Clinical Relevance of the Plasminogen Activator Inhibitor Type 1 – a Multifaceted Proteolytic Factor

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Key Words
Breast cancer · Invasion · Metastasis · PAI-1 · Proteolysis · Prognosis · Tumor-biological therapy

Schlüsselwörter
Mammakarzinom · Invasion · Metastase · PAI-1 · Proteolyse · Prognose · Tumorbiologische Therapie

Summary
The plasminogen activator inhibitor type-1 (PAI-1) is a multi-faceted proteolytic factor. It not only functions as an inhibitor of the protease uPA (urokinase-type plasminogen activator), but also plays an important role in signal transduction, cell adherence, and cell migration. Thus – an apparent paradox considering its name –, although it inhibits uPA during blood coagulation, it actually promotes invasion and metastasis. In the early 1990s, clinical evidence associated elevated PAI-1 levels in tumor tissue with poor clinical outcome in primary breast cancer. These clinical data have since been supported by experimental evidence that the concerted action of uPA, its cell surface receptor uPA-R, and PAI-1 facilitates invasion and metastasis. The strong prognostic impact of PAI-1 in primary breast cancer has been validated by international research groups assessing fresh tumor tissue extracts by ELISA. There is clinical evidence that high-risk patients with elevated PAI-1 in their tumor benefit from adjuvant systemic therapy. uPA also has a strong prognostic impact in primary breast cancer. In node-negative breast cancer, risk-group selection for adjuvant systemic therapy based on tumor levels of both PAI-1 and uPA is close to routine clinical use. Also in other malignancies such as ovarian, esophageal, gastric, colorectal or hepatocellular cancer, elevated PAI-1 is associated with tumor aggressiveness and poor patient outcome. This abundant clinical evidence implicating PAI-1 as a key factor for tumor invasion and metastasis renders it a promising target for tumor therapy. Novel therapeutic approaches targeting the PAI-1/uPA interaction are already in pre-clinical testing.

Zusammenfassung
Der Plasminogen-Aktivator-Inhibitor Typ 1 (PAI-1) ist ein Proteolysefaktor mit vielfältigen Eigenschaften. Er wirkt nicht nur als Inhibitor der Serinprotease uPA (Plasminogenaktivator vom Urokinasetyp), sondern spielt auch bei Signaltransduktion, Zelladhäsion und -migration eine wichtige Rolle. Obwohl er uPA bei Blutgerinnung hemmt, wirkt PAI-1 eher fördernd auf Tumorinvasion und Metastasierung – ein scheinbarer Widerspruch zu seinem Namen. Anfang der 90er Jahre zeigten zunächst klinische Studien einen Zusammenhang zwischen erhöhtem PAI-1 im Primärtumor und schlechter Prognose beim primären Mammakarzinom. Diese klinischen Daten wurden dann durch Ergebnisse aus der Grundlagenforschung untermauert: Das Zusammenspiel von uPA, seinem Oberflächenrezeptor uPA-R und PAI-1 fördert Tumorinvasion und Metastasierung. Die signifikante prognostische Bedeutung von PAI-1 – bestimmt mittels ELISA im Primärtumorextrakt – beim primären Mammakarzinom wurde von vielen internationalen Forschergruppen bestätigt. Klinische Daten deuten darauf hin, dass Hochrisikopatientinnen mit erhöhtem PAI-1 im Primärtumor von einer adjuvanten Systemtherapie profitieren. Auch uPA hat eine signifikante prognostische Bedeutung beim primären Mammakarzinom. Durch Bestimmung von PAI-1 *und* uPA im Primärtumorgewebe wird die Risikogruppeneinteilung für adjuvante Therapieentscheidungen beim nodal-negativen Mammakarzinom verbessert. Ergebnisse einer prospektiven randomisierten multizentrischen Therapiestudie bestätigen die klinische Relevanz von PAI-1 und uPA und legen ihre routinemäßige Bestimmung beim nodal-negativen Mammakarzinom nahe. Auch bei anderen Malignomen, wie beim Ovarial-, Ösophagus-, Magen-, Kolon- oder Leberzellkarzinom, korreliert hohes PAI-1 mit Tumoraggressivität und schlechter Prognose. Diese überzeugenden klinischen Daten, die auf eine Schlüsselrolle von PAI-1 bei Invasion und Metastasierung hinweisen, machen PAI-1 zu einer vielversprechenden Zielstruktur für tumorbiologische Therapieansätze. Neue Therapiemöglichkeiten zur Hemmung der PAI-1/uPA-Interaktion werden bereits in präklinischen Modellen getestet.

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First Reports on Clinical Relevance of PAI-1

There is abundant experimental evidence that the plasminogen activator (PA) system with the key components, the serine protease uPA (urokinase-type plasminogen activator), its inhibitor PAI-1 (plasminogen activator inhibitor type-1), and its cell surface receptor (uPA-R, CD 87), plays a fundamental role in tumor invasion and metastasis [1, 2]. In breast cancer, increased levels of uPA and PAI-1 have been found in carcinoma tissues compared to benign lesions or normal tissue [3]. The role of constituents of the PA system in cancer prognosis was first reported by Duffy et al. [4] who demonstrated that measurement of the enzymatic activity of uPA (serine protease *u*rokinase-type *p*lasminogen *a*ctivator) provides prognostic information in primary breast cancer [4]. This information was then strengthened by our finding that not only the enzymatic activity, but to an even greater degree the *tumor tissue antigen level* of uPA is of prognostic relevance. In the early 1990s, our group was the first to report that in addition to uPA, PAI-1 also has a significant prognostic impact in node-positive and node-negative breast cancer [3, 5]: Patients with high antigen levels of PAI-1 in their tumors have a significantly worse survival time than patients with low PAI-1. The prognostic impact of PAI-1 on disease-free (DFS) and overall survival (OS) in primary breast cancer has since been confirmed by several investigators (table 1). These clinical data, indicating a key role of the uPA/uPA-R/PAI-1 system in tumor invasion and metastasis, are supported by experimental evidence.

Tumor-Biological Properties of PAI-1

Initially, the clinical finding that an enzyme inhibitor does not have a protective function but is an indicator of bad prognosis was somewhat surprising. In the meantime, sufficient data from basic research about a concerted tumor-biological role of uPA and PAI-1 (fig. 1) explain these clinical findings [1]. uPA is produced by various normal and cancer cells and facilitates metastasis by directly acting on tumor cells via uPA-R or by activating plasminogen to plasmin, which degrades the extracellular matrix (ECM). After interaction of the cell surface uPA-R – uPA complex with PAI-1, this ternary complex is internalized into the cell, thereby initiating signal transduction and cell proliferation. Only the internalized uPA-R is recycled to the cell surface, thus focusing the proteolytic system to the invasive front of the cell [1, 2]. Apart from being a uPA inhibitor, PAI-1 plays an important role in cell adhesion and migration. It interferes with uPA-R/uPA-mediated tumor cell adhesion to the ECM component vitronectin [16]. Selective attachment to and detachment from the ECM may then promote tumor cell migration [17]. Thus, a critical balance of uPA, uPA-R, and PAI-1 is the prerequisite for efficient focal proteolysis, migration and hence subsequent tumor invasion and metastasis [1, 18].

Prognostic and Predictive Impact of PAI-1 in Solid Malignant Tumors

Most of the research on the clinical relevance of PAI-1 has been performed in breast cancer. All studies assessing PAI-1 antigen content in tumor tissue extracts have shown a strong prognostic impact of PAI-1 in primary breast cancer (table 1). Tumor tissue cytosols and detergent extracts (TX-100) yield equivalent results [19]. The ELISA assay for PAI-1 is robust enough for routine clinical use, and international quality assurance is guaranteed [20]. For analysis, only a small amount of tissue is needed. The ELISA can also be applied to extracts prepared from core biopsy specimens or cryostat sections. Extracts prepared from 100 μg tumor tissue corresponding to about 1 μg protein extract are sufficient. So far, no consistent clinically relevant data have been generated applying other determination techniques such as immunohistochemistry (IHC). A recent IHC study indicated that strong PAI-1 expres-

Table 1. Prognostic impact of PAI-1 in primary breast cancer as determined by ELISA: Literature overview

Authors	Year	Assay[1]	Cutoff	Number of patients	Follow-up, months (median)	Prognostic impact[2]
Jänicke et al. [3]	1991	ELISA[ADI]	optimized	115	25	yes (u, m)
Jänicke et al. [5]	1993	ELISA[ADI]	optimized	247	30	yes (u, m)
Grøhndahl-Hansen et al. [6]	1993	ELISA[Monozyme]	median	191	102	yes (u, m)
Foekens et al. [7]	1994	ELISA[ADI]	optimized	657	48	yes (u, m)
Kim et al. [8]	1998	ELISA[Biopool]	optimized	130	53	yes (u)
Kute et al. [9]	1998	ELISA[Monozyme]	median	162	58	yes (u, m)
Knoop et al. [10]	1998	ELISA[Monozyme]	median	429	61	yes (u, m)
Eppenberger et al. [11]	1998	ELISA[ADI]	optimized	305	37	yes (u)
Harbeck et al. [12]	1999	ELISA[ADI]	optimized	316	77	yes (u, m)
Bouchet et al. [13]	1999	ELISA[ADI]	quartiles	499	72	yes (u, m)
Foekens et al. [14]	2000	ELISA[ADI]	optimized	2,780	88	yes (u, m)
Harbeck et al. [15]	2000	ELISA[ADI]	optimized	276	109	yes (u, m)

[1] ELISA Assays: assays: ADI: American Diagnostica Inc., Greenwich, CT, USA; Biopool: Biopool, Umea, Sweden; Monozyme: Monozyme, Horsholm, Denmark; Santec: Santec, Bromma, Sweden.
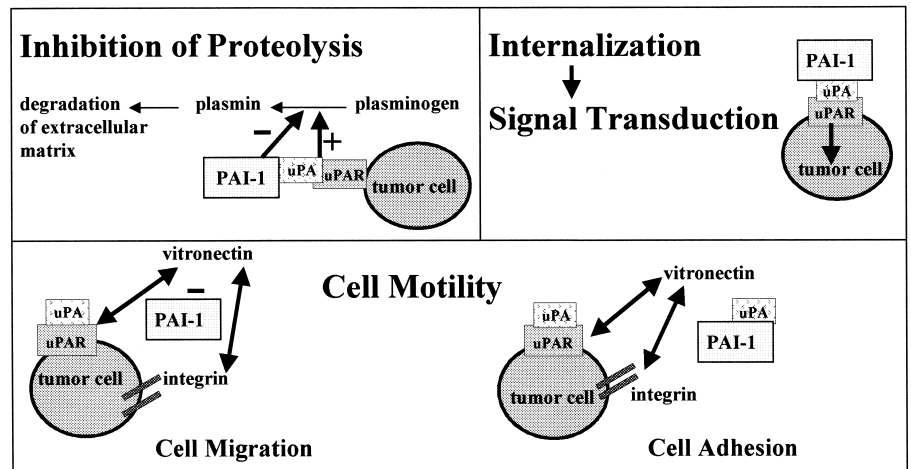[2] Univariate (u) and/or multivariate (m) analysis.

Fig. 1. Tumor-biological role of the plasminogen activator inhibitor type 1 (PAI-1) – a multifaceted proteolytic factor.

sion in fibroblasts is of more clinical relevance than PAI-1 expression in tumor cells, at least for the antibodies used [21].

In primary breast cancer, the *prognostic impact* of PAI-1 has been validated by numerous research groups (table 1). The fact that there is no contradictory published evidence on the prognostic impact of PAI-1 in breast cancer supports its tumor-biological role. Knoop et al. [10] showed that PAI-1 is a predictor for distant but not for local recurrence. Its prognostic strength shows evidence of a significant increase over time [22]. PAI-1 enables risk group assessment even within risk groups defined by established prognostic factors (fig. 2). The prognostic strength of PAI-1, particularly with regard to identification of low-risk patients, is enhanced by combination with uPA. Node-negative breast cancer patients with low levels of both PAI-1 *and* uPA in their primary tumor, i.e. about 50% of all node-negative patients, have a rather low relapse risk of less than 5% at 5 years [23]. Such a good characterization of a low-risk group cannot be achieved by either factor alone and is independent of that achieved by established or new prognostic factors such as HER2 [23, 24]. Thus, PAI-1 and uPA have fulfilled LOE I evidence for tumor marker utility [25] and are ready for transfer into clinical practice in primary breast cancer; First, there is undisputed evidence for their prognostic value from many unicenter studies using robust and quality-assured ELISA assay procedures [26]. Second, their utility as a selection marker for therapy decisions has been proven in a prospective randomized multicenter trial ('Chemo N_0') [27]. Third, results from a pooled analysis covering about 9,000 patients and looking at the prognostic value of PAI-1 and uPA will be available shortly [28].

Retrospective data suggest a *predictive impact* of PAI-1 with regard to therapy response in breast cancer. The prognostic impact of PAI-1 on DFS reflects effects of *adjuvant* systemic therapy: Whereas PAI-1 levels in the primary tumor strongly and significantly discriminate between a high-risk and a low-risk group in patients who did not receive any adjuvant systemic therapy, this risk group discrimination disappears in patients with adjuvant systemic therapy (fig. 3). This obvious difference suggests that high-risk *primary* breast cancer patients, as identified by elevated PAI-1 in their tumor, benefit from adjuvant systemic therapy. In *metastatic* breast cancer, high PAI-1 in the primary tumor is associated with poor response to *palliative* endocrine therapy [29, 30]. PAI-1 levels in the primary tumor also predict OS after first relapse [12], indicating that they reflect a rather aggressive tumor biology. Prospective evidence for the predictive impact of PAI-1 is still scarce. Pierga et al [31] did not observe a correlation between PAI-1 in primary tumor core biopsies and local response to anthracycline-containing neoadjuvant chemotherapy. PAI-1 tumor levels were not altered by chemotherapy; however, the authors did not present data on correlation of PAI-1 with patient outcome [31]. In the 'Chemo N_0' trial, node-negative breast cancer patients with high PAI-1 and/or uPA did benefit from adjuvant CMF chemotherapy [27]. A European follow-up study will compare different chemotherapy regimens for these high-risk patients.

In various other malignancies, such as ovarian, esophageal, gastric, colorectal or hepatocellular cancer, an increase in PAI-1 is also associated with tumor aggressiveness and poor patient outcome (table 2). In gastric cancer, Allgayer et al. [52] recently showed that PAI-1 and HER2 are independent prognostic factors. The extent to which PAI-1 determination will be used for routine patient care in malignancies other than breast cancer will depend on the clinical need for a tumor-biological prognostic or predictive factor. Nevertheless, the abundant clinical evidence implicating PAI-1 as a key factor for tumor invasion and metastasis in a variety of malignancies renders PAI-1 a promising target for tumor-biological therapy.

PAI-1 as a Target for Tumor Therapy

Early research on the role of PAI-1 in oncology was based on experimental evidence merely describing its uPA-inhibiting features. Thus, the PAI-1 molecule was initially used as a *tool* rather than a *target* for therapeutic intervention in cancer. The first therapeutic concept was to provoke overexpression of PAI-1 in the tumor or its vicinity in order to abrogate enzymatic uPA function. Transfection of the prostate carcinoma cell line PC3 with PAI-1 led to significant reduction of primary

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Schmitt/Jänicke

Fig. 2. Prognostic impact of PAI-1 on disease-free survival in clinically relevant subgroups of *node-negative* breast cancer patients *without* adjuvant systemic therapy (median follow-up 60 months): Relative risk of recurrence as a function of high PAI-1 (>14 ng/mg protein) vs. low PAI-1 (≤14 ng/mg protein) levels determined in primary tumor tissue extracts. Please note that for some patients not all of the information was available.
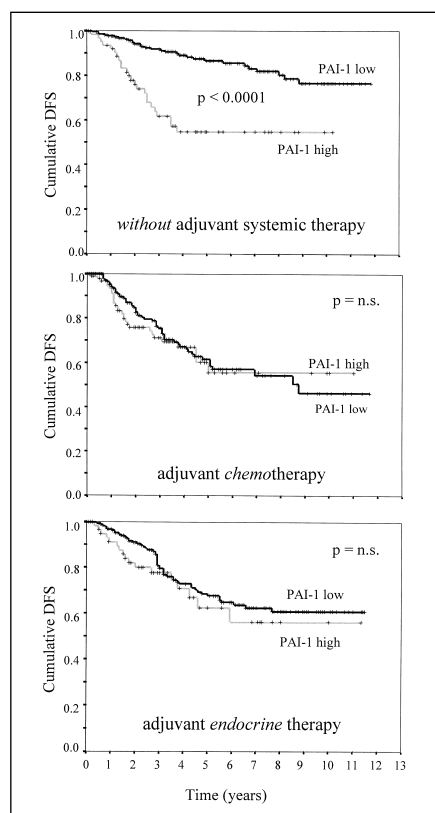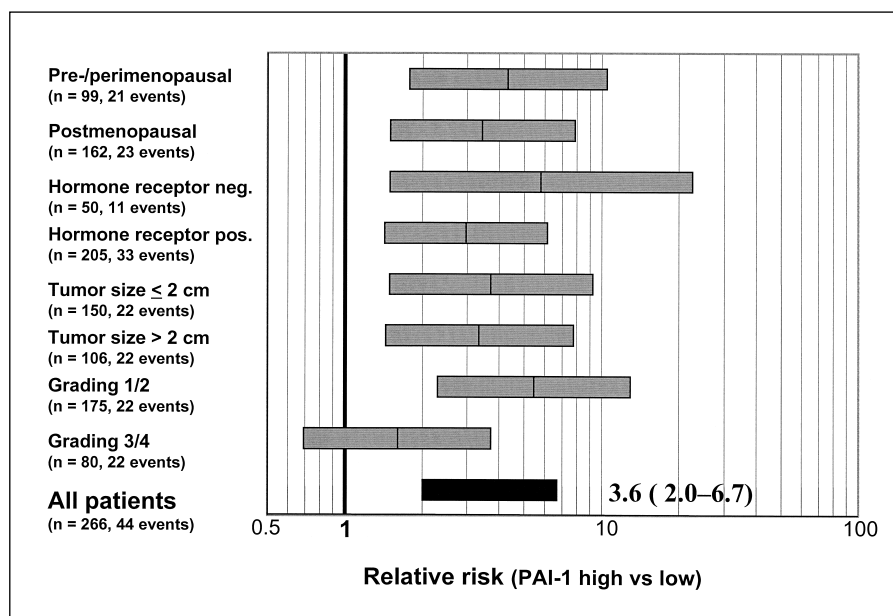

Fig. 3. Impact of PAI-1 disease-free survival reflects effects of adjuvant systemic therapy in *primary* breast cancer patients (median follow-up 48 months). A previously optimized and re-evaluated cutoff of 14 ng PAI-1 / mg protein was used to discriminate between high (>14) and low (≤14) PAI-1 levels in primary tumor tissue extracts [12]. n.s. = Not significant. *Top* (patients *without* adjuvant systemic therapy): PAI-1 low: 235 patients, 30 events; PAI-1 high: 64 patients, 24 events. *Middle* (patients *with* adjuvant *chemo*therapy): PAI-1 low: 186 patients, 56 events; PAI-1 high: 103 patients, 29 events. *Bottom* (patients *with* adjuvant *endocrine* therapy): PAI-1 low: 203 patients, 54 events. PAI-1 high: 79 patients, 17 events.

tumor growth and metastasis in *nude* mice [53]. A preclinical study employing prostate cancer xenografts showed that binding of PAI-1 to uPA reduced tumor size in *SCID* mice, while mutated PAI-1, unable to bind to uPA's active site, was ineffective [54]. Next, a gene delivery system to transfect cells with PAI-1 cDNA was developed: A replication-defective adenoviral vector AdCMV-PAI-1 expressing PAI-1 was used in an uveal melanoma mouse model; human or mouse uveal melanoma cells followed by the vector were inoculated into eyes of *nude* mice. The number of animals developing liver metastases was reduced by 50%, and the tumor burden in animals with metastases by 78% [55]. While these data support the feasibility of impairing the PA system using PAI-1 gene transfer as a therapeutic strategy, limitations of this concept became evident in another model: Fibrosarcoma cells (HT1080) were in vitro transduced with an adenoviral vector expressing PAI-1. The infected cells were tested in an in vitro migration assay and inoculated into nude mice [56]. Gene transfer of PAI-1 dose-dependently reduced cell migration in the invasion assay and the incidence of lung metastasis. In the same study, non-transduced HT1080 cells were inoculated into mice after intravenous injection of the PAI-1-expressing adenovirus, leading to PAI-1 expression in the liver cells and systemic PAI-1 elevation. In mice receiving the PAI-1-expressing adenovirus, primary tumor growth was reduced whereas lung metastasis was not affected. In contrast, intravenous injection of a PAI-2 expressing adenovirus led to efficient lung metastasis reduction. These data indicate that PAI-1, in contrast to PAI-2, cannot exert its invasion-inhibiting function by systemic application. The impact of murine host PAI-1 on the invasive and metastatic capacity of the B16 murine melanoma cell line was explored by Eitzman et al. [57]. Local tumor growth and lung metastasis were analyzed in tissues of mice overexpressing murine PAI-1 and of PAI-1-deficient mice. Surprisingly, neither tumor growth nor experimental lung metastasis was significantly affected in either of these mouse models. In another PAI-1 knockout

Table 2. Clinical relevance of PAI-1 in malignancies other than breast cancer: Literature overview

Tumor type	Assay	Number of patients	Clinical relevance[1]	Authors	Year
Ovarian cancer	ELISA	86	prognostic impact (u*, m*)	Kuhn et al. [32]	1999
Ovarian cancer	IHC	131	prognostic impact (u, m)	Chambers et al. [33]	1998
Cervical cancer	ELISA, IHC	62	prognostic impact (u)	Kobayashi et al. [34]	1994
Endometrial cancer	ELISA	64	correlation with aggressive phenotype	Kohler et al. [35]	1997
Gastric cancer	ELISA	76	prognostic impact (u, m)	Nekarda et al. [36]	1994
Gastric cancer	ELISA	25	prognostic impact (u)	Plebani et al. [37]	1995
Gastric cancer	ELISA	50	prognostic impact (u, m)	Ganesh et al. [38]	1996
Gastric cancer	IHC	203	prognostic impact (u, m)	Allgayer et al. [39]	1998
Colon cancer	ELISA	100	correlation with aggressive phenotype	Abe et al. [40]	1999
Colon cancer	ELISA	60	prognostic impact (u)	Herszenyi et al. [41]	1999
Colon cancer	IHC	97	prognostic impact (u, m)	Fujii et al. [42]	1999
Hepatocellular carcinoma	IHC, ELISA	19	correlation with aggressive phenotype	Zhou et al. [43]	2000
Hepatocellular carcinoma	ELISA	46	correlation with aggressive phenotype	Itoh et al. [44]	2000
Upper urinary tract carcinoma	IHC	154	prognostic impact (u)	Nakanishi et al. [45]	1998
Kidney cancer	ELISA	152	prognostic impact (u)	Hofmann et al. [46]	1996
Lung tumors: NSCLC[2]/NE[3]	IHC	84 / 72	correlation with aggressive phenotype	Robert et al. [47]	1999
Lung adenocarcinoma	ELISA	106	prognostic impact (u, m)	Pedersen et al. [48]	1994
Head-and-neck SCC[4]	ELISA	58	correlation with aggressive phenotype	Strojan et al. [49]	1998
Brain tumor	ELISA	64	correlation with aggressive phenotype	Arai et al. [50]	1998
Neuroblastoma	ELISA	64	prognostic impact (u)	Sugiura et al. [51]	1999

[1] Univariate (u) and/or multivariate (m) analysis.

[2] Non-small-cell lung carcinoma.

[3] Neuroendocrine lung tumor.

[4] Squamous cell carcinoma.

mouse model, absence of PAI-1 displayed a significant effect on keratinocyte invasion and angiogenesis [18]. After intravenous injection of a replication-defective adenovirus expressing human PAI-1, the capacity for tumor cell invasion and angiogenesis returned. This study implies that host-produced PAI-1 is essential for cancer cell invasion and angiogenesis. This body of experimental evidence further underlines the role of PAI-1 as a multifunctional protein. The feasibility of using PAI-1 as a *target* in tumor therapy was shown by Jankun et al. [58]. Internalization of the ternary uPA/uPA-R/PAI-1 complex with PAI-1 linked to cholera toxin resulted in selective killing of a fibrosarcoma cell line. Another approach for targeting PAI-1 activity consists of application of PAI-1-specific antibodies that interfere with PAI-1 activity toward uPA [59]. In addition to PAI-1, the other uPA-inhibitor, PAI-2, is also being explored as an anticancer tool [55, 60].

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