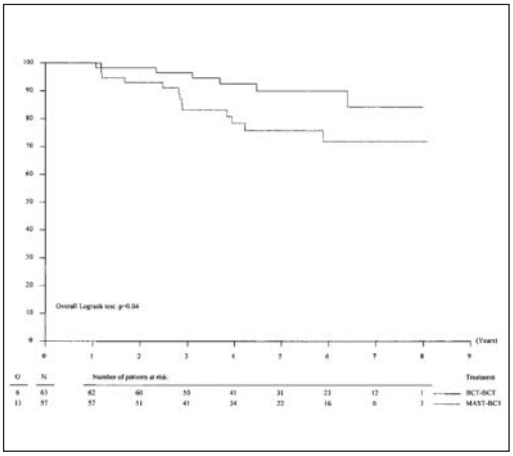

Figure 4. Survival in planned versus downstaged mastectomy

Characteristic	BCT ⇒ BCT No.	BCT ⇒ BCT %	MRM ⇒ BCT No.	MRM ⇒ BCT %
Total patients, N = 120	63		57	
Deaths	6	9.5	13	23
Age				
≤ 50 years	31	49	35	61
> 50 years	32	51	22	39
Clinical nodal status				
Negative	29	46	20	35
Positive	34	54	37	65
Clinical tumor size				
≤ 2 cm	49	78	44	77
> 2 cm	14	22	13	23
Pathologic nodal status				
Negative	32	51	36	63
Positive	31	49	21	37
Pathologic tumor size*				
≤ 2 cm	49	79	41	75
> 2 cm	13	21	14	25
ER status				
Negative	8	13	9	15
Positive	34	54	26	46
No data	21	33	22	39
Tumor response				
Overall response, cCR + cPR	39	62	44	77
No change	19	30	12	21
Progression of disease	1	2	1	2
No data	4	6	–	

Abbreviation: FEC, fluorouacil, epirubicin, and cyclophosphamide.
*For three patients pathologic tumor size was unknown.

Table 9. Planned versus performed type of surgery in patients who received 4 cycles of pre-operative FEC

was already indicated before the administration of chemotherapy. After chemotherapy, the pathologic node-negative rates were 63% and 51%, respectively. This indicates that axillary nodal response to preoperative chemotherapy was actually better in downstaged patients than in the others who underwent breast-conserving surgery. Remarkably, there was an obvious similarity in both clinical tumor size before preoperative chemotherapy and pathologic tumor size between the two groups (Table 9). These differences may well be a result of differences in initial tumor-node-metastasis system classification between both of these patient groups. However, the fact that clinical T and N stages were to a large extent similar does not support this hypothesis. Conclusively, these findings do support the assumption that radical conservative surgery, especially after downstaging, may be more difficult because of the fact that tumor-free margins are more difficult to assess after preoperative chemotherapy.

Quality of Life

Unfortunately, only one institution has collected quality-of-life data on a total number of 20 patients. These data are insufficient to report here or to draw any conclusion on the effect of preoperative chemotherapy on quality of life.

Discussion

In 1986, the Breast Cancer Cooperative Group started a trial to study the effectiveness of one course of fluorouracil, doxorubicin, and cyclophosphamide administered perioperatively within 36 hours after surgery in early breast cancer patients. This regimen resulted in significantly improved locoregional control and disease-free survival rates [10]. Therefore, the logical next step was to start a trial that would study the qualities of preoperative chemotherapy.

EORTC trial 10902 was designed to address whether preoperative chemotherapy yields the same or better results in terms of overall and disease-free survival compared with the same type of chemotherapy administered postoperatively and whether preoperative chemotherapy allows more breast-conserving therapies. A third objective was to assess the value of tumor response to preoperative chemotherapy as a predictor of disease outcome. Several randomized, clinical, phase III trials that compared postoperative adjuvant chemotherapy and preoperative chemotherapy were performed in the past two and a half decades. These trials have been listed in Tables 10 to 12. A comparison of the results of these trials is difficult because of the fact that the study protocols differ substantially in design and chemotherapeutic regimen. However, to date none of these trials, including EORTC trial 10902, has been able to show a positive effect of preoperative chemotherapy in terms of progression-free or overall survival.

Two trials, conducted by Mauriac and Scholl [15-20] initially reported a significant positive effect of preoperative chemotherapy on progression-free and overall survival, but after a longer period of follow-up, the significant benefit of preoperative chemotherapy on survival had disappeared. In addition, in the Mauriac trial, locoregional control was worse in the preoperative arm compared with the standard arm. Of the trials listed in Tables 10 through 12, the National Surgical Adjuvant Breast and Bowel Project B-18 [21,22] trial is similar in terms of study design to the EORTC trial. Fisher et al [21,22] studied the efficacy of four courses of preoperative doxorubicin and cyclophosphamide in 1,523 women with primary operable breast cancer. Contrary to the EORTC trial, the first course of postoperative chemotherapy was not administered directly after surgery. Overall survival, progression-free survival, and recurrence rates were not significantly different between the study and the postoperative population. Interestingly, the authors observed a significantly higher rate of ipsilateral breast recurrences in patients who underwent a lumpectomy as a result of downstaging compared with the rate in patients who underwent a lumpectomy as planned. We observed a similar effect on overall survival in patients who underwent breast-conserving surgery as a result of downstaging. However, we did not find such an effect on locoregional recurrence rates. Nevertheless, these results demonstrate an important potential danger induced by tumor downstaging. Even if clinical assessment of tumor response demonstrates tumor shrinkage as a result of chemotherapy, there is no absolute proof that the tumor has actually shrunken in size. Several authors demonstrated a loss of density in tumors treated by chemotherapy but no shrinkage [23-25]. In our trial, the mammographies before and after preoperative chemotherapy of 83 patients were revised and correlated with histologic data, and a similar effect on the assessment of tumor response was found (data not shown). Thus, treating downstaged tumors with more breast-conserving modalities may result in a higher false-negative rate of tumor-negative surgical margins. In the B18 trial, a clinical overall response was seen in 79%, whereas 35% of the preoperative chemotherapy group experienced a cCR. A pCR, however, occurred in only 9% of these patients. Disease-free, relapse-free, distant disease-free, and overall survival were better in women whose tumors showed a pCR compared with those patients with residual disease. This is in accordance with the results from the EORTC 10902 study and the experience of other investigators [19,26].

In the EORTC trial, tumor response to preoperative chemotherapy was low in

Study	No. of Patients	Median Follow-up (months)	Clinical TNM (%)	Diagnosis	Treatment
Ragaz, 1979-1988, phase II	212		Unknown	FNA	I: 1× CMF → Mast/BCT → 8× CMF
					II: Mast/BCT → 9× CMF
Mauriac, 1985-1989, phase III	272	124	II-IIIa	Drill biopsy	I: 3× EVM + 3× MTV → T0; RT/T1; BCT/>T1; Mast
			T2: 82		
			T3: 18		
					II: Mast; if ER−/N+ → 3× EVM + 3× MTV
			N0: 44		
Semiglazov, 1985-1990, phase III	271	53	IIb-IIIa	FNA	I: 1-2× TMF + RT → Mast → 4-5× TMF
			T1-2: 18		
			T3: 82		II: RT → Mast → 6× TMF
			N0: 31		
Scholl, 1986-1990, phase III	414; 390 assessable	105	T2-3 N0-1	Drill biopsy	I: 4-6× FAC → RT ± Mast/BCT (if T ≠ 0)
			T2: 73		
			T3: 27		II: RT ± Mast/BCT (if T ≠ 0) → 4× FAC
			N0: 41		
NSABP B-18, 1988-1993, phase III	1523	60	T1-3 N0-1	FNA	I: 4× AC → Mast/BCT
			T1: 28		
			T2: 59		II: Mast/BCT → 4× AC
			T3: 13		
			N0: 74		
Makris, Powles, 1990-1995, phase III	309; 293 assessable	48	T1: 12	Trucut biopsy/FNA	I: 4× 3M/2M + TAM → Mast/BCT ± RT → 4× 3M/2M
			T2: 82		
			T3: 5		
			N0: 81		II: Mast/BCT ± RT → 8× 3M/2M + TAM
EORTC 10902, 1991-1999, phase III	698	56	T1c-4b N0-1	Core needle biopsy/FNA	I: 4× FEC → Mast/BCT ± RT
			T1: 14		
			T2: 58		II: Mast/BCT → 4× FEC → ± RT
			T3: 21		
			T4: 5		
			N0: 48		

Abbreviations: Mast, mastectomy; RT, radiotherapy; T, tumor; CMF, cyclophosphamide, methotrexate, and fluorouracil; EVM, epirubicin, vincristine, and methotrexate; MTV, mitomycin, thiopeta, and vinblastine; TMF, thiopeta, methotrexate, and fluorouracil; FAC, fluorouracil, doxorubicin, and cyclophosphamide; AC, doxorubicin and cyclophosphamide; 3M/2M, methotrexate, mitoxanthrone, ± mitomycin; Tam, tamoxifen; TNM, tumor-node-metastasis; FNA, fine-needle aspiration.

Table 10. Similar trials

comparison with the response rates described in the literature [15-22,26-36]. Clinical overall tumor responses after four to six cycles of preoperative polychemotherapy range approximately between 65% and 90%. The cCR rates vary between 10% and 30%. However, the pCR rate usually is much lower, resulting in a poor correlation. Controversially, in the EORTC 10902, trial 49% of the patients who received preoperative chemotherapy experienced a clinical overall response as assessed by palpation and 7% of the study-population had a cCR. The remarkable discrepancy between our results and those of other authors is difficult to explain. The vast majority of patients received the chemotherapeutic dose stipulated by the protocol, ie, four courses of FEC containing epirubicin 60 mg/m^2, which means that the regimen was well tolerated. Now, one could argue that total cumulative doses of epirubicin lower than 300 mg/m^2 are suboptimal. However, higher doses of epirubicin in a combined chemotherapy schedule have so far not been demonstrated to be more effective in terms of overall or relapse-free survival in primary operable breast cancer [37-40]. Several studies [23-25] have addressed this discrepancy between cCR and pCR

Study	Clinical Overall Response (%)	cCR (%)	pCR (%)	Initial Type of Surgery (%)
Ragaz, 1979-1988, phase II				
Mauriac, 1985-1989, phase III	NA	I: 33	NA	I: 30 BCT 33 RT alone 37 Mast II: All Mast
Semiglazov, 1985-1990, phase III	I: 72 II: 60 (palpation & mammography)	I: 35 II: 28	I: 29 II: 19	I: All Mast II: All Mast
Scholl, 1986-1990, phase III	I: 82 II: 85	I: 30 II: 41	NA	I: 51 RT alone 31 BCT 18 Mast II: 46 RT alone 32 BCT 23 Mast
NSABP B-18, 1988-1993, phase III	I: 80	I: 36	I: 13	I: 67 BCT 33 Mast II: 60 BCT 40 Mast ($P = .002$)
Makris, Powles, 1990-1995, phase III	I: 84	I: 22	I: 7 (7 DCIS alone)	I: 90 BCT 10 Mast II: 77 BCT 22 Mast ($P < .003$)
EORTC 10902, 1991-1999, phase III	I: 49 (palpation & mammography)	I: 7	I: 4	I: 35 BCT 60 Mast II: 22 BCT 74 Mast

Abbreviation: NA, not available.

Table 11. Similar trials

Study	5-Year LRR (%)	5-Year DFS (%)	5-Year DDFS (%)	5-Year OS (%)
Ragaz, 1979-1988, phase II				I: 74 (10 years) II: 73 (10 years) $P = .9$
Mauriac, 1985-1989, phase III	I: 74 (10 years) II: 91 (10 years) $P = ?$	NA	Δ NS/NA (10 years)	Δ NS/NA (10 years)
Semiglazov, 1985-1990, phase III	NA	I: 81 II: 72 $P < .05$	NA	I: 86 II: 78 $P > .05$
Scholl, 1986-1990, phase III	I: 73 II: 81 Δ NS	I: 59 II: 55 $P = .4$	I: 73 II: 68 $P = .09$	I: 65 (10 years) II: 60 (10 years) $P = .18$
NSABP B-18, 1988-1993, phase III	I: 86 II: 89 $P = .23$ IBTR	I: 67 II: 67 $P = .99$	I: 73 II: 73 $P = .7$	I: 80 II: 80 $P = .83$
Makris, Powles, 1990-1995, phase III	I: 97 (4 years) II: 96 (4 years) Δ NS	I: 82 (4 years) II: 80 (4 years) $P = .8$	NA	I: 78 (4 years) II: 78 (4 years) $P = 1.0$
EORTC 10902, 1991-1999, phase III	I: 89 (4 years) II: 92 (4 years) $P = .61$ all events	I: 64 (4 years) II: 69 (4 years) $P = .27$	I: 69 (4 years) II: 72 (4 years) $P = .23$	I: 81 (4 years) II: 85 (4 years) $P = .38$

Abbreviations: LRR, time to locoregional recurrence; DFS, disease-free survival; DDFS, distant disease-free survival; OS, overall survival; Δ NS, no significant difference; IBTR, ipsilateral breast tumor recurrence.

Table 12. Similar trials

and compared clinical measurements with mammographic and ultrasonographic measurements. These studies generally demonstrate an overestimation of tumor response by palpation alone. In the majority of clinical trials that study the effects of preoperative chemotherapy, clinical measurements are commonly used to assess the amount of tumor response, as described by the guidelines of the International Union Against Cancer. In EORTC trial 10902, tumor response was measured by using the data of both palpation and mammographic tumor measurements. Unfortunately, we were not able to demonstrate this confounding error induced by using palpation measurements alone because of a poor registration of measurements. Conclusively, mammographic measurements should be implemented in the assessment of tumor response on a standard basis to give an objective idea of the degree of tumor response. Moreover, in the case of a cCR, it may be helpful to perform ultrasonography in selecting those patients who do not require surgery after preoperative chemotherapy and in localizing abnormalities in those who do [41,42].

In locally advanced and primary inoperable breast cancer, the purpose of preoperative treatment is to enable adequate local treatment, favorably leading to breast conservation. In patients with stage I or II breast cancer who are candidates for breast-conserving therapy irrespective of preoperative chemotherapy, the goal of preoperative chemotherapy is unclear. Some investigators argue that tumor response to preoperative chemotherapy is an independent predictor of treatment outcome. Therefore, it could be of benefit for breast cancer patients to adjust systemic adjuvant treatment at an early stage if tumor response to preoperative chemotherapy is inadequate.

Controversially, preoperative chemotherapy might lead to overtreatment of breast cancer patients. This can be explained by the fact that patients receive systemic treatment regardless of histologic staging of the tumor and axillary nodal status. The breast-conserving therapy rate was higher in the preoperative chemotherapy group in comparison with the postoperative chemotherapy group. This finding, together with the equal locoregional control rate in both groups, advocates the advantageous role of primary chemotherapy in breast-conserving management. On the other hand, 14% of patients who initially were supposed to undergo breast-conserving surgery received a modified radical mastectomy. This suggests that a delay in surgical treatment as a result of the use of primary chemotherapy can result in a more aggressive type of surgical management of breast cancer in a considerable number of patients. In addition, a hypothesis-generating analysis that compared survival rates of patients who underwent breast-conserving surgery as a result of downstaging of the tumor with patients who underwent breast-conserving surgery as initially was planned showed a favorable trend for the latter group of patients. Therefore, it can be hypothesized that patient prognosis is determined by the initial tumor stage and not tumor stage after preoperative chemotherapy.

The idea that changes in surgical management after preoperative chemotherapy are solely because of either increase or decrease of tumor volume is arguable. Because preoperative chemotherapeutic regimens take approximately 3 months to complete, patient or doctor surgical preferences may be altered for subjective reasons during this period of time.

The use of preoperative or primary chemotherapy was introduced approximately three decades ago in locally advanced breast cancer. Since then, its role in the

management of locally advanced breast cancer has been firmly established. However, the advantages are not clear in early breast cancer. Despite the fact that preoperative chemotherapy may permit more breast-conserving treatment modalities, there may be problems, for instance in achieving adequate locoregional control as a result of the difficulty of assessing tumor margins after the administration of preoperative chemotherapy.

Moreover, it has become clear that the supposed survival benefits of preoperative chemotherapy based on preclinical data are not exerted in primary operable breast cancer patients. Although benefits of preoperative chemotherapy in early breast cancer patients are less clear compared with the locally advanced breast cancer patients, the potential to enhance breast-conserving therapy makes it an attractive treatment modality. Postmenopausal early breast cancer patients especially might benefit from preoperative chemotherapy, although preoperative chemotherapy may be less beneficial for young breast cancer patients who are at a higher risk of recurrence of disease, especially after primary conservative therapy. Although this trial did not show such differences (data not shown), other investigators have found young age to be a strong independent prognostic factor for recurrence after breast-conserving therapy [11,43,44].

Moreover, the possibility of studying the effects of chemotherapy on well-established tumor characteristics as well as experimental tumor markers makes chemotherapy in the preoperative setting highly attractive for translational research purposes [45]. The comparison of core needle biopsies with the same tumor after systemic treatment is a worthwhile reason to continue preoperative chemotherapy trials in early breast cancer.

Unfortunately, not many data concerning quality-of-life issues in relation to preoperative chemotherapy are available in the literature. Quality-of-life studies, however, have been performed to investigate the effects of breast-conserving therapy versus mastectomy and fail to show a clear benefit for the conservative treatment modality, except for a slightly less impaired body image [46-49]. Considering the fact that preoperative as well as postoperative chemotherapy seems to yield similar results in terms of prognosis, this might be a conclusive factor on the decision of which chemotherapeutic strategy should be chosen. Therefore, the role of preoperative chemotherapy should be studied in future trials that focus on translational research, equivalence, quality of life, and local control, rather than trying to detect overall and progression-free survival differences.

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CHAPTER 4

Pathological complete response to preoperative anthracycline-based chemotherapy in operable breast cancer: the predictive role of p53 expression

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Abstract

The aim of this retrospective study was to identify markers capable of predicting pathological complete (pCR) and overall clinical tumour response to preoperative anthracycline-based chemotherapy and clinical outcome in women with operable breast cancer. Therefore, we used the pre-treatment core biopsies from 107 patients who were enrolled in the EORTC trial 10902 to analyse tumour characteristics and the oncogenic markers *bcl-2*, *p53*, ER, PgR, *HER2*, and *p21*. Median follow-up was 7 years (95% confidence interval [CI], 6.89-7.45). pCR was seen in seven patients (6.5%) and was associated with improved overall survival (hazards ratio, 0.39; 95% CI, 0.05-2.56; P = 0.30). In multivariate logistic regression analysis, pCR was independently predicted by *p53* overexpression estimated by immunohistochemistery (odds ratio [OR], 16.83; 95% CI, 1.78-159.33; P = 0.01). Fifty-eight patients showed clinical tumour response (>50% decrease in tumour size), however responders experienced no benefit in clinical outcome. Clinical tumour response was independently predicted by *p53* overexpression (OR, 5.57; 95% CI, 1.58-19.65; P = 0.008) and small clinical tumour size (OR, 10.26; 95% CI, 2.01-52.48; P= 0.005). In multivariate Cox regression analysis, negative pathological lymph node status, low tumour grade and use of tamoxifen showed improved overall survival. In conclusion, our data suggest *p53* expression is of predictive significance in anthracycline containing chemotherapeutic regimens.

Introduction

Preoperative chemotherapy for large but early stage breast cancer has been subject of interest for over two decades. The efficacy of preoperative chemotherapy has been demonstrated in several prospective randomized trials showing similar survival and locoregional control rates in patients receiving preoperative chemotherapy and postoperative chemotherapy. Tumour downstaging due to preoperative chemotherapy was found to increase breast-conserving therapy rates [1,2].

Response of breast tumours following preoperative chemotherapy can be assessed either clinically or pathologically. Patients with responding tumours showed an improved overall and disease-free survival and particularly pathological complete response (complete disappearance of malignant cells on microscopic examination; pCR) is suggested as a surrogate marker for these clinical endpoints [2-5].

Translational research using preoperative tumour tissue biopsies is an excellent study model to analyse the predictive value of different tumour characteristics for response to chemotherapy [6]. To date, a large number of oncogenic markers in breast cancer have been studied using classical survival analyses [7,8]. However, published data on the relation between tumour characteristics and pathological and clinical tumour response are still limited.

We used data from a prospective randomized trial comparing pre- versus postoperative chemotherapy to study the correlation between pathological and clinical tumour response and patient and tumour characteristics. Tumour characteristics included oncogenic markers analysed on pre-treatment biopsy specimens and classic tumour characteristics. In addition, we assessed the prognostic significance of these clinical characteristics including pathological and clinical tumour response on overall and distant disease-free survival.

Patients and Methods

Patients
All patients participated in a prospectively randomized trial (EORTC 10902) that compared preoperative chemotherapy versus the same chemotherapeutic regimen administered postoperatively in patients with operable breast cancer [1]. This trial accrued 698 women with early stage breast cancer between 1991 and 1999. The eligibility criteria for this trial have been described previously [1]. Efforts were made to obtain diagnostic biopsy material from all patients randomized to preoperative chemotherapy. For the present analysis we included patients who had received preoperative chemotherapy with known pathological and clinical tumour response and from whom biopsy material were available for pathological evaluation. We used pre-treatment biopsy material for immunohistochemical analyses in order to avoid interference of the chemotherapeutic regime on the expression levels of the oncogenic markers [9,10].

Treatment
Chemotherapy consisted of four cycles of preoperative fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 (FEC) administered intravenously, at intervals of every 3 weeks. Surgical therapy followed within 4 weeks of the fourth course of chemotherapy. Surgery consisted of either a modified radical mastectomy or breast-conserving surgery (wide local excision of the tumour or quadrantectomy plus axillary dissection and adjuvant radiotherapy). Recommended guidelines for radiotherapy have been described previously [1]. If radiotherapy was indicated, it was administered after surgery. Patients older than 50 years also received tamoxifen 20 mg daily for at least 2 years, regardless of their oestrogen receptor and nodal status.

Pathological tumour response
Surgical tumour specimens were examined for the presence of microscopic residual tumour. If no signs of residual malignant cells at the primary site were seen with histological examination, this was scored as a pathological complete response (pCR). The specimens still containing invasive malignant cells were graded as pINV.

Clinical tumour response
The tumour response classification system used in EORTC 10902 was according to the UICC [11]. Clinical tumour size was scored by the local investigators before the start of chemotherapy as well as at the time of surgery by both clinical examination and mammography. The product of the two greatest perpendicular diameters was used to compare tumour size before and after chemotherapy.
Clinical complete response (cCR) was defined as complete disappearance of all clinically detectable malignant disease by palpation and mammography. Clinical partial response (cPR) was defined as $\geq$ 50% decrease in total tumour size after four cycles of preoperative chemotherapy. An increase of $\geq$ 25% in tumour size after a minimum of two courses of preoperative chemotherapy was considered to be progressive disease (cPD). If patients did not meet one of the above-mentioned criteria after four cycles of chemotherapy, they were classified as having stable

disease (cSD). For the purpose of this analysis, we distinguished between patients with overall clinical response (cCR and cPR) and patients with non-responding tumours (cSD and cPD).

Histology and immunohistochemistery
Blocks were collected from core needle biopsies taken before the start of chemotherapy. All immunohistochemical (IHC) analyses were performed in one reference laboratory by two pathologists who were unaware of the clinical outcome of the patients.
Invasive carcinomas were histologically graded according to the method of Bloom and Richardson, adapted by Elston and Ellis [12]. *BCL-2* was assessed using Clone 124 (Boehringer Mannheim, Germany) and scored according to van Slooten and colleagues (staining ≥ 3 indicates positive status) [13]. *P53* accumulation was detected using Do-7 monoclonal antibody (NovaCastra, Newcastle on Tyne, United Kingdom) and a semi-quantitative system based on the sum of the mean staining intensity (0 to 3; none to strong) and an estimation of the percentage of positive cell nuclei (0 to 4; 0% to > 75%); this allowed a sum score of 0 to 7, with staining ≥ 4 being considered positive [14]. Oestrogen receptor status (ER) was estimated immunohistochemically using the monoclonal antibody DAKO-ER 1D5 (Dako, Glostrup, Denmark; staining indicates positive status) [14]. Progesterone receptor status (PgR) was measured using mPRI monoclonal antibody (Transbio, Paris, France; staining indicates positive status) [14]. *HER2* expression was assessed using the monoclonal antibody 3B5 (staining score 0, 1 and 2 indicates a negative result and ≥ 3 resembles a positive result) [15]. *P21* was measured using the monoclonal antibody EA10 (Calbiochem, Cambridge, MA, USA; ≥ 3 indicates a positive result) [13,14].

Statistical Methods
Overall survival time was defined as the time between randomization and death from any cause. Distant disease-free survival was defined as the time between the date of randomization and the date of distant disease relapse or death from any cause whichever came first. Correlations between the two tumour response classification systems and patient and tumour characteristics were tested using the Pearson's Chi-square test or the Fisher's Exact test. A multivariate logistic regression model was fitted that was based on all characteristics that had a P value up to 0.10 in the univariate analysis. The effect of patient and tumour characteristics on the survival endpoints was assessed using the Cox proportional hazards regression model to estimate hazard ratios and their 95% confidence intervals. A multivariate Cox regression model was fitted that was based on all characteristics that had a P value up to 0.10 in the univariate analysis. Survival curves of the tumour response groups were estimated using the Kaplan-Meier technique. The statistical analyses were performed using SPSS software (SPSS Inc., Chicago, II, USA). A two-sided significance level of 0.05 was used.

Results

Patient and tumour characteristics
EORTC 10902 trial randomised 350 patients to preoperative chemotherapy and 321

patients received this allocated treatment. Tumour response was assessable in 301 patients. For 194 of these patients no data was available on histological and immunohistochemical analyses. Thus, we were able to include 107 patients in this study. Patient and tumour characteristics are listed in Table 1.
The median age at diagnosis was 49.8 years. Seven (6.5%) pathological complete responses following preoperative chemotherapy were seen and 58 (54%) patients had clinically responding tumours. All but one of the patients with pCR were clinically graded as responders. At the time of analysis, the median follow-up period was seven years (95% confidence interval [CI], 6.89-7.45); thirty-one (29%) patients have died and of the patients alive, ten (9.3%) have experienced a distant relapse. Although otherwise stipulated in the treatment protocol, nine (17%) women older than 50 years were not administered to tamoxifen treatment and four (7.4%) women in the younger group did use tamoxifen.

Prognostic value of pathological tumour response
The association of pathological tumour response with overall survival and distant disease-free survival is shown in Figure 1 and 2, respectively. Patients with complete pathological response had an overall survival rate after 7 years of 86% compared with 68% for patients with residual disease (pINV) on pathological examination (hazards ratio [HR], 2.87; 95% CI, 0.39-21.14; P = 0.30). Patients with a complete pathological response had a distant disease-free survival rate at 7 years follow-up of 86%, compared to 59% for patients with pINV (HR, 3.62; 95% CI, 0.50-26.33; P = 0.21).

Prognostic value of clinical tumour response
Patients with a clinical tumour response had an overall survival rate after 7 years of 67% (Figure 3). Non-responders had an overall survival rate of 75% (HR, 0.71; 95% CI, 0.34-1.45; P = 0.35). Patients with clinical response had a distant disease-free survival rate after 7 years of 61% compared to 61% for patients with non-responding tumours (HR, 0.94; 95% CI, 0.51-1.74; P = 0.84; Figure 4).

Predictive characteristics for pathological and clinical response
We assessed the predictive value of patient and tumour characteristics and expression of oncogenic markers in pre-treatment core needle biopsies.
Table 2 lists the relationships between dichotomized characteristics and pathological and clinical tumour response. Pathological lymph node status and *p53* status were significantly correlated with pathological tumour response. Including both variables in the multivariate analysis (Table 3) revealed an independent relationship of positive *p53* expression with pCR (odds ratio [OR], 16.83; 95% CI, 1.78-159.33; P = 0.01) and a non-significant association of negative pathological lymph node status. Clinical tumour response was predicted by clinical tumour size, tumour grade, *p53* status, PgR status, and *HER2* status (Table 2). In multivariate analysis, positive *p53* expression (OR, 5.57; 95% CI, 1.58-19.65; P = 0.008) and small clinical tumour size (OR, 10.26; 95% CI, 2.01-52.48; P = 0.005) remained as independent predictive factors of clinical tumour response (Table 3).

Prognostic characteristics for overall survival and distant disease-free survival
Table 4 shows the prognostic value of patient and tumour characteristics in

Table 1. Patient and tumour characteristics

Characteristic	N	%
Age at diagnosis		
< 40 years	11	10
≥ 40 years	96	90
Type of surgery		
mastectomy	57	53
BCT	50	47
Tamoxifen		
no	59	55
yes	48	45
Radiotherapy		
no	20	19
yes	87	81
Clinical tumour size†		
T1	18	17
T2	64	60
T3	21	19
T4	4	3
Clinical tumour response‡		
complete	7	7
partial	51	48
stable disease	47	44
progressive disease	2	2
Pathological tumour size‡		
pT0/pCR	7	7
pT1	43	40
pT2	48	45
pT3	7	7
pT4	2	2
Clinical lymph node status†		
negative	65	58
positive	45	42
Pathological lymph node status‡		
negative	45	42
positive	65	58
Grade†		
I	13	12
II	69	64
III	19	18
unknown	6	6
BCL-2 expression†		
negative	25	23
positive	59	55
unknown	23	22
P53 expression†		
negative	73	68
positive	26	24
unknown	8	8
ER status†		
negative	21	20
positive	71	66
unknown	15	14
PgR status†		
negative	50	47
positive	49	46
unknown	8	7
HER2 expression†		
negative	92	86
positive	10	9
unknown	5	5
P21 expression†		
negative	45	42
positive	47	44
unknown	15	14

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; BCT= breast conservative treatment; pCR= pathological complete response

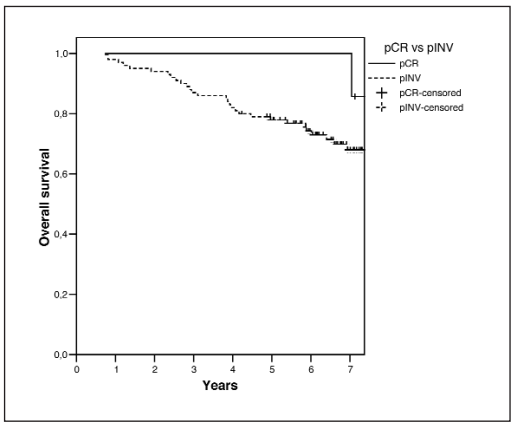

Figure 1. Pathological tumour response and overall survival.
pCR= pathological complete response;
pINV= invasive tumour cells on pathological examination

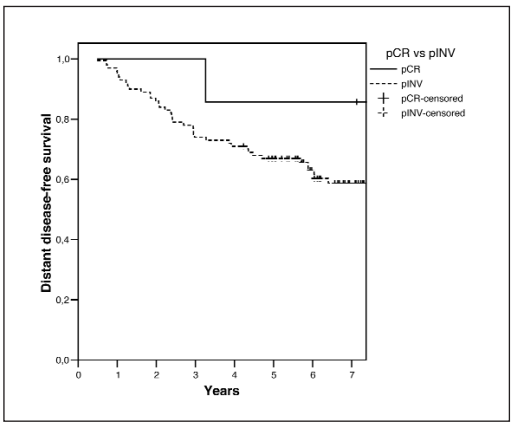

Figure 2. Pathological tumour response and distant disease-free survival.
pCR= pathological complete response;
pINV= invasive tumour cells on pathological examination

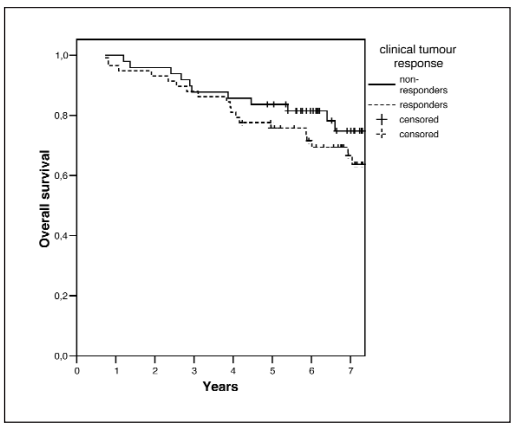

Figure 3. Clinical tumor response and overall survival

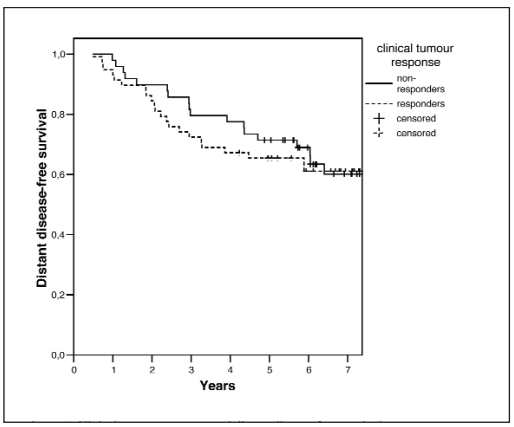

Figure 4. Clinical tumor response and distant disease-free survival

predicting clinical outcome. In this univariate analyses, significant prognostic variables for overall and distant disease-free survival were age, use of tamoxifen, and pathological lymph node status. In addition, histological tumour grade was significantly associated with overall survival. Overexpression of *p53* was non-significantly related with poorer overall (HR, 1.72; 95% CI, 0.82-3.62; P = 0.15) and distant disease-free survival (HR, 1.39; 95% CI, 0.70-2.74; P = 0.35).
The prognostic factors found to be trend significant in the univariate analyses were included in multivariate analyses to identify independent prognostic factors of overall and distant disease-free survival (Table 5). Negative pathological lymph node status and use of tamoxifen were both independently associated with improved overall and distant disease-free survival. In addition, histological tumour grade III was an independent prognostic factor of poorer overall survival.

Characteristic	Pathological tumour response					Clinical tumour response				
	pCR		pINV			responders		non-responders		
	N	%	N	%	P value	N	%	N	%	P value
Age at diagnosis										
< 40 years	0	0	11	100		8	73	3	27	
≥ 40 years	7	7	89	93	1.00	50	52	46	48	.22
Clinical tumour size†										
≤ 2 cm	0	0	18	100		16	89	2	11	
> 2 cm	7	8	82	92	.60	42	47	47	53	.001
Clinical lymph node status†										
negative	4	7	58	93		36	58	26	42	
positive	3	7	42	93	1.00	22	49	23	51	.43
Pathological lymph node status‡										
negative	6	13	39	87		28	62	17	38	
positive	1	2	61	98	.04	30	48	32	52	.17
Grade†										
I & II	5	6	77	94		40	49	42	51	
III	2	11	17	89	.61	14	74	5	26	.05
BCL-2 expression†										
negative	3	12	22	88		15	60	10	40	
positive	3	5	56	95	.36	27	46	32	54	.23
P53 expression†										
negative	1	1	72	99		32	44	41	56	
positive	5	19	21	81	.004	21	81	5	19	.001
ER status†										
negative	3	14	18	86		14	67	7	33	
positive	3	4	68	96	.13	34	48	37	52	.13
PgR status†										
negative	4	8	46	92		33	66	17	34	
positive	2	4	47	96	.68	19	39	30	61	.007
HER2 expression†										
negative	5	5	87	95		46	50	46	50	
positive	1	10	9	90	.47	8	80	2	20	.09
P21 expression†										
negative	3	7	42	93		25	56	20	44	
positive	3	6	44	94	1.00	23	49	24	51	.53

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; pCR= pathological complete response; pINV= invasive tumour cells on pathological examination

Table 2. Pathological and clinical tumour response and dichotomized patient and tumour characteristics

Characteristic	Pathological complete response			Clinical response		
	Odds Ratio	95 % CI	P value	Odds Ratio	95 % CI	P value
Negative pathological lymph node status‡	8.47	0.88-81.82	.07			
Positive *p53* expression†	16.83	1.78-159.33	.01	5.57	1.58-19.65	.008
Tumour size ≤ 2 cm†				10.26	2.01-52.48	.005
Grade III†				1.58	0.41-6.13	.51
Negative PgR status†				2.37	0.89-6.31	.08
Positive *HER2* expression†				2.93	0.47-18.14	.25

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; CI= confidence interval

Table 3. Multivariate logistic regression analyses of correlation between dichotomized tumour characteristics and pathological complete tumour response (N=99) and clinical response (N=94)

Discussion

In this analysis, we demonstrated a significant independent association between *p53* overexpression and pathological complete and clinical tumour response to 4 cycles of preoperative FEC. However, pCR as a prognostic factor for overall survival as well as for distant disease-free survival did in this patient population not reach statistical significance although a clear trend was demonstrated (Figures 1 and 2). This finding is in accordance with other randomised controlled trials studying preoperative chemotherapy in primary operable breast cancer while pCR was in these studies a significant prognostic factor [2, 16-18].

In this study, clinical tumour response showed no prognostic benefit (Figures 3 and 4). This result is in discordance with other reports [2,16,17] and most probably resembles a selection bias as the data derived from our larger study population suggest an association of non-response with poorer overall survival (HR, 1.43; 95% CI, 0.91-2.24; P = 0.12). However, the fact that clinical responders in the current group had no favourable prognosis implies that the results concerning the predictive value of characteristics for clinical response must be interpreted with caution. Moreover,

Characteristic	Overall Survival					Distant Disease-Free Survival				
	N/O	7-years percent	Hazards ratio	95% CI	P value	N/O	7-years percent	Hazards ratio	95% CI	P value
Age at diagnosis										
< 40 years	11/7	45	1.00			11/7	36	1.00		
≥ 40 years	96/24	73	0.34	0.14-0.78	.01	96/34	64	0.40	0.18-0.92	.03
Type of surgery										
mastectomy	57/17	66	1.00			57/24	58	1.00		
BCT	50/14	74	0.83	0.41-1.69	.62	50/17	64	0.72	0.36-1.33	.29
Tamoxifen										
no	59/24	60	1.00			59/30	48	1.00		
yes	48/7	84	0.34	0.15-0.79	.01	48/11	77	0.39	0.19-0.77	.01
Radiotherapy										
no	20/8	56	1.00			20/9	51	1.00		
yes	87/23	74	0.52	0.23-1.16	.11	87/32	63	0.69	0.33-1.44	.32
Clinical tumour size†										
≤ 2 cm	18/4	72	1.00			18/5	67	1.00		
> 2 cm	89/27	70	1.30	0.45-3.72	.63	89/36	59	1.57	0.61-4.00	.35
Clinical tumour response‡										
responders	58/19	67	1.00			58/22	61	1.00		
non-responders	49/12	75	0.71	0.34-1.45	.35	49/19	61	0.94	0.51-1.74	.84
Pathological tumour size‡										
≤ 2 cm	50/13	75	1.00			50/17	64	1.00		
> 2 cm	57/18	66	1.41	0.69-2.88	.35	57/24	58	1.43	0.77-2.67	.26
Pathological tumour response‡										
pCR	7/1	86	1.00			7/1	86	1.00		
pINV	100/30	68	2.87	0.39-21.14	.30	100/40	59	3.62	0.47-26.33	.21
Clinical lymph node status†										
negative	62/17	73	1.00			62/22	64	1.00		
positive	45/14	67	1.27	0.62-2.57	.51	45/19	56	1.33	0.72-2.55	.37
Pathological lymph node status‡										
negative	45/8	84	1.00			45/8	81	1.00		
positive	62/23	61	2.82	1.23-6.44	.01	62/33	46	4.15	1.90-9.06	.00
Grade†										
I & II	82/20	74	1.00			82/29	64	1.00		
III	19/9	55	2.23	1.01-4.91	.05	19/9	50	1.58	0.75-3.33	.23
BCL-2 expression†										
negative	25/8	70	1.00			25/11	54	1.00		
positive	59/12	79	0.62	0.26-1.53	.30	59/16	73	0.55	0.25-1.18	.12
P53 expression†										
negative	73/19	73	1.00			73/27	62	1.00		
positive	26/11	58	1.72	0.82-3.62	.15	26/12	52	1.39	0.70-2.74	.35
ER status†										
negative	21/9	60	1.00			21/9	56	1.00		
positive	71/19	71	0.57	0.26-1.26	.16	71/27	61	0.81	0.38-1.74	.59
PgR status†										
negative	50/19	62	1.00			50/23	52	1.00		
positive	49/12	75	0.58	0.28-1.19	.14	49/16	68	0.64	0.34-1.20	.16
HER2 expression†										
negative	92/27	70	1.00			92/37	59	1.00		
positive	10/3	69	1.11	0.34-3.66	.87	10/3	70	0.82	0.25-2.66	.74
P21 expression†										
negative	45/12	72	1.00			45/16	65	1.00		
positive	47/17	64	1.56	0.74-3.28	.24	47/12	53	1.44	0.75-2.76	.28

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy;
N/O= number of patients/ observed number of events; CI= confidence interval; BCT= breast

Table 4. Univariate Cox regression analyses of characteristics predicting for overall and distant disease-free survival

Characteristic	Overall survival			Distant disease-free survival		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Positive pathological lymph node status[‡]	4.30	1.71-10.82	.002	5.19	2.35-11.46	.000
Use of tamoxifen	0.41	0.17-1.00	.05	0.34	0.17-0.69	.003
Age younger than 40 years	2.13	0.81-5.65	.13	2.28	0.98-5.32	.06
Grade III[†]	3.02	1.28-7.12	.01			

N= number of patients; CI= confidence interval; [‡] Assessed after the delivery of chemotherapy; [†] Assessed prior to the delivery of chemotherapy

Table 5. Multivariate Cox regression analyses of characteristics predicting for overall (N=101) and distant disease-free survival (N=107)

determining clinical tumour response after preoperative chemotherapy is difficult and can be either under- or overestimated due to fibrosis, weakening of the tumour margins and resolution of oedema, suggesting prognostic superiority of pathologically evaluated tumour response [19-22].

Although pCR in our study was associated with *p53* overexpression and higher survival rate, positive *p53* status was not translated in improved clinical outcome. In contrast, *p53* overexpression was non-significantly related with poorer overall and distant disease-free survival. Hypothetically, the short-lived benefits of better response of *p53* positive tumours may be overcast by rapid regrowth of micro-metastases after initial remission of the primary tumour, reflecting their aggressive biology. Though, analysis of this hypothesis that survival in the pCR subgroup is dependent on *p53* status was not possible due to the limited power of the current study.

P53, a nuclear protein, plays an essential role in the regulation of cell cycle and functions as a tumour suppressor. Breast cancer patients with *p53* mutations or protein accumulation measured by IHC in their tumours have worse survival [23-26]. Meanwhile, the literature of the predictive value of *p53* status on tumour response to preoperative anthracycline-based chemotherapy is conflicting.(7) Most studies find no association between *p53* expression and tumour response to anthracyclines [27-32]. Others have associated *p53* overexpression with both resistance [14, 33-35] and sensitivity [10,36] to preoperative anthracycline containing chemotherapy. Interpretation of the above literature is complicated since the definition of response various across studies, the correlation between *p53* protein accumulation and the presence of mutations is not absolute and numerous non-standardized IHC techniques have been used, limiting the possibility to draw valid conclusions [37].

The pathological lymph node status after preoperative chemotherapy is in our data an independent prognostic factor for both overall and distant disease-free survival. This finding is confirmed by others [3, 38-40]. However, the pre-treatment clinical lymph node status was poorly correlated with clinical outcome. At the time this trial was conducted, the pre-treatment nodal status was determined by palpation. Nowadays, imaging techniques such as ultrasound are more feasible in establishing nodal status [41]. Future trials should include this technique to provide more reliable information of the actual response of lymph node metastases to preoperative chemotherapy and to determine the subsequent prognostic significance of such a response.

At this time, it is not possible to select patient who will benefit from chemotherapy. However, data have begun to emerge from micro-array studies which may lead to the introduction of tailored treatment strategies based upon custom made risk profiles rather than the classic guidelines derived from traditional RCT's [42-45].

In conclusion, our data derived from a prospective randomized trial suggest that *p53* overexpression estimated by immunohistochemistery is an independent predictive factor of tumour response after preoperative anthracycline-based chemotherapy in operable breast cancer patients. However, this conclusion must be limited to the regime used in this trial (FE60C) which is probably suboptimal today [46]. Moreover, the relatively small sample size requires conformation in larger studies and the use of *p53* measurements should be restricted to clinical trial settings. Prospectively derived data on the predictive and prognostic value of *p53* is on the way from the neoadjuvant EORTC trial 10994 [47,48].

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Part II

CHAPTER 5

Overexpression of P70 S6 kinase protein is associated with increased risk of locoregional recurrence in node-negative premenopausal early breast cancer patients

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Abstract

The RPS6KB1 gene is amplified and overexpressed in approximately 10% of breast carcinomas and has been found associated with poor prognosis. We studied the prognostic significance of P70 S6 kinase protein (PS6K) overexpression in a series of 452 node-negative premenopausal early-stage breast cancer patients (median follow-up: 10.8 years). Immunohistochemistry was used to assess PS6K expression in the primary tumour, which had previously been analysed for a panel of established prognostic factors in breast cancer. In a univariate analysis, PS6K overexpression was associated with worse distant disease-free survival as well as impaired locoregional control (HR 1.80, P = 0.025 and HR 2.50, P = 0.006, respectively). In a multivariate analysis including other prognostic factors, PS6K overexpression remained an independent predictor for poor locoregional control (RR 2.67, P = 0.003). To our knowledge, P70 S6 kinase protein is the first oncogenic marker that has prognostic impact on locoregional control and therefore may have clinical implications in determining the local treatment strategy in early-stage breast cancer patients.

Introduction

The treatment of breast cancer is guided by risk factors. Approximately 70% of all node-negative breast cancer patients can be cured by locoregional therapy alone. This automatically implies that the remaining 30% of these patients will develop a recurrence despite adequate locoregional therapy. Currently, treatment strategy in breast cancer is based upon tumour stage, grade, and hormone receptor status. Additional prognostic factors are greatly needed, first to select those patients who might benefit from adjuvant systemic therapy and second to optimise locoregional therapy in order to avoid locoregional recurrences.

The prognostic significance of a considerable number of tumour markers has already been investigated but to date, none of these factors can be used to guide the treatment of primary breast cancer.

A recent study by Barlund et al [1] demonstrated that amplification of a putative tumour marker called P70 S6 kinase protein (PS6K) might be associated with poor outcome in breast cancer. In addition, the authors reported that RPS6KB1 gene amplification and PS6K overexpression are significantly correlated. The RPS6KB1 gene is located at 17q23 and amplified in approximately 10% of all primary breast cancer cases. PS6K is a ribosomal protein that is involved in the progression from the G1 to S phase of the cell cycle. It is rapidly activated in response to mitogenic stimuli, for example, growth factors, cytokines, and oncogene products [2-15].

To study the significance of P70 S6 kinase protein compared with other established prognostic factors in breast cancer; we have tested the prognostic significance of PS6K overexpression in a subset of node-negative premenopausal early breast cancer patients. In this series, we have shown previously that premenopausal node-negative breast cancer patients whose tumours show p53 accumulation have a poor response to one cycle of adjuvant chemotherapy, whereas patients whose tumours have no accumulation of p53 benefit from adjuvant chemotherapy. In addition, we showed Ki-67 overexpression, negative ER status, and young age (< 43 years) to be associated with worse prognosis [16].

Patients and Methods

Patients

All patients were drawn from EORTC trial 10854 (1986–1991, median follow up 10.8 years). This trial, which randomised 2795 patients, studied whether one course of peri-operative chemotherapy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Peri-operative chemotherapy consisted of one single course of doxorubicin 50 mg/m^2, 5- fluorouracil 600 mg/m^2, and cyclophosphamide 600mg/m^2 (FAC), administered intravenously within 36 h after surgery. Axillary lymph node-positive premenopausal patients in the peri-operative chemotherapy group were recommended to receive five additional cycles of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) postoperatively. Node-positive patients, younger than 50 years, who did not receive peri-operative chemotherapy, were advised to be treated with one conventional course of FAC followed by five cycles of CMF after surgery. At randomisation, patients were stratified for institution, age ($\leq$ 50 years or $>$ 50 years) and surgical procedure (breast conserving therapy and modified radical mastectomy). Prolonged adjuvant systemic treatment was left to the discretion of the local investigator [17].
In total, 676 node-negative premenopausal patients were enrolled in this trial and representative tumour material was collected for 452 patients. Tumour material consisted of formalin fixed, paraffin-embedded tumour blocks. Tumours were histologically typed and graded [18] centrally by one pathologist; immunohistochemistry to assess the expression of various proteins has been performed. Results of these studies have been reported previously [16]. For the present study, assays were reviewed simultaneously by two investigators (M.J. van de Vijver, J.A. van der Hage) who had to come to an agreement in case of any uncertainties. During the evaluation of the results, the investigators were blinded for the clinical outcome of the patients.

p70 S6 kinase protein expression

A standard indirect immunoperoxidase protocol with a 3,3'-diaminobenzidine / imidazole solution as a chromogen was used. Before incubation with the primary antibody, antigen retrieval was done by boiling the sections in 10mM citrate buffer for 10 min using a microwave oven. PS6K expression was determined using a polyclonal anti-p70 s6k antibody (sc-230 Santa Cruz Biotechnology, Santa Cruz, USA) (1 : 1000 dilution in PBS containing 1% bovine serum albumin). PS6K staining was evaluated in tumour cells and in normal ductal epithelial cells. PS6K staining was scored categorical as: 0 = no staining; 1= weak cytoplasmic staining; 2 = moderate cytoplasmic staining; 3 = strong cytoplasmic staining. In all cases analysed, the staining was homogeneously distributed in the normal cells and also in the tumour cells. If the difference in staining score between the tumour cells and the normal epithelial ducts was greater or equal than two, tumours were deemed PS6K positive.

RPS6KB1 gene amplification

Two-colour FISH of tumor interphase nuclei was performed according to the ERBB2 short protocol of Ventana Medical Systems, Inc. (Tucson, AZ, USA). The Spectrum Orange-labelled chromosome 17 centromeric probe was purchased from Vysis, Inc.

	N = 452
Age (year)	
Median (range)	44 (24 – 63)
Local treatment (N (%))	
Breast-conserving therapy	368 (81)
Mastectomy	84 (19)
Tumour diameter (N (%))	
≤ 2 cm	278 (62)
> 2 cm	148 (33)
Unknown	26 (6)
Histologic tumour type (N (%))	
Infiltrating ductal	316 (70)
Infiltrating lobular	34 (8)
Other	91 (20)
Unknown	11 (2)
Histologic tumour grade (N (%))	
I	155 (34)
II	144 (32)
III	131 (21)
Unknown	22 (5)
ER status (N (%))	
Positive	390 (86)
Negative	46 (10)
Unknown	16 (4)
PgR status (N (%))	
Positive	329 (73)
Negative	106 (23)
Unknown	17 (4)
HER2 overexpression (N (%))	
Negative	380 (84)
Positive	60 (13)
Unknown	12 (3)
p53 expression (N (%))	
Negative	359 (79)
Positive	81 (18)
Unknown	12 (3)
Ki-67 (N (%))	
Negative	217 (48)
Positive	215 (48)
Unknown	20 (4)
PS6K	
Negative	391 (87)
Positive	39 (9)
Unknown	22 (5)

Table 1. Patient characteristics

(Downers Grove, IL, USA), the unlabelled bacterial artificial chromosome (BAC) clones for PS6K was isolated from a BAC library (RPCI-13 BAC library, Research Genetics, Inc.). Fluorescent signals were counted in 2 20 non-overlapping nuclei per component. Mapping of the PS6K BAC was verified by FISH on metaphase chromosomes.

Other tumour markers

Previously, tumour sections had been stained and analysed for oestrogen and progesterone receptor status, Ki-67 positivity, P53 expression, HER2 expression, and mitotic index [16].

Statistical methods

This analysis was based on locoregional control, distant-disease free survival, and overall survival. Locoregional recurrence was defined as the time to locoregional recurrence as a first event. Locoregional recurrences occurring simultaneously or after the diagnosis of distant metastasis or contralateral breast cancer or a secondary primary tumour were censored. Distant disease-free survival was defined as the time to distant metastasis or death, whichever of the events happened first. All variables were first analysed for their prognostic importance in a univariate analysis.

Eight potential prognostic variables were considered: PS6K (negative versus positive), ER status (negative versus positive), PgR status (negative versus positive), HER2 overexpression (negative versus positive), Ki67 (negative, i.e. ≤ 20% of positive tumour cells, versus positive, > 20% positive tumour cells), histologic tumour grade (grade I versus grade II versus grade III), tumour diameter (T ≤ 2cm versus T>2cm), and p53 (negative versus positive).

To test the independent prognostic significance of PS6K overexpression, we included PS6K together with the previously tested markers into a multivariate Cox regression analysis for overall survival, progression-free survival, distant disease-free survival, and locoregional control. Only markers that were significant predictors in the univariate analysis were included in the multivariate analysis. A Cox proportional hazards model was used for the univariate and multivariate analyses [19]. For factors with only two levels the second one was compared to the first one, while for factors with more than two levels dummy variables were used to compare each level to the first one. Patients who had missing information for any of the variables in the

analysis were excluded when this variable was included in the model. All tests were two-sided with a 5% alpha level.

Results

Patient characteristics are listed in Table 1. At the time of the analysis, the median follow-up period was 10.8 years, 80 (18%) of the 452 patients had died, 126 (29%) patients had experienced distant metastases or death, and 67 (15%) patients experienced a locoregional recurrence as first event (see Table 2).
PS6K expression levels could be assessed in 430 tumours. In all, 39 tumours (9%) showed PS6K overexpression (Table 1). Examples of PS6K overexpression are shown in Figure 1A & B.

Univariate analyses

In the univariate analyses, we could not confirm a significant association between PS6K overexpression and overall survival (Table 3). However, PS6K overexpression was a significant predictor for increased risk of locoregional recurrence (HR 2.50, 95% CI 1.30–4.81, P = 0.006) and of developing distant metastases (HR 1.80, 95% CI 1.08–3.01, P = 0.025).

Multivariate analyses

Apart from PS6K, p53 was the only significant risk factor for locoregional recurrence in the univariate analysis. When including these two factors in a multivariate model, PS6K appears as the only independent prognostic factor for locoregional control predicting a poor control rate in PS6K overexpressing cases (HR 2.67, 95% CI 1.39–5.14, P = 0.003, Table 4). Variables significantly associated with distant disease-free survival in the univariate analysis were PS6K, ER status, Ki67, grade, and tumour diameter. In a multivariate model including all these factors, Ki-67 overexpression was the only independent prognostic factor associated with poor distant disease-free survival (HR 1.79, 95% CI 1.11–2.91, P = 0.018, Table 4). PS6K as a prognostic factor did not remain significant in the multivariate analysis.
In addition, Ki-67 overexpression was an independent significant predictor for poor overall survival.

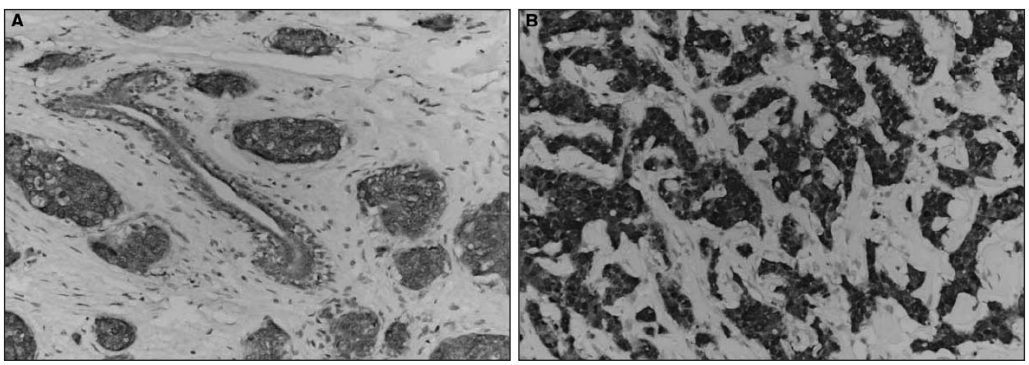

Figure 1. (A & B) Two cases of PS6K overexpressing breast cancer tumours

		Number of events		
Patient characteristics	**N = 452**	**Overall survival**	**Locoregional recurrence (first event)**	**Distant disease-free survival**
Local treatment (N (%))				
Breast-conserving therapy	368 (81)	65 (18)	58 (16)	102 (28)
Mastectomy	84 (19)	15 (18)	9 (11)	24 (29)
Tumour diameter (N (%))				
⩽2 cm	278 (62)	34 (12)	41 (15)	68 (24)
>2 cm	148 (33)	40 (27)	21 (14)	51 (34)
Unknown	26 (6)	6 (23)	5 (19)	7 (27)
Histologic tumour type (N (%))				
Infiltrating ductal	316 (70)	60 (19)	48 (15)	91 (29)
Infiltrating lobular	34 (8)	4 (12)	9 (26)	7 (21)
Other	91 (20)	14 (15)	8 (9)	25 (27)
Unknown	11 (2)	2 (18)	2 (18)	3 (27)
Histologic tumour grade (N (%))				
I	155 (34)	10 (6)	23 (15)	30 (19)
II	144 (32)	30 (21)	27 (19)	45 (31)
III	131 (21)	36 (27)	14 (11)	47 (36)
Unknown	22 (5)	4 (18)	3 (14)	4 (18)
ER status (N (%))				
Positive	390 (86)	64 (16)	60 (15)	104 (27)
Negative	46 (10)	13 (28)	4 (9)	18 (39)
Unknown	16 (4)	3 (19)	3 (19)	4 (25)
PgR status (N (%))				
Positive	329 (73)	5 (16)	53 (16)	90 (27)
Negative	106 (23)	27 (25)	12 (11)	33 (31)
Unknown	17 (4)	2 (12)	2 (12)	3 (18)
HER2 overexpression (N (%))				
Negative	380 (84)	66 (17)	52 (14)	107 (28)
Positive	60 (13)	12 (20)	13 (22)	16 (27)
Unknown	12 (3)	2 (17)	2 (17)	3 (25)
P53 expression (N (%))				
Negative	359 (79)	59 (16)	47 (13)	99 (28)
Positive	81 (18)	19 (23)	18 (22)	24 (30)
Unknown	12 (3)	2 (17)	2 (17)	3 (25)
Ki-67 (N (%))				
Negative	217 (48)	19 (9)	36 (17)	43 (20)
Positive	215 (48)	59 (27)	27 (13)	78 (36)
Unknown	20 (4)	2 (10)	4 (20)	5 (25)
PS6K				
Negative	391 (87)	66 (17)	52 (13)	102 (26)
Positive	39 (9)	8 (21)	11 (28)	17 (44)
Unknown	22 (5)	6 (27)	4 (18)	7 (32)

Table 2. Event rates

PS6K overexpression in patients who underwent breast-conserving treatment

In all, 368 patients underwent breast-conserving therapy. Event rates are shown in Table 5. The prognostic impact of PS6K was similar to that of the overall population. PS6K remained a predictor of poor locoregional control (HR 2.83, 95% CI 1.42–5.62, P = 0.003) but not for overall survival (HR1.44, 95 CI 0.66–3.18, P = 0.36) (Table 6). In the multivariate analyses, Ki67 remained an independent predictor for distant disease (RR 1.78, 95% CI 1.03–3.07, P = 0.038). Tumour grade remained an independent prognostic factor for poor survival (RR 1.63, 95% CI 1.04–2.53, P = 0.032) (Table 7).

FISH

A tissue microarray (TMA) was constructed from 12 tumours that showed PS6K overexpression, as assessed by immunohistochemistry. Amplification was studied using FISH by hybridising the TMA to a PS6K BAC probe and a CEP17 chromosome 17 centromeric probe. Probe signals and CEP17 signals were counted in each nucleus

	Locoregional control			Distant disease-free survival			Overall survival		
Marker	**Hazard ratio**	**95% CI**	***P*-value**	**Hazard ratio**	**95% CI**	***P*-value**	**Hazard ratio**	**95% CI**	***P*-value**
PS6K+	2.50	1.30–4.81	**0.006**	1.80	1.08–3.01	**0.025**	1.27	0.61–2.64	0.526
ER+	1.38	0.50–3.79	0.536	0.54	0.33–0.90	**0.017**	0.50	0.27–0.90	**0.021**
PgR+	1.31	0.70–2.45	0.403	0.81	0.54–1.20	0.287	0.55	0.35–0.88	**0.013**
HER2+	1.64	0.89–3.01	0.111	0.84	0.56–1.60	0.95	1.17	0.63–2.17	0.617
Ki 67+	0.96	0.58–1.58	0.856	2.24	1.54–3.25	**<0.0001**	3.74	2.22–6.27	**<0.0001**
Grade									
I *vs* I	1.42	0.81–2.48	0.216	1.75	1.10–2.77	**0.018**	3.46	1.70–7.08	**0.0007**
II *vs* I	0.93	0.48–1.80	0.820	2.28	1.41–3.60	**0.0004**	5.09	2.53–10.26	**<0.0001**
Diameter									
r>2 cm	1.13	0.68–1.91	0.651	1.61	1.12–2.31	**0.011**	2.43	1.54–3.85	**0.0001**
p53+	1.85	1.07–3.18	**0.027**	1.07	0.69–1.67	0.763	1.47	0.87–2.46	0.148

Table 3. Univariate analyses all patients (N = 430)

	Locoregional recurrence		
Marker	**Hazard ratio**	**95% CI**	***P*-value**
PS6K+	2.67	1.39–5.14	**0.003**
p53 positivity	1.67	0.95–2.96	0.076
Marker	**Risk ratio**	**95% CI**	***P*-value**
Distant disease-free survival			
PS6K+	1.52	0.87–2.64	0.139
ER+	0.93	0.50–2.91	0.810
Ki67 (⩾20%)	1.79	1.11–2.91	**0.018**
Grade III *vs* I/II	1.07	0.78–1.45	0.689
Diameter >2 cm	1.29	0.86–1.94	0.221
Overall survival			
ER+	1.17	0.56–2.43	0.685
PgR+	1.01	0.57–1.80	0.972
Ki67 (⩾20%)	2.84	1.44–5.59	**0.003**
Grade III *vs* I/II	1.33	0.88–2.00	0.174
Diameter >2 cm	1.63	0.99–2.69	0.055

Table 4. Multivariate analyses all patients (N = 430)

and a ratio of mean probe signal to mean CEP17 signal was calculated. Ratios of X2 were scored as amplification. Eight of the 12 tumours with PS6K overexpression (75%) showed PS6K gene amplification, which is in accordance with the data shown by Barlund et al [1].

Correlation between HER2 and PS6K

As the PS6K gene and the HER2 gene are both located on chromosome 17, and amplification has been reported to occur in both genes simultaneously, we studied the correlation of PS6K expression and HER2 expression and between PS6K expression and Ki67 expression, respectively. Based on available data, we found a significant association between PS6K and HER2 expression (Fisher's exact test (two sided) P = 0.01), whereas no significant association was found between PS6K positivity and Ki67 positivity (Fisher's exact test (two sided) P = 0.24).

Discussion

We have found that P70 S6 kinase protein overexpression in breast cancer is associated with increased risk of locoregional recurrence. To our knowledge, no other oncogenic markers as predictors of locoregional recurrence have been identified previously. At present, the common risk factors for local control after breast-conserving treatment are: patient age, margin status, and the presence of an extensive intraductal component [20-23].

The addition of new predictive markers for locoregional recurrence may help in guiding the optimal type of local therapy. This is of particular importance since local therapy does not only have an impact on locoregional control but also on survival [24, 25]. P70 S6 kinase protein overexpression was associated with an increased risk of locoregional recurrence when all patients were analysed. The majority of the patients

		Number of events		
Patient characteristics	**N = 368**	**Overall survival**	**Locoregional recurrence (first event)**	**Distant disease-free survival**
Tumour diameter (N (%))				
≥2 cm	249 (68)	32 (13)	38 (15)	59 (24)
>2 cm	100 (27)	27 (27)	15 (15)	36 (36)
Unknown	19 (5)	6 (32)	5 (26)	7 (37)
Histologic tumour type (N (%))				
Infiltrating ductal	260 (71)	48 (18)	41 (16)	73 (28)
Infiltrating lobular	22 (6)	2 (9)	7 (32)	3 (14)
Other	79 (21)	13 (16)	8 (10)	23 (29)
Unknown	7 (2)	2 (29)	2 (29)	3 (43)
Histologic tumour grade (N (%))				
I	124 (34)	6 (2)	19 (15)	21 (17)
II	121 (33)	26 (21)	25 (21)	40 (33)
III	107 (29)	30 (28)	11 (10)	37 (35)
Unknown	16 (4)	3 (19)	3 (19)	4 (25)
ER status (N (%))				
Positive	319 (87)	52 (16)	51 (16)	85 (27)
Negative	38 (10)	10 (26)	4 (11)	13 (34)
Unknown	11 (3)	3 (27)	3 (27)	4 (37)
PgR status (N (%))				
Positive	268 (73)	45 (17)	45 (17)	77 (29)
Negative	87 (24)	18 (21)	11 (13)	22 (25)
Unknown	13 (4)	2 (15)	2 (15)	3 (23)
HER2 overexpression (N (%))				
Negative	312 (85)	54 (17)	44 (14)	87 (28)
Positive	48 (13)	9 (19)	12 (25)	12 (25)
Unknown	8 (2)	2 (25)	2 (25)	3 (38)
p53 expression (N (%))				
Negative	290 (79)	47 (16)	42 (14)	77 (27)
Positive	70 (19)	16 (23)	14 (20)	22 (31)
Unknown	8 (2)	2 (25)	2 (25)	3 (38)
Ki-67 (N (%))				
Negative	175 (48)	16 (9)	32 (18)	33 (19)
Positive	181 (49)	47 (26)	23 (13)	65 (36)
Unknown	12 (3)	2 (17)	3 (25)	4 (33)
PS6K				
Negative	322 (88)	54 (17)	45 (14)	83 (26)
Positive	30 (8)	7 (23)	10 (33)	13 (43)
Unknown	16 (4)	4 (25)	3 (19)	6 (38)

Table 5. Pts who underwent breast-conserving therapy

	Locoregional control			Distant disease-free survival			Overall survival		
Marker	**Hazard ratio**	**95% CI**	***P*-value**	**Hazard ratio**	**95% CI**	***P*-value**	**Hazard ratio**	**95% CI**	***P*-value**
PS6K+	2.83	1.42–5.62	**0.003**	1.80	1.00–3.23	**0.049**	1.44	0.66–3.18	0.360
ER+	1.22	0.44–3.38	0.701	0.65	0.36–1.16	0.146	0.54	0.27–1.06	0.071
PgR+	1.28	0.66–2.48	0.461	1.09	0.68–1.75	0.728	0.77	0.45–133	0.346
HER2+	1.79	0.94–3.39	0.075	0.86	0.47–1.57	0.617	1.07	0.53–2.16	0.862
Ki 67+	0.86	0.50–1.47	0.582	2.31	1.52–3.52	**<0.0001**	3.40	1.93–6.02	**<0.0001**
Grade									
I *vs* I	1.58	0.87–2.86	0.136	2.18	1.29–3.70	**0.004**	4.94	2.03–12.01	**0.0004**
II *vs* II	0.86	0.41–1.80	0.683	2.52	1.47–4.30	**0.0007**	7.12	2.96–17.11	**<0.0001**
Diameter									
R>2 cm	1.14	0.63–2.07	0.670	1.72	1.14–2.60	**0.011**	2.24	1.34–3.75	**0.002**
p53+	1.47	0.81–2.70	0.209	1.20	0.74–1.92	0.460	1.44	0.82–2.54	0.209

Table 6. Univariate analyses (Pts who received breast-conserving therapy n = 368)

Marker	Risk ratio	95% CI	*P*-value
Locoregional recurrence			
PS6K+	2.83	1.42–5.62	**0.003**
Distant disease-free survival			
PS6K+	1.54	0.83–2.88	0.174
Ki67 (≥20%)	1.78	1.03–3.07	**0.038**
Grade III *vs* I/II	1.17	0.83–1.64	0.369
Diameter >2 cm	1.24	0.78–1.98	0.365
Overall survival			
Ki67+	2.01	0.96–4.22	0.066
Grade III *vs* I/II	1.63	1.04–2.53	**0.032**
Diameter >2 cm	1.44	0.83–2.48	0.194

Table 7. Multivariate analyses (Pts who received breast-conserving therapy n = 368)

(N = 368) underwent breast-conserving treatment. When these patients were analysed separately, PS6K remained an independent predictor of locoregional recurrence. In the univariate analysis, p53 overexpression was also associated with an increased risk of locoregional recurrence (HR 1.85, P1= 0.027); however, this was not the case for the subset of patients who underwent breast-conserving therapy (data not shown). In addition, 5-year follow-up results concerning the impact of P53 and PS6K status on locoregional control demonstrate similar results.

Locoregional control rates at 5 years of follow-up are 93% (95% CI 92.3–94.2) in P53 negative vs. 84% (95% CI 81.7–87.2) in P53-positive patients and 93% (95% CI 91.8–93.7) vs 83.3% (95% CI 79.0–87.5), respectively.

Several studies have examined the relation between P53 overexpression and local breast tumour recurrence. A case–control study of 66 women with local breast tumour relapse following lumpectomy and radiation therapy showed that p53 overexpression was an independent predictive factor for ipsilateral breast tumour recurrence (IBTR) [26]. Recent studies conducted by Turner et al [26] and Zellars et al [27] demonstrated predictive significance of P53 overexpression for locoregional recurrence in patients who underwent breast-conserving therapy, as well as in patients who underwent mastectomy. Turner and colleagues showed in a matched case-control study comprising 47 cases and 47 controls that overexpression of P53 had prognostic significance in respect to IBTR following lumpectomy and radiotherapy (P = 0.003). Zellars and co-workers demonstrated in 1530 mastectomy-treated breast cancer patients of whom 259 received adjuvant radiotherapy that P53 overexpression was independently associated with a significantly increased local failure rate in patients treated with mastectomy, with (RR 2.5, 95% CI 1.1–5.7) or without (RR 1.7, 95% CI 1.2–2.4) radiotherapy. Although, in our series, P53 lost its prognostic significance in the multivariate analysis, a trend still remained, suggesting worse locoregional recurrence rates in P53-overexpressing tumours (RR 1.67, 95% CI 0.95–2.96).

Barlund et al [1] analysed RPS6KB1 amplification using FISH in 668 informative primary breast tumours. In all, 9% of the tumours showed amplification of the RPS6KB1 gene. In their series, PS6K was significantly associated with poor survival (P = 0.0021). In addition, the authors analysed overexpression in a subset of 445 primary breast tumours. P70 S6 kinase protein staining of cytoplasm was subjectively scored into four groups: negative (no staining), weak, moderate, or strong staining. For statistical analyses, the data were combined into two groups: low expression (negative or weak staining) and high expression (moderate or strong staining). High expression was seen in 15.6%. There was a statistically significant association between RPS6KB1 amplification and high P70 S6 kinase protein expression (P = 0.0004), with 41% of the amplified tumours (FISH) exhibiting high PS6K expression, and overexpression of PS6K was associated with poor survival (P = 0.0083) as well. Our results suggest an even stronger association between amplification and expression, albeit

with not enough data to make a sound statistical comparison. Moreover, the authors found that patients showing both PS6K and HER2 amplification had a significant worse prognosis in terms of breast cancer-specific survival than those with no amplification or amplification of only one of the genes. These results together with our data suggest that P70 S6 kinase protein overexpression may be an important predictor of not only worse survival but also of poor locoregional control.

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CHAPTER 6

Impact of locoregional treatment on the early-stage breast cancer patients: a retrospective analysis

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Abstract

Although adequate locoregional treatment improves local and regional control in early-stage breast cancer, uncertainty still exists about the role of locoregional therapy with respect to survival.

To study the impact of surgery and radiotherapy on locoregional control and survival, we combined the data of three European Organisation for Research and Treatment of Cancer (EORTC) Breast Cancer Group trials including early-stage breast cancer patients with long-term follow-up. Risk ratios (RR) were estimated for locoregional recurrence and overall survival using Cox regression models. All analyses were adjusted for tumor size, nodal status, age, adjuvant radiotherapy, adjuvant chemotherapy and trial.

The combined data set consisted of 3648 patients. The median follow-up period was 11 years. 5.9% of the patients who underwent mastectomy and 10.8% of the patients who underwent breast-conserving therapy had a locoregional recurrence ($P < 0.0001$). The risk of death after breast-conserving therapy was similar compared with mastectomy (RR 1.07, $P = 0.37$). Adjuvant radiotherapy after mastectomy was associated with a lower risk for locoregional recurrence (RR 0.43, $P < 0.001$) and death (RR 0.73, $P = 0.001$). Patients with 1–3 positive nodes benefited the most from radiotherapy after mastectomy. Breast-conserving therapy was associated with an impaired locoregional control. However, breast- conserving therapy was not associated with a worse overall survival. Adjuvant radiotherapy in mastectomised patients was associated with both a significantly superior locoregional control and overall survival. The effect of adjuvant radiotherapy was most profound in patients who had 1–3 positive nodes.

Introduction

It has long been accepted that adequate locoregional therapy can delay or prevent local or regional recurrence in women with early breast cancer. In addition, the detrimental impact of locoregional recurrence on disease outcome has been firmly established [1,2].

Many investigators have studied the role of locoregional control and its impact on disease outcome. The predominating assumption is that locoregional recurrence is an independent prognostic factor that is associated with a poor outcome. However, more aggressive locoregional treatment has not been reported to result in better survival despite improved locoregional control.

Therefore, locoregional recurrence is not regarded as an instigator of subsequent systemic disease. Locoregional therapy is based on surgery and radiation therapy. Trials that studied breast-conserving surgery versus mastectomy have failed to detect a difference in overall survival, despite demonstrating a superior locoregional control after mastectomy [1,3–5]. However, randomised trials that studied the role of adjuvant radiotherapy after mastectomy in patient samples that were at a high risk of recurrence demonstrated superior locoregional control as well as superior overall survival rates after adjuvant radiotherapy [6,10–13]. The fact that radiotherapy may influence disease outcome, but more aggressive surgery may not, is intriguing. The

most recent follow-up of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG 2000 update) demonstrated a significant overall survival difference of 6.0% in favour with patients who underwent mastectomy compared to patients who underwent breast-conserving surgery without radiotherapy at 15 years of follow-up (survival rates of 53 and 47%, respectively).
This effect was observed in 2489 randomised patients. However, in 4463 women randomised between mastectomy and conservative surgery plus radiotherapy, the survival patterns were very similar after 15 years of follow-up (overall survival of 61 and 60.8%, respectively; EBCTCG 2000 update).
Adjuvant radiotherapy trials have demonstrated a beneficial effect for radiotherapy on overall survival after mastectomy in high-risk early breast cancer patients. However, data from the 2000 update of the EBCTCG concerning the effects of radiotherapy on overall survival are still inconclusive, in that the beneficial effect of radiotherapy on breast cancer mortality is balanced by its negative impact on cardiac mortality [6].
We hypothesised that any improvement in survival through locoregional therapy has to be accompanied by an improvement in local control. The rationale behind this is, of course, that locoregional therapy is directed against locoregional disease and not against systemic micrometastases. The combination of data from different trials provides a larger sample size, which increases the possibility of finding small, but clinically relevant, differences between locoregional treatment modalities. Therefore, we conducted a retrospective analysis combining the data of three trials with sufficient follow-up, which enrolled early breast cancer patients who either underwent mastectomy or breast-conserving therapy, to study whether more aggressive surgery would result in better overall survival rates in a large set of early breast cancer patients. It was decided to select patients with T1 and T2 tumors since these patients can generally be treated by either mastectomy or breast-conserving surgery.

Patients and methods

Selection of the trials
Patients were selected from trials that randomized early-stage breast cancer patients. The European Organisation for Research and Treatment (EORTC) has conducted several large randomised phase III trials concerning the management of breast cancer patients with stage I or stage II/III breast cancers. These trials, EORTC trial 10801, 10854, and 10902 have enrolled a total of over 4018 early breast cancer patients. Median follow-up periods ranged from 6.1 to 13.4 years in these studies. From these trials, all patients who had clinical T1 or T2 tumors at the time of diagnosis were selected. Patient characteristics are listed in Table 1. A brief description of these trials follows below:

EORTC trial 10801 (1980–1986, median follow-up of 13.4 years) was conducted in order to assess the safety of breast-conserving treatment. In this trial, patients were randomised between breast-conserving surgery combined with radiotherapy and radical mastectomy. Six cycles of adjuvant chemotherapy with cyclophosphamide 100 mg/m^2 given orally on days 1–14, methotrexate 40 mg/m^2 given intravenously (i.v.)

on days 1 and 8, and 5-fluorouracil (5-FU) 600 mg/m^2 given i.v. on days 1 and 8, were indicated for all patients under the age of 55 years with positive nodes. No information was collected on hormonal therapy. In this study, 902 patients were randomised [3].

EORTC trial 10854 (1986–1991, median follow-up of 10.8 years) studied the question whether one course of perioperative chemotherapy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Perioperative chemotherapy consisted of one single course of doxorubicin 50 mg/m^2, 5-FU 600 mg/m^2 and cyclophosphamide 600 mg/m^2 (FAC), administered i.v. within 36 h after surgery. Axillary lymph node-positive premenopausal patients in the perioperative chemotherapy group were recommended to receive an extra five cycles of cyclophosphamide, methotrexate and 5-FU (CMF). Node-positive patients, younger than 50 years, who did not receive perioperative chemotherapy, were advised to receive one conventional course of FAC followed by five cycles of CMF after surgery. Patients were stratified for breast-conserving therapy and modified radical mastectomy. Prolonged adjuvant systemic treatment was left to the discretion of the local investigators. 2795 patients were included in this trial [7].

EORTC trial 10902 (1991–1999, median follow-up of 6.1 years) was set up to determine the value of preoperative chemotherapy. Patients were randomised to receive four cycles of chemotherapy either before or after surgery. Chemotherapy consisted of four cycles of 5-FU 600 mg/m^2, epirubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (FEC) administered i.v., at 3-weekly intervals. In the preoperative chemotherapy group, surgical therapy followed within 4 weeks of the fourth course of chemotherapy. In the postoperative chemotherapy group, the first cycle was given within 36 h after surgery. Stratification was performed for planned type of surgery instead of performed type of surgery. This was done because of the expected effect of preoperative chemotherapy on downstaging of the tumor. A total number of 698 patients were randomised [8].

Selection of data

All of eligible patients from all the trials were included in the analysis, with the exception of patients who underwent preoperative chemotherapy in EORTC trial 10902. These patients would have introduced a selection bias since preoperative chemotherapy influences the choice of locoregional treatment due to tumor downstaging.

Selection of covariates

To study the independent impact of surgery and radiotherapy on locoregional control and overall survival, we included the following covariates; clinical tumor size, pathological nodal status, age, type of surgery, adjuvant radiotherapy, adjuvant chemotherapy, and the trial in which a patient participated. Clinical tumor size was measured taking the largest diameter using callipers. Pathological tumor size, hormone receptor status and tamoxifen use were not taken into account as these tumor- and treatment-related characteristics were poorly reported in some of the trials. Specifications on the radiotherapeutic regimens used differed between the trials and the institutions in which patients were treated. Therefore, it was decided

that any type of radiotherapy given to a patient after surgery should be regarded as adjuvant radiotherapy without specification of radiation fields and doses.

Locoregional treatment

In all of the trials, patients were selected for breast-conserving therapy if a wide local excision could be performed provided that at least a 1-cm margin around the macroscopic dimension of the tumor could be achieved. Patients who received breast-conserving therapy underwent lumpectomy plus axillary lymph node dissection and radiotherapy to the whole breast, with or without a boost. Radiotherapy to the axilla was given in cases of extensive lymph node metastasation (pN1-bii/ pN2) or in cases of positive nodes in level III of the axilla. All patients who underwent mastectomy underwent axillary lymph node dissection. Postoperative radiotherapy to the breast was always indicated after breast-conserving surgery. In EORTC trials 10854 and 10902, postoperative radiotherapy to the chest wall and parasternal lymph node chain after mastectomy was indicated if surgery was considered not to be radical, if the tumor was >5 cm, or if a positive infraclavicular node was found after surgery. In EORTC trial 10801, microscopically-incomplete excision was not a reason for exclusion. Lumpectomy was followed by radiotherapy (50 Gy over a 5-week period), with an additional booster dose of 25 Gy directed to the lumpectomy site via an Iridium-192 implant. If implants could not be used for technical reasons, patients were given an equivalent booster dose with external irradiation. Postoperative irradiation to the chest wall was indicated after a microscopicallyincomplete operation. General guidelines concerning adjuvant radiotherapy were as follows: for patients both after mastectomy or breast-conserving therapy, irradiation of the parasternal lymph node region was indicated for patients with a centrally or medially localised tumor and for patients with a lateral tumor and histologically-proven axillary lymph node metastases. Postoperative radiation was always given in cases in which surgery was considered not to be radical. In cases of breast-conserving surgery, microscopically incomplete or not, the whole breast was irradiated using a dose of at least 50 Gy followed by a boost on the initial tumor of at least 16 Gy.

Statistical methods

To compare different locoregional treatment modalities, type of surgery was divided into two states; breast-conserving therapy (lumpectomy plus axillary lymph node dissection followed by radiotherapy) and (modified) radical mastectomy, with or without radiotherapy. All analyses were performed for overall survival and locoregional recurrence. Survival time was defined as the time between randomisation and death from any cause. A locoregional recurrence was defined as any recurrence in the breast or axilla. Only recurrences, which occurred before the diagnosis of a distant metastasis and/or a new primary tumor, were regarded as a locoregional recurrence as the first event included in the analysis. In EORTC trial 10854, any chemotherapy (1x perioperative FAC) was scored as having received chemotherapy. Cox proportional-hazard regression models [9] were used to estimate the hazard ratios with their 99% confidence intervals (CIs). Since the number of patients is high, a 1% significance level was used. All tests are two-sided. To control for possible differences in the study populations, we added study as a factor in the multivariate Cox regression analysis, after testing the proportional hazards assumption.

	10801	10854	10902	Total
Mastectomy (%)	49	41	71	45
BCT (%)	51	59	29	55
T1 (%)	20	32	17	28
T2 (%)	80	68	83	72
N–(%)	59	54	36	54
N+ (%)	41	46	64	46
⩽50 years (%)	38	40	59	41
>50 years (%)	62	60	41	59
No adjuvant CT (%)	83	41		48
Adjuvant CT (%)	17	59[a]	100	52
No. of deaths	368 (42.5)	672 (26.3)	51 (22.3)	1091 (29.9)
No. of locoregional recurrences	80 (9.2)	223 (8.7)	11 (4.8)	314 (8.6)

CT, chemotherapy; BCT, breast-conserving therapy; peri-Op FAC, perioperative 5-fluorouracil, doxorubicin, cyclophosphamide. *N*–, node-negative; *N*+, node-positive.
[a] 70% of these patients only received 1×peri-OpFAC.

Table 1. Patient characteristics (N=3648)

Results

Patient's characteristics

In total, 4018 primary operable breast cancer patients were randomised to one of the trials. Of these patients, 3886 breast cancer patients were deemed eligible. 3648 patients had cT1 or cT2 tumors and were subsequently included in the analysis. At the time of the analysis, the median follow-up period in this subset of patients was 11 years, 1091 patients have died, and 314 patients have developed a locoregional recurrence as their first event. Other patient characteristics are listed in Table 1. 2011 patients (55%) underwent breast-conserving therapy. Breast-conserving therapy consisted of lumpectomy and axillary lymph node dissection followed by adjuvant radiation therapy. 1633 patients underwent a (modified) radical mastectomy. In total, 804 (49%) patients received adjuvant radiotherapy to the chest wall and/or the axilla after mastectomy (Table 2).

Overall analysis

Overall, 5.9% of the patients who underwent mastectomy and 10.8% of the patients who underwent breast-conserving therapy experienced a locoregional recurrence (as the first event) (Chi square test $P < 0.0001$). Overall survival rates were slightly better for patients who underwent breast-conserving therapy, 72.3% versus 67.5%, respectively.

In the multivariate analysis, breast-conserving therapy was significantly associated with a poor locoregional control (Risk Ratio (RR) 2.25, $P < 0.001$, Table 3). Age <50 years at the time of diagnosis was an independent predictor of a poor locoregional control (Table 3). Additional covariates associated with an improved locoregional control were adjuvant radiotherapy and chemotherapy. Although breast-conserving therapy was associated with a poor locoregional control, there was no association with poor outcome in terms of overall survival (BCT: RR 1.07, $P = 0.37$). Significant independent predictors of a poor overall survival were involved axillary nodes, tumor size >2 cm and age >50 years at the time of diagnosis (Table 3). Again, adjuvant radiotherapy and chemotherapy were associated with an improved overall survival. In addition, in 452 patients aged <40 years at the time of diagnosis, breast-conserving therapy was not associated with an impaired locoregional control or overall survival. The RRs for locoregional recurrence and overall mortality after breast-conserving therapy were 1.31 (99% CI 0.49–3.56, $P = 0.48$) and 0.76 (99%CI 0.45–1.29, $P = 0.18$), respectively (Table 4). To study the effect of (prolonged) adjuvant chemotherapy alone, we repeated the analysis excluding

Radiotherapy, *N*	No radiotherapy, *N*	Total, *N*
804 (49%)	829 (51%)	1633

Table 2. Patients who underwent mastectomy to the chest wall and/or axilla

	Locoregional recurrence (as the first event)			Overall survival		
	RR	99% CI	*P* value	RR	99% CI	*P* value
BCT	2.25	1.47–3.44	<0.001	1.07	0.88–1.29	0.37
pN+	1.25	0.91–1.72	0.07	2.38	2.00–2.83	<0.001
T2	1.11	0.80–1.54	0.40	1.54	1.25–1.89	<0.001
Age > 50 years	0.66	0.49–0.89	<0.001	1.18	1.00–1.40	0.01
Adjuvant radiotherapy	0.60	0.38–0.95	0.005	0.77	0.62–0.96	<0.001
Adjuvant chemotherapy	0.63	0.45–0.89	0.001	0.77	0.64-0.93	<0.001
EORTC trial						
10801 versus 10854	1.13	0.78–1.63	0.40	0.76	0.63–0.91	<0.001
10801 versus 10902	1.22	0.51–2.96	0.56	1.09	0.71–1.66	0.61

99% CI, 95% Confidence Interval; EORTC, European Organisation for Research and Treatment of Cancer; RR, relative risk.

Table 3. Multivariate Cox regression analysis all patients (N = 3648)

	Locoregional recurrence (as the first event)			Overall survival		
	RR	99% CI	*P* value	RR	99% CI	*P* value
BCT	1.31	0.49–3.56	0.48	0.76	0.45–1.29	0.48
pN+	1.03	0.48–2.21	0.94	2.75	1.63–4.63	<0.001
T2	1.43	0.70–2.92	0.20	1.77	1.04–3.01	<0.01
Adjuvant radiotherapy	1.04	0.34–3.20	0.92	1.63	0.84–3.15	0.06
Adjuvant chemotherapy	0.78	0.35–1.73	0.42	0.67	0.39–1.15	0.06
EORTC trial						
10801 versus 10854	2.01	0.71–5.78	0.09	0.70	0.42–1.18	0.08
10801 versus 10902	1.10	0.13–9.24	0.91	1.08	0.42–2.78	0.83

99% CI, 95% Confidence Interval; EORTC, European Organisation for Research and Treatment of Cancer; RR, relative risk.

Table 4. Multivariate Cox regression analysis patients younger than or equal to 40 years (N = 452)

patients who received perioperative chemotherapy. Breast-conserving therapy remained the strongest predictive factor for locoregional recurrence (RR 2.31, P < 0.001). In addition, young age remained a significant predictor of poor locoregional control and the effect of adjuvant radiotherapy on locoregional control remained unchanged (data not shown). In the overall survival multivariate analysis, nothing changed except for the fact that age lost its prognostic significance (data not shown).

Mastectomy with or without radiotherapy

Forty-nine percent of patients who underwent mastectomy subsequently received radiotherapy. Adjuvant radiotherapy after mastectomy decreased locoregional recurrence rates independent of the TNM stage, patient's age, and whether they received adjuvant chemotherapy (RR 0.43, P < 0.001) (Table 5). Furthermore, it was the only independent predictor of a better locoregional control among these covariates. In addition, patients who received radiotherapy had a lower risk of dying (RR 0.73, P = 0.001) compared with patients who did not receive adjuvant radiotherapy (Table 5).

Adjuvant chemotherapy was also independently associated with a better outcome in terms of decreased mortality (RR 0.77, P = 0.01). Independent predictors for a poor overall survival were a positive nodal status and tumor size >2 cm.

	Locoregional recurrence (as the first event)			Overall survival		
	RR	99% CI	*P* value	RR	99% CI	*P* value
Adjuvant radiotherapy	0.43	0.23–0.80	<0.001	0.73	0.57–0.94	0.001
pN+	1.25	0.68–2.28	0.34	2.40	1.83–3.15	<0.001
T2	1.40	0.69–2.87	0.22	1.49	1.07–2.08	0.002
Age > 50 years	0.67	0.39–1.17	0.06	1.11	0.87–1.43	0.28
Adjuvant chemotherapy	0.89	0.47–1.67	0.64	0.77	0.59–1.00	0.01
EORTC trial						
10801 versus 10854	1.08	0.56–2.11	0.76	0.87	0.66–1.14	0.18
10801 versus 10902	0.52	0.12–2.27	0.25	1.11	0.65–1.90	0.63

Table 5. Multivariate Cox regression analysis mastectomized patients (N = 1633)

	Locoregional recurrence (as the first event)			Overall survival		
	RR	99% CI	*P* value	RR	99% CI	*P* value
(a) 1–3 positive nodes						
Adjuvant radiotherapy	0.28	0.09–0.85	0.003	0.48	0.31–0.75	<0.001
T2	2.04	0.40–10.28	0.26	1.77	0.94–3.31	0.02
Age > 50 years	0.46	0.14–1.47	0.08	0.98	0.62–1.54	0.90
Adjuvant chemotherapy	0.52	0.15–1.79	0.18	0.68	0.42–1.08	0.03
EORTC trial						
10801 versus 10854	0.77	0.22–2.69	0.59	0.69	0.43–1.12	0.05
10801 versus 10902	0.89	0.09–8.48	0.89	1.04	0.43–2.50	0.92
(b) 4 or more positive nodes						
Adjuvant radiotherapy	0.48	0.16–1.40	0.08	1.15	0.77–1.73	0.37
T2	0.88	0.17–4.37	0.84	1.14	0.60–2.14	0.60
Age > 50 years	1.28	0.40–4.04	0.59	1.05	0.68–1.63	0.76
Adjuvant chemotherapy	0.57	0.45–5.57	0.36	0.76	0.50–1.16	0.39
EORTC trial						
10801 versus 10854	0.57	0.15–2.17	0.27	0.87	0.53–1.41	0.46
10801 versus 10902	0.44	0.05–4.15	0.35	0.61	0.24–1.55	0.17

Table 6.
A. Multivariate Cox regression analysis mastectomized patients with 1-3 positive nodes (N = 507)
B. Multivariate Cox regression analysis mastectomized patients with 4+ positive nodes (N = 381)

Subgroup analyses were undertaken in order to study whether the effect of adjuvant radiotherapy after mastectomy could be demonstrated in node-positive, as well as node-negative, breast cancer patients. Node-positive patients benefited in terms of an improved locoregional control (RR 0.36, 99% CI 0.17–0.77, P = 0.001). However, in node-negative patients, radiotherapy was not associated with a better locoregional control (RR 0.56, 99% CI 0.22–1.45, P = 0.12). In terms of overall survival, node-positive patients who received radiotherapy had better overall survival rates than patients who did not (RR 0.70, 99% CI 0.52–0.94, P = 0.002). This could not be shown in the node-negative patient group in which no advantageous effect of adjuvant radiotherapy could be demonstrated (RR 0.87, 99% CI 0.56–1.34, P = 0.40). In patients who underwent mastectomy and had 1–3 positive nodes, radiotherapy was associated with significantly improved survival rates (RR 0.48, 99% CI 0.31–0.75, P = <0.001, Table 6a). However, in patients with four or more positive nodes, no significant association between radiotherapy and overall survival was found (Table 6b).

Discussion

The central question regarding locoregional therapy (i.e. surgery and radiotherapy) for early breast cancer remains; that is, whether more aggressive locoregional treatment significantly reduces long-term mortality from breast cancer. For example, the EORTC trial 10801 [3], which randomised between modified radical mastectomy and breast-conserving surgery demonstrated a significant difference in terms of local control in favour of the modified radical mastectomy arm after a long-term follow-up. The respective local control rates were 87% after radical mastectomy and 77% after breast-conserving therapy at 13 years of follow-up. However, overall survival was not significantly different between the arms. To study both treatment modalities in a large non-randomised sample, we combined the data of three controlled clinical trials conducted by the EORTC Breast Cancer Group studying different treatment regimens in early breast cancer patients. As mentioned before, the analyses in this study are based upon non-randomised comparisons. In our series, the most important predictor of locoregional recurrence was undergoing breast-conserving surgery. This is a striking finding considering the fact that breast-conserving therapy is well established in the management of early-stage breast cancer. The underlying cause for this effect may be due to inadequate surgical margins, which are known to impair local control after breast-conserving surgery [14–16]. Unfortunately, we were not able to retrieve this information from the case report forms, so a definite answer to this question cannot be given. However, the increased risk for locoregional recurrence after breast-conserving therapy did not result in an increased risk for worse overall survival. This is in accordance with the results of the randomised trials comparing breast-conserving surgery and mastectomy [3-5], as well as with the meta-analyses conducted by the EBCTCG. This underlines the fact that the majority of early locoregional recurrences after breast-conserving therapy are associated with a poor prognosis, but are not instigators of the subsequent systemic spread [1,17]. In addition, late recurrences and second ipsilateral primary tumors can be treated well with salvage mastectomy and have a much less detrimental effect on prognosis compared with early recurrences. Although the median follow-up of this analysis was 11 years, this period may be to short to detect a survival benefit if the impact of more aggressive surgery on survival occurs after a longer follow-up, i.e. 15–20 years. Therefore, it must be stressed that the impact of surgery on outcome in breast cancer has to be followed-up in the future. Breast cancer at a young age, i.e. younger than 35/40 years at the time of diagnosis, is often associated with a poor prognosis [18–21]. In addition, young breast cancer patients have been reported to be at a higher risk of local recurrence, especially after breast-conserving therapy [22,23]. In line with these data, our results also demonstrate that young age, i.e. lower than 50 years, is associated with a poor locoregional control. Remarkably, in our analysis, breast-conserving therapy was not associated with a higher risk for locoregional recurrence or death in women younger than or equal to 40 years. Patients that underwent mastectomy and subsequently received adjuvant radiotherapy were at a lower risk for locoregional recurrence in our analysis and this resulted in a lower risk of death as well. Two randomized trials in high-risk breast cancer patients conducted by the Danish Breast Cancer Study Group and one Canadian trial have previously demonstrated a survival benefit for adjuvant radiotherapy after mastectomy [10–12].

However, the quality of surgery in these trials was very poor, especially the management of the axilla, resulting in recurrence rates exceeding 20% after 10 years. Therefore, radiotherapy may have compensated for inadequate surgery in these trials. In addition, results from a meta-analysis performed by the EBCTCG demonstrated a trade-off effect due to an increase in cardiac-related mortality after adjuvant radiotherapy, which equalled the decrease in breast cancer-related mortality induced by radiotherapy [6]. It has been proposed that the detrimental effects of radiotherapy are mainly attributable to older trials (conducted before 1975) that used obsolete radiotherapy regimens causing excessive damage to the heart (EBCTCG 2000 update). In our analysis, patients were included who had participated in trials conducted between 1980 and 1999. The median follow-up was 11 years at the time of the diagnosis and our results show a definite favourable effect for radiotherapy after mastectomy in terms of overall survival. This suggests that, in this series, in which the radiotherapeutic regimen that was given to patients varied widely, adjuvant radiotherapy directed at either the chest wall or the axilla resulted in an absolute gain in survival. However, adjuvant radiotherapy only contributed to locoregional control and overall survival in node-positive patients. In the node-positive group, an association between radiotherapy and a favourable outcome was seen, especially in patients with 1–3 positive nodes as opposed to patients with four or more positive lymph nodes. This remarkable finding is in accordance with the results from the Danish radiotherapy trials [7,8] in which patients with limited nodal involvement benefited more from adjuvant radiotherapy than patients with extensive nodal disease. A possible explanation for this difference is that node-positive patients benefit from radiotherapy due to the local eradication of residual tumor cells. In patients with four or more positive lymph nodes, systemic spread of tumor cells may be much more extensive compared with patients with 1–3 positive nodes and therefore locoregional therapy will not have a significant impact on survival in these patients.

This stresses the need for trials studying the management of the axilla. The EORTC Breast Cancer Group is currently conducting a trial in which sentinel node-positive patients are randomised between axillary lymph node dissection and radiotherapy [24].

Many investigators have tried to divide locoregional recurrences into a category that are merely associated with distant disease and a category that are the instigators of distant disease [25–35]. Although these were retrospective analyses, a short disease-free interval between primary therapy and adverse primary tumor characteristics have been identified as predictors for poor outcome after locoregional recurrence. In addition, a small locoregional recurrence (<1 cm) was associated with a favourable prognosis, suggesting an advantageous effect for early detection [31]. Finally, it must be emphasised that this is was a non-randomised, retrospective analysis and, therefore, our results should be interpreted with caution and be considered hypotheses rather than conclusions. Nevertheless, the apparent lack of benefit of mastectomy in young patients, in terms of locoregional control, and the lack of benefit in the general population, in terms of overall survival, once again raises the question of whether breast cancer patients should receive more aggressive surgery. In addition, the differences in the efficacy of radiotherapy between patients with only a few involved nodes (1–3) and those with more involved nodes (54) have to be evaluated more thoroughly. However, any recurrence, either an associative factor or

an instigator of distant spread, is an emotionally devastating event for the patient [36]. Therefore, any treatment strategy against breast cancer should include an adequate local therapy in order to avoid unnecessary locoregional recurrences. The fact that an adequate local therapy may improve survival provides further support for this argument.

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CHAPTER 7

Isolated loco-regional recurrence of breast cancer is more common in young patients and following breast conserving therapy: Long-term results of European Organization for Research and Treatment of Cancer studies

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Abstract

The aim of this study was to evaluate prognostic factors for isolated loco-regional recurrence in patients treated for invasive stage I or II breast cancer. The study population comprised 3602 women who had undergone primary surgery for early stage breast cancer, who were enrolled in European Organisation for Research and Treatment of Cancer (EORTC) trials 10801, 10854, or 10902, by breast conservation (55%) and mastectomy (45%). The median follow-up time varied from 5.3 (range: 0.6–9.5) to 11.9 years (range: 0.6–17.4). Main outcome was the occurrence of isolated loco-regional recurrence. The results of multivariate analysis showed that younger age and breast conservation were risk factors for isolated loco-regional recurrence (breast cancer under 35 years of age versus over 50 years of age: hazard ratio 2.80 (95% CI 1.41–5.60)); breast cancer age 35–50 years versus over 50 years: hazard ratio 1.72 (95% CI 1.17–2.54); breast conservation (hazard ratio: 1.82 (95% CI 1.17–2.86)). After perioperative chemotherapy, less isolated loco-regional recurrences were observed (hazard ratio 0.63 (95% CI 0.44–0.91)). No significant interaction effects were observed. It is concluded that young age and breast conserving therapy are both independent predictors for isolated loco-regional recurrence. As an isolated loco-regional recurrence is a potentially curable condition, women treated with breast conservation or diagnosed with breast cancer at a young age should be monitored closely to detect local recurrence at an early stage.

Introduction

Loco-regional recurrence of breast cancer is of concern in breast cancer treatment, as it is a well-established independent risk factor for distant metastases and death [1,2]. Many studies have looked for factors associated with the increased risk of loco-regional recurrence [3]. A well-known risk factor is breast conserving surgery, being associated with a higher risk of loco-regional recurrence, compared with mastectomy [4–8].

Risk factors for local recurrence frequently reported in patients treated with breast conserving therapy are: positive margin status, extensive intraductal component and young age of diagnosis of primary tumour [3, 9–12]. Risk factors commonly reported for loco-regional recurrence in patients treated primarily with mastectomy are histological grade, and extensive axillary node involvement [13,14]. We studied risk factors at primary diagnosis of early breast cancer associated with isolated loco-regional recurrence and other recurrences, including distant metastases or death irrespective of primary treatment. We focused on isolated loco-regional recurrences, because these recurrences are not associated with distant metastases and are, in principle, curable, To do this, we re-analysed the data of 3602 patients with early stage breast cancer surgically treated and recruited in three European Organization for Research and Treatment of Cancer (EORTC) trials (studies 10801, 10854 and 10902). Within all three studies, patients were treated with mastectomy or with breast conserving therapy, which allowed us to study prognostic factors in relation to primary treatment.

Characteristics	Study			Total
	10801	10854	10902	
	n (%)	*n* (%)	*n* (%)	*n* (%)
Age at diagnosis (years)				
>50	502 (58.5)	1429 (56.8)	86 (37.7)	2017 (56.0)
35–50	317 (36.9)	970 (38.6)	122 (53.5)	1409 (39.1)
⩽35	39 (4.5)	117 (4.7)	20 (8.8)	176 (4.9)
Tumour size (cm)				
<2	167 (19.5)	801 (31.8)	38 (16.7)	1006 (27.9)
2–5	691 (80.5)	1715 (68.2)	190 (83.3)	2596 (72.1)
Nodal state				
Node-negative	502 (58.5)	1360 (54.1)	83 (36.4)	1945 (54.0)
Node-positive	356 (41.6)	1156 (45.9)	145 (63.6)	1657 (46.0)
Surgical therapy				
Mastectomy	414 (48.3)	1030 (40.9)	162 (71.1)	1606 (44.6)
Breast conserving therapy	444 (51.7)	1486 (59.1)	66 (28.9)	1996 (55.4)
Perioperative chemotherapy				
No	858 (100)	1261 (50.1)	228 (100)	2347 (65.2)
Yes	–	1255 (49.9)	–	1255 (34.8)
Adjuvant chemotherapy				
No	709 (82.6)	2061 (81.9)	–	2770 (76.9)
Yes	149 (17.4)	455 (18.1)	228 (100)	832 (23.1)
Adjuvant radiotherapy				
No	243 (28.3)	533 (21.2)	83 (36.4)	859 (23.8)
Yes	615 (71.7)	1983 (78.8)	145 (63.6)	2743 (76.2)
Tamoxifen				
No	858 (100)	1827 (72.6)	137 (60.1)	2822 (78.3)
Yes	–	689 (27.4)	91 (39.9)	780 (21.7)

Table 1: Characteristics of the patients (3,602) included in this analysis per study and total; n (%)

Patients and methods

Selection of trials and patients

Patients were selected from trials that randomised early stage breast cancer patients. EORTC has conducted several large randomised phase III trials concerning the optimal management of breast cancer in patients with stage I or II breast cancer. A total of 4395 breast cancer patients have been enrolled for these trials; EORTC trials 10801, 10854 and 10902. Patients treated with pre-operative chemotherapy (n = 377), those not eligible for the study (due to false inclusion or severe protocol violation, n = 88), those with stage III breast cancer (n = 238) and those without full information on all co-variates (n = 90) were excluded from the analysis. A summary of the 3602 included patients is given in Table 1. For a short summary of the outcomes, the median overall follow-up times, and the median follow-up times to first event, see Table 2. A brief description of these trials follows.

EORTC trial 10801 (1980–1986)was conducted in order to assess the safety of breast conserving treatment. In this trial, patients were randomised between breast conserving surgery combined with radiotherapy, and modified radical mastectomy. Six cycles of adjuvant chemotherapy with cyclophosphamide (100 mg/m^2) given orally on days 1–14, methotrexate (40 mg/m^2) given intravenously on days 1 and 8, and 5-fluorouracil (600 mg/m^2) given intravenously on days 1 and 8, were indicated for all

patients under the age of 55 years with positive nodes. No information was collected on hormonal therapy. In this study, 902 patients were randomised [5,15,16].

EORTC trial 10854 (1986–1991) considered the question of whether one course of perioperative chemotherapy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Perioperative chemotherapy consisted of one single course of doxorubicin (50 mg/m^2), 5-fluorouracil (600 mg/m^2) and cyclophosphamide (600 mg/m^2) (FAC), administered intravenously within 36 h after surgery. It was recommended that axillary lymph node-positive pre-menopausal patients in the perioperative chemotherapy group received an extra five cycles of cyclophosphamide, methotrexate and 5-fluorouracil (CMF). The advice for node-positive patients, younger than 50 years, who did not receive perioperative chemotherapy, was one conventional course of FAC followed by five cycles of CMF after surgery. Patients were stratified for breast conserving therapy and modified radical mastectomy. Prolonged adjuvant systemic treatment was left to the discretion of the local investigators. A total of 2795 patients were included in this trial [17–19].

EORTC trial 10902 (1991–1999) was set up to determine the value of pre-operative chemotherapy. Patients were randomized to receive four cycles of chemotherapy either before or after surgery. Chemotherapy consisted of four cycles of 5-fluorouracil (600 mg/m^2), epirubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) (FEC) administered intravenously, at 3-weekly intervals. In the pre-operative chemotherapy group, surgical therapy followed within 4 weeks of the fourth course of chemotherapy. In the postoperative chemotherapy group, the first cycle was given within 36 h after surgery. A total of 698 patients were randomised [20].

Assessments and statistical methods

Endpoints for this study were: (i) isolated loco-regional recurrences, (ii) all other events, including distant metastases or death. Non-isolated loco-regional recurrences were considered as distant metastases. A loco-regional recurrence was defined as any recurrence in the ipsilateral breast, axilla or chest wall. A loco-regional recurrence was considered isolated if for a period of 2 years after the loco-regional recurrence occurred, no distant metastasis or death was observed. A loco-regional recurrence was considered non-isolated if distant metastasis was observed before, or concomitantly with, or within a period of 2 years after the occurrence of the loco-regional recurrence. In the database we could not discern breast cancer specific death from other causes of death, so all causes of death were considered as one group. The following characteristics were considered: tumour size (62 cm, 2–5 cm), axillary nodal status positivity (no, yes), oestrogen receptor positive (yes, no), age at diagnosis (635, 36–50, >50 years), surgical therapy (mastectomy, breast conserving therapy), margins free (yes, no), perioperative chemotherapy (yes, no), adjuvant chemotherapy (yes, no), adjuvant radiotherapy (yes, no) and tamoxifen (yes, no) (see Table 1). The values for all characteristics were based on clinical observations. Survival time was defined as the time between randomisation and the occurrence of the first events (loco-regional recurrence, distant metastasis or death from any cause) or last date of follow-up. Multivariate Cox proportional-hazard regression models were used to estimate hazard ratios with their 95% confidence intervals (CIs). All tests were two-sided. To

Outcomes (first events)	Study			Total
	10801	10854	10902	
	n (%)	*n* (%)	*n* (%)	*n* (%)
Isolated loco-regional recurrences, 2 years event-free[a]	47 (5.5)	122 (4.8)	3 (1.3)	172 (4.8)
Distant metastasis or death, or loco-regional recurrences with events in 2 years follow-up[b]	318 (37.1)	803 (31.9)	61 (26.8)	1182 (32.8)
Median (range) follow-up time (years)	11.9 (0.6–17.4)	10.2 (0.2–14.2)	5.3 (0.6–9.5)	10.2 (0.2–17.4)
Median (range) follow-up time to first event	11.0 (0.1–17.4)	9.5 (0.1–14.1)	4.9 (0.5–9.5)	9.2 (0.1–17.4)

a Without distant metastasis or death within 2 years of follow-up.
b With distant metastasis or death within 2 years of follow-up.

Table 2: Follow-up and first events for the patients included in this analysis per study and total

test the assumption of proportional hazards, an interaction term of a prognostic variable and a time-dependent covariate was added [21]. To control for unmeasured possible differences in study populations, we added study as a factor in the multivariate Cox regression analysis. Two years disease-free follow-up was taken as cut-off for an isolated loco-regional recurrence because the incidence of metastases lowers after that [22]. Because this point is not clear-cut, in a sensitivity analysis we varied this cut-off point between 3 months, 1 year and 5 years.

Results

In all, 55% of the patients underwent breast conserving therapy (Table 1). An isolated loco-regional recurrence without distant metastasis or death within 2 years of follow-up was observed in 172 (4.8%) of the patients (Table 2). Another event (a distant metastasis or death) occurred in 1182 (32.8%) of the patients. A total of 55 (32%) of the isolated loco-regional recurrences were seen in patients treated with mastectomy, and 117 (68%) were seen in patients treated with breast conserving therapy (data not in table).
From the multivariate Cox regression analyses (Table 3) it appeared that significant risk factors for isolated loco-regional recurrence were: younger age at diagnosis of breast cancer, breast conserving therapy and no perioperative chemotherapy. Risk for isolated loco-regional recurrence for women under 35 years of age was compared with over 50 years of age: hazard ratio 2.34 (1.30–4.24); 35–50 years: hazard ratio 1.60 (1.14–2.25). Risk for loco-regional recurrence for breast conserving therapy compared with mastectomy: hazard ratio 1.82 (1.17–2.86). Less frequent isolated loco-regional recurrences were observed after perioperative chemotherapy (hazard ratio 0.63 (0.44–0.91)).

In a model predicting loco-regional recurrence including age at diagnosis, surgical therapy and an interaction effect between these two, no statistically significant effects other than already reported, were observed (results not presented). In the multivariate Cox regression analyses more distant metastases and deaths were observed in very young patients (under 30 years of age, hazard ratio: 1.55 (1.20–2.00)); in patients with larger tumour size (hazard ratio 1.56 (1.35–1.80)); and in patients with positive nodal status (hazard ratio 2.12 (1.81–2.47)). In patients treated with adjuvant chemotherapy less distant metastases or deaths were also observed (hazard ratio 0.66 (0.54–0.79)).

Subsequently the definition of an isolated loco-regional recurrence was varied: (a loco-regional recurrence was considered isolated if for a period of 3 months, 1 year and 5 years (instead of 2 years) after the loco-regional recurrence occurred, no distant metastasis or death was observed). It was found that a less restrictive definition (a shorter timeframe without any event after loco-regional recurrence was observed) reduced the prognostic effects of age and perioperative chemotherapy; meanwhile, it enhanced the prognostic effects of surgical therapy and adjuvant radiotherapy (see Table 4). A more restrictive definition (a longer time-frame after loco-regional recurrence without any event was observed) enhanced the prognostic effects of age and perioperative chemotherapy. Due to smaller numbers of patients, the confidence intervals are wider. With regard to distant metastasis, death, or non-isolated loco-regional recurrences, the hazards were not influenced, mainly due to the fact that the relative change in number was very small (results not presented).

Characteristics	Isolated loco-regional recurrences, 2 years event-free[a] $n = 172$	Distant metastasis or death[b] $n = 1073$
	HR (95% CI)	HR (95% CI)
Age at diagnosis (years)		
>50	1	1
35–50	1.60 (1.14–2.25)	1.01 (0.87–1.16)
⩽35	2.34 (1.30–4.24)	1.55 (1.20–2.00)
Tumour size (cm)		
<2	1	1
2–5	1.12 (0.81–1.56)	1.56 (1.35–1.80)
Nodal state		
Node-negative	1	1
Node-positive	0.87 (0.55–1.38)	2.12 (1.81–2.47)
Surgical therapy		
Mastectomy	1	1
Breast conserving therapy	1.82 (1.17–2.86)	0.98 (0.86–1.13)
Perioperative chemotherapy		
No	1	1
Yes	0.63 (0.44–0.91)	0.94 (0.82–1.08)
Adjuvant chemotherapy		
No	1	1
Yes	0.63 (0.35–1.12)	0.66 (0.54–0.79)
Adjuvant radiotherapy		
No	1	1
Yes	0.66 (0.41–1.07)	1.00 (0.85–1.18)
Tamoxifen		
No	1	1
Yes	0.77 (0.42–1.41)	0.89 (0.73–1.07)

a Without distant metastasis or death within 2 years of follow-up.
b Including loco-regional recurrences with distant metastasis or death within 2 years of follow-up.

Table 3: Multivariate analyses of all patients related to outcomes (first events) (Hazard Ratio's and 95% C.I.)

Discussion

The major risk factor for an isolated loco-regional recurrence in this analysis was younger age as well as breast conservation (breast cancer under 35 years of age: hazard ratio 2.80 (1.41–5.60)); breast cancer between 35 and 50 years of age: hazard ratio 1.72 (1.17–2.54); breast conservation (hazard ratio: 1.82 (1.17–2.86)). No significant interaction effects between these two variables were observed. After perioperative chemotherapy, less isolated loco-regional recurrences were observed (hazard ratio 0.63 (0.44–0.91)), which has been published earlier [20]. Prognostic factors for distant metastases or deaths were larger tumour size (hazard ratio 1.56 (1.35–1.80)), positive nodal status (hazard ratio 2.12 (1.81–2.47)), and breast cancer under 35 years (hazard ratio 1.55 (1.20–2.00)). After adjuvant chemotherapy less distant metastases or death were observed (hazard ratio 0.66 (0.54–0.79)). No significant interaction effects were observed. Young age (breast cancer diagnosed before 35 years) was a predictor for isolated loco-regional recurrence as well for other recurrences. Young age is generally accepted as being a prognostic factor for worse loco-

Characteristics	Isolated loco-regional recurrences, 3 months event-free[a] $n = 280$	Isolated loco-regional recurrences, 1 year event-free[b] $n = 228$	Isolated loco-regional recurrences, 5 years event-free[c] $n = 88$
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age at diagnosis (years)			
>50	1	1	1
35–50	1.46 (1.10–1.93)	1.47 (1.08–2.00)	2.04 (1.27–3.30)
⩽35	2.35 (1.48–3.74)	2.27 (1.36–3.80)	2.71 (1.17–6.28)
Tumour size (cm)			
<2	1	1	1
2–5	1.14 (0.88–1.49)	1.09 (0.82–1.45)	1.04 (0.66–1.64)
Nodal state			
Node-negative	1	1	1
Node-positive	1.14 (0.81–1.61)	1.08 (0.73–1.58)	0.66 (0.35–1.27)
Surgical therapy			
Mastectomy	1	1	1
Breast conserving therapy	2.14 (1.52–3.00)	2.10 (1.43–3.09)	1.85 (0.99–3.47)
Perioperative chemotherapy			
No	1	1	1
Yes	0.69 (0.53–0.93)	0.67 (0.49–0.92)	0.50 (0.29–0.87)
Adjuvant chemotherapy			
No	1	1	1
Yes	0.81 (0.53–1.22)	0.72 (0.47–1.15)	1.03 (0.43–2.47)
Adjuvant radiotherapy			
No	1	1	1
Yes	0.60 (0.41–0.87)	0.60 (0.40–0.91)	0.56 (0.29–1.09)
Tamoxifen			
No	1	1	1
Yes	1.01 (0.66–1.54)	0.88 (0.54–1.42)	1.34 (0.60–2.96)

a Without distant metastasis or death within 3 months of follow-up.
b Without distant metastasis or death within 1 year of follow-up.
c Without distant metastasis or death within 5 years of follow-up.

Table 4: Results of sensitivity analysis; characteristics of all patients related to isolated loco-regional recurrences (multivariate, Hazard Ratio's and 95% C.I.)

regional control in breast cancer [3,9–12]. However, it has been reported that this is not the case for radical mastectomy [14]. Although the absolute number of isolated loco-regional recurrences was higher after breast conserving therapy than after mastectomy in our series, the effect of young age on the occurrence of isolated loco-regional recurrences was not different in patients treated with breast conserving therapy or mastectomy. Arriagada and colleagues found the same negative effect of young age as we did on loco-regional control irrespective of the type of surgery [14]. Other reported risk factors for local recurrences in patients treated with breast conserving therapy are positive margin status and extensive intraductal component [3,9–12]. Because these measurements were not consistently assessed in the included studies, they could not be studied. Risk factors reported for loco-regional recurrences in patients primarily treated with mastectomy are histological grade, and extensive axillary node involvement (10 nodes or more) [13,14]. We could not confirm these findings in our study, which might be explained by the more restricted definition of loco-regional recurrences we used (i.e., not followed by distant metastases within 2 years of follow-up). The impact of loco-regional recurrences on overall survival has not been demonstrated in trials which randomised between breast conserving therapy and mastectomy [13,23,24]. This means that some loco-regional recurrences are potentially curable, as they are not followed by further distant spread of the

disease. Whether adjuvant chemotherapy is effective in these women is the subject of a randomized trial of the National Cancer Institute and the Breast International Group [25].

As outlined earlier, more loco-regional recurrences were observed after breast conserving therapy. It can be expected that, due to improvement in patient selection and treatment techniques, the differences will decrease between breast conserving therapy and mastectomy, with regard to the occurrence of loco-regional recurrences after breast conserving therapy [26]. This is also in accordance with the results of the EORTC trial that randomised between a conventional therapeutic regimen and an extra boost to the tumour bed after breast conserving surgery [27]. Local control was significantly improved by adding a radiation boost for patients with breast conservation.

This analysis shows that young age and breast conserving therapy are both independent predictors for isolated loco-regional recurrences. To reach optimal local control, young women and patients treated with breast conserving therapy should be monitored closely to detect local breast cancer recurrences at an early stage.

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Part III

CHAPTER 8

Impact of established prognostic factors in early stage breast cancer in very young breast cancer patients; a translational research project using pooled datasets derived from 4 EORTC Breast Group Trials

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Abstract

Young age at time of diagnosis of breast cancer is an independent prognostic factor associated with unfavorable outcome in terms of survival and locoregional control. This has led to the general recommendation to administer adjuvant systemic chemotherapy to patients aged 35 years or less at time of diagnosis regardless of other tumor characteristics like tumor size and axillary lymph node status.

However, since breast cancer at a very young age, i.e. < 41 years is a relative rare event, evidence concerning prognostic factors within this subgroup of patients is lacking. Therefore the data of four EORTC Breast Group Trials concerning primary operable breast cancer were combined to study prognostic factors on long term outcome in very young breast cancer patients. The total dataset consisted of 9938 early breast cancer patients. Tumor material was collected from 549 patients aged under 41 years of age at time of diagnosis. In the multivariate analyses, only histological grade remained a significant prognostic factor for both overall survival (Grade II HR 2.67; 95% CI 0.91 to 7.80; P = 0.07, Grade III HR 3.92; 95%CI 1.38 to 11.16; P = 0.01) and distant metastasis free survival (Grade II HR 2.04; 95% CI 1.07 to 3.88; P = 0.03, Grade III HR 2.38; 95%CI 1.29 to 4.39; P < 0.01). However, large tumor size remained an independent unfavorable prognostic factor on outcome in terms of distant metastasis free survival as well (HR 1.64 (1.17-2.31) P < 0.01). In the subgroup of node negative very young breast cancer patients, histological grade remained an independent prognostic factor for both overall survival (Grade III HR 8.92; 95%CI 1.17 to 68.20; P = 0.04) and distant disease-free survival respectively (Grade III HR 4.12; 95%CI 1.42 to 11.98; P < 0.001). Histological grade is a strong independent prognostic factor, even in young breast cancer patients. These findings support the fact that histological grade is an excellent diagnostic tool to assess disease outcome in this specific subset of very young breast cancer patients.

Introduction

The incidence of early stage breast cancer in very young women is increasing. At present breast cancer at young age, i.e. under age 35, does account for approximately 5% of the total number of cases diagnosed each year in the US.

Based upon multiple retrospective analyses demonstrating the independent unfavorable prognostic impact of young age on prognosis in breast cancer, current consensus guidelines have included young age (< 35) as an absolute indication for adjuvant systemic chemotherapy after primary removal of the tumor irrespective of other tumor characteristics [1-4]. Such guidelines imply that young patients with favorable tumor features such as small tumor size and a negative axillary nodal status will receive chemotherapy as well although absolute treatment benefits for these patients are not well known which is the result of the fact that breast cancer at very young age remains a relatively infrequent event.

Retrospective analyses have demonstrated breast cancer at a very young age to be associated with higher grade, ER negative tumors and later stage disease at time of diagnosis [5,6].

However, other yet unknown factors may be responsible for the poorer outcome in this subset of patients and this hypothesis is emphasized by the fact that BRCA I and II mutation carriers only account for 10% in this population [7-9].

Therefore, two questions remain still very much open for discussion to date. First, do all very young breast cancer patients require adjuvant systemic chemotherapy, and second, by which means can subsets of patients within this group of very young women be identified who have an excellent or poor prognosis.
To study these questions we pooled the data of four randomized trials conducted by the EORTC Breast Cancer Group and the EORTC radiotherapy Group and collected tumor material of patients under age 41 who participated in one of these trials.

Patients and Methods

The data used in this study was obtained from 4 randomized phase III EORTC trials that included patients with early stage breast cancer. Two trials randomized between two types of locoregional therapy whereas two trials randomized between different timing of the same type of systemic therapy. The detailed features of these trials have been described in detail previously (ref). In summary, the trial protocols are listed below:

EORTC trial 10801 (1980-1986, median follow up 13.4 years) was conducted in order to assess the safety of breast conserving treatment. In this trial, patients were randomized between breast conserving surgery combined with radiotherapy and radical mastectomy. Six cycles of adjuvant chemotherapy with cyclophosphamide 100 mg/m^2 given orally on days 1-14, methotrexate 40mg/m^2 given intravenously on days 1 and 8, and 5-fluorouracil 600 mg/m^2 given intravenously on days 1 and 8, were indicated for all patients under the age of 55 with positive nodes. No information was collected on hormonal therapy. In this study, 902 patients were randomized [10].

EORTC trial 10854 (1986-1991, median follow up 10.8 years) studied the question whether one course of peri-operative chemotherapy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Peri-operative chemotherapy consisted of one single course of doxorubicin 50 mg/m^2, 5-fluorouracil 600 mg/m^2, and cyclophosphamide 600 mg/m^2 (FAC), administered intravenously within 36 hours after surgery. Axillary lymph node-positive premenopausal patients in the peri-operative chemotherapy group were recommended to receive an extra 5 cycles of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). Node-positive patients, younger than 50 years, who did not receive peri-operative chemotherapy, were advised to give one conventional course of FAC followed by five cycles of CMF after surgery. Patients were stratified for breast conserving therapy and modified radical mastectomy. Prolonged adjuvant systemic treatment was left to the discretion of the local investigators. 2795 patients were included in this trial [11].

EORTC trial 10902 (1991-1999, median follow up 6.1 years) was set up to determine the value of pre-operative chemotherapy. Patients were randomized to receive four cycles of chemotherapy either before or after surgery. Chemotherapy consisted of four cycles of 5-fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 (FEC) administered intravenously, at 3-weekly intervals. In the pre-operative chemotherapy group, surgical therapy followed within four weeks of the fourth course of chemotherapy. In the postoperative chemotherapy group, the first

	Patient characteristics (N = 9938)									
	10801		10854		10902		22881		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age (median 53.6 years)										
≤ 40 years	113	13	396	14	125	18	558	10	1192	12
≤ 30 years	14	2	34	1	18	3	44	1	*110*	*1*
31-35 years	26	3	101	4	33	5	158	3	*318*	*3*
35-40 years	73	8	261	9	74	10	356	6	*763*	*8*
> 40 years	789	87	2399	86	573	82	4985	90	8746	88
Tumor size*										
T1	175	19	823	30	96	14	2868	52	3962	40
T2	725	81	1759	64	403	59	2662	48	5549	56
T3	-		166	6	188	27	13	0.2	367	4
Missing	2		47		11				60	
Nodal status**										
N-	514	59	1467	53	253	39	4351	79	6585	67
N+	361	41	1327	47	401	61	1192	21	3281	33
Missing	27		1		44				72	
ER status***										
Positive	-		1823	73	337	70	2930	73	5090	73
Negative	-		673	27	141	30	1092	27	1906	27
Missing	902		299		220		1521		2942	
Surgery										
Breast conserving	466	52	1544	56	198	30	5543	100	7751	78
Mastectomy	436	48	1235	44	475	70	-		2128	22
Missing			16		43				59	
Adjuvant CT****										
No	753	83	2227	82	-		4792	87	7772	79
Yes	149	17	502	18	698	100	699	13	2028	21
Missing			66				52		118	

* Clinical tumor size
** Pathological nodal status
*** EORTC trial 10801 did not report estrogen receptor status, all patients (N=902 have been deemed missing)
**** EORTC trial 10854 randomized between 1 course of peri-operative chemotherapy. One course of peri-operative chemotherapy was not deemed as prolonged chemotherapy

Table 1. All patients

cycle was given within 36 hours after surgery. Stratification was performed for planned type of surgery instead of performed type of surgery. This was done because of the expected effect of pre-operative chemotherapy on downstaging of the tumor. A total number of 698 patients were randomized [12].

EORTC trial 22881 (1989 – 1996, median follow up 5.1 years) studied the value of a boost dose after primary breast conserving surgery. Patients with breast cancer of clinical stage T1–2,N0–1,M0 were eligible for the trial. Patients with stage I or II breast cancer who had undergone macroscopically complete surgical removal of the tumor and axillary dissection were randomly assigned to undergo 50-Gy irradiation of the whole breast with or without an additional dose of 16 Gy to the tumor bed. Patients with a microscopically incomplete excision were assigned to receive booster doses of 10 or 26 Gy. Patients with axillary lymph-node involvement received adjuvant systemic therapy: premenopausal patients received chemotherapy, and postmenopausal patients received tamoxifen. Patients not given adjuvant chemotherapy began radiotherapy within nine weeks after lumpectomy. For patients who received adjuvant chemotherapy, a delay of up to six months before irradiation was allowed. This study enrolled 5569 patients [13].
In all trials if adjuvant chemotherapy was indicated, patients either received CMF or an anthracyclin-based regimen (FAC or FEC). Adjuvant hormonal therapy for premenopausal ER or PgR positive patients was not yet recommended at the time when these trials were conducted. No information concerning estrogen receptor status and tamoxifen use was available for patients who participated in EORTC trial 10801. In the trials where tamoxifen use was recorded, less than 5% of patients ≤ 41 years received tamoxifen.

Collection of tumor material and immunohistochemistry
A questionnaire was send to participating institutions to collect paraffin tumor specimens from all patients aged under 41 at time of diagnosis except for those who had participated in EORTC trial 10902 and received neoadjuvant chemotherapy. Tumor tissue was collected and processed for immunohistochemistry using a tissue microarray. Three core biopsies were taken from every tumor specimen and put in a so-called donor block. On average, one tissue array donor block consisted of three biopsies from sixty tumor specimens. This procedure has been described in debt by others previously [14-17].

Histological grading, scoring of the extent of intraductal carcinoma and lymph vessel invasion was performed on H&E colored slides according to Bloom and Richardson [18,19]. ER, PgR, Her2 and P53 expression levels were estimated by immuno-histochemistry. Detailed procedures have been described previously [20-22]. In summary, a tissue microarray slide was stained and scored counting the percentage of positive nuclei and taking the mean value of the three tumor biopsies. For estrogen- and progesterone receptor expression, Tumors with >10% of the tumor cells showing nuclear staining were considered positive. Tumor were deemed p53 positive if there was > 50% nuclear staining. Her2 expression was scored estimating the level of membranous staining. Strong membranous staining in > 10% of tumor cells was considered positive. Estimation of tumor grade and protein expression levels were scored by two investigators (MJ vd V & JA vd H) simultaneously who had to come to an agreement in case of different views.

Selection of endpoints

Since this study was set up to study the impact of potential prognostic factors in very young breast cancer patients on long term outcome, endpoints studied were overall survival and distant metastasis free survival. Survival time was defined as the time between randomization and death from any cause. Distant metastasis free survival time was defined as time to distant metastasis or death if the latter event occurred before a distant metastasis was diagnosed. Breast cancer specific survival was not included since exact information concerning the cause of death was lacking in three out of four trials.

Statistical analyses

All analyses were performed for overall survival and distant metastasis free survival. Apart from patient age, covariates included consisted of tumor-, and treatment related characteristics. Tumor characteristics were tumor size, nodal status, tumor grade, hormone receptor status, Her2 overexpression, p53 overexpression, and lymphangio invasion. Treatment characteristics consisted of type of surgery and the administration of chemotherapy. Tamoxifen use was not included because of the high rate of missing data for this covariant. Cox proportional-hazard regression models [23] were used to estimate hazard ratios with 95% confidence intervals. A 5 % significance level was used and all tests are two-sided. Survival analyses were performed using the Kaplan Meyer method [24].

Results

Patient characteristics

A total of 9938 early stage breast cancer patients participated in one of four trials. The majority of these patients, i.e. approximately 67%, were node negative. In addition, approximately 70% of the patients whose hormone receptor status was available had estrogen receptor positive breast cancer. Further patient characteristics are listed in Table I. 1192 patients were aged under 41 years at time of diagnosis. Paraffin embedded tumor material was successfully obtained and processed into a tissue micro array for 549 patients younger than 41 years. This subgroup of patients had

	Patient characteristics (N= 549)	
	Age (median 36.7 years)	
	No.	%
Tumor type		
Ductal	497	96
Lobular	17	3
Other	5	1
Missing	30	
Clinical Tumor size		
T1	219	40
T2	308	56
T3	20	4
Missing	2	
Pathological tumor size		
T1	333	67
T2 / T3	164	33
Missing	52	
Nodal status*		
N-	341	63
N+	204	37
Missing	4	
Tumor grade		
I	76	15
II	165	32
III	276	53
Missing	32	
Lymfangio invasion		
None	357	69
2-5 vessels	86	17
> 5 vessels	76	14
Missing	30	
ER status		
Positive	288	61
Negative	180	39
Missing	81	
PgR status		
Positive	223	48
Negative	241	52
Missing	85	
HER2 status		
Negative	349	74
Positive	121	26
Missing	79	
P53 status		
Negative	331	71
Positive	133	29
Missing	85	
Surgery		
Breast conserving therapy	446	81
Mastectomy	102	19
Missing	1	
Prolonged adjuvant chemotherapy		
No		
Yes	326	60
Missing	221	40
	2	

*Pathological nodal status

Table 2. Patients < 41 years

similar characteristics in terms of nodal status and hormone receptor status as compared to the whole group of patients. Patient characteristics of this subgroup are listed in Table 2. At time of the analysis, 1837 patients have died and 603 patients developed distant disease and were still alive. The median follow up period was 7 years.

Young patients versus older patients

Univariate prognostic factor analyses were performed using age as a covariant to determine whether age had significant prognostic impact on disease outcome in this patient population. First, patients aged under 41 years of age were compared with patients older than 41 years and secondly this group of patients was further divided into three subgroups; < 30 years, 31-35 years and 36-40 years.

In the univariate analysis, age under 41 was a significant prognostic factor for overall survival (HR 1.34 (1.18-1.52) P < 0.01) (Figure 1) and distant metastasis free survival (HR 1.48 (1.33-1.65) P < 0 .01) associated with unfavorable prognosis. The unfavorable prognostic impact was most profound in patients aged under 31 for overall survival (HR 1.77 (1.25-2.51) P < 0.01) (Figure 2) and distant metastasis free survival (HR 2.16 (1.63-2.86) P < 0.01).

To test whether the observed prognostic impact of young would remain significant when other tumor characteristics are taken into account, we first performed univariate analyses for overall and distant metastasis free survival including tumor size, nodal status, estrogen receptor status, type of surgery, and the administration of adjuvant chemotherapy. To prevent potential confounding due to selection bias as a result of the different trials in which patients participated; we also inserted trial as a covariant. Trial 22881 was defined as reference trial. All the above mentioned covariates were significantly associated with outcome for overall survival and distant metastasis free survival (Table 3).

Next, we included all covariates, including patient age, into a multivariate analysis to test the independent effect of age on outcome. Estrogen receptor status was not included in the multivariate analysis since no information was available for 2942 patients including all patients who participated in EORTC trial 10801 and therefore

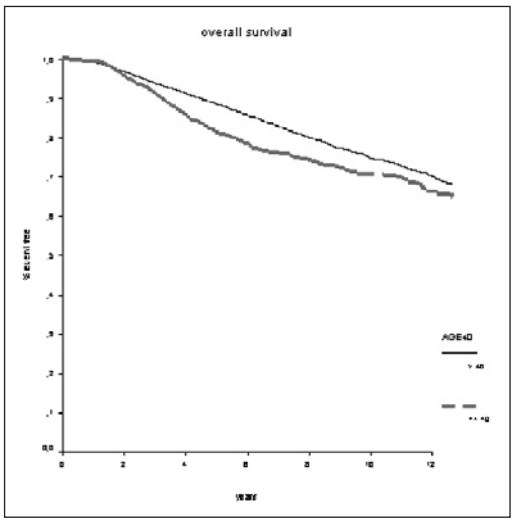

Figure 1. Overall survival

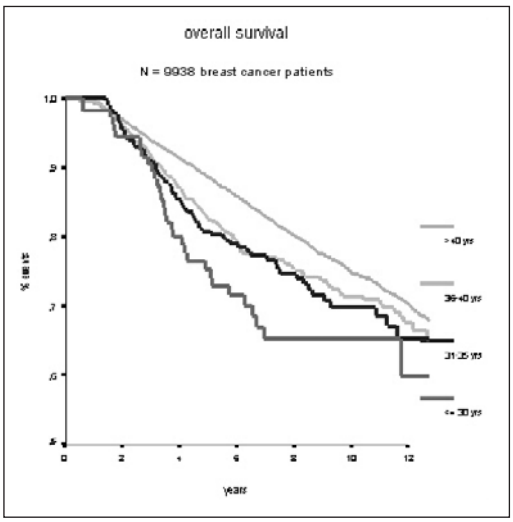

Figure 2. Overall survival

these data would be lost. The prognostic impact of all covariates except type of surgery remained significant (See table 3). Tumor size > 5cm and positive axillary lymph nodes were strong prognostic factors for poor prognosis, risk ratio's being 2.28 and 2.37 for overall survival and 2.25 and 1.97 for distant metastasis free survival respectively. In addition, young age remained an independent prognostic factor for overall (RR 1.43 (1.25-1.63) P < 0.01) and distant metastasis free survival (RR 1.58 (1.41-1.77 P < 0.01).

Prognostic factor analyses within the young age group

Next, we studied the prognostic impact of several different tumor characteristics in the subset of 549 patients aged under 41 of which tumor material was collected. Patient characteristics and immunohistochemistry results are listed in Table 2. To test whether these covariant had significant impact on prognosis in young breast cancer patients, univariate analyses for overall survival and distant metastasis free survival were performed. Large tumor size, positive nodal status, poorly differentiated histological grade, extensive lymphangio invasion and negative hormone receptor status were all associated with poor survival (Table 4). In addition, adjuvant chemo-therapy was associated with poor outcome (HR 1.90 (1.34-2.71) P < 0.01). Her2 over expression (HR 1.09 (0.70-1.69) P = 0.71) and P53 overexpression (HR1.53 (0.90-2.04) P = 0.15) were not significantly associated with poor overall survival in this group of patients.

For distant metastasis free survival, large tumor size, nodal status, poorly differentiated histological grade, and adjuvant chemotherapy were associated with poor outcome (Table 4). Positive ER status (HR 0.90 (0.65-1.24) P = 0.51) did not have a significant impact on distant metastasis free survival. Similar results were found for progesterone receptor status. In addition, Her2 and P53 overexpression did not have a significant impact on distant metastasis free survival.

Subsequently, we tested the independent significant covariates in the univariate

	Overall survival			Distant disease-free survival		
	HR	95% CI	P	HR	95% CI	P
≤ 40 years	1.34	1.18-1.52	<0.01	1.48	1.33-1.65	<0.01
≤ 30 years	*1.77*	*1.25-2.51*	*<0.01*	*2.16*	*1.63-2.86*	*<0.01*
31-35 years	*1.34*	*1.07-1.69*	*0.01*	*1.54*	*1.27-1.87*	*<0.01*
36-40 years	*1.29*	*1.10-1.50*	*<0.01*	*1.38*	*1.20-1.57*	*<0.01*
cT2 (vs cT1)	1.77	1.59-1.97	<0.01	1.66	1.52-1.81	<0.01
cT3 (vs cT1)	3.70	3.06-4.48	<0.01	3.18	2.68-3.78	<0.01
pN+	2.40	2.18-2.63	<0.01	1.99	1.84-2.16	<0.01
ER+	0.65	0.57-0.73	<0.01	0.78	0.71-0.87	<0.01
BCT	0.61	0.55-0.67	<0.01	0.66	0.60-0.72	<0.01
Adj CT*	1.46	1.31-1.62	<0.01	1.34	1.23-1.47	<0.01
Trial (ref trial: 22881)						
10801	2.13	1.85-2.46	<0.01	1.59	1.40-1.81	<0.01
10854	1.58	1.41-1.78	<0.01	1.48	1.34-1.62	<0.01
10902	2.51	2.11-3.0	<0.01	1.92	1.65-2.23	<0.01
	Multivariate analyses all patients					
	Overall survival			Distant disease-free survival		
	RR	95% CI	P	RR	95% CI	P
≤ 40 years	**1.43**	**1.25-1.63**	**<0.01**	**1.58**	**1.41-1.77**	**<0.01**
cT2 (vs cT1)	1.46	1.30-1.64	<0.01	1.47	1.34-1.62	<0.01
cT3 (vs cT1)	2.28	1.82-2.86	<0.01	2.25	1.84-2.75	<0.01
pN+	2.37	2.12-2.64	<0.01	1.97	1.80-2.17	<0.01
BCT	**0.93**	**0.82-1.04**	**0.19**	**0.97**	**0.87-1.08**	**0.51**
Adj CT*	0.69	0.60-0.79	<0.01	0.71	0.62-0.80	<0.01
Trial (ref trial: 22881)						
10801	1.55	1.32-1.81	<0.01	1.25	1.09-1.45	<0.01
10854	1.03	0.90-1.18	0.68	1.07	0.95-1.19	0.26
10902	1.62	1.28-2.06	<0.01	1.45	1.18-1.77	<0.01

* Prolonged chemotherapy

Table 3. Univariate and multivariate analyses all patients

	Overall survival			Distant disease-free survival		
	HR	95% CI	P	HR	95% CI	P
cT2	2.15	1.42-3.24	< 0.01	1.92	1.38-2.67	<0.01
cT3	4.11	1.95-8.64	<0.01	5.10	2.82-9.28	<0.01
pT2/3	2.00	1.37-2.93	<0.01	2.01	1.47-2.74	<0.01
pN+	2.13	1.50-3.03	<0.01	1.91	1.43-2.55	<0.01
Gr II	2.65	1.10-6.39	0.03	2.59	1.39-4.85	<0.01
Gr III	4.69	2.04-10.76	<0.01	3.04	1.67-5.54	<0.01
Lymfangio invasion	1.29	0.88-1.89	0.19	1.33	0.97-1.83	0.07
> 5 vessels	*1.81*	*1.17-2.80*	*<0.01*	*1.82*	*1.25-2.64*	*<0.01*
ER +	0.63	0.43-0.93	0.02	0.90	0.65-1.24	0.51
PgR +	0.59	0.40-0.88	0.01	0.78	0.57-1.08	0.14
HER2 +	1.09	0.70-1.69	0.71	1.00	0.69-1.45	0.99
P53 +	1.35	0.90-2.04	0.15	1.03	0.72-1.46	0.89
BCT	0.67	0.45-0.99	0.04	0.71	0.51-1.00	0.05
Adj CT*	1.90	1.34-2.71	<0.01	1.58	1.18-2.12	<0.01

*Prolonged chemotherapy

Table 4. Univariate analyses young patients

analyses. Therefore, we performed a multivariate survival analysis for the endpoints overall survival and distant metastasis free survival. In the multivariate analysis we selected tumor size as assessed by pathological examination and discarded clinical tumor size.

In the multivariate analyses, only histological grade remained a significant prognostic factor for both overall survival and distant metastasis free survival (Table 5). However, large tumor size remained an independent unfavorable prognostic factor on outcome in terms of distant metastasis free survival as well (HR 1.64 (1.17-2.31) P < 0.01).

Node negative patients who did not receive chemotherapy

Young versus Old

To detect whether differences in prognosis between young and older patients would still exist in node negative patients, we selected all axillary node negative patients who had not received adjuvant-prolonged chemotherapy. This subgroup consisted of 6060 patients of whom characteristics are listed in Table 6. Except for estrogen receptor status, patients characteristics were not significantly different between both groups. Young age remained of significant prognostic impact for patients bearing cT2 or cT3 tumors in terms of distant metastasis free survival (Table 7). In terms of overall survival, young age still showed a trend significant effect on outcome for smaller tumors but not for larger tumors (Table 7).

	Overall survival			Distant disease-free survival		
	HR	95% CI	P	HR	95% CI	P
pT2/3	1.43	0.92-2.23	0.11	1.64	1.17-2.31	<0.01
pN+	1.70	0.81-3.56	0.16	1.67	0.92-3.01	0.09
Gr II	**2.67**	**0.91-7.80**	**0.07**	2.04	1.07-3.88	0.03
Gr III	**3.92**	**1.38-11.16**	**0.01**	2.38	1.29-4.39	<0.01
Lymfangio invasion				1.06	0.74-1.51	0.77
ER +	0.83	0.48-1.45	0.51			
PgR +	0.90	0.52-1.58	0.72			
BCT	0.82	0.49-1.36	0.44	0.90	0.59-1.36	0.61
Adj CT*	0.98	0.47-2.05	0.96	0.81	0.45-1.43	0.46

*Prolonged chemotherapy

Table 5. Multivariate analyses young patients

Patient characteristics	≤ 40 years	> 40 years
	No / %	No / %
Clinical Tumor size		
T1	312 / 49	2580 / 48
T2	320 / 50	2772 / 51
T3	6 / 1	53 / 1
ER status* **		
Positive	**260 / 61**	**2785 / 75**
Negative	**167 / 39**	**938 / 25**
Surgery		
Breast conserving therapy	573 / 89	4773 / 88
Mastectomy	67 / 11	646 / 12

*Missing data not shown, **significant difference between both groups

Table 6. Node negative patients who did not receive prolonged CT (N= 6060)

	Overall survival			Distant disease-free survival		
	HR	95%CI	P	HR	95%CI	P
≤ 40 years vs. > 40 years						
cT1 (312 pts vs. 2579 pts)	1.38	0.99-1.92	0.06	1.50	1.16-1.94	<0.01
cT2 (319 pts vs. 2765 pts)	1.13	0.85-1.50	0.39	1.44	1.17-1.79	<0.01

Table 7. Multivariate analyses node negative patients who did not receive prolonged CT

Prognostic factors within young node negative patients

The subgroup of young node negative patients of whom tumor material was collected consisted of 341 women. Patient characteristics are listed in Table 8. In this subgroup, univariate analyses were performed, including tumor size, histological grade, vessel invasion, hormone receptor status, Her2 status, P53 status, and type of surgery and chemotherapy. In the univariate analyses, tumor size, grade and hormone receptor status demonstrated to be significant prognostic factors on overall and distant metastasis-free survival (See Table 9).

Next, in the multivariate analyses, histological grade remained an independent prognostic factor for both overall survival (Gr II vs Gr I NS, Gr III vs Gr I HR 8.92 (1.17-68.20) P 0.04) and distant disease-free survival respectively (Gr II vs Gr I NS, Gr III vs Gr I HR 4.12 (1.42-11.98) P <0.001). Further results are listed in Table 10 and univariate Kaplan Meyer curves for overall survival and distant disease free survival concerning histological grade are depicted in Figures 3 and 4.

Discussion

In this study we performed a retrospective analysis to gain further insight in tumor characteristics of young breast cancer patients. Young age at onset of breast cancer is a well-known independent prognostic factor but a genotypical explanation for this phenomenon is still lacking. Part of the more aggressive behavior of breast cancer at a young age may be attributable to hereditary

	Patient characteristics, (N = 341)*	
	No.	%
Clinical Tumor size		
T1	167	49
T2	169	50
T3	4	1
Pathological tumor size		
T1	231	73
T2 / T3	84	27
Tumor grade		
I	54	17
II	93	29
III	172	54
Lymfangio invasion		
None	243	76
2-5 vessels	49	15
> 5 vessels	27	9
ER status		
Positive	165	59
Negative	115	41
PgR status		
Positive	136	49
Negative	141	51
HER2 status		
Negative	216	77
Positive	64	23
P53 status		
Negative	198	72
Positive	78	28
Surgery		
Breast conserving therapy	229	88
Mastectomy	42	12
Prolonged adjuvant chemotherapy		
No	304	89
Yes	37	11

* Missing data not shown

Table 8. Node-negative patients aged < 41

	Overall survival			Distant disease-free survival		
	HR	95% CI	P	HR	95% CI	P
cT2	2.47	1.39-4.38	< 0.01	1.84	1.19-2.84	<0.01
cT3	6.44	1.48-27.97	<0.01	15.5	4.57-52.59	<0.01
pT2/3	2.38	1.37-4.13	<0.01	1.81	1.15-2.83	0.01
Gr II	1.80	0.48-6.81	0.38	2.35	0.95-5.84	0.07
Gr III	5.64	1.74-18.23	<0.01	3.89	1.67-9.05	<0.01
Lymfangio invasion	1.13	0.61-2.13	0.69	1.41	0.68-2.26	0.16
> 5 vessels	*1.12*	*0.44-2.83*	*0.82*	*1.71*	*0.87-3.34*	*0.12*
ER +	0.43	0.24-0.78	<0.01	0.61	0.39-0.96	0.03
PgR +	0.44	0.24-0.82	<0.01	0.64	0.40-1.02	0.06
HER2 +	0.75	0.35-1.60	0.45	0.90	0.51-1.59	0.71
P53 +	1.54	0.82-2.87	0.18	1.15	0.69-1.9	0.59
BCT	1.14	0.53-2.43	0.74	1.44	0.72-2.88	0.30
Adj CT*	2.73	1.36-5.46	<0.01	1.19	0.60-2.38	0.62

*37 pts in this subset received prolonged adjuvant chemotherapy

Table 9. Univariate analyses node-negative patients aged < 41

factors. However, at present only approximately 10% of young breast cancer cases have a documented BRCA I or BRCA II mutations or have a strong positive family history of breast cancer [7-9, 25].

We demonstrated in approximately 10000 early stage breast cancer patients that age > 41 years is a strong prognostic factor on disease outcome independent of other covariates. This is in accordance with previous data, which have led to the recommendation that all patients aged ≤ 35 years at time of diagnosis should receive adjuvant chemotherapy irrespective of other tumor characteristics. In this study the effect was most profound for patients aged under 31. However, the finding that patients aged between 35 and 41 still had a poor prognosis compared to older patients as well could raise the question whether or not these patients should also receive adjuvant chemotherapy.

In the subgroup of node negative patients who did not receive prolonged adjuvant chemotherapy the prognostic effect of young age was less clear. In terms of overall survival, young age as a prognostic factor failed to reach statistical significance. However, there was a significant effect on distant disease free survival. Hazard ratios varied between 1.13 and 1.50 in these analyses which could be roughly converted in NNT's (numbers needed to treat) varying between 11 and 38 hypothesizing an expected 30% event rate at 10 years. In addition, in this study young node negative patients bearing grade I tumors had excellent 10 years survival and distant disease-free survival rates of approximately 90% for both endpoints.

This raises the discussion whether or not all young node negative patients should

	Overall survival			Distant disease-free survival		
	HR	95% CI	P	HR	95% CI	P
pT2/3*	1.50	0.80-2.81	0.20	1.39	0.84-2.30	0.21
Gr II	4.11	0.49-34.57	0.19	2.49	0.81-7.66	0.11
Gr III	**8.92**	**1.17-68.20**	**0.04**	**4.12**	**1.42-11.98**	**<0.01**
ER +	0.73	0.32-1.70	0.47	0.90	0.46-1.76	0.76
PgR +	0.97	0.40-2.34	0.95	1.08	0.55-2.09	0.83
Adj CT	1.87	0.77-4.57	0.17			

*Pathological tumor size was included in the multivariate analysis and clinical tumor size was left out

Table 10. Multivariate analyses node-negative patients aged < 41

receive chemotherapy. Probably two subgroups can be defined comprising young patients who do not require adjuvant chemotherapy. First, young early breast cancer patients who have an excellent prognosis and second patients with chemotherapy-resistant tumors who do not benefit from chemotherapy anyway. Current research using microarray based prognostic and predictive risk models [26-28] may further elucidate this challenge of so-called treatment tailoring. In this study, histological grade was the strongest prognostic factor of the covariates studied, distinguishing young patients with a favorable prognosis from young patients with an unfavorable prognosis. The majority of young patients had grade III tumors (53%). In addition, large tumor size remained an independent risk factor for distant disease free survival as well. Axillary nodal status was a prognostic factor in the univariate analyses but did not remain significant in the multivariate analyses. Her 2 overexpression and p53 overexpression failed to be of prognostic significance in this subset of young patients. This is not in accordance with previous reports [29, 30]. Maru et reported a positive p53 status in 22 of 44 patients (50%), and a positive HER-2/*neu* status in 18 of 41 patients (44%) scored by FISH. In our study, the p53 and Her 2 positive rates were 29% and 26% respectively estimated by immunohistochemistry. Although Her2 overexpression is a well-known risk factor associated with poor prognosis, we were not able to demonstrate a significant effect. This could be due to insufficient sample size since only 121 patients had Her2 overexpressing tumors

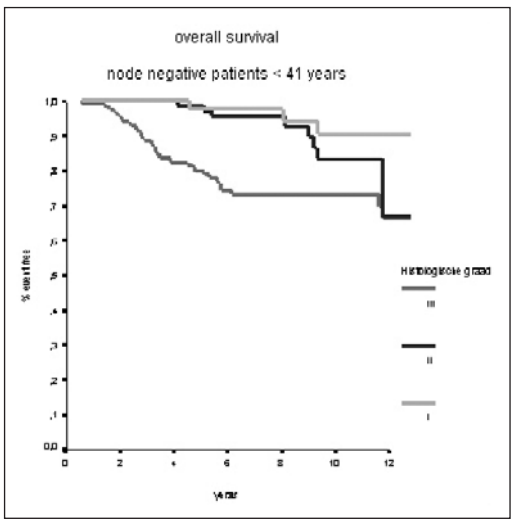

Figure 3. Overall survival and grade

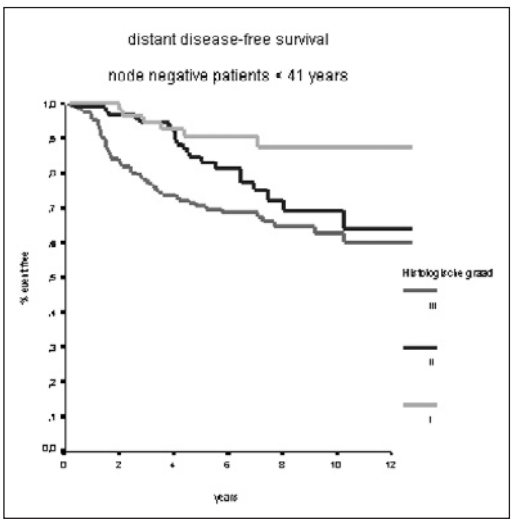

Figure 4. Distant disease-free survival and grade

estimated by immunohistochemistry. It may also be due to other unknown factors in young breast cancer patients, which result in a more aggressive genotype, which is much less influenced by Her2 expression. These plausible unknown factors yet have to be discovered [31].

In conclusion, well known established prognostic factors as tumor size and histologic grade still remain independent prognostic factors on disease outcome in young breast cancer patients and therefore can be a valuable tool in patient information and education. Treatment guidelines concerning young breast cancer patients should be refined in the future based on tumor characteristics, probably derived from microarray driven translational research projects, and not based upon age alone.

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CHAPTER 9

Efficacy of adjuvant chemotherapy according to hormone receptor status in young breast cancer patients

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Abstract

Breast cancer at a young age is associated with an unfavorable prognosis. We studied the effect of adjuvant chemotherapy in young breast cancer patients in relation to hormone receptor status. Paraffin embedded tumor material was collected from 480 early stage breast cancer patients younger than 41 years who participated in one of four EORTC trials. Estrogen receptor- and progesterone receptor status were assessed using immunohistochemistry.. The median follow up period was 7.3 years. Patients that received chemotherapy did not have significant differences in OS (HR 0.87, P = 0.63) and DMFS (HR 1.36, P = 0.23) rates according to ER status. Patients with ER-positive tumors who did not receive adjuvant chemotherapy had better OS (HR 0.41, P < 0.01) and DMFS (HR 0.59, P = 0.02) rates than those with ER-negative tumors. Patients with ER-positive tumors benefit less from adjuvant systemic chemotherapy than patients with ER-negative tumors. These differences were similar for PgR status. In conclusion, young patients with ER positive tumors seem to benefit less from adjuvant systemic chemotherapy than patients with ER negative tumors.

Introduction

Breast cancer in premenopausal women is associated with worse outcome compared to postmenopausal patients [1]. Approximately 7% of women diagnosed with breast cancer are aged below 40 years [2]. Especially very young women, i.e. < 35 years are at a high risk of developing distant metastases and therefore are recommended to receive adjuvant systemic chemotherapy regardless of tumor stage [3]. In addition, high local regional recurrence rates after breast conserving therapy have been reported in young premenopausal breast cancer patients [4]. Although it is clear that young age is an independent prognosticator of adverse outcome in breast cancer, controversies exist regarding the optimal treatment in this population.
Adjuvant systemic chemotherapy in premenopausal patients has been shown to improve survival [5], but controversy still exists about the role of chemotherapy in hormone receptor positive patients. Since chemotherapy alone in estrogen receptor (ER) and/or progesterone receptor (PgR)- positive breast cancer patients may not be sufficient [6], several trials in premenopausal ER and/or PgR- positive breast cancer patients have studied the role of ovarian ablation using LHRH-analogues [7,8,9,10]. One study by Aebi et al. [6] very clearly showed that the endocrine effects of chemotherapy alone might not be sufficient for very young breast cancer patients. In this study, it was shown that estrogen receptor positive tumors in patients younger than 35 years and treated with CMF had a significantly worse disease-free survival compared to estrogen receptor negative patients.
To detect whether we could confirm these data by finding similar results, we studied the efficacy of chemotherapy in young breast cancer patients according to estrogen receptor and progesterone receptor status, we selected patients younger than or equal to 40 years of age at time of primary diagnosis from 4 European Organization for Research and Treatment of Cancer (EORTC) trials, 10801, 10854, 10902, and 22881, that were conducted by the EORTC Breast Cancer- and Radiotherapy Group.

Patients and Methods

Data was collected from four EORTC trials. In total, 9938 patients participated in these trials and 934 of these patients were younger or equal to 40 years of age at time of diagnosis. The trial designs are summarized below:

EORTC trial 10801 (1980-1986, median follow up 13.4 years) was conducted in order to assess the safety of breast conserving treatment. In this trial, patients were randomized between breast conserving surgery combined with radiotherapy and radical mastectomy. Six cycles of adjuvant chemotherapy with cyclophosphamide 100 mg/m^2 given orally on days 1-14, methotrexate 40mg/m^2 given intravenously on days 1 and 8, and 5-fluorouracil 600 mg/m^2 given intravenously on days 1 and 8, were indicated for all patients under the age of 55 with positive nodes. In this study, 902 patients were randomized [11].

EORTC trial 10854 (1986-1991, median follow up 10.8 years) studied the question whether one course of peri-operative chemotherapy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Peri-operative chemotherapy consisted of one single course of doxorubicin 50 mg/m^2, 5-fluorouracil 600 mg/m^2, and cyclophosphamide 600 mg/m^2 (FAC), administered intravenously within 36 hours after surgery. For axillary lymph node-positive premenopausal patients in the peri-operative chemotherapy group adjuvant chemotherapy consisting of 5 cycles of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) was recommended. For node-positive patients younger than 50 years who did not receive peri-operative chemotherapy, one conventional course of FAC followed by five cycles of CMF after surgery was recommended. Postmenopausal patients were recommended to receive tamoxifen. 2795 patients were included in this trial [12].

EORTC trial 10902 (1991-1999, median follow up 6.1 years) was set up to determine the value of pre-operative chemotherapy. Patients were randomized to receive four cycles of chemotherapy either before or after surgery. Chemotherapy consisted of four cycles of 5-fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 (FEC) administered intravenously, at 3-weekly intervals. In the pre-operative chemotherapy group, surgical therapy followed within four weeks of the fourth course of chemotherapy. In the postoperative chemotherapy group, the first cycle was given within 36 hours after surgery. Patients $\geq$ 50 years received tamoxifen for 2 years. A total number of 698 patients were randomized [13].

EORTC trial 22881 enrolled 5569 patients between 1989 and 1996. Stage I/ II breast cancer patients were randomized between to undergo 50 Gy irradiation of the whole breast with or without an additional dose of 16 Gy to the tumor bed after lumpectomy. Patients with a microscopically incomplete resection were assigned to receive a boost dose of 10 Gy or 26 Gy. Premenopausal patients with axillary lymph node involvement received chemotherapy and postmenopausal patients received tamoxifen [14].

In all trials if adjuvant chemotherapy was indicated, patients either received CMF or

Number of patients	N	480
Clinical tumor size	cT1	185
	cT2	276
	cT3	17
	missing data	2
Histological tumor size	pT1	292
	pT2/3	151
	missing data	37
Nodal status	N –	288
	N +	188
	missing data	4
Surgery	BCS*	393
	Mastectomy	86
	missing data	1
Adjuvant chemotherapy	No	279
	Yes	200
	missing data	1
Tamoxifen**	No	273
	Yes	9
	missing data	198
Histological grade	I	70
	II	145
	III	255
	missing data	10
Estrogen receptor	+	288
	-	180
	missing data	12
Progesterone receptor	+	223
	-	241
	missing data	16

* Breast Conserving Surgery
** During the period of time in which these trials were conducted, tamoxifen was not routinely given to premenopausal ER-positive patients

Table 1. Patient & Tumor characteristics

an anthracyclin-based regimen (FAC or FEC). Adjuvant hormonal therapy for premenopausal hormone receptor positive patients was not yet recommend at the time when these trials were conducted. In the oldest two trials tamoxifen administration was not even recorded. This explains the high number of patients for which no information was found on tamoxifen use. In the trials where tamoxifen use was recorded, less than 5% of patients ≤ 41 years received tamoxifen.

ER staining and PgR staining
Paraffin embedded tumor material was collected from 549 patients ≤ 40 years. Tumors were histologically graded using H&E slides as described previously [15]. Immunohistochemical staining for estrogen receptor and progesterone receptor status was performed using a tissue micro array [16,17,18,19]. Three core biopsies were taken from each tumor block and inserted into a donor block. Immuohistochemical staining for estrogen receptor was performed using the monoclonal antibody DAKO-ER, 1D5 (Dakopatts, Glostrup, Danmark); for progesterone receptor using the monoclonal antibody mPRI (TRANSBIO, Paris, France. Immunohistochemical staining was scored using a semiquantative system based on the percentage of positive nuclei. After counting the percentage of positive nuclei in three core biopsies the mean value was taken. For estrogen- and progesterone receptor, tumors with >10% of the tumor cells showing nuclear staining were considered positive.

Statistical analysis
Analyses were performed for distant metastasis-free survival (DMFS) and overall survival (OS). Distant metastasis-free survival was defined as the interval from time of randomization until time of distant metastasis or death, whichever event came first. Overall survival was defined as time from randomization to death from any cause. Survival curves were estimated using the Kaplan-Meier method [20]. Differences in survival were analysed using Cox proportional hazard models [21]. All statistical analyses were performed using SPSS software. A direct comparison of patients who received chemotherapy versus patients who did not receive chemotherapy per hormone receptor status group was not feasible since this would

	No adjuvant chemotherapy N = 279	Adjuvant chemotherapy N = 200
ER +	161	126
ER -	110	70
PgR +	135	88
PgR -	135	105
T1	187	105
T2 / T3	76	75
N –	259	29
N +	18	170
BCT	247	146
Mastectomy	32	53

*Missing data not shown

Table 2. Patient characteristics specified by adjuvant chemotherapy*

	No adjuvant chemotherapy	Adjuvant chemotherapy
Deaths (N = 102)		
ER +	19	35
ER -	29	19
Distant metastasis or death (N = 150)		
ER +	37	54
ER -	38	21

Table 3. Type of events

introduce a selection bias in this retrospective analysis. This due to the fact that the vast majority of patients receiving chemotherapy had positive axillary lymph nodes. Therefore, conclusions in this explorative analysis were based upon indirect comparisons.

Results

Paraffin embedded tumor specimens were collected for 480 patients ≤ 40 years at time of diagnosis. Patient characteristics are listed in Table 1. For 12 patients, ER status could not be scored and for 16 patients PgR status could not be scored. 288 patients were deemed ER positive whereas 223 patients were PgR positive. Two hundred patients received prolonged adjuvant systemic chemotherapy and 279 patients did not receive adjuvant systemic chemotherapy. Ninety-four percent of patients that did not receive chemotherapy were node-negative and eighty-five percent of patients that did receive chemotherapy were node-positive. Characteristics related to adjuvant systemic chemotherapy treatment are listed in Table 2.

At time of the analysis, 102 patients had died and 150 patients developed a distant recurrence or died. The number of events stratified by estrogen receptor status is listed in Table 3. The median-follow-up period at time of analysis was 7,3 years. Overall, patients with ER-positive tumors had better OS rates compared to ER-negative patients, (HR 0.63, 95%CI 0.43 - 0.93, P= 0.02, Figure 1). Survival rates after a median follow up of 7 years were approximately 82% for the ER positive group and 77% for the ER negative group. DMFS rates were 70% and 66% respectively which was not statistically significant (HR 0.90, 95% CI 0.65 - 1.24, P = 0.51, Figure 2). Progesterone receptor status yielded similar results in terms of overall survival and distant metastasis-free survival. Patients with progesterone positive tumors had better OS (HR 0.59, 95%CI 0.4 - 0.88, P = 0.01) but for DMFS this difference was not of statistical significance (HR 0.78, 95%CI 0.57 - 1.01, P = 0.14).

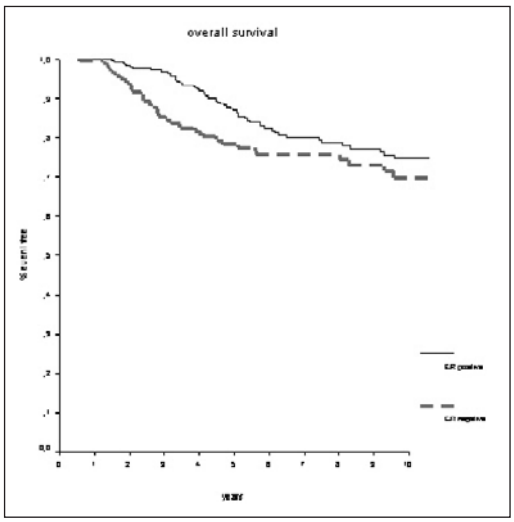

Figure 1. Overall survival for all patients

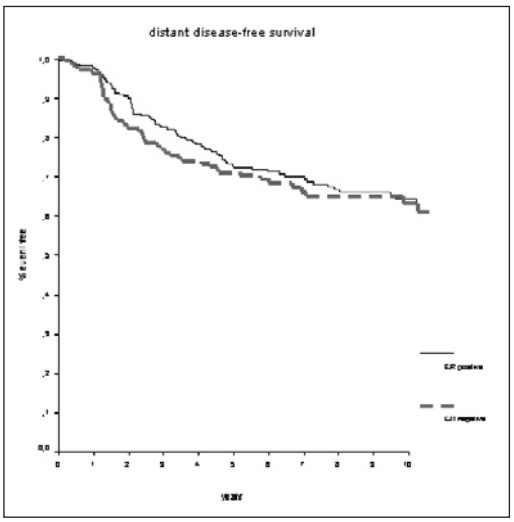

Figure 2. Distant metastasis-free survival for all patients

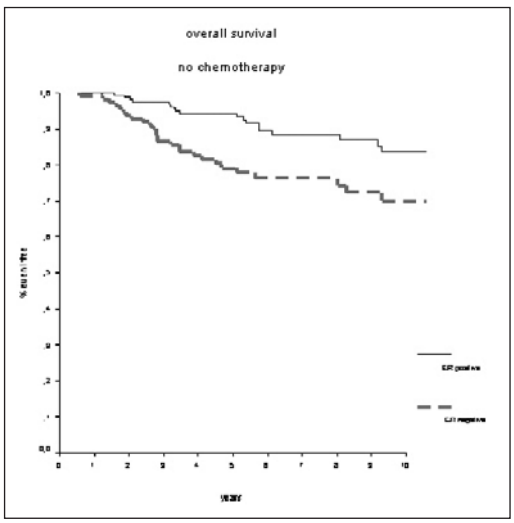

Figure 3. Overall survival in patients who did not receive adjuvant chemotherapy

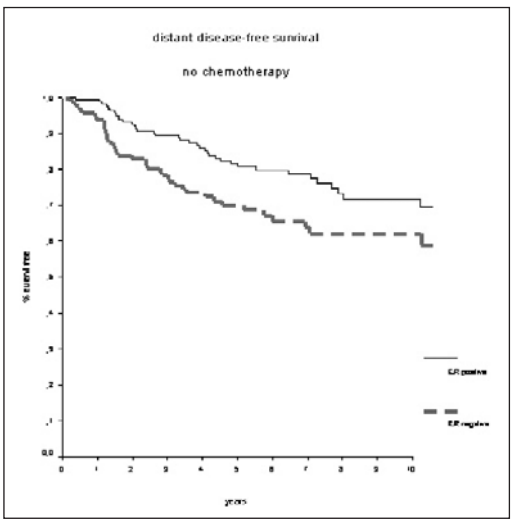

Figure 4. Distant metastasis-free survival in patients who did not receive adjuvant chemotherapy

Patients that did not receive prolonged adjuvant chemotherapy

Estrogen receptor status

In the subset of patients that did not receive adjuvant systemic chemotherapy, positive ER status was associated with better OS (HR 0.41, 95%CI 0.23 - 0.74, P < 0.01, figure 3). Survival rates at 7 years were 90% for the ER positive group and 77% for the

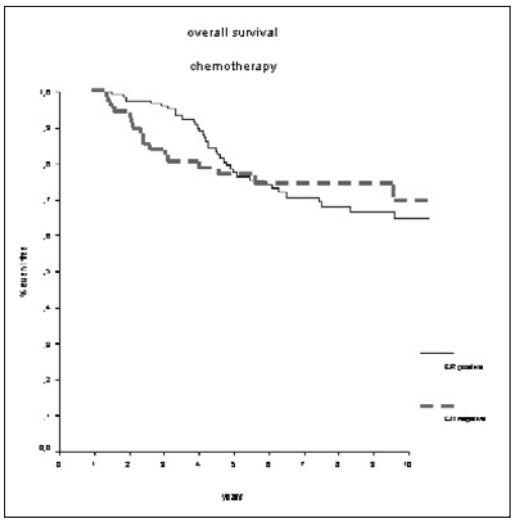

Figure 5. Overall survival in patients who received adjuvant chemotherapy

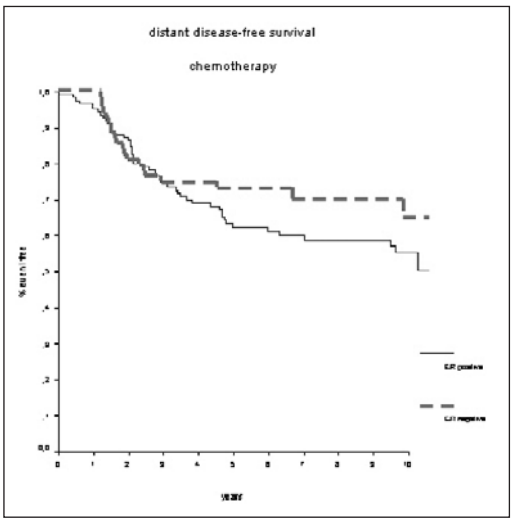

Figure 6. Distant metastasis-free survival in patients who received adjuvant chemotherapy

ER negative group. DMFS rates in the subset of patients that did not receive adjuvant systemic chemotherapy were significantly better for ER positive patients as well, 80% and 64% respectively (HR 0.59, 95%CI 0.37 - 0.92, P = 0.02, figure 4).

Progesterone receptor status
PgR positive patients who did not receive adjuvant chemotherapy had better OS (HR 0.44, 95%CI 0.24 - 0.8, P < 0.01). OS rates were 88% and 75% for ER positive and ER negative patients. DMFS rates were 79% for PgR positive patients and 67% for PgR negative patients respectively. However, this difference did not reach statistical significance (HR 0.66, 95%CI 0.42 - 1.04, P = 0.07).

Patients who received prolonged adjuvant systemic chemotherapy

Estrogen receptor status
In the group of two hundred patients that did receive adjuvant systemic chemotherapy treatment outcome was not significantly different between ER positive- and ER negative breast cancer patients. OS rates were 70% for the ER positive group and 75% for the ER negative group (HR 0.87, 95%CI 0.50 - 1.52, P = 0.63, figure 5) and DMFS rates were 59% for the ER positive group and 70% for the ER negative group (HR 1.36, 95% CI 0.82 - 2.26, P = 0.23, figure 6).

Progesterone receptor status
Patients who had PgR negative tumors and received adjuvant systemic chemotherapy did not have significant differences in terms of OS and DMFS rates. Both in the PgR positive and PgR negative patient group, OS was 72% at 7 years of follow up (HR 0.84, 95%CI 0.49 - 1.43, P = 0.51). DMFS did not differ significantly between PgR positive

patients and PgR negative patients who received adjuvant chemotherapy. DMFS rates were 59% for the PgR positive group and 64% for the ER negative group (HR1.02, 95%CI 0.65 - 1.6, P = 0.93).

Multivariate analysis

Multivariate Cox regression overall survival analyses were performed for ER status and PgR status separately. Other covariates included nodal status, tumor size, and the administration of prolonged adjuvant chemotherapy. Both ER status (RR 1.65) and PgR (1.56, data not shown) status remained independent prognostic factors with a significant impact on overall survival (Table 4).

Discussion

Adjuvant systemic chemotherapy is a well-established treatment modality in premenopausal breast cancer. In patients younger or equal to 35 years, chemotherapy is advocated regardless of nodal status and tumor size and grade [3]. However, several reports have questioned the efficacy of chemotherapy in premenopausal patients with ER-positive breast cancer [6,7,10].

We demonstrated that ER–positive and/or PgR positive patients ≤ 40 years who received prolonged adjuvant chemotherapy showed no advantage in treatment outcome compared with ER-/PgR-negative patients, whereas ER-/PgR- positive patients who did not receive adjuvant chemotherapy had better overall survival rates compared with their ER-/PgR- negative counterparts. In terms of survival, figure 5 even suggests that the proportional hazards assumption is not justified in the assessment of the effect of chemotherapy according to hormone receptor status. Therefore we conclude that treatment efficacy of adjuvant chemotherapy is less in young hormone receptor positive patients compared to young hormone receptor negative patients. We did not perform direct comparisons between patients who received chemotherapy versus patients who did not receive chemotherapy according to hormone receptor status. Axillary lymph node status would have induced a confounding error and since the majority of patients that did receive chemotherapy also had positive axillary lymph nodes. Hormone receptor status therefore, may not have been of significant impact on outcome in this subgroup.

However, in the multivariate analysis including ER status, axillary lymph node status, tumor size and the administration of prolonged adjuvant chemotherapy, ER status remained an independent prognostic factor for overall survival (RR1.65, 95%CI 1.09 - 2.5 P = 0.02, Table 4). Since these patients participated in trials in which adjuvant tamoxifen was not routinely given to hormone receptor positive premenopausal patients, less than 5% of the study population received tamoxifen, the effect of adjuvant tamoxifen could not be measured.

Similar findings were recorded if ER status was replaced by PgR status (RR 1.56, 95%CI 1.02 - 2.39, P = 0.04).

We realize that this is a retrospective analysis using heterogeneous data from different randomized trials and therefore any conclusions have to be drawn with

Overall survival			
	RR	95% CI	P – value
ER negative	1.65	1.09 – 2.5	0.02
pN+	1.7	0.79 – 3.66	0.17
pT2/3	1.66	1.09 – 2.52	0.02
Adjuvant chemotherapy	1.02	0.48 – 2.17	0.96

Table 4. Multivariate Cox overall survival regression analysis

caution. Preferably, we should have liked to compare chemotherapy versus not in hormone receptor positive patients and then compare chemotherapy versus not in hormone receptor negative patients. However, since this is not a randomized comparison, the confounding effect of axillary lymph node status would have induced a significant selection bias. On the other hand, our findings are in accordance with data from Aebi et al. [6] who demonstrated that young premenopausal breast cancer patients treated with adjuvant CMF chemotherapy had a higher risk of relapse and death than older premenopausal patients, especially if their tumors were ER-positive. In this study, ER was assessed using a ligand-binding assay; in our study ER has been assessed using immunohistochemistry. By analyzing ER status centrally, we have provided standardized ER measurements for all tumors in the study.

In order to optimize adjuvant systemic treatment in premenopausal breast cancer patients, several investigators have studied the role of ovarian suppression by LHRH agonists.

The Zoladex‰ Early Breast Cancer Research Association (Zebra) trial [7,8] compared goserelin and CMF in1640 node-positive, premenopausal and perimenopausal patients, aged 50 years or less, with early breast cancer. After a median follow-up of 6 years, goserelin and CMF showed equivalent disease-free survival rates in ER positive patients (HR 1.01, 95%CI 0.84 - 1.20). However in the ER negative subgroup, a significant advantage in favor of CMF was found (HR 1.76, 95%CI 1.27 - 2.44). A recent update of the analysis demonstrated similar results. In addition, patients who received LHRH agonists suffered less from treatment related side effects than patients who received chemotherapy [22].

Other trials studying the effect of goserelin with or without tamoxifen versus CMF in premenopausal hormone receptor positive breast cancer patients also demonstrate equivalent or even better disease-free survival rates but this has not yet resulted in better overall survival rates [23,24].

Although these results underline the fact that chemotherapy may be equivalent to hormonal ovarian suppression in terms of treatment outcome in ER positive patients, these results fail to demonstrate a superior effect of LHRH agonists over adjuvant chemotherapy.

In conclusion, we have demonstrated in a subset of patients aged 40 years or less at time of diagnosis that hormone receptor status is an independent prognostic factor on distant metastasis-free survival and overall survival. Moreover, we showed that hormone receptor status influences response to chemotherapy. Therefore, we can conclude that chemotherapy alone is not sufficient hormone receptor positive young breast cancer patients.

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CHAPTER 10

General Discussion

This thesis consists of three parts.

In part I, we demonstrate that neoadjuvant and perioperative chemotherapy are very feasible treatment options in early stage breast cancer patients. Both treatment strategies result in equal or better results in terms of disease outcome as compared to conventional postoperative adjuvant chemotherapy. In addition, the higher breast conserving therapy rate after neoadjuvant chemotherapy described in Chapter 3 and the potential to assess tumor response as a prognostic factor as stipulated in Chapter 4 are attractive characteristics of this type of treatment.

In part II, we demonstrate that locoregional treatment strategy may be based on tumor cell characteristics and patient age. Next, we describe the significant impact of adequate locoregional treatment on locoregional control as well as overall survival. For example in *Chapter 6*, we show in a selected subgroup of patients bearing 1 to 3 metastatic axillary lymph nodes, that adjuvant radiotherapy after mastectomy was associated with superior locoregional control and survival rates. In addition, in *Chapter 7* we attempt to identify baseline risk factors, i.e. factors assessed at time of diagnosis of the primary tumor, for locoregional recurrence.

In part III, we demonstrate that very young breast cancer patients can be divided in good- and bad prognosis groups based upon tumor characteristics. The current guideline that all very young breast cancer patients should receive chemotherapy irrespective of tumor characteristics can therefore be questioned. Next, we demonstrate that tumor grade is a strong and independent prognostic factor for distant metastasis-free survival and overall survival in this specific subgroup of very young breast cancer patients. Finally in *Chapter 9*, a trend is described suggesting inferior chemosensitivity in estrogen receptor (ER) positive and/or progesterone receptor (PgR) positive very young breast cancer patients as compared to their ER and/or PgR negative counterparts.

Breast cancer treatment is making progress. New therapies are introduced and existing ones are further modified. One of these modifications is the result of studies that focused on timing of administration of adjuvant systemic therapy which has resulted into the introduction of neoadjuvant chemotherapy in the treatment of breast cancer. Level I evidence is currently available for this type of treatment for both locally advanced breast cancer patients and early stage breast cancer patients [1-3].

While survival and progression free survival have not yet been improved by neoadjuvant chemotherapy in early breast cancer patients, breast conserving rates have risen with acceptable locoregional control rates when surgery is not omitted from the locoregional regime after neoadjuvant chemotherapy [1,4,5].

In the Netherlands however, neoadjuvant chemotherapy in early stage breast cancer patients is still not being administrated on a routine basis although these patients might definitely benefit from this treatment strategy. One of the potential reasons for this conduct could be the reluctance of doctors to administrate systemic treatment before definitive staging has been performed. However, the decision whether or not

systemic chemotherapy will be indicated in a case of early breast cancer can to a large extent very well be established by preoperative core needle biopsy and/or fine needle aspiration of tumor and potential suspect axillary lymph nodes in combination with physical examination and diagnostic imaging. In addition, the indications for the administration of adjuvant chemotherapy have widened which has resulted in a higher a priori probability for receiving chemotherapy. Therefore, a shift in paradigm concerning treatment strategy of early breast cancer patients in the Netherlands is needed.
Although the Dutch situation may cause some concern, research concerning neoadjuvant treatment in breast cancer has gained a lot of interest and many trials studying different neoadjuvant chemotherapy regimens are being conducted. Research concerning neoadjuvant trials in early stage breast cancer should be focused on four major topics:

1) Translational research. It is important to note that the response to neoadjuvant chemotherapy in vivo could provide a useful prediction of prognosis and help define strategies for an individual patient's future treatment with alternative chemotherapy regimens or molecular-targeting agents. Furthermore, the discovery of predictive markers for tumor response to neoadjuvant chemotherapy through the analysis of complementary DNA microarrays and proteomics may also help facilitate individualized chemotherapy, particularly by improving survival in patients with breast cancer with a poor prognosis. Therefore, translational research has to be focussed on classical and molecular tumor characteristics and their response, i.e. up- or downregulation, to established and experimental chemotherapeutic regimens and the assessment of chemosensitivity in terms of tumor response [6,7].

2) Tumor monitoring modalities. Adequate assessment of tumor response and pretreatment staging are vital in the neoadjuvant chemotherapy setting. Imaging of tumor response has several implications;
First, tumor response is considered as an independent prognostic factor on treatment outcome and therefore should therefore be monitored meticulously [8].

Second, diagnostic modalities such as MRI and CT need to be prospectively evaluated to study whether or not they yield superior results over classical ways of imaging like ultrasonography and mammography. Breast MRI has been assuming an important role in the assessment of the extent of cancer and may be more accurate than conventional modalities such as mammography and ultrasonography. On the other hand, MRI is associated with an increase in invasive therapeutic and diagnostic procedures for benign abnormalities due to high false-positive rates. Therefore, MRI may be feasible in a population of high risk patients but not in all early stage breast cancer patients. In conclusion, the exact role of MRI in breast cancer and the assessment of neoadjuvant chemotherapy needs to be determined [9-15].

Finally, imaging of tumor response is of significance considering optimalization of subsequent breast conserving surgery. Tumor margins after neoadjuvant chemotherapy have been a matter of concern. Tumor response does not always lead to a decrease in tumor volume but can result in less tumor density. Although EORTC

trial 10902 did not demonstrate a higher locoregional recurrence rate in downstaged patients who underwent breast conserving surgery, meta-analyses which included trials in which surgery was omitted after neoadjuvant chemotherapy demonstrated inferior local control rates. Therefore the diagnostic preoperative assessment of residual tumor after neoadjuvant chemotherapy is important [16,17].

3) Studies addressing the relation between locoregional treatment and neoadjuvant chemotherapy, for instance the feasibility of sentinel node procedure after neoadjuvant chemotherapy and quality of life studies concerning the psychological effect of breast conserving therapy after tumor downstaging. Sentinel node biopsy after neoadjuvant chemotherapy has been a matter of debate. Retrospective series have demonstrated acceptable accuracy rates comparable to sentinel node biopsies in the primary surgery setting. Recently, the first meta-analysis concerning sentinel node biopsy after neoadjuvant chemotherapy has been published and the accuracy rates in this study are in accordance with previous reports suggesting satisfactory feasibility of this surgical treatment modality [18,19].

4) The efficacy of neoadjuvant hormonal therapy either by tamoxifen or by aromatase inhibitors. With recent advances in endocrine therapy, and rapid and routine assessment of predictive factors of response such as estrogen (ER), progesterone (PR) and Her2 neu receptor status, endocrine therapy has come to the forefront of research investigating a neoadjuvant alternative to chemotherapy. Early studies of neoadjuvant endocrine therapy mainly evaluated the role of tamoxifen in the treatment of elderly postmenopausal women with LABC who were unselected for ER/PR status and who were unsuitable for either surgery or chemotherapy. Response rates in these patients were found to be inferior to those traditionally obtained from trials with neoadjuvant chemotherapy. Parallel to the superiority that third-generation aromatase inhibitors have shown over tamoxifen in the metastatic and adjuvant settings however, AIs have also demonstrated superiority in the neoadjuvant setting. Recent studies have shown response rates for neoadjuvant treatment with aromatase inhibitors in carefully selected hormone receptor positive patients to be comparable to those seen with neoadjuvant chemotherapy. This is particularly important as hormone receptor positive tumors have repeatedly been shown to have lower response rates to neoadjuvant chemotherapy than hormone receptor negative tumors [20-22].

Next, when neoadjuvant chemotherapy is not feasible and adjuvant chemotherapy will be administrated postoperatively, the first course of chemotherapy can be given in a perioperative setting which means that the patient receives the first course of chemotherapy within 36 hours after surgery. Perioperative chemotherapy, as mentioned previously in Chapter 2, is based principally upon evidence from murine models demonstrating surgery-induced proliferation of tumor cells that responded well to early administration of chemotherapy [23,24].

EORTC trial 10854, of which the long term results are presented in this thesis demonstrated that this is a safe and feasible treatment modality which may have an impact on locoregional control as well as on survival in selected groups of patients [25].

Concerning locoregional therapy, different strategies should be employed in different risk groups, for instance based upon age. Young breast cancer patients who are at a high risk for locoregional recurrence, especially with histologically aggressive tumors should be offered mastectomy with immediate or delayed reconstruction. Locoregional control rates and patient satisfaction could be improved [26-29].

On the other hand, standard administration of chemotherapy in young patients with node negative breast cancer can be questioned. Since risk ratios between young and older breast cancer patients have moderate differences, subgroups within the young age group could be identified where chemotherapy should not have been applied irrespective of other patient and tumor characteristics. For instance, node negative breast cancer patients bearing small grade I tumors have an excellent prognosis and might not receive a clinically relevant benefit from adjuvant chemotherapy but they do receive the burdens.

Thus, translational research concerning risk groups of young breast cancer patients who might benefit from chemotherapy is needed. Recently, translational research has been accelerated due to the introduction of micro-array analysis [30-33].

This highly promising technique using high throughput gene chips is not yet fully validated but may enable treatment tailored strategies in the future. However, until thorough validation of microarray is established and demonstrated, classical tumor prognostic factors have to be used. Currently, neoadjuvant chemotherapy trials are already being conducted with the incorporation of tumor markers in their study design [34-36].

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CHAPTER 11

Summary

In *Chapter 1*, a general introduction and an outline of the present thesis are given.

In *Chapter 2*, the eleven year follow up results of EORTC study 10854 are presented, comparing one short intensive course of polychemotherapy (fluorouracil, doxorubicin, cyclophosphamide; FAC) to no peri-operative chemotherapy in 2795 stage I en II breast cancer patients, randomized between 1986 and 1991. Peri-operative chemotherapy was associated with significant better progression free survival rates (59% vs 53% without peri-operative chemotherapy) and locoregional control rates (91% vs 86%) respectively. In the subgroup of patients that did not receive prolonged systemic chemotherapy, one course of peri-operative chemotherapy led to significant higher overall survival rates as well (HR=0.80; 95% CI: 0.64–0.98; P = 0.035).

In *Chapter 3*, the five-year results of EORTC study 10902 are presented, comparing neoadjuvant anthracyclin based polychemotherapy versus the same chemotherapeutic regimen given postoperatively. EORTC trial 10902 randomized 698 patients between 1991 and 1999. No significant differences between the two treatment arms were observed for progression-free and overall survival. Overall survival after 5 years was 82% in the preoperative group and 84% in the postoperative group (HR 1.16; 95% CI: 0.83 to 1.63; P = 0.38). Progression-free survival rates after 5 years for the preoperative and postoperative groups were 65% and 70%, respectively (HR 1.15; 95% CI, 0.89 to 1.48; P = 0.27). Time to locoregional recurrence was not significantly different between the two treatment arms (HR 1.13; 95% CI, 0.70 to 1.81; P = 0 .61).

In *Chapter 4*, the predictive role of p53 expression is studied in patients receiving neoadjuvant chemotherapy. Using tumor response as a surrogate endpoint, associations between p53 expression as well as other tumor markers like Her2 and outcome were studied. Tumor biopsy specimens were taken from 107 patients prior to the administration of neoadjuvant chemotherapy. In a multivariate logistic regression analysis, pCR was independently predicted by *p53* overexpression estimated by immunohistochemistry (OR 16.83; 95% CI, 1.78 to 159.33; P = 0.01) and negative pathological lymph node status (OR 8.47; 95% CI, 0.88 to 81.82; P = 0.07). In multivariate Cox regression analysis, positive pathological lymph node status and no use of tamoxifen showed unfavourable prognosis for overall and distant disease-free survival.

In *Chapter 5,* the potential prognostic impact of a putative tumor marker called PS6K on locoregional recurrence is presented. The PS6K protein is encoded by the RPS6KB1 gene is located at chromosome and amplified in approximately 10% of all primary breast cancer cases. PS6K is a protein that is involved in the cell cyclus. It is rapidly activated in response to mitogenic stimuli, for example growth factors, cytokines, and oncogene products. In a series of 452 node-negative premenopausal early-stage breast cancer patients PS6K overexpression was associated both with worse distant disease-free survival and with impaired locoregional control (HR 1.80, P = 0.025 and HR 2.50, P = 0.006, respectively). In a multivariate analysis including other prognostic factors, PS6K overexpression remained an independent predictor for poor locoregional control (RR 2.67, P = 0.003). Therefore, PS6K could be a putative predictive and prognostic factor to be used in the planning of less or more aggressive locoregional therapy.

In *Chapter 6*, a retrospective analysis is presented concerning the impact of loco-regional treatment on disease outcome. Breast-conserving surgery and mastectomy with or without radiotherapy to the axilla and / or breast are compared in terms of locoregional control and disease outcome. The combined data set consisted of 3648 patients. 5.9% of the patients who were treated with mastectomy and 10.8% of the patients who were treated with breast-conserving therapy had a locoregional recurrence ($P < 0.0001$). The risk of death after breast-conserving therapy was similar compared with mastectomy (RR 1.07, $P = 0.37$). Adjuvant radiotherapy after mastectomy was associated with a lower risk for locoregional recurrence (RR 0.43, $P < 0.001$) and death (RR 0.73, $P = 0.001$). The effect of adjuvant radiotherapy after mastectomy was most profound in patients who had 1–3 positive nodes (RR 0.48, 99% CI 0.31–0.75, $P < 0.001$).

In *Chapter 7*, risk factors for locoregional recurrence and the relationship between locoregional recurrence and subsequent metastatic disease are studied in more detail. To that end, different time intervals between locoregional recurrence and subsequent metastatic disease are defined and compared in sensitivity analyses. The study population comprised 3602 women who had undergone primary surgery for early stage breast cancer. The results of multivariate analysis showed that younger age and breast conservation were risk factors for isolated loco-regional recurrence; breast cancer under 35 years of age versus over 50 years of age: HR 2.80 (95% CI 1.41 to 5.60); breast cancer age 35–50 years versus over 50 years: HR 1.72 (95% CI 1.17 to 2.54); breast conservation: HR 1.82 (95% CI 1.17 to 2.86). After perioperative chemotherapy, less isolated loco-regional recurrences were observed (HR 0.63; 95% CI 0.44 to 0.91).
Therefore we hypothesised that, assuming an isolated loco-regional recurrence to be a potentially curable condition, women treated with breast conservation or diagnosed with breast cancer at a young age should be monitored closely to detect local recurrence at an early stage.

In *Chapter 8*, a translational research project is presented concerning very young breast cancer patients. The total dataset consisted of 9938 early breast cancer patients. Tumor material was collected from 549 patients aged under 41 years of age at time of diagnosis. In the multivariate analyses, only histological grade remained a significant prognostic factor for both overall survival (Grade II HR 2.67; 95% CI 0.91 to 7.80; $P = 0.07$, Grade III HR 3.92; 95%CI 1.38 to 11.16; $P = 0.01$) and distant metastasis free survival (Grade II HR 2.04; 95% CI 1.07 to 3.88; $P = 0.03$, Grade III HR 2.38; 95%CI 1.29 to 4.39; $P < 0.01$). However, large tumor size remained an independent unfavorable prognostic factor on outcome in terms of distant metastasis free survival as well (HR 1.64 (1.17-2.31) $P < 0.01$). In the subgroup of node negative very young breast cancer patients, histological grade remained an independent prognostic factor for both overall survival (Gr III HR 8.92; 95%CI 1.17 to 68.20; $P = 0.04$) and distant disease-free survival respectively (Gr III HR 4.12; 95%CI 1.42 to 11.98; $P < 0.001$).

In *Chapter 9*, the efficacy of chemotherapy in early breast cancer is studied according to hormone receptor status in patients aged less than 41 years. The median follow up period was 7.3 years. Patients that received chemotherapy did not have significant

differences in overall survival (HR 0.87, P = 0.63) and distant metastasis-free survival (HR 1.36, P = 0.23) rates according to ER status. Patients with estrogen receptor (ER) positive tumors who did not receive adjuvant chemotherapy had better overall survival (HR 0.41, P < 0.01) and distant metastasis-free survival (HR 0.59, P = 0.02) rates than those with ER-negative tumors. Therefore, it was concluded that very young early stage breast cancer patients with ER-positive tumors benefit less from adjuvant systemic chemotherapy than patients with ER-negative tumors. Similar results were demonstrated for progesterone receptor status.

In *Chapter 10*, the results of this thesis are discussed within the scope of current breast cancer therapy and research. Finally, suggestions are made concerning future directions in translational and clinical breast cancer research.

CHAPTER 12

Nederlandse samenvatting

Dit proefschrift behelst een aantal klinische en translationele studies met betrekking tot de behandeling van het primair operabel mammacarcinoom. Zowel aspecten van de locoregionale behandeling als van de systemische behandeling worden belicht. Alle hoofdstukken uit dit proefschrift zijn studies gebaseerd op EORTC onderzoeken.

In Hoofdstuk 1 wordt een overzicht van de geschiedenis van de behandeling van het primair operabel mammacarcinoom gegeven en worden de rationales waarop dit proefschrift is gebaseerd nader uiteengezet.

In Hoofdstuk 2 wordt een gerandomiseerde trial beschreven (EORTC trial 10854) waarin het effect van een eenmalige gift polychemotherapie, gegeven direct na de operatie, bij patiënten met primair operabel mammacarcinoom is bestudeerd. In totaal participeerden bijna drieduizend patiënten in deze studie. Na een mediane follow-up van elf jaar was er sprake van een significant betere ziektevrije overleving alsmede een betere locoregionale controle voor alle patiënten die deze vorm van zogenaamde perioperatieve chemotherapie ontvingen. Voor patiënten bij wie geen verdere systemische behandeling werd gegeven leidde een eenmalige gift perioperatieve chemotherapie zelfs tot betere overlevings-ratios.

In Hoofdstuk 3 worden de resultaten van EORTC trial 10902 gepresenteerd. Het betreft een gerandomiseerde studie waarin het effect van neoadjuvant polychemotherapie wordt vergeleken met postoperatieve polychemotherapie bij patiënten met primair operabel mammacarcinoom. In totaal deden 698 vrouwen mee aan deze studie. Na vijf jaar waren er geen verschillen tussen de experimentele en de controle arm in deze studie wat betreft (ziektevrije-) overleving en locoregionale controle. Wel werden patiënten die neoadjuvant chemotherapie ontvingen vaker mammasparend geopereerd ten gevolge van zogenaamde “tumor downstaging”.

In Hoofdstuk 4 wordt een translationele studie gepresenteerd die de voorspellende waarde bestudeert van “p53 expressie” op het effect van neoadjuvant chemotherapie. Tumor materiaal van patiënten die participeerden in EORTC studie 10902 werd verzameld en onderzocht middels immunohistochemie op de expressie van p53 en Her2. Overexpressie van p53 en de afwezigheid van okselkliermetastasen waren geassocieerd met een complete respons van de tumor op neoadjuvant chemotherapie onafhankelijk van andere factoren. Ook was de aanwezigheid van okselklier-metastasen na neoadjuvant chemotherapie een ongunstige prognostische factor wat betreft (metastase vrije) overleving.

In Hoofdstuk 5 wordt de prognostische rol van een experimentele tumor marker, PS6K genaamd, bestudeerd. Tumor weefsel van 452 patiënten met borstkanker zonder oksel-kliermetastasen die participeerden in EORTC trial 10854 werd verzameld en onderzocht middels immunohistochemie op overexpressie van dit eiwit dat een rol speelt bij de cel cyclus. PS6K expressie was verhoogd bij patiënten met een slechte prognose wat betreft metastase vrije overleving. Tevens was overexpressie van PS6K onafhankelijk van andere factoren geassocieerd met een hoog locoregionaal recidief risico. De resultaten in dit hoofdstuk onderschrijven de mogelijke rol van deze tumor marker bij het bepalen van een meer of minder agressieve vorm van locoregionale behandeling.

In Hoofdstuk 6 wordt de rol van de locoregionale behandeling op de prognose bij patiënten met primair operabel mammacarcinoom bestudeerd middels een retrospectieve studie. De data van een drietal EORTC studies werden gecombineerd (EORTC trials 10801, 10854, en 10902) en geanalyseerd. In totaal betrof het 3648 patiënten.
5.9% van de vrouwen die een mastectomie ondergingen versus 10.8% van de vrouwen die een mammasparende operatie ondergingen kregen een locoregionaal recidief ($P < 0.0001$). Ondanks het verschil in locoregionale controle was de lange termijn prognose (na correctie voor andere factoren) gelijk voor beide chirurgische behandelingsmodaliteiten. Echter, het geven van adjuvant radiotherapie na mastectomie was zowel geassocieerd met een betere locoregionale controle als met een betere lange termijn prognose dan mastectomie zonder radiotherapie. Dit effect was het meest opvallend bij patiënten met een beperkt aantal okselklier metastasen.

In Hoofdstuk 7 worden mogelijke risicofactoren voor het ontwikkelen van een locoregionaal recidief en de prognostische impact van het locoregionale recidief op het ontwikkelen van metastasen op afstand nader bestudeerd. De studie populatie bestond uit 3602 vrouwen met primair operabel mammacarcinoom. Uit multivariate analyses bleken zowel jonge leeftijd als mammasparende chirurgie onafhankelijke factoren voor het ontwikkelen van een locoregionaal recidief te zijn. Perioperatieve chemotherapie was onafhankelijk geassocieerd met een lagere kans op het krijgen van een locoregionaal recidief.

In Hoofdstuk 8 wordt een retrospectieve analyse gepresenteerd waarin risicofactoren zijn geanalyseerd in een subgroep van zeer jonge vrouwen met primair operabel mammacarcinoom. De data van 4 EORTC trials werden gecombineerd (EORTC trial 10801, 10854, 10902, 22881). In totaal participeerden 9938 vrouwen in deze studies. Ongeveer 10 % van de vrouwen was 40 jaar of jonger ten tijde van de diagnose. Van 549 van deze jonge vrouwen was tumor materiaal beschikbaar. De mate van expressie van verscheidene tumor markers werd door middel van immunohistochemie bepaald. Histologische gradering was een onafhankelijke prognostische factor voor zowel totale overleving als metastase vrije overleving. Tumor grootte was een onafhankelijke prognostische factor voor metastase vrije overleving. Ook in de subgroep van jonge vrouwen zonder okselklier metastasen bleef histologische gradering een onafhankelijke prognostische factor wat betreft totale en metastase vrije overleving.

In Hoofdstuk 9 wordt het effect van adjuvant systemische polychemotherapie vergeleken bij vrouwen van 40 jaar of jonger ten tijde van de diagnose met hormoonreceptor-positieve tumoren en hormoonreceptor-negatieve tumoren. De mediane follow-up bedroeg 7.3 jaar ten tijde van de analyse. In de groep patiënten die adjuvant systemische chemotherapie kregen was er geen verschil wat betreft overleving en metastase vrije overleving tussen patiënten met hormoonreceptor-positieve en hormoonreceptor-negatieve tumoren. In de groep patiënten die geen chemotherapie ontvingen hadden patiënten met hormoonreceptor-positieve tumoren een significant betere prognose wat betreft totale overleving en ziektevrije overleving. Derhalve lijken jonge vrouwen met hormoonreceptor-positieve tumoren minder baat

te hebben bij chemotherapie dan vrouwen met hormoonreceptor-negatieve tumoren.

In Hoofdstuk 10 worden de resultaten van dit proefschrift geanalyseerd en worden toekomstperspectieven van klinisch en translationeel onderzoek met betrekking tot de behandeling van het primair operabel mammacarcinoom besproken.

Curriculum Vitae

The author was born on September 20th 1971 in Hilversum. In 1989 he graduated from secondary school at The Baarnsch Lyceum in Baarn. Subsequently, he was admitted to medical school in the same year at the State University of Leiden. During his studies, he worked at the Department of Neurology and was actively involved in clinical trials concerning migraine research. After his graduation in 1998, he started working as a surgical resident in The Red Cross Hospital in The Hague. In 1999, he was awarded a Koningin Wilhelmina Fonds (KWF) fellowship at the EORTC Data Center in Brussels in Belgium. It was here where he laid the foundation for this thesis. In 2000, he returned to Leiden to continue his research at the Departments of Surgery and Pathology under supervision of Prof. Dr. C.J.H. van de Velde and Prof. Dr. G.J. Fleuren and Dr. M.J. van de Vijver after being granted a surgical research fellowship (AGIKO). In 2002, he started his surgical training at the Leiden University Medical Center under supervision of Prof. Dr. O.T. Terpstra. At present, he continues his surgical training at the Red Cross Hospital in The Hague, initially under supervision of Dr. H. Boutkan and as of January 2006 under supervision of Dr. P.J. Breslau. He is living happily in the "Benoordenhout" together with his lovely girlfriend and motivator Léontine, and his two beautiful little ladies Annebeth and Josephine.

Curriculum Vitae

De auteur van dit proefschrift werd op 20 september 1971 geboren te Hilversum. Hij groeide op in Baarn waar hij in 1989 zijn eindexamen behaalde aan Het Baarnsch Lyceum. In datzelfde jaar startte hij met de medicijnen studie aan de Rijksuniversiteit Leiden. Gedurende zijn studie, werkte hij als studentonderzoeker op de afdeling Neurologie alwaar hij betrokken was bij fase III studies op het gebied van de behandeling van migraine. Na zijn artsexamen in 1998, was hij tien maanden werkzaam als AGNIO Heelkunde in het Rode Kruis Ziekenhuis in Den Haag. In 1999 ontving hij een EORTC fellowship gefinancierd door het Koningin Wilhelmina Fonds (KWF). Hiermee werd aan de EORTC in Brussel de basis gelegd voor dit proefschrift. In 2000 keerde hij terug naar Leiden waar hij onder supervisie van Prof. Dr. C.J.H. van de Velde, Prof. Dr. G.J. Fleuren, en Dr. M.J. van de Vijver aan de afdelingen Heelkunde en Pathologie zijn onderzoeksactiviteiten voortzette als AGIKO Heelkunde. In 2002 startte hij zijn opleiding Heelkunde in het Leids Universitair Medisch Centrum onder leiding van Prof. Dr. O.T. Terpstra. Vanaf 2004 tot heden is hij werkzaam in het Rode Kruis Ziekenhuis te Den Haag, eerst onder auspiciën van Dr. H. Boutkan, vanaf 2006 onder auspiciën van Dr. P.J. Breslau. Hij woont met veel plezier in het Benoordenhout samen met zijn lieve vriendin en stok achter de deur Léontine, en zijn twee schitterende kleine dames Annebeth en Josephine.