Urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1: novel tumor-derived factors with a high prognostic and predictive impact in breast cancer

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Summary
Urokinase-type plasminogen activator (uPA) and its inhibitor, PAI-1, play a key role in tumor invasion and metastasis. They were the first novel tumor biological factors to be validated at the highest level of evidence (LOE I) regarding their clinical utility in breast cancer. Their antigen levels are determined in tumor tissue extracts by standardized, quality-assured immunometric assays (ELISA). Since the late 1980s, numerous independent studies have demonstrated that patients with low levels of uPA and PAI-1 in their primary tumor tissue have a significantly better survival than patients with high levels of either factor. These prognostic data have recently been validated by an EORTC (European Organization for Research and Treatment of Cancer) pooled analysis comprising more than 8,000 breast cancer patients. In addition, results from a multicenter prospective randomized therapy trial in node-negative breast cancer ("Chemo N₀") showed that node-negative breast cancer patients with low levels of uPA and PAI-1 in their primary tumor have a very good prognosis, and may thus be candidates for being spared the burden of adjuvant chemotherapy. In contrast, node-negative patients with high uPA/PAI-1 are at substantially increased risk of disease recurrence, comparable to that of patients with three or more tumor cell positive axillary lymph nodes. The "Chemo N₀" trial as well as retrospective data also indicate that these high-risk patients benefit from adjuvant chemotherapy. In conclusion, over a period of about 15 years sufficient evidence has been put forward to demonstrate that determination of uPA and PAI-1 in primary breast cancer patients supports risk-adapted individualized therapy decisions, particularly in patients with node-negative disease.

Keywords
Adjuvant therapy, metastasis, node-negative breast cancer, PAI-1, prognosis, prediction of therapy response, risk group assessment, uPA

Tumor biology
The serine protease urokinase-type plasminogen activator (uPA) and its inhibitor, PAI-1, are key players in a proteolytic cascade involved in physiological and pathophysiological degradation and remodelling of the extracellular matrix. uPA, when bound to its cellular receptor uPA-R (CD87) efficiently converts plasminogen into the broad spectrum serine protease plasmin; its action on plasminogen is controlled by the serine protease inhibitors PAI-1 and PAI-2. Plasmin disintegrates various components of the connective tissue, and in malignancy, the tumor stroma surrounding the tumor cell nests. Interaction of PAI-1 with the uPA/uPA-R-complex induces internalization of the ternary complex uPA-R/uPA/PAI-1 via help of transmembrane
receptors of the LDL-receptor family, which subsequently results in intracellular degradation of uPA and PAI-1, while uPA-R is recycled to the cell surface. By this, the proteolytic activity is efficiently reorganized on the cell surface enabling pericellular proteolysis. PAI-2 also forms a ternary cell surface-associated complex with uPA and uPA-R (uPA-R/uPA/PAI-2), but this is not internalized; in contrast, PAI-2 is degraded once bound to uPA-R/uPA (1). Besides its proteolytic potential, the uPA-system, in concert with other proteins, exerts several other important biological effects including chemotaxis, migration/invasion, adhesion, proliferation, and angiogenesis (2-5). Binding of uPA to cell surface-associated uPA-R leads to activation of various intracellular signalling molecules such as tyrosine- and serine-protein kinases (4). uPA-R interacts with many other proteins such as members of the mannose receptor family and the extracellular matrix protein vitronectin as well as certain types of integrins (3), thereby modulating cell adhesion and migration (5). Interestingly, PAI-1 modulates these uPA-R-mediated processes by competing with uPA-R for binding to vitronectin and to integrins. Thus, the biological role of PAI-1 goes beyond that of a mere protease inhibitor (6). PAI-2 lacks a cleavable signal peptide and, thus, is mainly present intracellularly. Only a small amount of PAI-2 (≈ 20%) is secreted. In contrast to PAI-1, no other functions in addition to the inhibition of urokinase have been attributed to extracellular PAI-2. A well-balanced production and cellular assembly of cellular and pericellular uPA, uPA-R, and PAI-1 is the prerequisite for efficient focal proteolysis, angiogenesis, cell adhesion and migration, and hence, tumor cell invasion and metastasis (3, 7-8) (Fig. 1). In solid malignant tumors, including breast cancer, uPA, uPA-R, and PAI-1 may be localized to a varying extent to both tumor cells and nonmalignant host cells.

### Determination method

Antigen levels of uPA and PAI-1 are determined by standardized immunometric assays (ELISA) in extracts of the primary tumor tissue. For this, commercially available ELISAs are used (Table 1) which are robust enough for clinical routine determination of uPA and PAI-1. International quality assurance of the kits is guaranteed, and kits have been assessed by the Receptor and Biomarker Group of the EORTC (European Organization for Research and Treatment of Cancer) (9, 10). Extraction of tumor tissue in the presence of the non-ionic detergent Triton X-100 is recommended, as such a detergent-based extraction method yields about twice as much uPA antigen and provides a considerably better assessment of disease-free patient survival than uPA measured in detergent-free tumor extracts (cytosol fraction) (11). Extracts prepared from as little as 100 µg tumor tissue corresponding to about 1 µg protein suffice for routine ELISA testing. Therefore, the ELISAs can also be applied to extracts prepared from core biopsy specimens or cryostat sections.

At present, no clinically relevant data have been published applying immunohistochemistry (IHC) or other techniques for determination of uPA and PAI-1 protein expression in breast carcinoma tissue. In light of this, for clinical routine use, the ELISA procedure is highly recommended to determine uPA and PAI-1 in tumor tissue extracts.

### Prognostic impact of uPA and PAI-1

In 1988, Duffy and coworkers were the first to show that measurement of the enzymatic activity of the serine protease uPA provides prognostic information in patients with primary breast cancer (12). Shortly afterwards, this initial report was strengthened by our finding that not only the enzymatic activity, but to an even greater extent the tumor tissue antigen level of uPA is of prognostic relevance (13). Moreover, in 1990, our group was the first to report that, in addition to uPA, its inhibitor PAI-1 is of significant prognostic impact in both node-positive and node-negative breast cancer patients: patients with high tumor antigen levels of either factor have a significantly worse survival probability than patients with low levels of these antigens (14-16). At that time, the clinical finding that high levels of a protease inhibitor would indicate poor prognosis seemed surprising, but this finding has since been explained by thorough basic research on the concerted interaction of uPA and PAI-1 in tumor invasion and metastasis (3, 6, 7, 17). The strong prognostic impact of both uPA and the inhibitor PAI-1 on disease-free (DFS) and overall survival (OS) in patients with primary breast
cancer has since been confirmed in all of the published studies using immunometric biochemical assays (Table 1). The lack of contradictory evidence on the prognostic impact of uPA and PAI-1 in breast cancer is quite unique for any tumor biological factor and is remarkable considering the variety of demographic conditions covered by the studies (Europe, USA, and Japan).

In breast cancer, uPA and PAI-1 are predictors for distant metastasis, but not for loco-regional disease recurrence (18). In this disease, both factors are only weakly correlated with other traditional prognostic factors (19) and the prognostic impact of uPA and PAI-1 is independent of these other prognostic factors.

With regard to risk group assessment, the particular combination of both factors, uPA/PAI-1 (both low vs either or both factors high), is superior to either factor taken alone as well as to established prognostic factors such as tumor size, grade, hormone receptor or menopausal status (20). uPA/PAI-1 thus enable optimal distinction between high-risk and low-risk patients (21, 22) and allows risk group assessment even in patient risk groups as defined by established prognostic factors (23). Moreover, uPA/PAI-1 outperforms other tumor biological factors such as cathepsins B, D, L, p53, S-phase, MIB1 or DNA ploidy with regard to prognostic importance (20, 24). Interestingly, HER2 status and uPA/PAI-1 reveal independent but complementary information in breast cancer (21, 25). In node-negative patients, HER2 status defined either by gene amplification or by protein overexpression does not add significant information to risk-group selection determined by uPA/PAI-1 (25) (Fig. 2).

### Table 1: Individual key studies supporting the prognostic impact of uPA and PAI-1 in primary breast cancer.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Assays (method of tissue extraction)</th>
<th>Patients (N0)</th>
<th>Follow up (median, months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duffy (1988)*</td>
<td>Ireland</td>
<td>Activity (Cyt)</td>
<td>52 (25)</td>
<td>17</td>
<td>Cancer 62:531 (12)</td>
</tr>
<tr>
<td>Fernö (1996)*</td>
<td>Sweden</td>
<td>LIA SIM (Cyt)</td>
<td>688 (265)</td>
<td>42</td>
<td>Ear J Cancer 32:793 (43)</td>
</tr>
<tr>
<td>Eppenberger (1998)</td>
<td>Switzerland</td>
<td>ELISA ADR (Cyt)</td>
<td>305 (159)</td>
<td>37</td>
<td>J Clin Oncol 16: 3129 (44)</td>
</tr>
<tr>
<td>Kim (1998)</td>
<td>Japan</td>
<td>ELISA BIO (Cyt)</td>
<td>130 (130)</td>
<td>53</td>
<td>Clin Cancer Res 4:177 (45)</td>
</tr>
<tr>
<td>Kute (1998)</td>
<td>USA</td>
<td>ELISA MG (Cyt)</td>
<td>168 (168)</td>
<td>58</td>
<td>BCRT 47:9 (46)</td>
</tr>
<tr>
<td>Knoop (1998)</td>
<td>Denmark</td>
<td>ELISA MG (TX)</td>
<td>429 (178)</td>
<td>61</td>
<td>Br J Cancer 77:932 (18)</td>
</tr>
<tr>
<td>Bouchet (1999)</td>
<td>France</td>
<td>ELISA ADR (Cyt)</td>
<td>499 (233)</td>
<td>72</td>
<td>J Clin Oncol 17:3048 (47)</td>
</tr>
<tr>
<td>Jänicke (2001)</td>
<td>Germany</td>
<td>ELISA UD (TX)</td>
<td>556 (556)</td>
<td>32</td>
<td>JNCI 93:913 (27)</td>
</tr>
<tr>
<td>Konecny (2001)</td>
<td>USA / Germany</td>
<td>ELISA ADI (TX)</td>
<td>587 (283)</td>
<td>26</td>
<td>Clin Cancer Res 7:2448 (21)</td>
</tr>
<tr>
<td>Harbeck (2002)</td>
<td>Germany</td>
<td>ELISA DI (TX)</td>
<td>761 (269)</td>
<td>60</td>
<td>J Clin Oncol 20: 1000 (23)</td>
</tr>
<tr>
<td>Hansen (2003)</td>
<td>Denmark</td>
<td>ELISA DI (TX)</td>
<td>228 (101)</td>
<td>150</td>
<td>Br J Cancer 88:102 (49)</td>
</tr>
</tbody>
</table>

* only determination of uPA, † Assays: ADI (American Diagnostica Inc., Greenwich, CT, USA), Mo (Monozyme, Horsholm, Denmark), San (Sangtec, Bromma, Sweden), Bio (Biopool, Umea, Schweden), ih (in-house assay). ‡ Tissue extraction: Cyt (cytosol), TX (Triton-X-100, i.e. detergents extraction).

**Obtaining highest level of evidence: EORTC RBG pooled analysis and Chemo N₀ trial**

A pooled analysis performed by the Receptor and Biomarker Group (RBG) of the European Organization for Research and Treatment of Cancer (EORTC) validated published data on the prognostic impact of uPA and PAI-1 in primary breast cancer. The analysis comprised 18 data sets provided by members of the EORTC RBG with a total of 8,377 primary breast cancer patients and a median follow-up of 6.5 years (26). Next to nodal status, uPA and PAI-1 were the strongest prognostic factors for disease-free and overall survival. High uPA and/or PAI-1 levels in primary tumor tissue more than double a patient’s risk of suffering disease recurrence or dying from breast cancer. Within the clinically relevant subgroup of node-negative patients without any adjuvant systemic therapy (n=3,362), uPA and PAI-1 were the strongest prognostic factors.

A prospective randomized multicenter therapy trial in node-negative breast cancer (Chemo N₀) randomizing high-uPA/PAI-1 patients to adjuvant CMF or observation only, was performed between 1993-1999 in 13 German academic centers and 1 cen-
ter in Slovenia (27). In this study, uPA and PAI-1 were prospectively determined in detergent extracts of primary tumor tissue using commercially available ELISA assays (American Diagnostica Inc., Stanford, CT). The first scheduled interim analysis of this study confirmed the independent prognostic impact of uPA/PAI-1 with regard to disease-free survival and validated the previously optimized cut-off values for uPA and PAI-1 (19) used to discriminate between low and high uPA and PAI-1. Even after a short median follow-up time of 32 months, a considerable and statistically significant benefit from adjuvant CMF chemotherapy (per protocol analysis) was observed in high-risk patients (27). A second scheduled interim analysis of the trial comprising 647 patients with a median follow-up of 50 months strongly confirmed the results of the first interim analysis. After this longer follow-up period, the second analysis validated the prognostic impact of uPA/PAI-1, not only with regard to disease-free but also to overall survival and showed that node-negative breast cancer patients with low levels of uPA and/or PAI-1 have an excellent prognosis with an approximately 95% 5-year overall survival (28).

Predictive impact of uPA and PAI-1 on therapy response

The observed treatment benefit in the Chemo N0 trial is consistent with retrospective data suggesting a benefit from adjuvant systemic therapy in high-risk patients according to uPA/PAI-1 (22, 24). This benefit pertains to adjuvant endocrine as well as adjuvant chemotherapy (22). Moreover, recent observations suggest that high-risk patients as determined by uPA/PAI-1 benefit in particular from adjuvant chemotherapy (22). Consequently, chemotherapy seems to be an ideal complement to novel therapeutic agents targeted against the uPA/uPA-R system (29). In metastatic breast cancer, retrospective studies have suggested that high uPA or PAI-1 tumor levels at primary therapy are associated with poor response to later palliative endocrine therapy (11, 30). Taken together with the aforementioned data from the adjuvant setting, these two findings are consistent with the tumor biology of uPA and PAI-1: high levels of uPA and/or PAI-1 do reflect an aggressive tumor phenotype which may be overcome or suppressed by early systemic therapy in the adjuvant setting but may be far too advanced for response to palliative therapy at a later stage.

Current clinical trials

Taking into account the prognostic data presented above, an interesting and still unanswered clinical question remains the optimal systemic therapy for those high-risk patients with high uPA/PAI-1, and in particular for the node-negative ones. Currently, two clinical trials seek to answer this question: First, prospective uPA and PAI-1 determinations are being performed in a randomized adjuvant therapy trial in Germany (ADEBAR), evaluating the added benefit from an anthracyline-docetaxel sequence vs an anthracylin-containing polychemotherapy in primary breast cancer patients with more than 3 tumor cell positive lymph nodes. With 137 actively participating centers and a median recruitment of 24.5 patients/month, ADEBAR is currently the best recruiting adjuvant chemotherapy trial in this specific risk group in Germany. The prospective uPA and PAI-1 determinations will help to resolve the question of whether high-risk patients, according to uPA/PAI-1, do benefit from the addition of a taxane (i.e. docetaxel) to a standard anthracycline chemotherapy.

Second, as a follow-up to the Chemo N0 trial, a European prospective multicenter therapy trial in node-negative breast cancer (NNBC-3), supported by the German AGO (Working Group for Gynecological Oncology) and the EORTC RBG, was initiated in October 2002. This trial compares classical prognostic factors (i.e. age, grade, steroid hormone receptor status,
tumor size) and uPA/PAI-1 for risk group stratification and evaluates the optimal chemotherapy for high-risk node-negative patients.

Ultimately, the data from both trials will help to define the most promising “conventional” chemotherapeutic regimen which can then be combined with the novel targeted therapeutics interfering with the uPA/uPA-R system.

**Clinical consequences of uPA/PAI-1 determination in breast cancer**

uPA and PAI-1 are the first tumor biological prognostic factors that have been validated at the highest level of evidence with regard to their clinical utility in breast cancer management. Level of evidence I (LOE I, defined according to reference 31) is reached either by a prospective clinical trial or a metaanalysis, both of which have been performed for uPA and PAI-1. Standardized and quality-assured immunometric test kits (ELISA) are available for routine determination of both factors. uPA and PAI-1 now fulfill all criteria for evaluation of prognostic markers prior to their clinical application (32, 33).

In breast cancer, uPA and PAI-1 are of greatest clinical relevance in node-negative disease where adequate risk group assessment is particularly important considering the rising percentage of node-negative breast cancer patients, and the increasingly important issue of over-treatment in potentially cured women. Strictly following the St. Gallen consensus guidelines (34), more than 90% of node-negative patients would be candidates for adjuvant systemic therapy, even though only 30% of node-negative patients will eventually experience disease recurrence. The low-risk group identified by uPA/PAI-1, comprising about 50% of node-negative breast cancer patients is substantially larger than that characterized by employing the St. Gallen criteria, and hence much closer to the actual 70% of node-negative patients cured by loco-regional treatment alone. As demonstrated by the prospective Chemo N₀ trial, node-negative patients with low uPA and PAI-1 have a very low risk of disease recurrence (27, 28), and thus are candidates for being spared the burden of adjuvant chemotherapy. Node-negative patients with high-uPA/PAI-1, however, have a considerably increased risk of relapse despite the negative axillary lymph nodes, approximately comparable to that of patients with three or more tumor cell positive axillary lymph nodes (23). Hence, for these high-risk patients, adjuvant systemic therapy, particularly chemotherapy, is clearly indicated. The Chemo N₀ trial as well as recent retrospective data show a treatment benefit from adjuvant chemotherapy in these high-risk node-negative patients.
Since breast cancer management routines differ substantially between countries, and in particular, unfixed tissue for the immunomunometric measurement is not always available, in current international breast cancer management guidelines uPA and PAI-1 are favorably mentioned (34, 35) but not generally proposed. Still, in Germany, in their annually updated evidence-based guidelines, the AGO breast cancer expert panel recommends using uPA and PAI-1 for therapy decisions in node-negative breast cancer (www.ago-online.de).

Clinical relevance of uPA and PAI-1 in other solid malignancies

High tumor tissue levels of uPA and/or PAI-1 correlate with tumor aggressiveness and poor patient outcome, not only in breast cancer but also in other malignancies such as ovarian, esophageal, gastric, colorectal, lung or liver cancer (8). In gastric cancer patients, preliminary clinical data suggest that the uPA-system plays an important role in early tumor cell dissemination. Heiss et al (36) were able to show that the mere presence of disseminated tumor cells in the bone marrow but rather the presence of uPA-R on these cells does correlate with disease aggressiveness. So far, no meta-analysis or other level I evidence for clinical utility of uPA and PAI-1 in other malignancies has been reported. In addition, next to statistical significance, clinical relevance with regard to the potential to influence therapy decisions is important before any tumor biomarker will become part of clinical routine. Such potential may not be present in other solid malignancies. Thus, at present, immediate clinical consequences derived from determination of uPA and PAI-1 are still limited to breast cancer.

Prospects and future directions

Over a period of about 15 years, sufficient evidence has been put forward that determination of uPA and PAI-1 in primary breast cancer supports risk-adapted individualized therapy decisions, particularly in patients with node-negative disease (Fig. 3). Furthermore, the convincing experimental and clinical data demonstrating a key role of uPA and PAI-1 in tumor cell invasion and metastasis render these two factors promising targets for tumor biological therapy (8, 29, 37). A first compound, a synthetic serine protease inhibitor (WX-UK1), has already entered phase-I/II clinical cancer trial (38). Other novel therapeutics are receptor ligand analogues designed to interfere with the cellular uPA-R/uPA interaction which are currently under investigation in preclinical tumor models (39, 40). In addition, PAI-1 is also tested as a potential target for therapy by development of antibodies or small molecules which are able to inhibit/modulate its function (6).

References


38. Wilex AG, Munich, Germany. Press release 10-14-02. www.wilex.de
