Adjuvant Chemotherapy in Node-negative Breast Cancer: UPA/PAI-1 Determinations for 163 Cases

LAURENCE VÉNAT-BOUVET[1], VERONIQUE FERMEAUX[2], SOPHIE LEOBON[1], NADIRA SAIDI[3], JACQUES MONTEIL[4], JOELLE MOLLARD[5], YVES AUBARD[5], ISABELLE JAMMET[6] and NICOLE TUBIANA-MATHIEU[1]

Departments of [1]Medical Oncology, [2]Pathology, [3]Radiotherapy and [4]Nuclear Medicine, University Hospital, Limoges, France; Departments of [5]Gynaecology, [6]Radiology and Medical Imaging, Mother and Child Hospital, Limoges, France

Abstract. *Background: The urokinase-type plasminogen activator (UPA) and its main inhibitor plasminogen activator inhibitor-1 (PAI-1) are involved in tumor interactions with the microenvironment. The UPA/PAI-1 content in tumor tissue can be used to identify populations at low-or high-risk of recurrence of breast cancer, even without other standard prognostic markers. Materials and Methods: The purpose of the present study was to compare adjuvant chemotherapy decisions made by a multi-disciplinary board for 163 node-negative breast cancer cases, based on clinicopathological (CP) and UPA/PAI-1 risk assessment. Results: The UPA/PAI-1 levels identified 37% of the population as being at low risk. Adjuvant chemotherapy indication was spared in high-CP risk in 17%, but maintained in low-CP risk in 33%. Conclusion: The use of UPA/PAI-1 data did not consistently result in a decrease of adjuvant chemotherapy. This study highlighted the difficulties encountered in a local multi-disciplinary board in determining appropriate roles and weights of new prognostic markers (UPA/PAI-1 was not routinely employed in France) when no data are available for assessing their prognostic and predictive power compared to other prognostic factors.*

The urokinase-type plasminogen activator (UPA) and its inhibitor, plasminogen activator inhibitor-1 (PAI-1), are proteases critically involved in tumor invasiveness. On the one hand, they degrade the extracellular matrix and on the other, they promote cell adhesion and migration. They are also involved in neo-angiogenesis during tumor development (1). The prognostic value of these biomarkers in breast cancer was established (2, 3) and validated with the highest level of evidence (LOE-1) by the American Society of Clinical Oncology in 2007 (4) and by the National Cancer Institute in association with the French Society of Senology and Breast Pathology (SFSPM) in 2009 (5). The UPA/PAI-1 content in tumor tissue has been correlated with disease aggressiveness; thus, it impacts disease-free survival and overall survival in patients with primary breast tumors.

Node-negative (N0) breast carcinomas are heterogeneous diseases with different potential to metastasize. Adjuvant chemotherapy may improve progression-free survival and overall survival, but chemotherapy can be avoided in a large majority of patients with N0 status that are at low risk of recurrence. One of the major clinical challenges is to identify these types of patients. Clinicopathological (CP) parameters for assessing the risk of recurrence include age, tumor size, grade of tumor differentiation, steroid hormone receptor status, Human Epidermal Growth Factor Receptor-2 (HER2) overexpression, and the level of proliferative markers Antigen Ki-67 (Ki67). These markers are useful for identifying subgroups of patients, but they are limited in their usefulness for deciding adjuvant chemotherapy without a risk of overtreatment.

UPA/PAI-1 content in primary breast tumor tissue can be used to identify sub-groups of patients with N0 breast cancer according to the risk of relapse; thus, the UPA/PAI-1 content can be used to refine adjuvant chemotherapy indications (6, 7). The thresholds for identifying low-and high-risk sub-groups are 3 ng/mg of cytosolic protein for UPA and 14 ng/mg for PAI-1; these can be used regardless of whether CP criteria are used (3). An increase in both proteins indicates a worse prognosis than an increase in only one (8).

Based on UPA/PAI-1 content, 55% of patients with N0 disease have an extremely low risk of relapse (93% disease-free survival after three years without adjuvant therapy) (9). For these patients, chemotherapy is not usually indicated.

Correspondence to: Prof. Nicole Tubiana-Mathieu, Department of Medical Oncology, University Hospital, 2 avenue Martin Luther King, 87042 Limoges cedex, France. Tel: +33 555056100, Fax: +33 555056398, e-mail: oncologie@chu-limoges.fr

Key Words: UPA-PAI-1, molecular biomarkers, adjuvant chemotherapy, node-negative breast cancer.

The purpose of the present study was to assess the impact of using the UPA/PAI-1 content for assessing N0 breast carcinomas in daily clinical practice. This study was conducted by a monocentric French team. We describe the decision-making process of a multi-disciplinary board that determined whether adjuvant chemotherapy was indicated based on UPA/PAI-1 levels and CP factors. The CP factors had been defined by national recommendations and recommendations from the St. Gallen International Consensus 2011 (10, 11).

Materials and Methods

Study design. This prospective study was performed between January 2010 and December 2011 at the Limoges University Hospital, France.

Population. We enrolled patients with invasive N0 breast cancer that were newly diagnosed, had undergone surgery, and had known UPA/PAI-1 tumor expression. We excluded patients with tumors that had macroscopic or microscopic lymph node involvement, who had been treated with neoadjuvant chemotherapy, or had exhibited isolated cells in the sentinel node (pN0i+).

A total of 285 N0 breast carcinomas were surgically removed during the study time period. During that period, it was possible to freeze 163 invasive breast cancer tumors. Therefore, we determined UPA/PAI-1 content in tumors from 160 patients; out of these, two patients had synchronous bilateral breast cancer and one was a man.

Methods. In all cases, a minimum, 10-day interval was routinely observed between the breast biopsy (diagnosis) and the surgical treatment to prevent false-positive results secondary to the tissue repair process. The tissues were transported within 60 min of surgical excision to the Pathology Department. The tissues were histologically examined, then frozen in liquid nitrogen, and transferred to the Biological Oncology Transfer Laboratory at Marseille, France. The assays were conducted with Food and Drug Administration-approved and labeled Enzyme Linked ImmunoSorbent Assay (ELISA) technique, using the FEMTELLE® kit (American Diagnostica Laboratories, Stamford, CT, USA) (12).

The validated thresholds were adopted from the German group (Arbeitsgemeinschaft Gynäkologische Onkologie) (12, 13), which specified that UPA greater than 3 ng/mg cytosolic protein or PAI-1 greater than 14 ng/mg cytosolic protein indicated a high risk of relapse.

The results were acquired within 10 and 15 days from biopsy.

Main objective. This study aimed to explain the multidisciplinary board's decisions on chemotherapy indications based on a combination of data from St. Gallen recommended CP factors and UPA/PAI-1 levels. The St. Gallen severity criteria were based on the following risk factors: age ≤35 years, tumor grade II (tumor size over 2 cm), grade III tumor, a high Ki67 value, absence of hormone receptors, overexpression of HER2, and vascular emboli. Overall, the presence of one of these factors was considered a sufficient criterion to indicate the need for adjuvant chemotherapy. An unfavorable UPA/PAI-1 level was defined as an elevation of at least one of the two markers above the pre-set thresholds.

Table I. *Tumor characteristics of 163 patients with N0 breast cancer.*

	Patients with low UPA and low PAI-1 (n=64)	Patients with high UPA and/or high PAI-1 (n=99)
T stage		
T1a	2	1
T1b	15	12
T1c	24	57
T2	20	29
T3/T4	3	0
Grade		
1	16	20
2	39	57
3	9	22
Ki67 LI		
≤15%	50	69
16-30%	9	18
>30%	5	12
HER2 status		
–	63	94
+++ and/or FISH+	1	5
LVI		
+	0	14
–	64	85
ER and PR –	4	12
ER and/or PR +	60	87

Ki67: Antigen Ki-67; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor-2; FISH: fluorescence in situ hibridization; LVI: lymphovascular invasion; UPA: urokinase-type plasminogen activator; PAI1: plasminogen activator inhibitor-1.

Results

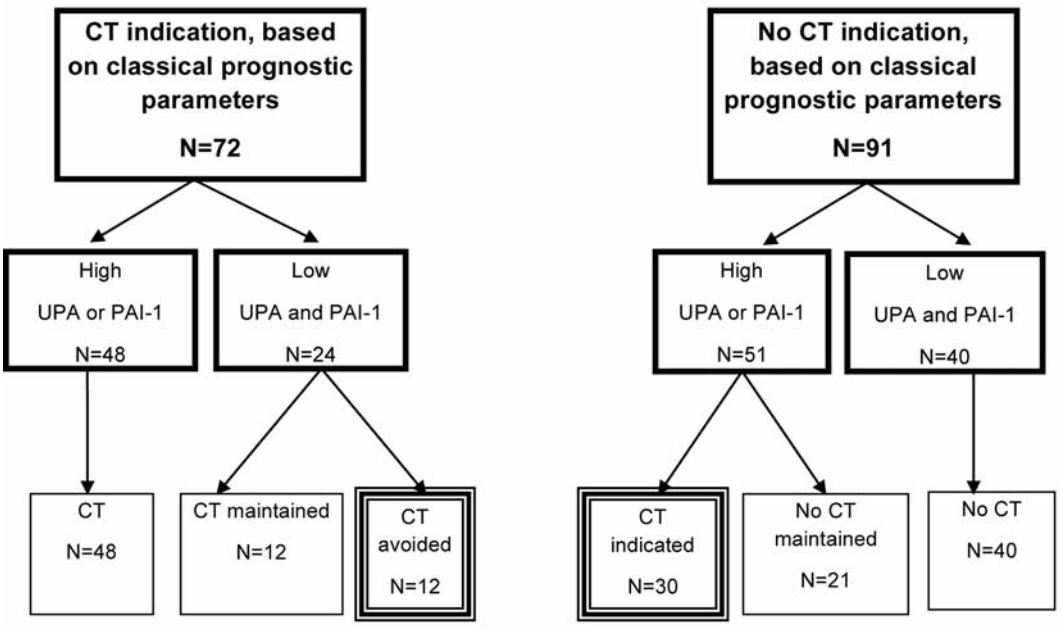
In total, 160 patients were included, with a median age of 61.4 (range=36-88) years. From these patients, 163 tumors were excised and frozen. Two patients had bilateral tumors, and 13 patients had multi-focal tumors.

The tumor characteristics, including CP and UPA/PAI-1 parameters, are reported in Table I. Sixty-four tumors presented low UPA/PAI-1 levels and 99 presented high UPA/PAI-1 levels.

The two patients with bilateral tumors had both tumors removed. Assays for UPA/PAI-1 factors were performed on both tumors in each case, and both tumors had similar characteristics.

Table II shows a comparison of adjuvant chemotherapy indications according to CP and UPA/PAI-1 parameters. In 72 cases, adjuvant chemotherapy was indicated based on the presence of at least one CP prognostic factor. Out of these 72 cases, UPA/PAI-1 was unfavorable in 48 and favorable in 24. Based on the UPA/PAI-1 results, adjuvant chemotherapy was avoided in 12 cases. However, in 12 other cases with favorable UPA/PAI-1 results, the multi-disciplinary board maintained the initial decision to administer adjuvant chemotherapy. The latter 12 patients (under 74 years old) had tumors with the following characteristics: Scarff Bloom and Richardson (SBR) grade III

Table II. *Comparison of adjuvant chemotherapy (CT) indications according to classical clinical parameters (top row) and urokinase-type plasminogen activator (UPA) + plasminogen activator inhibitor-1 (PAI-1) levels (middle row). Final decisions (bottom row) were based on consideration of both sets of data. Double-lined boxes show the number of times that the initial decision (top row) was reversed based on the UPA/PAI-1 data (middle row).*



(8 cases) or SBR grade II (4 cases) with 30% Ki67 (1 case), tumor size of 5 cm (1 case), negative hormone receptor (1 case), or positive HER2 (1 case).

In 91 cases, CP prognostic factors were favorable. Among these, 40 cases had low UPA/PAI-1 levels, which supported the decision of no chemotherapy, but 51 cases had high UPA/PAI-1 levels. This prompted a discussion in the multi-disciplinary board, and adjuvant chemotherapy was recommended for 30 out of the 51 cases. In the other 21 cases, the board did not consider the UPA/PAI-1 results sufficiently compelling to override the CP findings. These 21 cases (Table III) concerned Estrogen Receptors (ER)+++ tumors, without emboli, and HER2-negative; 10 tumors were SBR grade I, and 11 cases were SBR grade II. Moreover, in three cases, the unfavorable UPA/PAI-1 result reflected only a slightly high level of UPA. Table IV shows the median values of UPA/PAI-1 of the different tumors.

Discussion

We showed that evaluating the UPA/PAI-1 content in primary breast tumor tissue was feasible, and could be implemented as a routine procedure. However, the ELISA assay technique was limited by the fact that it required a minimum of 50 mg of frozen tissue. Thus, in our routine practice, the test was performed on only 57% of tumors. In the present study, low UPA/PAI-1 levels were observed in 64 cases (39.7%). Our results were very close to those from the population of patients in the N0 chemotherapy trial (9); 43% of those patients had low UPA/PAI-1 levels. The clinical conclusion from the N0 chemotherapy data was that at least 44% of patients with N0 status could potentially be spared adjuvant chemotherapy (9, 13-16). An updated report after a 10-year follow-up validated the independent prognostic value of the tumor grade and the UPA/PAI-1 level (17).

In the present study, our board decisions considered the UPA/PAI-1 data, but this did not consistently result in a decrease of adjuvant chemotherapy counter to the indication from CP markers. Among our population, 55% were considered as being of low risk based on CP markers and 44% based on UPA/PAI-1 markers; but only 32% were spared adjuvant chemotherapy. Disagreements between CP and UPA/PAI-1 indications corresponded to a positive HER2 status, triple-negative tumors, and grade III tumors. Thus, the board based their final decisions on the true value of UPA/PAI-1, not simply on the threshold values. In our study, increases in both factors were observed in 43 cases and increases in one of the factors were observed in 56 cases (43 for PAI-1 and 13 for UPA). Many abnormal UPA/PAI-1 values were close to the threshold level, which led to a difficult interpretation. The median PAI-1 value was high (21.42 ng/mg) and far from the threshold, but the median UPA value was close to the threshold (4.69 ng/mg). Based on the UPA/PAI-1 values, we decided that adjuvant chemotherapy was indicated for 10 patients with

Table III. *Details of cases where the initial decision for adjuvant chemotherapy (CT) was upheld, despite urokinase-type plasminogen activator (UPA) / plasminogen activator inhibitor-1 (PAI-1) indications.*

UPA/PAI-1 levels (n=163)	No. of tumors (n)	CT decisions	Cases where CT decision based on classical parameters was upheld
Low UPA and PAI-1	64	52 no CT 12 CT	12 Cases: 8 Grade III 4 Grade II: 1 ER–/PR– 1 HER2+++ 2 Tumor size 3.8 cm and 5 cm
High UPA and PAI-1	43	39 CT 4 no CT	4 Cases: T1c, ER+++/ PR+++, Ki67 low 2 Grade I 2 Grade II 3≥70 Years 1 Man, 56 years
Low UPA, high PAI-1	43	30 CT 13 no CT	13 Cases: Menopausal status, no LVI, ER /PR +++ 7 Grade I 6 Grade II with low Ki67 5≥75 Years 2 Tumor size ≤1 cm 1 Value slightly higher than normal (14.45 ng/ml)
High UPA, low PAI-1	13	9 CT 4 no CT	4 Cases: 1 Grade I 3 Grade II with low UPA (between 3.2 and 3.78 ng/ml)

ER: Estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor-2; LVI: lymphovascular invasion; Ki67: antigen Ki-67.

Table IV. *Urokinase-type plasminogen activator (UPA) / plasminogen activator inhibitor-1 (PAI-1) levels in tumors assessed as having high values (n=99).*

UPA/PAI-1 levels[a]	Number of tumors	Tumor protein levels that were above threshold. Median (range) (ng/mg protein)
Low UPA, high PAI-1	43	PAI-1=21.42 (14.36-98.78)
High UPA, low PAI-1	13	UPA=4.69 (3.03-7.32)
High UPA, high PAI-1	43	UPA=4.64 (3.06-10.77) PAI-1=24.32 (14.05-167.59)

[a]Thresholds for high classification were: UPA >3 ng/mg cytosolic protein, PAI-1 >14 ng/mg cytosolic protein.

isolated high UPA values; however, the standard CP factors only indicated adjuvant chemotherapy for five of the patients.

Tissue UPA and PAI-1 levels are independent significant factors that indicate a poor prognosis for patients with breast cancer. The work of Duffy (18) and Jänicke *et al*. (19) showed in multivariate analyses that PAI-1 was the most relevant factor for indicating the risk of relapse and survival. Several more recent studies have confirmed the prognostic value of PAI-1; the highest PAI-1 values were related to the shortest survival times (20-24). Therefore, it remains very difficult to determine the roles and weights of these parameters in the decision-making process.

Regardless of whether a sufficient amount of published evidence exists on the superiority of UPA/PAI-1 factors compared to CP criteria (2), at present, there is a lack of data on the outcomes of survival and relapse for patients at low risk who did not receive adjuvant chemotherapy based on the selection criteria employed (25). The Node Negative Breast Cancer III (NNBC-3) test initiated in Germany in 2003, and later in Europe (closed to inclusions since 15/01/2009), was conducted while taking this evidence into account. However, several stratification levels have been provided for evaluating patients according to the type of CP or clinico-biological risk and risk status (low or high risk of relapse).

Conclusion

The primary aim of the present study was to highlight the difficulty that clinicians face in their efforts to decide on whether to recommend adjuvant chemotherapy and to evaluate the reliability of new validated prognostic markers that are not used in standard practice in France. The multiple clinical situations that may be encountered generally extend beyond the context of published studies. Results from the NNBC-3 study will be used to confirm the usefulness of these biological markers; this information may improve the integration of these new markers into routine treatment procedures for SBR grade II, HER2-negative, N0 breast cancer. Above all, the new data will indicate the level of importance (weighting) of these biomarkers in adjuvant chemotherapy decision-making.

References

1 Tetu B, Trudel D and Wang CS: Proteases by reactive stromal cells in cancer: An attractive therapeutic target. Bull Cancer *93*: 944-948, 2006 (in French).

2 Harbeck N, Kates R, Look M and Foekens J: On behalf of the pooled analysis study of the EORTC Receptor and Biomarker Group (RBG): Pooled analysis (n=8,377) evaluates predictive impact of uPA and PAI-1 for response to adjuvant therapy in breast cancer. 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). J Clin Oncol *22(14S)*: 523, 2004.

3 Look MP, van Putten WL, Duffy MJ, Harbeck N, Christensen IJ, Thomssen C, Kates R, Spyratos F, Ferno M, Eppenberger-Castori S, Sweep CG, Ulm K, Peyrat JP, Martin PM, Magdelenat H,

Brunner N, Duggan C, Lisboa BW, Bendahl PO, Quillien V, Daver A, Ricolleau G, Meijer-van Gelder ME, Manders P, Fiets WE, Blankenstein MA, Broet P, Romain S, Daxenbichler G, Windbichler G, Cufer T, Borstnar S, Kueng W, Beex LV, Klijn JG, O'Higgins N, Eppenberger U, Janicke F, Schmitt M and Foekens JA: Pooled analysis of prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in 8377 breast cancer patients. J Natl Cancer Inst *94*: 116-128, 2002.

4 Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr. and American Society of Clinical Oncology: American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol *25*: 5287-5312, 2007.

5 Société française de sénologie et de pathologie mammaire (SFSPM) et Rapport 2009 sur l'état des connaissances relatives aux biomarqueurs tissulaires uPA/PAI-1 ODTeM: Résumé des conclusions des deux rapports publiés en novembre 2009 dans le cadre du partenariat avec l'INCA. La lettre du Sénologue *46*: 28-31, 2009 (in French).

6 Foekens JA, Schmitt M, van Putten WL, Peters HA, Bontenbal M, Janicke F and Klijn JG: Prognostic value of urokinase-type plasminogen activator in 671 primary breast cancer patients. Cancer Res *52*: 6101-6105, 1992.

7 Harbeck N, Kates RE, Schmitt M, Gauger K, Kiechle M, Janicke F, Thomassen C, Look MP and Foekens JA: Urokinase-type plasminogen activator and its inhibitor type 1 predict disease outcome and therapy response in primary breast cancer. Clin Breast Cancer *5*: 348-352, 2004.

8 Harbeck N, Dettmar P, Thomssen C, Berger U, Ulm K, Kates R, Hofler H, Janicke F, Graeff H and Schmitt M: Risk-group discrimination in node-negative breast cancer using invasion and proliferation markers: 6-year median follow-up. Br J Cancer *80*: 419-426, 1999.

9 Janicke F, Prechtl A, Thomssen C, Harbeck N, Meisner C, Untch M, Sweep CG, Selbmann HK, Graeff H, Schmitt M and German NSG: 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. J Natl Cancer Inst *93*: 913-920, 2001.

10 Gnant M, Harbeck N and Thomssen C: St. Gallen 2011: Summary of the Consensus Discussion. Breast Care (Basel) *6*: 136-141, 2011.

11 Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann B, Senn HJ and Panel M: Thresholds for therapies: Highlights of the St. Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol *20*: 1319-1329, 2009.

12 Sweep CG, Geurts-Moespot J, Grebenschikov N, de Witte JH, Heuvel JJ, Schmitt M, Duffy MJ, Janicke F, Kramer MD, Foekens JA, Brunner N, Brugal G, Pedersen AN and Benraad TJ: External quality assessment of trans-European multicentre antigen determinations (enzyme-linked immunosorbent assay) of urokinase-type plasminogen activator (uPA) and its type 1 inhibitor (PAI-1) in human breast cancer tissue extracts. Br J Cancer *78*: 1434-1441, 1998.

13 Janicke F, Schmitt M, Pache L, Ulm K, Harbeck N, Hofler H and Graeff H: Urokinase (uPA) and its inhibitor PAI-1 are strong and independent prognostic factors in node-negative breast cancer. Breast Cancer Res Treat *24*: 195-208, 1993.

14 Harbeck N, Alt U, Berger U, Kruger A, Thomssen C, Janicke F, Hofler H, Kates RE and Schmitt M: Prognostic impact of proteolytic factors (urokinase-type plasminogen activator, plasminogen activator inhibitor 1, and cathepsins B, D, and L) in primary breast cancer reflects effects of adjuvant systemic therapy. Clin Cancer Res *7*: 2757-2764, 2001.

15 Harbeck N, Dettmar P, Thomssen C, Henselmann B, Kuhn W, Ulm K, Janicke F, Hofler H, Graeff H and Schmitt M: Prognostic impact of tumor biological factors on survival in node-negative breast cancer. Anticancer Res *18*: 2187-2197, 1998.

16 Kantelhardt EJ, Vetter M, Schmidt M, Veyret C, Augustin D, Hanf V, Meisner C, Paepke D, Schmitt M, Sweep F, von Minckwitz G, Martin PM, Jaenicke F, Thomssen C and Harbeck N: Prospective evaluation of prognostic factors uPA/PAI-1 in node-negative breast cancer: Phase III NNBC3-Europe trial (AGO, GBG, EORTC-PBG) comparing 6 FEC *versus* 3 FEC/3 docetaxel. BMC Cancer *11*: 140, 2011.

17 Harbeck N, Schmitt M, Meisner C, Friedel C, Untch M, Schmidt M, Sweep CG, Lisboa BW, Lux MP, Beck T, Hasmuller S, Kiechle M, Janicke F, Thomssen C and Chemo NSG: Ten-year analysis of the prospective multicentre Chemo-N0 trial validates American Society of Clinical Oncology (ASCO)-recommended biomarkers uPA and PAI-1 for therapy decision making in node-negative breast cancer patients. Eur J Cancer *49*: 1825-1835, 2013.

18 Duffy MJ: Proteases as prognostic markers in cancer. Clin Cancer Res *2*: 613-618, 1996.

19 Janicke F, Schmitt M and Graeff H: Clinical relevance of the urokinase-type and tissue-type plasminogen activators and of their type 1 inhibitor in breast cancer. Semin Thromb Hemost *17*: 303-312, 1991.

20 Manders P, Tjan-Heijnen VC, Span PN, Grebenchtchikov N, Geurts-Moespot A, van Tienoven DT, Beex LV and Sweep FC: Complex of urokinase-type plasminogen activator with its type 1 inhibitor predicts poor outcome in 576 patients with lymph node-negative breast carcinoma. Cancer *101*: 486-494, 2004.

21 Harbeck N, Thomssen C, Berger U, Ulm K, Kates RE, Hofler H, Janicke F, Graeff H and Schmitt M: Invasion marker PAI-1 remains a strong prognostic factor after long-term follow-up both for primary breast cancer and following first relapse. Breast Cancer Res Treat *54*: 147-157, 1999.

22 Foekens JA, Peters HA, Look MP, Portengen H, Schmitt M, Kramer MD, Brunner N, Janicke F, Meijer-van Gelder ME, Henzen-Logmans SC, van Putten WL and Klijn JG: The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. Cancer Res *60*: 636-643, 2000.

23 Ferno M, Bendahl PO, Borg A, Brundell J, Hirschberg L, Olsson H and Killander D: Urokinase plasminogen activator, a strong independent prognostic factor in breast cancer, analysed in steroid receptor cytosols with a luminometric immunoassay. Eur J Cancer *32A*: 793-801, 1996.

24 Cufer T, Borstnar S and Vrhovec I: Prognostic and predictive value of the urokinase-type plasminogen activator (uPA) and its inhibitors PAI-1 and PAI-2 in operable breast cancer. Int J Biol Markers *18*: 106-115, 2003.

25 Penault-Llorca F, Coeffic D, Delozier T, Dohollou N, Freyer G, Gligorov J, Hardy-Bessard AC, Jacot W, Misset JL, Nabholtz JM, Petit T, Spielmann M and Namer M: Node negative breast cancer. Beyond international consensus: a pragmatic approach. Bull Cancer *98*: 807-825, 2011 (in French).

Received December 13, 2013
Revised January 23, 2014
Accepted January 24, 2014