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## Theme Issue Article

# The complex between urokinase-type plasminogen activator (uPA) and its type-I inhibitor (PAI-I) independently predicts response to first-line endocrine therapy in advanced breast cancer

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### Summary

It has been shown that urokinase-type plasminogen activator (uPA) and its main inhibitor (PAI-I) have predictive value for therapy success in advanced breast cancer. Levels of the complex between uPA and PAI-I, formed when both molecules are in their active form, might have superior predictive power. Here, we investigate the association between levels of uPA:PAI-I complex and rate of response to first-line systemic therapy for advanced breast cancer. Tumor tissues of 170 patients with advanced breast cancer were analyzed for uPA:PAI-I complex concentrations using a quantitative enzyme-linked immunosorbent assay. The patients received either endocrine therapy (n=96) or chemotherapy (n=74) as first-line treatment after diagnosis of advanced disease. Of the endocrine treated patients, those with high levels of uPA:PAI-I complex showed a shorter progression-free survival (PFS) compared to patients

with lower uPA:PAI-I complex levels ( $P=0.035$ ). Furthermore, in the multivariate regression analysis a significant lower rate of response to first-line endocrine therapy was found in patients with high uPA:PAI-I complex levels compared to patients with low uPA:PAI-I complex levels (odds ratio (OR)=0.27, 95% CI, 0.09-0.59,  $P=0.018$ ), in addition to the predictive impact of the steroid hormone receptor (ER/PgR) status (OR=2.68, 95% CI, 1.08-6.63,  $P=0.033$ ). Complex levels did not predict efficacy of chemotherapy in patients with advanced breast cancer. The results show that the plasminogen activation system affects the response to endocrine therapy independent of steroid hormone receptor status and may be of help to further refine the indication for this treatment in individual patients. Further studies are warranted to explain this underlying resistance to endocrine therapy when uPA:PAI-I levels are high.

### Keywords

Advanced breast cancer, plasminogen activator inhibitor type-I, predictive, uPA:PAI-I complex, urokinase-type plasminogen activator

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## Introduction

One of the most thoroughly studied metastasis-associated systems in patients with breast cancer is the plasminogen activation system. This system comprises of, among others, the

urokinase-type plasminogen activator (uPA) and its main inhibitor (PAI-1), and is primarily involved in extracellular matrix degradation (1). Most studies have focused on the prognostic value of uPA and PAI-1 (1-6). The potential predictive value of tumor levels of uPA and PAI-1, i.e. the power to predict therapy

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success, has been investigated in three breast cancer studies (7-9). Harbeck, et al. investigated the predictive value of uPA and PAI-1 (7, 8), showing that patients with high tumor tissue uPA levels in combination with high PAI-1 levels benefited more strongly from conventional adjuvant systemic therapy, particularly from chemotherapy, than those with low levels. So far, there is only one study addressing the correlation between tumor levels of uPA and PAI-1 and the response to first-line tamoxifen therapy in advanced breast cancer patients (9). The results of that study showed that increasing tumor levels of uPA and PAI-1 were predictive for poor response to tamoxifen in patients with advanced breast cancer.

The plasminogen activation system is a multifactorial system and in addition to uPA and PAI-1, uPA:PAI-1 complex is present in tumor tissue extracts (10, 11). As complex formation between uPA and PAI-1 occurs only when both molecules are in their active form, one might assume that the level of uPA:PAI-1 complex represents previously active uPA and PAI-1. Importantly, the active forms of these components are involved

in tumor growth, invasion and metastasis. Therefore, selective assessment of uPA:PAI-1 complex levels in tumor tissue could yield even more predictive information than total uPA and/or PAI-1 levels. The prognostic value of uPA:PAI-1 complex has been evaluated in invasive breast cancer in two retrospective studies (12, 13). To date, however, there are no studies on the predictive value of uPA:PAI-1 complex levels for response to systemic therapy in advanced breast cancer.

The purpose of this retrospective study was to assess the predictive value of tumor uPA:PAI-1 complex levels on the efficacy of systemic therapy in patients with advanced breast cancer and to compare it with the predictive impact of uPA and PAI-1. For this purpose, patients with breast cancer who developed distant metastases during follow-up and were treated with palliative first-line endocrine- or first-line chemotherapy were included in this study.

## Patients and methods

### Patients

A series of 170 patients with primary breast cancer who underwent resection of their primary tumor between January 1987 and December 1996, and who subsequently developed distant metastases were included. All patients were treated with first-line endocrine therapy (n=96) or chemotherapy (n=74). The median follow-up time of all patients and patients still alive from start of systemic therapy for advanced disease was 34 months (range 2-180 months) and 38 months (range 2-180 months), respectively. The median age was 55 years (range 28-86 years) at the time of surgery of the primary tumor. Further characteristics of patients and tumors are listed in Table 1.

### First-line systemic treatment

First-line endocrine therapy was given to 96 patients. These patients received tamoxifen (n=82), ovarian ablation (n=10), aromatase inhibitors (n=3) or medroxy-progesterone acetate (n=1). Nineteen of the patients who received first-line endocrine therapy had received prior adjuvant chemotherapy. However, none of these patients had received adjuvant endocrine therapy nor had become postmenopausal due to the adjuvant chemotherapy. Further, none of the patients were exposed to neoadjuvant therapy.

At the start of first-line endocrine therapy, 61 of the patients (64%) had an estrogen receptor positive (ER+)/progesterone receptor positive (PgR+) tumor, 11 (11%) had an ER+/PgR negative (PgR-) tumor, 7 (7%) had an ER negative (ER-)/PgR+ tumor, 15 (16%) had an ER-/PgR- tumor and the ER/PgR status of 2 (2%) was unknown. The patients who received first-line endocrine therapy had a median age of 58 years (31-86 years) at start of first-line therapy and the median follow-up time from this point of patients still alive was 46 months (range 2-180 months).

**Table 1:** Categorical distributions of baseline characteristics in patients treated with first-line endocrine therapy or first-line chemotherapy.

Characteristics	Patients treated with endocrine therapy		Patients treated with chemotherapy	
	N <sup>A</sup>	(%)	N <sup>B</sup>	(%)
Age (years) <sup>C</sup>				
≤40	16	(16.7)	21	(28.4)
41-55	29	(30.2)	21	(28.4)
56-70	34	(35.4)	26	(35.1)
>70	17	(17.7)	6	(8.1)
Menopausal status <sup>C</sup>				
Pre	34	(35.4)	39	(52.7)
Post	62	(64.6)	35	(47.3)
ER/PgR				
(fmol/mg protein)				
both <10	15	(15.6)	36	(50.0)
both or one ≥10	79	(82.3)	36	(50.0)
Primary tumor size				
pT1	34	(35.4)	14	(18.9)
pT2	44	(45.8)	42	(56.8)
pT3+4	17	(17.7)	16	(21.6)
Histological grade				
I	6	(6.3)	1	(1.4)
II	20	(20.8)	12	(16.2)
III	27	(28.1)	39	(52.7)
Lymph node status				
Negative	45	(46.9)	18	(24.3)
Positive	43	(44.8)	52	(70.3)
First site of relapse <sup>D</sup>				
Soft tissue	4	(4.2)	35	(47.3)
Bone	55	(57.3)	18	(24.3)
Viscera	37	(38.5)	21	(28.4)
Disease-free interval				
<1 year	24	(25.0)	31	(41.9)
≥1 year	72	(75.0)	43	(58.1)

<sup>A</sup>Because of missing values, numbers do not always add up to 96 (100%).

<sup>B</sup>Because of missing values, numbers do not always add up to 74 (100%).

<sup>C</sup>At time of primary surgery. <sup>D</sup>In case of multiple sites, the site with the worst prognosis was considered dominant.

Seventy-four patients were treated with first-line chemotherapy for advanced disease. This therapy consisted of classical cyclophosphamide, methotrexate, 5-fluorouracil (CMF, n=43) or 5-fluorouracil, epirubicin/adriamycin, and cyclophosphamide (FEC/FAC, n=31). Of these patients, 36 (50%) had an ER-/PgR- tumor, 3 (4%) had an ER-/PgR+ tumor, 11 (15%) had an ER+/PgR- tumor and 22 (31%) had an ER+/PgR+ tumor. Fifteen of the patients who were treated with first-line chemotherapy had received prior adjuvant chemotherapy, and fifteen adjuvant endocrine therapy. The patients who received first-line chemotherapy had a median age of 48 years (28-81 years) at start of first-line chemotherapy. The median follow-up time of patients still alive was 28 months (range 5-178 months).

### **Steroid hormone receptor and total protein assays**

Tumor specimens were drawn from a pool of frozen specimens (stored in liquid nitrogen) originally submitted to our laboratory for steroid-hormone receptor analysis. Processing of the tumors was performed as described previously (14). ER and PgR levels were determined with ligand binding assay in cytosols (14). The cut-off level used to classify tumors as ER or PgR positive or negative was 10 fmol/mg cytosolic protein, the protein was measured according to Lowry (15) with bovine serum albumin as a standard.

### **Determination of the antigen levels of uPA, PAI-1 and uPA:PAI-1 complex**

We used ELISAs developed by our department for uPA and PAI-1 (16) and for the uPA:PAI-1 complex (10). These ELISAs are based on a combination of four polyclonal antibodies (raised in four different animal species, i.e. duck, chickens, rabbits and goat), which were employed in a sandwich assay format. The polyclonal antibodies raised in chickens and rabbits are specifically directed against uPA and PAI-1. The details of the assays, including those of the specificity and performance, have been described elsewhere (10, 16).

### **Data analysis**

To analyze interrelations between uPA, PAI-1 and uPA:PAI-1 complex and various traditional parameters, Spearman rank correlations ( $r_s$ ) were calculated for continuous variables and the Kruskal-Wallis test for ordered variables, followed by a Wilcoxon-type test for trend if appropriate.

The three markers were categorized in order to study their possible relationship with progression-free survival (PFS) time (here defined as the time from the start of the first-line therapy until the start of a next therapy or until the time of death). The median value of uPA, PAI-1 and uPA:PAI-1 complex was used as the cut off value in the statistical analyses. Further division of the marker values in tertiles or quartiles resulted in progressively smaller subgroups, which lead to a significant loss of power.

uPA, PAI-1 and uPA:PAI-1 complex were also analyzed as log-transformed continuous variables.

In univariate and multivariate logistic regression analysis, the relation with response-to-therapy was examined in the group of 96 patients who received first-line endocrine therapy and in the group of 74 patients that received first-line chemotherapy. These analyses were also performed in a subset of 82 of the endocrine treated patients that received tamoxifen as part of first-line endocrine therapy, to test also a more uniform group. Multivariate analysis was performed for all factors that were analyzed in univariate analysis, with stepwise removal of non-significant factors based on the likelihood ratio. Odds ratios (OR) were calculated and presented with their 95% confidence intervals (95% CI). Overall response was defined as either a complete remission (disappearance of disease), partial remission (more than 50% decrease in product of longest diameter times its perpendicular diameter of measurable parameters) or minor response (more than 25%, but less than 50%, decrease in size of measurable parameters).

For the survival rate analyses PFS time was used as follow-up parameter. The method of Kaplan and Meier was used to generate the survival curves (17). The log-rank test was used to test for differences.

For the analyses, classifications were made for the steroid hormone receptor status (ER and PgR <10 fmol/mg protein, i.e. negative receptor-status, *versus* ER and/or PgR ≥10 fmol/mg protein, i.e. positive receptor-status). All computations were done with the SPSS statistical package (release 10.0.5, November 1999). Two-sided *P*-values below 0.05 were considered to be statistically significant.

## **Results**

### **Distribution of uPA, PAI-1 and uPA:PAI-1 complex**

ELISAs were used to determine the antigen levels of uPA, PAI-1 and uPA:PAI-1 complex in the tumor cytosols. The cytosolic uPA content varied from 0.00 to 9.03 ng/mg protein (median 0.50 ng/mg protein) in the 170 tumors. The cytosolic levels of PAI-1 complex ranged from 0.00 to 30.00 ng/mg protein, with a median value of 1.88 ng/mg protein. A wide range of concentrations of uPA:PAI-1 complex, ranging from 0.000 to 1.635 ng/mg protein, was observed (median 0.091 ng/mg protein). Table 2 shows the median levels and quartiles of uPA, PAI-1 and uPA:PAI-1 complex in subgroups of tumors.

### **Correlations**

The correlation between the cytosolic uPA levels and the PAI-1 levels was statistically significant ( $r_s=0.490$ ,  $P<0.001$ ). Significant correlations were also found between the levels of uPA:PAI-1 complex and uPA levels ( $r_s=0.531$ ,  $P<0.001$ ) and uPA:PAI-1 complex and PAI-1 levels ( $r_s=0.665$ ,  $P<0.001$ ).

**Table 2:** Correlations of uPA, PAI-I and uPA:PAI-I complex with patients and tumor characteristics.

Characteristics	Frequency <sup>A</sup>	Median value (quartiles)		
		uPA	PAI-I	uPA:PAI-I complex
All patients	170	0.50 (0.22, 1.03)	1.88 (1.01, 3.41)	0.091 (0.001, 0.244)
Age (years) <sup>B</sup>				
≤40	37	0.44 (0.24, 1.12)	2.02 (0.99, 3.00)	0.104 (0.032, 0.179)
41-55	50	0.50 (0.24, 0.91)	1.88 (0.93, 3.20)	0.080 (0.001, 0.281)
56-70	60	0.39 (0.16, 0.97)	1.46 (0.77, 3.48)	0.088 (0.001, 0.260)
>70	23	0.83 (0.40, 1.11)	2.49 (1.64, 3.79)	0.102 (0.001, 0.293)
<i>P</i> -value <sup>C</sup>		0.518	0.401	0.781
Menopausal status <sup>B</sup>				
Pre	73	0.42 (0.23, 1.03)	1.88 (0.93, 3.12)	0.082 (0.001, 0.186)
Post	97	0.54 (0.20, 1.05)	1.88 (1.10, 3.70)	0.101 (0.001, 0.295)
<i>P</i> -value <sup>D</sup>		0.660	0.292	0.623
ER/PgR <sup>E</sup>				
both <10	83	0.54 (0.22, 0.96)	2.22 (1.00, 4.17)	0.152 (0.034, 0.300)
both or one ≥10	83	0.50 (0.23, 1.09)	1.79 (1.01, 3.05)	0.049 (0.001, 0.139)
<i>P</i> -value <sup>D</sup>		0.046	0.044	0.002
Primary tumor size				
pT1	48	0.42 (0.26, 1.15)	1.80 (0.86, 3.12)	0.057 (0.001, 0.175)
pT2	86	0.68 (0.22, 1.09)	2.01 (1.12, 3.44)	0.106 (0.001, 0.294)
pT3+4	33	0.27 (0.16, 0.66)	1.88 (0.99, 3.55)	0.070 (0.001, 0.208)
<i>P</i> -value <sup>F</sup>		0.158	0.509	0.826
Histological grade				
I	7	0.34 (0.10, 0.64)	1.89 (0.67, 9.98)	0.012 (0.001, 0.621)
II	32	0.54 (0.35, 1.15)	2.13 (1.46, 3.99)	0.094 (0.001, 0.281)
III	66	0.58 (0.22, 1.07)	2.13 (1.12, 3.60)	0.110 (0.022, 0.244)
<i>P</i> -value <sup>F</sup>		0.494	0.841	0.609
Lymph node status				
Negative	63	0.58 (0.30, 1.15)	1.98 (1.32, 3.61)	0.113 (0.001, 0.297)
Positive	95	0.48 (0.20, 0.96)	1.76 (0.92, 3.41)	0.084 (0.001, 0.244)
<i>P</i> -value <sup>D</sup>		0.104	0.600	0.454
First site of relapse <sup>G</sup>				
Soft tissue	55	0.54 (0.27, 0.99)	2.22 (1.07, 5.09)	0.140 (0.001, 0.265)
Bone	73	0.43 (0.19, 1.01)	1.76 (0.87, 3.06)	0.073 (0.001, 0.162)
Viscera	42	0.44 (0.20, 1.15)	1.86 (1.12, 4.24)	0.086 (0.001, 0.308)
<i>P</i> -value <sup>F</sup>		0.702	0.274	0.210
Disease-free interval				
<1 year	55	0.58 (0.25, 1.11)	2.41 (1.25, 3.61)	0.147 (0.042, 0.297)
≥1 year	115	0.43 (0.19, 0.96)	1.76 (0.90, 3.12)	0.078 (0.001, 0.197)
<i>P</i> -value <sup>D</sup>		0.051	0.049	0.046

<sup>A</sup> Because of missing values, numbers do not always add up to 170. <sup>B</sup> At time of primary surgery. <sup>C</sup> Spearman rank correlation. <sup>D</sup> Mann-Whitney *U* test. <sup>E</sup> in fmol/mg protein. <sup>F</sup> Kruskal-Wallis test, followed by a non-parametric test for trend when appropriate. <sup>G</sup> In case of multiple sites, the site with the worst prognosis was considered dominant.

Table 2 shows the relationships of uPA, PAI-I and uPA:PAI-I complex with patient and tumor characteristics. The uPA, PAI-I and uPA:PAI-I complex tumor levels were higher in ER and PgR-negative tumors compared with ER and/or PgR-positive tumors ( $P=0.046$ ,  $P=0.044$  and  $P=0.002$ , respectively). Within the ER and/or PgR positive tumors, no correlation was found between uPA, PAI-I and uPA:PAI-I complex levels and ER absolute levels ( $P=0.776$ ,  $P=0.456$  and  $P=0.801$ , respectively) or PgR absolute levels ( $P=0.077$ ,  $P=0.703$  and  $P=0.115$ , respectively). The tumor levels of all three markers, uPA, PAI-I and uPA:PAI-I complex, showed a significant association with disease-free interval, i.e. the levels were higher in patients with a relapse within 1 year after the primary diagnosis of breast cancer compared with patients with a disease-free interval of ≥1 year ( $P=0.051$ ,  $P=0.049$  and  $P=0.046$ , respectively).

### Patients treated with endocrine therapy

Of the 96 patients who had first-line endocrine therapy for advanced disease, 51% responded. At a median follow-up of 46 months, 11 (11%) of the patients were still on first-line endocrine therapy. The remaining 85 (89%) of the patients had tumor progression after a median duration of 8 months (range 1-68 months).

Table 3 shows the response rates to first-line endocrine therapy in subgroups defined by ER/PgR status and uPA:PAI-I complex tumor levels. The response rate to first-line endocrine therapy was 11% in patients with high uPA:PAI-I complex levels and ER and PgR negative tumors, 14% with low uPA:PAI-I complex and negative ER and PgR status, 35% in patients with high uPA:PAI-I complex levels and positive ER and/or PgR status and 72% in patients with low uPA:PAI-I complex levels

and ER and/or PgR positive tumors. The response rates to first-line endocrine therapy for patients with ER+/PgR+ patients (n=61) in subgroups defined by uPA:PAI-1 complex tumor levels, were 37% in patients with high uPA:PAI-1 complex levels and 70% in patients with low uPA:PAI-1 complex levels (data not shown).

The rate of response to endocrine therapy defined by each individual clinicopathological parameter is listed in Table 4. The response rate of patients with high tumor uPA:PAI-1 complex levels was 29% and in the group of patients with low tumor uPA:PAI-1 complex levels 62%.

Furthermore, Table 4 shows the results of the logistic regression analyses for response to first-line endocrine therapy in patients with advanced breast cancer. In the univariate analysis, postmenopausal patients and patients with positive ER and/or PgR tumors had a significant higher rate of response to endocrine therapy than patients who were premenopausal or had ER and PgR-negative tumors (OR=3.02,  $P=0.051$  and OR=2.85,  $P=0.022$ , respectively). In addition, increased cytosolic levels of uPA:PAI-1 complex were also related to a lower response rate (OR=0.43, 95% CI, 0.21-0.81,  $P=0.015$ ). There was also a significant association between higher uPA:PAI-1

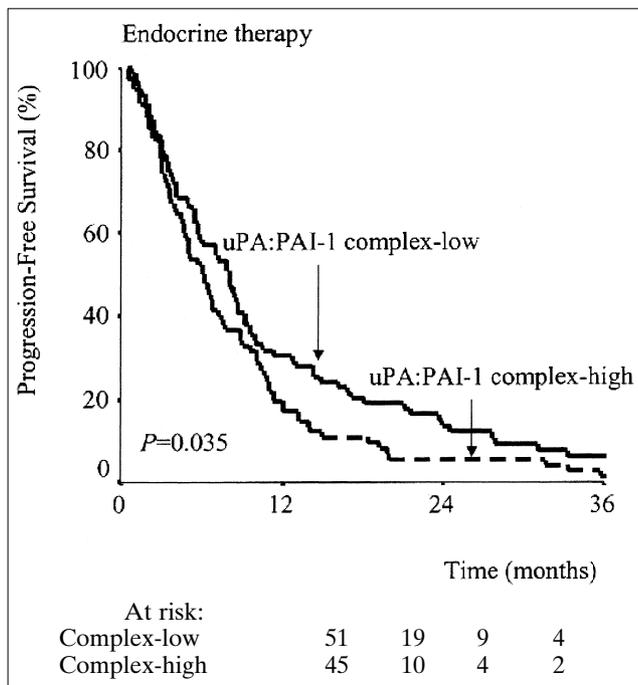
**Table 3:** Response rate to first-line endocrine therapy by ER/PgR status and tumor levels of uPA:PAI-1 complex.

		Response rate to first-line endocrine therapy by tumor levels of uPA:PAI-1 complex (ng/mg protein)		
		≥0.091	<0.091	all patients
Response rate to first-line endocrine therapy by ER/PgR (fmol/mg protein)	both <10	11% (n=8)	14% (n=7)	11% (n=15)
	both or one ≥10	35% (n=32)	72% (n=47)	59% (n=79)
	all patients	29% (n=40)	62% (n=54)	

**Table 4:** Univariate and multivariate logistic regression analysis for response to first-line endocrine therapy in patients with advanced breast cancer.

Factor	Response rate (%)	Univariate analysis		Multivariate analysis <sup>A</sup>	
		P-value	OR (95% CI) <sup>B</sup>	P-value	OR (95% CI) <sup>B</sup>
Age (years) <sup>C</sup>		0.325			
≤ 40	41		1		
41-55	49		1.06 (0.21-5.21)		
56-70	56		0.42 (0.09-1.96)		
> 70	62		0.71 (0.13-3.87)		
Menopausal status <sup>C</sup>		0.051			
Pre	47		1		
Post	67		3.02 (0.99-9.17)		
First site of relapse		0.940			
Soft	64		1		
Bone	59		1.32 (0.17-10.11)		
Viscera	50		1.43 (0.18-11.38)		
Disease-free interval		0.130			
<1 year	38		1		
≥1 year	58		2.15 (0.80-5.81)		
ER/PgR status <sup>D</sup>		0.022		0.033	
Both negative	11		1		1
Either positive	59		2.85 (1.61-6.98)		2.68 (1.08-6.63)
uPA levels <sup>E</sup>		0.482			
Low	58		1		
High	47		0.74 (0.32-1.72)		
PAI-1 levels <sup>F</sup>		0.647			
Low	56		1		
High	49		0.82 (0.35-1.91)		
uPA:PAI-1 complex levels <sup>G</sup>		0.015		0.018	
Low	62		1		1
High	29		0.43 (0.21-0.81)		0.27 (0.09-0.59)

<sup>A</sup>The final multivariate model with all the factors known included 96 patients. <sup>B</sup>Odds ratio (95% confidence interval) of multivariate analysis. <sup>C</sup>Age and menopausal status at start of first-line therapy. <sup>D</sup>Cut off point used for ER and PgR, 10 fmol/mg protein. <sup>E</sup>Low: <0.50 ng/mg protein; high: ≥0.50 ng/mg protein. <sup>F</sup>Low: <1.88 ng/mg protein; high: ≥1.88 ng/mg protein. <sup>G</sup>Low: <0.091 ng/mg protein; high: ≥0.091 ng/mg protein.



**Figure 1:** Progression-free survival (PFS) after the start of endocrine therapy as a function of cytosolic tumor levels of uPA:PAI-1 complex: low,  $<0.091$  ng/mg protein; high,  $\geq 0.091$  ng/mg protein.

"At risk" indicates the number of patients at risk in each group. Patients at risk at 0, 12, 24 and 36 months are indicated.

complex levels and reduced response rate when this marker was used as log-transformed continuous variable ( $P=0.031$ ). Neither uPA nor PAI-1 was significantly associated with rate of response to first-line endocrine therapy.

To investigate the independent relation of tumor levels of uPA, PAI-1 and uPA:PAI-1 complex with the rate of response to endocrine treatment in advanced breast cancer, multivariate logistic regression analysis was used (Table 4). Increased levels of uPA:PAI-1 complex levels were associated with a poor rate of response (OR=0.27, 95% CI, 0.09-0.59,  $P=0.018$ ). In addition to uPA:PAI-1 complex, ER and PgR negativity was associated with a poor response rate in the multivariate analysis (ER and/or PgR positive: OR=2.68, 95% CI, 1.08-6.63,  $P=0.033$ ). Subsequently, a logistic multivariate regression analysis was performed in which uPA, PAI-1 and uPA:PAI-1 complex were added as log-transformed continuous variables. In this second multivariate analysis, the contributions of ER/PgR status (OR=2.54, 95% CI, 1.24-6.41,  $P=0.026$ ) and uPA:PAI-1 complex (OR=0.22, 95% CI, 0.11-0.57,  $P=0.014$ ) were statistically significant as well.

A logistic multivariate model was also prepared separately for uPA and PAI-1 without inclusion of uPA:PAI-1 complex. In the final multivariate model only ER/PgR status was associated

with response rate (OR=2.66, 95% CI, 1.07-6.61,  $P=0.035$ ), while uPA and PAI-1 were both not related.

In the Kaplan-Meier analysis of all patients who received endocrine therapy, patients with high tumor levels of uPA:PAI-1 complex showed a significantly shorter PFS compared with patients with lower uPA:PAI-1 complex tumor levels (Fig. 1,  $P=0.035$ , log-rank). uPA and PAI-1 were not associated with PFS ( $P=0.998$ , log-rank and  $P=0.589$ , log-rank, respectively).

The rate of response was 48% in the subset of 82 patients who were treated with tamoxifen. In the multivariate analysis for response to tamoxifen, uPA:PAI-1 complex was again a significant predictor of a poor rate of response (OR=0.37, 95% CI, 0.13-0.76,  $P=0.023$ ), in addition to ER/PgR status (OR=2.41, 95% CI, 1.04-7.36,  $P=0.038$ ). In the multivariate analysis in which uPA:PAI-1 complex was added to the model as a log-transformed continuous variable instead of a dichotomized variable, the contribution of uPA:PAI-1 complex was still statistically significant (OR=0.43, 95% CI, 0.26-0.73,  $P=0.009$ ), together with ER/PgR status (OR=2.52, 95% CI, 1.27-6.19,  $P=0.031$ ).

### Patients treated with chemotherapy

Of the 74 patients treated with first-line chemotherapy, 38 (51%) responded. In the group of patients who were treated with CMF ( $n=43$ ) 46% responded and in the group of patient who were treated with FEC/FAC ( $n=31$ ) 58% responded. At a median follow-up of 28 months after start of first-line chemotherapy, 3% of the patients did not yet have progressive disease. The remaining 97% of the patients had tumor progression after a median duration of 5 months (range 1-67 months).

In Kaplan-Meier analysis of the 72 patients who were treated with first-line chemotherapy, cytosolic levels of uPA, PAI-1 and uPA:PAI-1 complex were not related with PFS ( $P=0.086$ ,  $P=0.678$  and  $P=0.701$ , log-rank, respectively; data not shown). The relation of uPA, PAI-1 and uPA:PAI-1 complex with response-to-therapy in advanced breast cancer was investigated using univariate logistic regression analysis. The cytosolic levels of uPA, PAI-1 and uPA:PAI-1 complex, neither when analyzed as log-transformed continuous ( $P=0.122$ ,  $P=0.537$  and  $P=0.336$ , respectively) nor as dichotomized variables ( $P=0.244$ ,  $P=0.167$  and  $P=0.274$ , respectively), were significantly related with the rate of response. Furthermore, none of the other investigated established prognosticators were significantly associated with the response rate to first-line chemotherapy (data not shown), except for the disease-free interval. That is, patients with a disease-free interval  $\geq 1$  year had a significant higher rate of response to chemotherapy than patients with disease-free interval  $< 1$  year (OR=2.74, 95% CI, 1.05-7.18,  $P=0.040$ ). Since uPA, PAI-1 and uPA:PAI-1 complex were shown not to be significantly associated with the rate of response in univariate analysis, logistic multivariate analysis was not performed. A similar result was found, when all aforementioned analyses were per-

formed with the exclusion of the 15 patients treated with adjuvant chemotherapy.

## Discussion

In this retrospective study, the predictive value of uPA:PAI-1 complex levels in tumor tissue with respect to efficacy of first-line endocrine therapy and first-line chemotherapy was evaluated in 170 patients with primary breast cancer who developed distant metastases during follow-up. Increased tumor levels of uPA:PAI-1 complex were significantly associated with a shorter PFS after start of first-line endocrine treatment. Furthermore, uPA:PAI-1 complex levels were significantly related with the rate of response to first-line endocrine therapy, in addition to the predictive impact of ER/PgR status. In contrast, tumor levels of uPA and PAI-1 were not associated with PFS during/with first-line endocrine therapy nor with the rate of response to first-line endocrine therapy.

There are two previous studies that focussed on the prognostic value of the uPA:PAI-1 complex (12, 13). These studies showed conflicting results. Pedersen, et al. reported that high levels of uPA:PAI-1 complex were associated with a favorable prognosis in the univariate analysis. They included 342 patients of whom more than 50% received adjuvant systemic therapy, which makes it difficult to draw conclusions about the true prognostic impact of uPA:PAI-1 complex, as actually a possible predictive impact has also been taken into account (12). In the study by Sten-Linder, et al., no information can be found on adjuvant systemic therapy, but since in their study 45% of the total group of patients (n=233) was node-positive, it is likely that at least a substantial part of these patients received adjuvant systemic therapy (13). Furthermore, the investigators did not perform a multivariate analysis. They found that high uPA:PAI-1 complex levels were associated with a poor prognosis.

Here, we showed that breast tumors with high uPA:PAI-1 complex levels were less sensitive to endocrine therapy. We and others (4-6) observed a significant inverse correlation between levels of components of the plasminogen activation system and the steroid hormone receptor status. Given the higher uPA:PAI-1 complex levels in ER/PgR-negative tumors, one might speculate that there may be a relative lower ER/PgR content in the ER/PgR-positive tumors with high uPA:PAI-1 complex levels compared to those with low uPA:PAI-1 complex levels, which might consequently also influence the response to endocrine therapy. For that reason, we also analyzed whether the absolute ER/PgR levels were correlated with the uPA:PAI-1 complex. We observed that within the ER and/or PgR positive tumors no correlation could be found between the absolute ER/PgR levels and uPA:PAI-1 complex levels. In our view, the exclusion of this correlation considerably strengthens the conclusion that the relation between uPA:PAI-1 complex and rate of response to first-line endocrine treatment is truly independent of ER/PgR

status. For endocrine therapy, the expectation is that slowly growing tumors better respond to endocrine therapy. Foekens, et al. found a significant negative correlation between the tumor levels of uPA and PAI-1 and the response to tamoxifen, but they did not analyze the uPA:PAI-1 complex levels (9). We found no relation between efficacy of endocrine therapy and the separate uPA and PAI-1 components, this relation was only found for the complex levels. This is not so controversial as it may seem, realizing that the predictive value of uPA:PAI-1 complex tumor levels on theoretical grounds is superior to that of either uPA or PAI-1, allowing statistical significant results in an even relatively small group of patients.

Previous studies showed that the expression of uPA is influenced by (anti-)estrogens (18-20). Xing and colleagues reported that the combination of tamoxifen and the synthetic uPA active site inhibitor B-428 had an additive anti-invasive effect in a model in which uPA and its receptor uPAR play a key role (18). In a study of Abidi, et al., it appeared that ER-mediated inhibition of uPA activity by anti-estrogens may limit the generation of plasmin and the activation of other proteases (19). In addition, Levenson, et al. found that 4-hydroxytamoxifen and raloxifene showed agonist activity by downregulation of uPA and PAI-1 genes in ER-transfectants (20). The exact role of (anti-)estrogens in these processes is still under investigation. Possibly, uPA:PAI-1 complexes might be involved in estrogen-independent growth signalling, thereby counteracting the efficacy of endocrine therapy. In the human breast cancer cell-line MCF-7, binding of PAI-1 to uPA has been shown to modify the promigratory effect of uPA into a mitogenic signal (21). uPA:PAI-1 complexes selectively promoted cellular growth through sustained phosphorylation of extracellular signal-regulated kinase (ERK), whereas either uPA or PAI-1 did not. Thus, uPA:PAI-1 complexes might form an autocrine growth regulatory pathway leading to endocrine (tamoxifen)-resistance.

In the current study, the included patients were treated with first-line endocrine therapy for advanced breast cancer between 1987 and 1996. In the late eighties and early nineties, first-line endocrine therapy was also given to patients with an ER and PgR negative (or ER and/or PgR unknown) tumor in case of high age or poor performance status. In the current study, almost one-fifth of the included patients treated with first-line endocrine therapy actually had an ER and PgR negative tumor. We observed in this group of patients a response rate of only 11%. A comparable low response rate was also reported in previous studies (22, 23). Moreover, a substantial percentage of ER and/or PgR positive patients did not respond (41%) to first-line endocrine treatment. Therefore, additional markers are needed to improve the ability to predict response to first-line endocrine therapy. The present findings indicate that the presence of high tumor levels of uPA:PAI-1 complex in ER and/or PgR positive tumors was associated with a reduced response to first-line endocrine treatment from 59% to 35%, while low levels of

uPA:PAI-1 complex in ER and/or PgR positive tumors resulted in an increased response rate from 59% to 72%. Therefore, advanced breast cancer patients with tumors that are ER and/or PgR positive and that have high levels of uPA:PAI-1 complex might benefit from a future approach of a combined therapy that consists of both endocrine therapy and specific therapy directed against the plasminogen activation system.

In the current study we also investigated whether the tumor levels of components of the plasminogen activation system were predictive for the responsiveness to first-line chemotherapy. Harbeck, et al. found an association between components of the plasminogen activation system and responsiveness to chemotherapy in the adjuvant setting, i.e. patients with high tumor tissue levels of both uPA and PAI-1 benefited more strongly from conventional adjuvant chemotherapy than those with low levels (7, 8). We found no such association with palliative chemotherapy.

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In conclusion, according to our results, increased tumor levels of uPA:PAI-1 complex predict a reduced response to first-line endocrine therapy in patients with advanced breast cancer, independent of steroid hormone receptor status. Understanding and influencing this underlying resistance to endocrine therapy may ultimately improve outcome in this specific group of patients.

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