Randomized Adjuvant Chemotherapy Trial in High-Risk, Lymph Node-Negative Breast Cancer Patients Identified by Urokinase-Type Plasminogen Activator and Plasminogen Activator Inhibitor Type 1

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For the German Chemo N_0 Study Group

Background: **Most patients with lymph node-negative breast cancer are cured by locoregional treatment; however, about 30% relapse. Because traditional histomorphologic and clinical factors fail to identify the high-risk patients who may benefit from adjuvant chemotherapy, other prognostic factors are needed. In a unicenter study, we have found that levels of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) in the primary tumor are predictive of disease recurrence. Thus, we designed the Chemo N_0 prospective randomized multicenter therapy trial to investigate further whether uPA and PAI-1 are such prognostic factors and whether high-risk patients identified by these factors benefit from adjuvant chemotherapy. After 4.5 years, we present results of the first interim analysis.** ***Methods:*** **We studied 556 patients with lymph node-negative breast cancer. The median follow-up was 32 months. All patients with low tumor levels of uPA (≤3 ng/mg of protein) and of PAI-1 (≤14 ng/mg of protein) were observed. Patients with high tumor levels of uPA (>3 ng/mg of protein) and/or of PAI-1 (>14 ng/mg of protein) were randomly assigned to combination chemotherapy or subjected to observation only. All statistical tests were two-sided.** ***Results:*** **A total of 241 patients had low levels of uPA and PAI-1, and 315 had elevated levels of uPA and/or PAI-1. The estimated 3-year recurrence rate for patients with low tumor levels of uPA and PAI-1 (low-risk group) was 6.7% (95% confidence interval [CI] = 2.5% to 10.8%). This rate for patients with high tumor levels of uPA and/or PAI-1 (high-risk group) was 14.7% (95% CI = 8.5% to 20.9%) (P = .006). First interim analysis suggests that high-risk patients in the chemotherapy group benefit, with a 43.8% lower estimated probability of disease recurrence at 3 years than high-risk patients in the observation group (intention-to-treat analysis: relative risk = 0.56; 95% CI = 0.25 to 1.28), but further follow-up is needed for confirmation.** ***Conclusions:*** **Using uPA and PAI-1, we have been able to classify about half of the patients with lymph node-negative breast cancer as low risk, for whom adjuvant chemotherapy may be avoided, and half as high risk, who appear to benefit from adjuvant chemotherapy. [J Natl Cancer Inst 2001;93: 913–20]**

Currently, about 50% of the patients with primary breast cancer do not have axillary lymph node involvement, and this percentage is increasing *(1,2).* It is not possible to reliably identify the high-risk patients (who will need adjuvant chemotherapy) and the low-risk patients (who can be spared adjuvant chemotherapy) by traditional histomorphologic and clinical characteristics, such as tumor size, histologic grade, age, steroid hormone receptor status, or menopausal status *(3).* If these characteristics were used to select therapies for patients, as recommended by the 1998 and 2001 St. Gallen consensus statements [*(4);* 7th International Consensus Conference on Adjuvant Therapy of Primary Breast Cancer, St. Gallen, Switzerland, February 2001], up to 90% of the patients with lymph node-negative breast cancer would be candidates for adjuvant chemotherapy, although only about 30% of the patients with lymph node-negative breast cancer will relapse and thus need adjuvant chemotherapy. This discrepancy has prompted a search for additional prognostic factors.

The plasminogen activator system plays an important role in tumor invasion and metastasis [reviewed in *(5–7)*]. Our group was the first, to our knowledge, to report that tumor levels of urokinase-type plasminogen activator (uPA) and of its inhibitor plasminogen activator inhibitor type 1 (PAI-1) appear to be prognostic factors for lymph node-positive *(8)* and lymph node-negative *(9)* breast cancer. Patients with high levels of uPA and/or PAI-1 in their primary tumors, determined by enzyme-linked immunosorbent assay (ELISA), had statistically significant shorter disease-free survival (DFS) and overall survival rates than patients with low tumor levels. The prognostic importance of uPA and PAI-1 in lymph node-negative breast cancer has since been confirmed by other investigators [reviewed in *(10)*].

Patients with lymph node-negative breast cancer who are at risk for disease recurrence (high-risk patients) can be identified

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See "Appendix" for centers and members of the German Chemo N_0 Study Group.

See "Notes" following "References."

by the levels of uPA and PAI-1 in their primary tumor. About 45% of patients with lymph node-negative breast cancer belong to this high-risk group as defined by high levels of uPA and/or PAI-1 in their primary tumor *(11).* Low-risk patients with lymph node-negative breast cancer have low levels of both uPA and PAI-1 in their tumor. This low-risk group, about 55% of all patients with lymph node-negative breast cancer, has an excellent prognosis, with a probability of relapse after 5 years of less than 5% *(11).* Thus, there is little reason to generally recommend adjuvant chemotherapy to this group *(12,13),* although, in an individual therapy decision, the patient's opinions on life-quality choices need to be considered *(14).* Finally, it is not known whether high-risk patients identified by high tumor levels of uPA and/or PAI-1 benefit from systemic adjuvant chemotherapy.

Chemo-N_0 is a prospective randomized multicenter therapy trial, initiated in Germany in June 1993, that uses tumor levels of uPA and PAI-1 to stratify patients. This trial was designed to answer the following two principal questions: 1) Can the reported prognostic importance of tumor levels of uPA and PAI-1 be validated in a prospective multicenter therapy trial (i.e., can low tumor levels of uPA and PAI-1 identify low-risk, lymph node-negative patients who might avoid adjuvant chemotherapy)? 2) Do high-risk patients, as identified by elevated tumor levels of uPA and/or PAI-1, benefit (as assessed by DFS) from cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) adjuvant chemotherapy? In this article, we report results of the first interim analysis, performed 4.5 years after the beginning of the trial.

Patients and Methods

Trial Profile

Patients with lymph node-negative breast cancer were included who had tumors with diameters between 1 and 5 cm and were undergoing standard locoregional treatment, independent of steroid hormone receptor and menopausal status. Patients were stratified by the levels of uPA and PAI-1 in their primary tumors. Statistically optimized cutoffs, as previously calculated and later re-evaluated *(15),* were used to define low-risk and high-risk patients. The cutoff for uPA was 3 ng/mg of protein, and the cutoff for PAI-1 was 14 ng/mg of protein. High-risk patients with lymph node-negative breast cancer (uPA levels >3 ng/mg of protein and/or PAI-1 levels >14 ng/mg of protein) were randomly assigned either to six courses of CMF (study arm B1) or to observation only (study arm B2). High-risk patients who refused randomization were followed-up and analyzed separately (study arm B3). Patients with low tumor levels of uPA and PAI-1 (uPA levels $\leq$3 ng/mg of protein and PAI-1 levels $\leq$14 ng/mg of protein) did not receive systemic adjuvant therapy but received observation only (study arm A; Fig. 1). Because of initial results indicating that the effectiveness of endocrine therapy was reduced in high-risk patients *(16,17),* a standard chemotherapy regimen (CMF) was selected for systemic adjuvant treatment. Other types of systemic adjuvant treatment were not permitted. Eligibility criteria are presented in Table 1. The trial has the following two goals: 1) to evaluate the prognostic importance of tumor levels of uPA and PAI-1 in a prospective multicenter therapy trial and 2) to determine whether CMF adjuvant chemotherapy increases the DFS of high-risk patients.

Fourteen breast cancer centers participate in the Chemo N_0 trial. The centers are associated with departments of obstetrics and gynecology at German universities or affiliated community hospitals, and the Comprehensive Cancer Center of Ljubljana, Slovenia, is a participating center. The trial was approved by ethics committees from all participating centers. The trial headquarters are located at the Department of Obstetrics and Gynecology, Technical University of Munich, Germany, and at the Department of Obstetrics and Gynecology, University of Hamburg, Germany. After giving signed, informed consent, patients with elevated tumor levels of uPA and/or PAI-1 were randomly assigned to treatment groups by the Statistical Evaluation Center at the Institute for Medical Information Processing, University of Tübingen, Germany. Results were communicated back to the participating centers. Randomization was performed by a computerized block randomization procedure that was stratified for each center in blocks of six treatment assignments. Patients randomly assigned to chemotherapy received six courses of intravenous CMF (i.e., cyclophosphamide at 500 mg/m^2, methotrexate at 40 mg/m^2, and 5-fluorouracil at 600 mg/m^2 on days 1 and 8; the protocol was repeated each 28 days). At the time of primary therapy, none of the patients had distant metastases, as verified by a clinical examination, chest x-ray, bone scan, and ultrasound examination of the liver. Follow-up data are obtained regularly every 6–12 months. Interim analyses are scheduled for 4.5, 6.5, 8.5, and 10 years after the start of the patient recruitment. Clinically evident disease recurrence is documented by cytology, histology, or image analysis. A recurrence in the breast is not classified as a relapse. Patient follow-up is carried out by the individual centers. On-site monitoring for data verification is carried out by an external monitor. Data are then collected by the Munich Trial Headquarters and reported to the Statistical Evaluation Center in Tübingen.

Patients

Patient recruitment began on June 24, 1993, and continued through December 29, 1998, when 689 patients had been enrolled, 249 of whom were randomly assigned to treatment. Patients were classified as postmenopausal 1 year after their last menstruation. If we were uncertain of a patient's menopausal status, serum levels of estradiol and follicle-stimulating hormone were determined. Examination of a minimum of 10 axillary lymph nodes was required. Histologic grade was scored as described previously by Bloom–Richardson *(18,19).* Tumor levels of estrogen receptor and progesterone receptor were determined immunohistochemically as an immunoreactive score *(20)* or biochemically by use of a dextran-coated charcoal assay or enzyme immunoassay. Estrogen receptors and progesterone receptors were classified as positive if the immunoreactive score was more than 0 or the dextran-coated charcoal assay/enzyme immunoassay found a value of 20 or more fmol/mg of protein. Steroid hormone receptor status was considered to be positive if results were positive for either or both of these receptors.

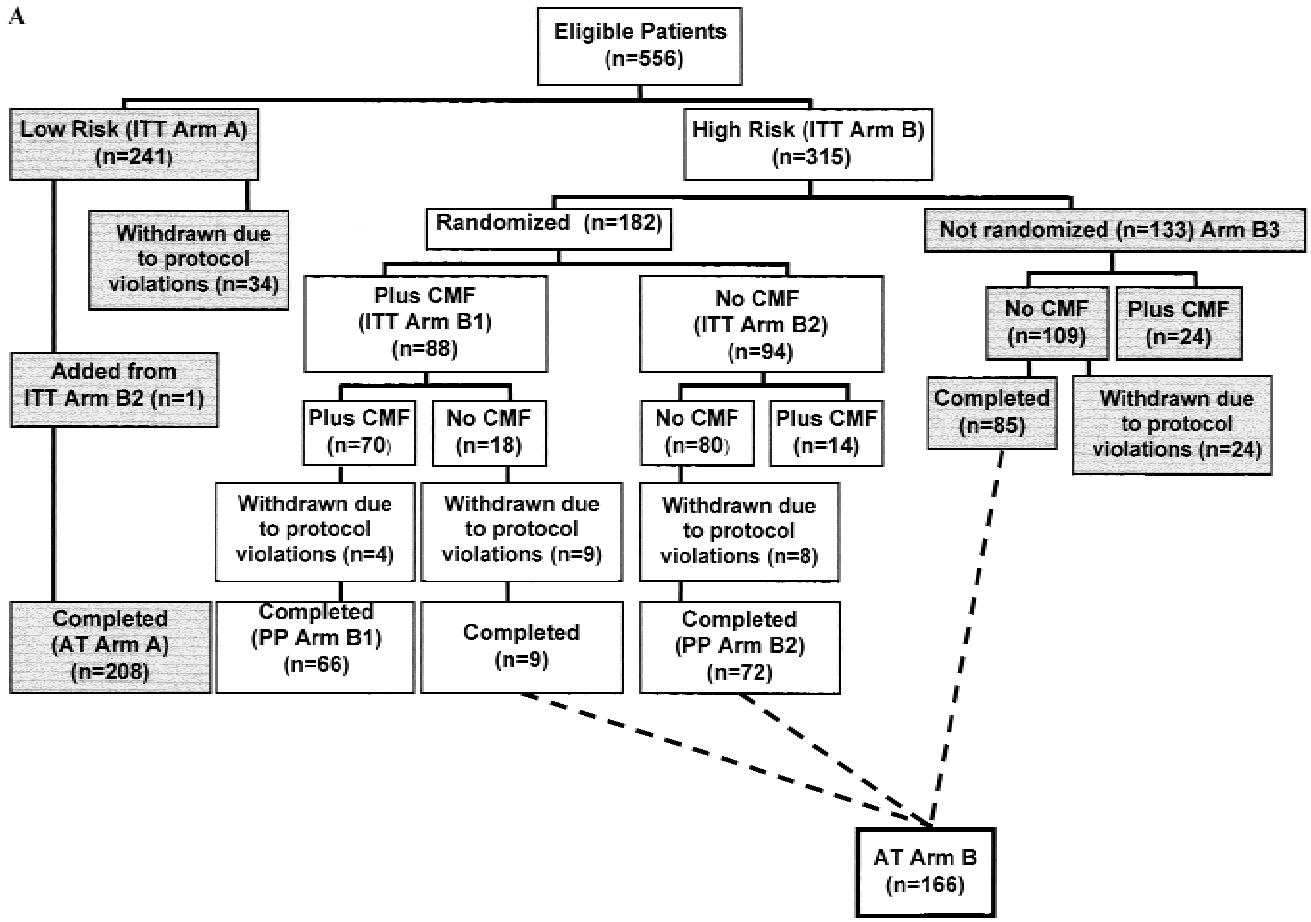
Laboratory Assays

Immediately after excision, the tumor tissue was placed on ice and transported on ice to the pathologist to examine frozen sections. Approximately 300 mg of tumor tissue was snap-frozen and stored in liquid nitrogen. For the preparation of the tumor tissue extracts, the still-frozen tumor tissue was pulverized, suspended in buffer (1 mL of Tris-buffered saline = 0.02 *M* Tris–HCl/0.125 *M* NaCl [pH 8.5]) containing 0.1% nonionic detergent Triton X-100, and centrifuged at 100 000*g* for 1 hour at 4 °C in an ultracentrifuge as described previously *(9).* The levels of uPA and PAI-1 in tumor extracts were determined by certified ELISA tests (uPA = Imubind 894; PAI-1 = Imubind 821; both from American Diagnostica Inc., Greenwich, CT) and were expressed as nanograms per milligram of tumor protein *(9).* Assays for uPA and PAI-1 were carried out by six centers. The performance of assays was controlled and assured by the Munich Study Headquarters and the Quality Assurance Center at the Department of Chemical Endocrinology, University Medical Center St. Radboud, University of Nijmegen, The Netherlands *(21).*

First Interim Analysis

The first interim analysis 4.5 years after the trial began was scheduled in the study protocol. The database for the interim analysis was closed on March 24, 1998. The 556 patients enrolled before March 31, 1997, were eligible for this analysis because of a sufficiently long follow-up period and, where applicable, the completion of CMF chemotherapy. The median age at primary surgery was 54 years (range, 28–71 years). Patients received a modified radical mastectomy (n = 160) or breast-conserving therapy (n = 396). The median follow-up time of patients still alive at the time of analysis was 32 months (range, 0–53 months). During the follow-up period, disease recurred in 60 patients (10.8%).

To evaluate whether tumor levels of uPA and PAI-1 have prognostic importance, we analyzed 374 patients with lymph node-negative breast cancer who did not receive CMF and did not violate the eligibility criteria (the as-treated population; Fig. 1 and Table 2). Of these 374 patients, 208 low-risk patients were assigned to study arm A and 166 high-risk patients were assigned to study arms B1, B2, or B3. The benefit of adjuvant CMF in the high-risk group was assessed as DFS in the following two populations: The intention-to-treat population



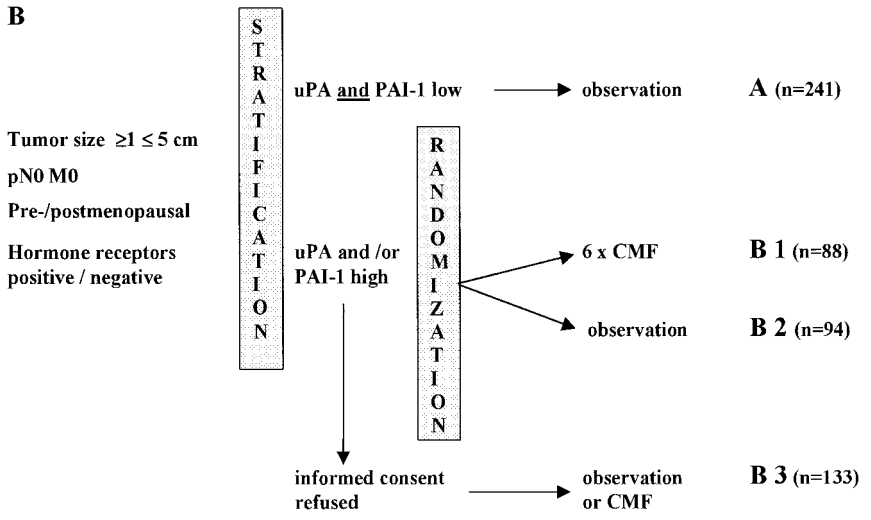


Fig. 1. A) Trial profile (CONSORT diagram) of the German multicenter Chemo N_0 trial: risk-adapted adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) chemotherapy in lymph node-negative breast cancer based on tumor biologic factors urokinase-type plasminogen activator (uPA), and plasminogen activator inhibitor type 1 (PAI-1). **B**) Study outline. ITT = intention-to-treat population; AT = as-treated population; PP = per-protocol population. High-risk patients with lymph node-negative breast cancer (uPA levels >3 ng/mg of protein and/or PAI-1 levels >14 ng/mg of protein) were randomly assigned either to six courses of CMF (study arm B1) or to observation only (study arm B2). High-risk patients who refused randomization were followed-up and analyzed separately (study arm B3). Patients with low tumor levels of uPA and PAI-1 (uPA levels ⩽3 ng/mg of protein and PAI-1 levels ⩽14 ng/mg of protein) did not receive systemic adjuvant therapy but received observation only (study arm A).

contained 182 patients (study arm B1 or B2; Fig. 1 and Table 3), and the per-protocol population contained 138 patients (study arm B1 or B2) who adhered to the study protocol after randomization and did not violate the eligibility criteria (Fig. 1).

Data Documentation and Statistics

The same database software programmed in dBASE IV/FOX-BASE was used for patient documentation in all of the recruiting centers. Plausibility of the clinical data was controlled by the Munich Study Headquarters. Statistical evaluation with the SAS program package, version 6.12 for Windows (SAS Institute, Inc., Cary, NC), was performed at the Institute for Medical Information Processing, University of Tübingen, Germany. The primary end point for statistical analyses is DFS; the secondary end point is overall survival, which is not reported in this interim report. Our target was 900 patients. We planned to have 203 patients in each high-risk group to detect a 30% reduction in the incidence of disease recurrence *(8,9,11).* A two-group Fisher exact test ($\alpha = .05$) would then have an 86% power to detect the difference between 45% disease recurrences in the high-risk, untreated group and 30% disease recurrences in the high-risk, CMF-treated group.

Tumor levels of uPA and PAI-1 were coded as binary variables, by use of optimized cutoffs as previously described *(15).* Three-year DFS rates were es-

Table 1. Eligibility criteria*

Criteria for inclusion
- Signed informed consent of the patient
- Premenopausal and postmenopausal patients ⩽70 y old
- Tumor diameter between 1 cm and 5 cm
- No. of investigated axillary lymph nodes ⩾10
- Breast-conserving therapy including radiation therapy or modified radical mastectomy
- Availability of fresh tumor tissue for uPA and PAI-1 antigen determination by ELISA

Criteria for exclusion
- Previous contralateral breast cancer or cancer of different origin; simultaneous second malignancy
- Radiation therapy, chemotherapy, or tamoxifen therapy before tissue removal
- Postoperative tamoxifen or progestin therapy
- WBC count <3000 WBCs per μL and platelets <100 000 platelets per μL
- Infectious diseases
- Pregnancy
- Participation in another primary breast cancer therapy trial

*uPA = urokinase-type plasminogen activator; PAI-1 = plasminogen activator inhibitor type 1; ELISA = enzyme-linked immunosorbent assay; WBC = white blood cell.

Table 2. Distribution of histomorphologic and clinical factors in low-risk patients (study arm A) and high-risk patients not receiving cyclophosphamide, methotrexate, and 5-fluorouracil (study arms B2 and B3)

	Low-risk patients (n = 208)		High-risk patients (n = 166)	
Factor	No.	%	No.	%
Age, y				
<50	67	32	56	34
⩾50	141	68	110	66
Menopausal status				
Premenopausal	79	38	74	45
Postmenopausal	129	62	92	55
pT stage*				
pT1	128	62	110	66
pT2	80	38	56	34
Type of locoregional treatment				
Breast-conserving therapy	144	69	126	76
Modified radical mastectomy	64	31	40	24
Histologic grade*				
G1	22	11	13	8
G2	146	70	108	65
G3	39	19	42	25
Unknown	1	0.5	3	2
Hormone receptor status				
Positive	190	91	132	80
Negative	18	9	34	20

*Staging system was described previously *(23)*. pT = tumor size as determined by the pathologist. Grading system was described previously *(18,19)*.

timated, and Kaplan–Meier survival curves were plotted *(22)*. Log-rank tests were used to compare the DFS of low-risk and high-risk patients and the DFS of the two groups of randomly assigned patients. The Cox proportional hazards regression model was used in univariate and multivariate analyses to calculate *P* values, relative risks (RRs), and 95% confidence intervals (CIs). Multivariate analyses were conducted in two steps: 1) Full models including all relevant factors were computed, and 2) all variables were tested for colinearity. When colinearity was observed, only one of the variables was included in the final model. Therefore, the variables menopausal status and tumor size were excluded from the final model in favor of the variables age and pT stage [tumor size as determined by the pathologist *(23)*]. Interactions and possible center effects were also investigated, but none were found. A maximum duration trial design was

Table 3. Distribution of histomorphologic and clinical factors in high-risk patients who received CMF* adjuvant chemotherapy (study arm B1) and in patients who did not (study arm B2)

	With CMF (B1; n = 88)		Observation only (B2; n = 94)	
Factor	No.	%	No.	%
Age, y				
<50	25	28	34	36
⩾50	63	72	60	64
Menopausal status				
Premenopausal	33	38	42	45
Postmenopausal	55	62	52	55
pT stage†				
pT1	44	50	53	56
pT2	44	50	41	44
Type of locoregional treatment				
Breast-conserving therapy	58	66	71	76
Modified radical mastectomy	30	34	23	24
Histologic grade†				
G1	10	11	3	3
G2	44	50	54	57
G3	33	38	36	38
Unknown	1	1	1	1
Hormone receptor status				
Positive	71	81	65	69
Negative	17	19	29	31

*CMF = cyclophosphamide, methotrexate, and 5-fluorouracil.

†Staging system was described previously *(23)*. pT = tumor size as determined by the pathologist. Grading system was described previously *(18,19)*.

selected *(24)*, and four periodic analyses were scheduled at 4.5, 6.5, 8.5, and 10.5 years after the start of patient recruitment. At the time of the first interim analysis, patient recruitment was still ongoing. Therefore, to account for a type I error probability of the planned repeated tests of statistical significance, a spending function procedure was used *(25)*. The information time was estimated as a function of calendar time, and the statistical significance level for this first analysis was computed as $4.5/10.5 \times 0.05$ and thus $\alpha = 0.021$. All statistical tests were two-sided.

RESULTS

Patient Population

Five hundred fifty-six patients were eligible for this first interim analysis, with a median follow-up of 32 months. Two hundred forty-one patients had low tumor levels of both uPA and PAI-1 (study arm A), and 315 patients had elevated tumor levels of uPA and/or PAI-1 and fulfilled the criteria for randomization (study arm B). Of these 315 high-risk patients, 88 were randomly assigned to adjuvant CMF (study arm B1), 94 were randomly assigned to observation only (study arm B2), and 133 refused randomization (study arm B3) (Fig. 1). After randomization to study arm B1, 18 patients (20%) refused CMF chemotherapy and thus were observed only. Fourteen patients (15%) randomly assigned to observation (study arm B2) opted for chemotherapy and received CMF. Of the 133 patients in study arm B3, 24 (18%) opted for chemotherapy and received at least three courses of CMF, and 109 (82%) preferred not to receive chemotherapy. The observed side effects of CMF chemotherapy were mostly nausea (6.8%), leukopenia (5.2%), anemia (2.8%), and alopecia (2.7%), all of which were World Health Organization (WHO) grade 1 or 2. WHO grade 3 side

effects were observed in only 0.7% of the CMF courses given to patients.

Validation of Prognostic Importance of uPA/PAI-1 Levels

This prospective multicenter therapy trial confirmed the previously reported strong prognostic importance of uPA and PAI-1 levels for patients with lymph node-negative breast cancer. Of the 374 patients in the as-treated population without systemic adjuvant therapy, 208 with low tumor levels of uPA and PAI-1 had an estimated 3-year recurrence rate of 6.7% (95% CI = 2.5% to 10.8%). The 166 patients with high tumor levels of uPA and/or PAI-1 had a rate that was more than twice as high (14.7%; 95% CI = 8.5% to 20.9%). This difference in DFS is highly statistically significant (log-rank; $P = .006$) (Fig. 2). We then compared these results from multiple centers with results from a long-term follow-up analysis of a unicenter prospective study from Germany *(11)* describing the prognostic importance of uPA and PAI-1 levels in patients with lymph node-negative breast cancer who did not receive systemic adjuvant therapy (Fig. 2). Only 101 patients in the unicenter study who fulfilled the eligibility criteria of the Chemo N_0 trial were included in this comparison. We found that the survival curves of the Chemo N_0 trial almost coincided with those of the unicenter study for low-risk and high-risk patients. The final Cox model (Table 4) includes the variables age, steroid hormone receptor status, pT stage, surgical technique, and histologic grade. This model showed that patients with high tumor levels of uPA and/or PAI-1 had a 2.83-fold higher risk of disease recurrence (95% CI = 1.3-fold to 6.0-fold; $P = .007$) than patients with low tumor levels of uPA and PAI-1. Histologic grade was also an independent statistically significant prognostic factor for DFS (RR = 3.38 [95% CI = 1.7 to 6.8]; $P = .001$). When tumor levels of uPA and PAI-1 were used to classify patients for the risk of disease recurrence, 208 (56%) of the patients were classified as low risk compared with only 35 (9%) when histologic grade (grade G1) was used.

In this therapy trial, we used the previously optimized cutoff levels for uPA (3 ng/mg of protein) and PAI-1 (14 ng/mg of protein) to stratify patients *(15)*. With these cutoffs, we observed that tumor levels of uPA and PAI-1 were strong prognostic factors, as in the prospective unicenter trial (Fig. 2). In this interim analysis, cutoff levels were re-evaluated with log-rank statistics for the group of patients who did not receive CMF. Optimal cutoffs for tumor levels of both uPA and PAI-1 were within the previously published ranges *(15)*.

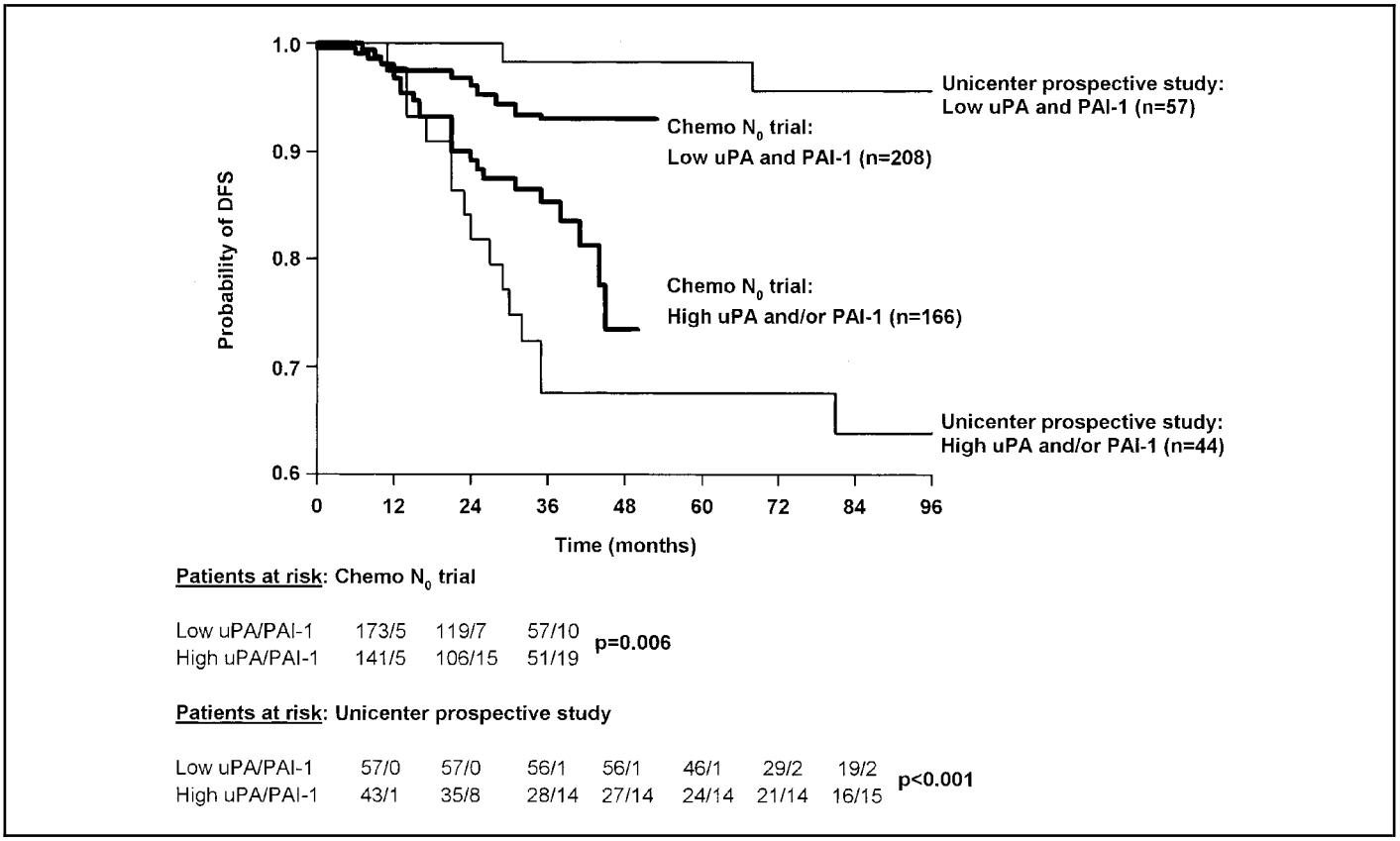


Fig. 2. Kaplan–Meier curves showing the impact of tumor levels of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (PAI-1) on the probability of disease-free survival (DFS). **Thick lines** = Chemo N_0 trial (as-treated population); **thin lines** = long-term follow-up from a previously published study *(11)* of patients with lymph node-negative breast cancer who had not received systemic adjuvant therapy and were enrolled in a prospective unicenter study. All 101 patients from the unicenter study whose data were used in this analysis fulfilled the eligibility criteria of the Chemo N_0 trial. The 95% confidence intervals (CIs) for probability of DFS are as follows: unicenter study for low uPA/PAI-1 levels (n = 57), 100% (no events) at 1 and at 2 years and 98.3% (95% CI = 94.8% to 100%) at 3 and at 5 years; Chemo N_0 trial for low uPA/PAI-1levels (n = 208), 97.5% (95% CI = 95.3% to 99.7%) at 1 year, 96% (95% CI = 93.1% to 99%) at 2 years, and 93.3% (95% CI = 89.2% to 97.5%) at 3 years; Chemo N_0 trial for high uPA/PAI-1 levels (n = 166), 96.8% (95% CI = 93.9% to 99.6%) at 1 year, 89.2% (95% CI = 84% to 94.4%) at 2 years, and 85.3% (95% CI = 79.1% to 91.5) at 3 years; and unicenter study for high uPA/PAI-1 levels (n = 44), 97.7% (95% CI = 93.3% to 100%) at 1 year, 81.8% (95% CI = 70.4% to 93.2%) at 2 years, and 67.6% (95% CI = 53.6% to 81.6%) at 3 and at 5 years. All statistical tests were two-sided.

Table 4. Univariate and multivariate analyses of histomorphologic, clinical, and tumor biologic factors for disease-free survival in 374 patients with lymph node-negative breast cancer who did not receive systemic adjuvant therapy*

	Univariate analysis		Final multivariate analysis	
Factor	RR (95% CI)	*P*	RR (95% CI)	*P*
Histologic grade	4.44 (2.3 to 8.5)	<.001	3.38 (1.7 to 6.8)	.001
uPA/PAI-1	2.71 (1.3 to 5.7)	.009	2.83 (1.3 to 6.0)	.007
Type of locoregional treatment	2.54 (1.3 to 5.0)	.008	2.79 (1.4 to 5.8)	.006
Age	0.40 (0.2 to 0.8)	.009	0.40 (0.2 to 0.8)	.010
pT stage	2.61 (1.3 to 5.2)	.007	1.65 (0.8 to 3.4)	.177
Hormone receptor status	2.53 (1.2 to 5.3)	.014	1.32 (0.6 to 2.9)	.481

*Four patients had missing values for grading. Multivariate analysis was thus performed on data from 370 patients. The factors were entered into the analysis as follows: histologic grade (G3 versus G2 versus G1) *(18,19),* urokinase-type plasminogen activator (uPA) and/or plasminogen activator inhibitor type 1 (PAI-1; either or both high versus both low), type of locoregional treatment (modified radical mastectomy versus breast-conserving therapy), age (≥50 year versus <50 year), pT (tumor size as determined by the pathologist) stage (pT2 versus pT1) *(23),* and steroid hormone receptor status (negative versus positive). RR = relative risk; 95% CI = 95% confidence interval. All *P* values are two-sided.

Benefit From CMF Adjuvant Chemotherapy in High-Risk Patients

High-risk patients with high uPA and/or PAI-1 levels were randomly assigned to receive either CMF adjuvant chemotherapy or observation alone. At this first interim analysis, the estimated 3-year risk probability for disease recurrence was 12% for the 88 patients who received CMF adjuvant chemotherapy and 18% for the 94 patients who were observed only (Fig. 3, A). Thus, in high-risk patients, CMF adjuvant therapy was associated with a 43.8% decrease in the RR of disease recurrence (RR = 0.56; 95% CI = 0.25 to 1.28). In the intention-to-treat population, the effect of CMF chemotherapy was influenced by patients not adhering to the study protocol after randomization or the eligibility criteria being violated. These patients were excluded in a per-protocol analysis. The effect of CMF adjuvant chemotherapy then increased (*P* = .016; RR = 0.27 [95% CI = 0.09 to 0.78]; Fig. 3, B).

Discussion

The first interim analysis of the Chemo-N_0 prospective multicenter therapy trial is presented. By using tumor levels of uPA and PAI-1, we can classify about half of the lymph node-negative patients as low risk, for whom adjuvant chemotherapy may be avoided, and about half as high risk, who appear to benefit from adjuvant chemotherapy.

We confirm the strong and independent prognostic importance of tumor levels of uPA and PAI-1 for patients with lymph node-negative breast cancer, which has been observed in numerous unicenter studies [reviewed in *(10)*]. After a median follow-up of 32 months, we observed that the risk of disease recurrence was statistically significantly lower for patients with low tumor levels of uPA and PAI-1 than for patients with high tumor levels. Because of their excellent prognosis, about one half of the patients with lymph node-negative breast cancer may thus avoid adjuvant chemotherapy. Moreover, we observed that high-risk patients receiving CMF adjuvant chemotherapy have a substantial DFS benefit.

ELISAs measuring the levels of uPA and PAI-1 performed consistently well, as demonstrated by the Quality Assurance Center in Nijmegen *(21).* Quality-control data from the European Organization for Research and Treatment of Cancer (EORTC) Receptor Biomarker Group *(26)* prove that these ELISAs perform well in routine clinical use.

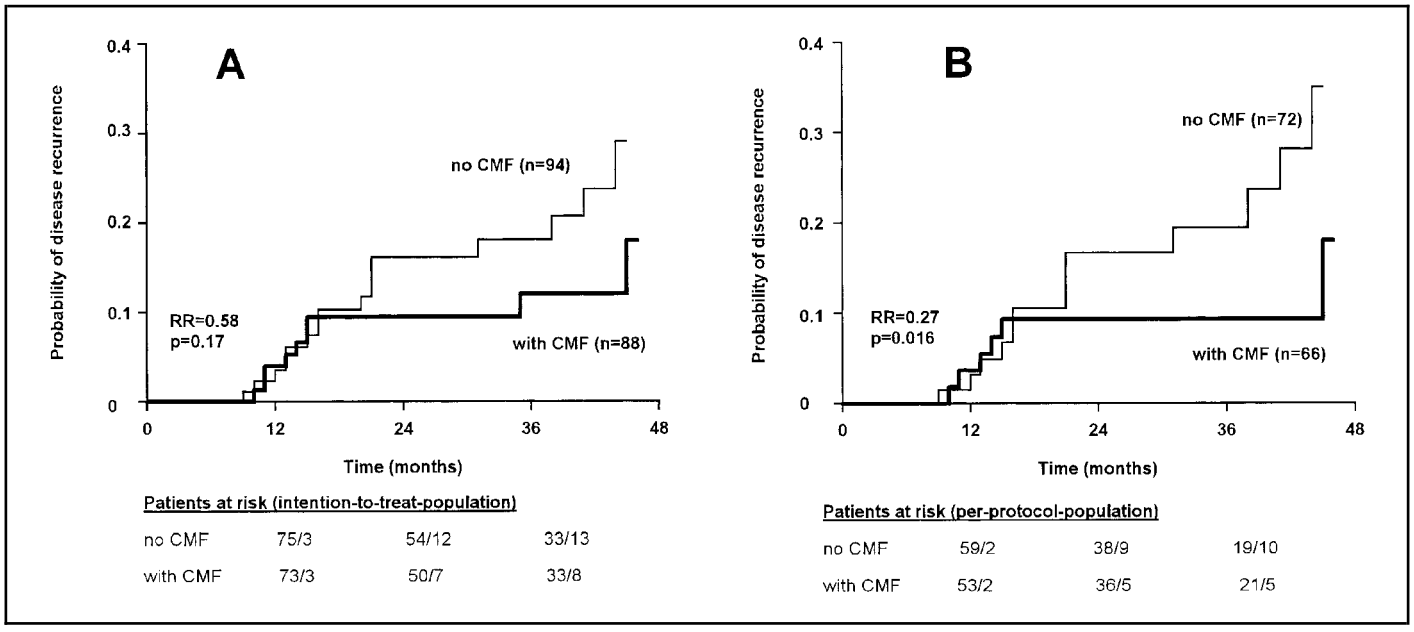


Fig. 3. Probability of distant disease recurrence in high-risk patients with and without adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). **A**) Intention-to-treat population. The 95% confidence intervals (CIs) for probability of distant disease recurrence are as follows: without CMF (n = 94), 3.5% (95% CI = 0% to 7.5%) at 1 year, 16.2% (95% CI = 7.7% to 24.6%) at 2 years, and 18.1% (95% CI = 9% to 27.1%) at 3 years; and with CMF (n = 88), 3.9% (95% CI = 0% to 8.3%) at 1 year, 9.5% (95% CI = 2.8% to 16.1%) at 2 years, and 12.0% (95% CI = 3.9% to 20.2%) at 3 years. **B**) Per-protocol population. The 95% CIs for probability of distant disease recurrence are as follows: without CMF (n = 72), 3.1% (95% CI = 0% to 7.4%) at 1 year, 16.6% (95% CI = 6.6% to 26.7%) at 2 years, and 19.4% (95% CI = 8.3% to 30.5%) at 3 years; and with CMF (n = 66), 3.6% (95% CI = 0% to 8.5%) at 1 year and 9.3% (95% CI = 1.5% to 17.0%) at 2 and at 3 years. All statistical tests were two-sided.

Rather strict criteria have been put forward for the evaluation of new prognostic factors for breast cancer before they are recommended for routine clinical use *(3,13).* In accordance with these criteria, we have validated for the first time in a prospective multicenter therapy trial that tumor levels of uPA and PAI-1 are prognostic factors for DFS of patients with breast cancer. To our knowledge, there is no contradictory information on the prognostic impact of uPA and PAI-1 levels in primary breast cancer. The Kaplan–Meier DFS curves in our trial are remarkably similar to those, after a long-term follow-up, in a single-center study of patients with lymph node-negative breast cancer not receiving systemic adjuvant therapy *(11)* (Fig. 2). Risk-group assessment by tumor levels of uPA and PAI-1 places 44% of the patients with lymph node-negative breast cancer into the high-risk group, for whom adjuvant chemotherapy is recommended. This percentage corresponds well to the actual estimate of about 30% of such patients who will eventually relapse *(2).* In contrast, when the high-risk group is defined by histologic grade alone or by the St. Gallen's Consensus Conference recommendations [*(4);* 7th International Consensus Conference on Adjuvant Therapy of Primary Breast Cancer, St. Gallen, Switzerland, February 2001], twice as many lymph node-negative patients (or almost every lymph node-negative patient) are candidates for adjuvant chemotherapy *(12).* The focus of the Chemo N_0 trial is on tumor levels of uPA and PAI-1, and patients were not stratified for type of locoregional treatment. Therefore, the results of our trial with regard to this variable cannot be compared with those of a large randomized trial addressing locoregional treatment *(27).*

When the Chemo N_0 trial started, systemic adjuvant treatment of patients with lymph node-negative breast cancer was not generally recommended. Thus, we could design a study in which patients in the target population were randomly assigned either to CMF treatment or to only observation to determine whether adjuvant chemotherapy was effective in high-risk patients as defined by uPA and/or PAI-1 tumor levels. Such a study design is no longer feasible for the following reasons: First, an increased emphasis on adjuvant chemotherapy for lymph node-negative patients was put forward by the 1998 St. Gallen's Consensus Conference *(4).* Second, the advantages of adjuvant tamoxifen therapy for patients with steroid hormone receptor-positive breast cancer were clearly demonstrated by the 1998 meta-analysis from the Early Breast Cancer Trialists' Collaborative Group *(28).* In addition, this first interim analysis of the Chemo N_0 trial showed that high-risk patients, defined by high tumor levels of uPA and/or PAI-1, appear to benefit from adjuvant CMF, although the benefit associated with this treatment lacked statistical significance in the intention-to-treat analysis. In the per-protocol analysis, the treatment benefit was even more pronounced. Moreover, the observed benefit was greater than the estimated 30% used to calculate the original sample size. Therefore, we expect that the treatment effect will become statistically significant when data from more patients, more events, and a longer median follow-up are examined in upcoming analyses. Thus, for these reasons and in agreement with members of the external review committee, we decided to stop patient recruitment at the end of December 1998, when 689 patients had been enrolled in the Chemo N_0 trial. Further follow-up data will be obtained as scheduled.

As a follow-up trial, to determine the optimal adjuvant chemotherapy protocol for the high-risk patients with lymph node-negative breast cancer, we have devised a new clinical trial (Euro Chemo N_0–European Node-Negative Breast Cancer Trial) that compares an anthracycline-containing adjuvant chemotherapy with a sequential taxane regimen. In this new study, all patients with steroid hormone receptor-positive breast cancer will receive adjuvant tamoxifen therapy. This prospective European multicenter clinical trial will use tumor levels of uPA and PAI-1 as well as HER2/neu status as stratification and randomization criteria. This trial has been approved and is being supported by the European Research Council BIOMED-2 Program and the EORTC Breast Cancer Group.

We anticipate that the results of the Chemo N_0 trial and the published clinical data on tumor levels of uPA and PAI-1 will alter the assessment of prognosis and risk-adapted treatment strategies for individual patients with lymph node-negative breast cancer. The highest level of evidence (LOE I) of the Tumor Marker Utility Grading System *(13,29)* has now been reached for tumor levels of uPA and PAI-1. We thus believe that our findings are strong enough to recommend larger scale testing of tumor levels of uPA and PAI-1 for patients with primary lymph node-negative breast cancer. By use of tumor levels of uPA and PAI-1 as stratification criteria, about one half of the patients with lymph node-negative breast cancer can be considered low risk, with a probability of less than 10% that the disease will recur. It seems reasonable to assume that the relapse rate among these low-risk patients can be further reduced by treatment with adjuvant tamoxifen *(28).* Although adjuvant chemotherapy for these low-risk patients may be overtreatment, high-risk patients, defined by high tumor levels of uPA and/or PAI-1, appear to benefit from adjuvant chemotherapy, but further follow-up is needed for confirmation. Furthermore, the fundamental role of uPA and PAI-1 in tumor invasion and metastasis indicates that these factors should be explored as targets for tumor biology-oriented therapies *(30).*

APPENDIX

German Chemo N_0 Study Group: Frauenklinik der Technischen Universität München (Professor Dr. H. Graeff, Dr. A. Prechtl, Dr. N. Harbeck, and Professor Dr. M. Schmitt); Frauenklinik der Universität Hamburg (Professor Dr. F. Jänicke, Professor Dr. C. Thomssen, and Dr. B. Lisboa); Frauenklinik-Klinikum Grosshadern der Ludwig-Maximilians-Universität München (Professor Dr. H. Hepp and Dr. M. Untch); Frauenklinik der Universität Mainz (Professor Dr. P. G. Knapstein and Dr. M. Mahlke); Frauenklinik der Universität Erlangen/Nürnberg (Professor Dr. N. Lang and Dr. G. Wieland); Frauenklinik-Klinikum Innenstadt der Ludwig-Maximilians-Universität München (Professor Dr. G. Kindermann and Dr. P. Hantschmann); Frauenklinik des St. Joseph Hospitals, Wiesbaden (Dr. G. Hoffmann and Dr. P. Scheler); Frauenklinik der Universität Ulm (Professor Dr. R. Kreienberg and Professor Dr. V. Möbus); Frauenklinik vom Roten Kreuz München (Professor Dr. W. Eiermann and Dr. U. Hamann); Frauenklinik des Klinikums Rosenheim (Professor Dr. T. Beck and Dr. E. Thurner-Hermanns); Institute of Oncology, Ljubljana, Slovenia (Professor Dr. T. Cufer and Dr. S. Borstnar); Frauenklinik Mannheim, Universität Heidelberg (Professor Dr. F. Melchert and Dr. R. Klose); Chirurgische Klinik der Technischen Universität München (Professor Dr. J. R. Siewert and Dr. H. Nekarda); Frauenklinik der Universität Heidelberg (Professor Dr. G. Bastert and Professor Dr. D. Wallwiener); Institut für Medizinische Informationsverarbeitung Universität Tübingen (Professor Dr. H. K. Selbmann and C. Meisner); and Department of Chemical Endocrinology, University of Nijmegen, The Netherlands (Professor Dr. C. G. Sweep and Professor Dr. T. Benraad).

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NOTES

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