Statistics say: 70% of these women with early stage breast cancer won’t relapse after surgery. They just don’t say which ones.

FEMTELLE does!
Now guide individual care with the test which has the highest prognostic value for risk assessment.
The Breast Cancer Challenge

- Due to breast cancer awareness, early screening and mammography about 60% of all breast cancers are detected at an early-stage of the disease.
- The majority (70%) of patients with Stage I and II breast cancer do not have recurrence of disease following removal of primary tumors. Still, 30% of patients diagnosed with early-stage breast cancer have disease recurrence following surgery.
- Clinico-pathological features of tumors cannot determine which individual tumor falls into the “no recurrence” group and which fall into the “recurrence” group.

The Challenge:

**Prognosis:** Identify those patients who likely will have recurrence of disease after surgery.

**Risk Assessment:** Identify patients at high risk for recurrence who would most likely benefit from post surgical adjuvant chemotherapy.
FEMTELLE Meets the Challenge!

There’s no more fundamental decision a woman diagnosed with Stage I or Stage II breast cancer can make than her post-surgical treatment course. What, if anything, will give her the best chance of long term survival? As shown, statistics are on the side of a considerable majority of women achieving a cure with surgery alone. But until now, thousands of American women have opted for aggressive treatments, including adjuvant chemotherapy, which may not have been necessary – because they could not predict their chances.

The scientists at American Diagnostica/Sekisui, in collaboration with international research and development partners, have now introduced FEMTELLE. This laboratory test can help women and their doctors accurately assess their risk of cancer recurrence – and make more rational treatment choices.

FEMTELLE uses the “gold standard” analytical technology – ELISA – that measures the levels of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) in tumor tissue. These are the most highly validated breast cancer biomarkers and FEMTELLE is the only test to achieve Level of Evidence-1, according to the 2007 American Society of Clinical Oncology (ASCO) Guidelines. In fact, the ASCO Guidelines support FEMTELLE as a reliable predictor of a woman’s likelihood of cancer recurrence.

Particularly in node-negative, hormone sensitive breast cancers, if uPA and PAI-1 levels are low, it is unlikely that cancer will recur. High levels indicate an aggressive cancer where a patient will benefit from adjuvant chemotherapy. FEMTELLE offers a real measure with no areas of doubt between high and low potential for recurrence – even in node-positive patients.

FEMTELLE is in routine usage in Europe where it has received the CE Marking. Clinical studies of 12,000 patients have validated its efficacy and reliability.

Now, FEMTELLE can help physicians guide their patients’ post-surgical care with high confidence based on individualized analysis of their breast cancer biomarkers.
**Why you should use FEMTELLE**

**Question:** Why is prognosis and risk assessment important for newly diagnosed early stage breast cancer patients and their oncologists?

**Answer:** Diagnosis of stage I and II breast cancer leads to fear in the patient and uncertainty for the oncologist. The patient is fearful of what will happen in the future. The oncologist has treatment choices to best manage the care of the patient.

**Question:** How can FEMTELLE help in the medical management of the breast cancer patient?

**Answer:** FEMTELLE provides vital information about each patient’s tumor. FEMTELLE will indicate with high confidence whether your patient’s tumor will recur up to 10 years post-surgery. FEMTELLE will also guide therapy choices based upon risk assessment of recurrence of your patient’s tumor.

**ASCO* Recommendations on Breast Cancer Biomarkers in 2007**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPA/PAI-1</td>
<td>To determine prognosis; For treatment planning; To guide CMF-based adjuvant chemotherapy</td>
</tr>
<tr>
<td>Proteomic Array Analysis</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>Multiparameter Gene Expression Analysis</td>
<td>Recommended</td>
</tr>
<tr>
<td>Gene Array</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>P53</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>Circulating Tumor Cells</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>Bone Marrow Micrometastasis</td>
<td>Not Recommended</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>Not Recommended</td>
</tr>
</tbody>
</table>

*ASCO: American Society of Clinical Oncology

Prognosis, Risk Assessment and Tumor Biomarkers

For the last twenty years prognosis and risk assessment in breast cancer have been determined using population statistics based upon clinical observation and pathological criteria such as tumor size and grade, hormone receptor status and age. These factors alone do not provide accurate prognosis and risk assessment for many patients.

In today’s post-genomic/proteomic era, tumor biomarkers have moved into the forefront as the newest and most accurate factors for diagnosis, prognosis and prediction of treatment outcome. The identification of key tumor markers has lead the way towards personalized prognosis and individualized medicine. Tumor biomarkers can provide vital information for determining the future course of disease and to guide individualized treatment options.

Clinicians are faced with the difficult task of determining who of their stage I and II breast cancer patients are likely to benefit from adjuvant therapy following removal of their primary tumor. For patients with early-stage breast cancer, multiple post-surgical treatment options are possible. Tumor biomarker assays are increasingly relied upon for prognosis of the future course of the cancer, selecting the best treatment options for each patient and predicting a patient’s response to particular therapies.

Characteristics of a validated biomarker: Level of Evidence-1

- The biomarker must be biologically relevant to the disease.
- The biomarker assay must be validated in preclinical and clinical applications.
- The utility of the marker must be designated (e.g. risk assessment, prognosis, diagnosis, screening and monitoring).
- The biomarker must be rigorously tested in randomized, prospective clinical trials.

uPA and PAI-1 have been validated at the highest level of evidence (LOE-1) American\(^1\) and German\(^2\) guidelines recommend uPA and PAI-1 for prognosis in node-negative breast cancer.

Guideline Recommendations:

- \(^1\)ASCO (American Society of Clinical Oncology)
- \(^1\)NACB (National Academy of Clinical Biochemistry)
- \(^2\)AGO (German Working Group for Gynecological Oncology)
- \(^2\)S3 (German Cancer Society)
The Role of uPA and PAI-1 in Breast Cancer

The urokinase plasminogen activator system is comprised of urokinase-type plasminogen activator (uPA), urokinase-type plasminogen activator receptor (uPAR), and the uPA inhibitor, plasminogen activator inhibitor type-1 (PAI-1). uPAR binds uPA but also interacts with a variety of extracellular matrix and cell-surface proteins, such as vitronectin and integrins. These interactions can be modulated by the binding of uPA to uPAR. PAI-1 modulates the activity of uPA and uPAR.

When bound to uPAR, the serine protease uPA converts plasminogen to plasmin, which is a broad spectrum protease that activates various proteases involved with degradation of the extracellular matrix.

The uPA-system plays a key role in tumor-related processes such as metastasis and cellular migration/invasion, adhesion, and proliferation, as well as angiogenesis. Currently, the uPA/PAI-1 system has been targeted for the development of cancer therapies.

Twenty-years of research has shown that measuring uPA and PAI-1 protein levels by ELISA in detergent extracts from primary early stage breast tumors provides vital information about the biology and metastatic potential of each person’s tumor ten years after surgical excision.
Clinical Laboratory Routine

Biopsy/Excised Tumor Tissue

PATHOLOGY

1. Inspection of unfixed tissue
2. Removal of a representative piece of tumor tissue (> 50 mg)
3. Snap freezing
4. Storage of the frozen tissue (-20°C)

Histology, IHC

DIAGNOSTIC LABORATORY

1. Pulverization of tumor tissue
2. Extraction of uPA and PAI-1
3. Perform FEMTELLE uPA/PAI-1 ELISA

Transfer of test results to Physician

PHYSICIAN

Prognosis/Therapy Discussion
Proven in randomized and prospective clinical trials

Prospective randomized multicenter therapy trial
“Chemo-N0” in node-negative breast cancer prognosis

Chemo-N0 Study

Primary breast cancer patients
T-size: ≥ 1 cm ≤ 5 cm.
pN₀ / M₀
Pre- and/or Postmenopausal
positive or negative hormone
receptor status

Stratification

Randomization

Observation
Arm A

PAI -1 and uPA low

Observation
Arm B 1

6 x CMF

PAI -1 and/or uPA high

Observation
Arm B 2

Observation or CMF
Arm B 3

Randomization Refused

• 12 Centers (11 x Germany, 1x Slovenia)
• 5 Laboratories, external quality assurance (Nijmegen)
• Total: 647 patients (recruitment June 1993 - December 1998)
• Final analysis after 10 years

• Median follow-up 113 (5-167) months
• Low risk arm (uPA and PAI-1 low): n = 283
• High risk arm (uPA and PAI-1 high): n = 364
• 242 patients randomized (117 CMF, 125 observation only)

Chemo-N0 Trial: 10 year follow-up validates uPA and PAI-1 as prognostic factors for therapy decisions in node-negative breast cancer.
10 year follow-up of Chemo-N0 trial

**Univariate (n=409) vs. Multivariate (n=406)**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Univariate (n=409)</th>
<th>Multivariate (n=406)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade (G1 vs. G2 vs. G3)*</td>
<td>HR 2.67, P&lt;0.0001</td>
<td>HR 2.33 (1.5 - 3.7), P=0.0004</td>
</tr>
<tr>
<td>uPA/PAI-1</td>
<td>HR 1.87, P=0.012</td>
<td>HR 1.84 (1.1 - 3.0), P=0.016</td>
</tr>
<tr>
<td>Local regional hospital treatment</td>
<td>HR 1.64, P=0.059</td>
<td>HR 1.57 (0.9 - 2.7), -</td>
</tr>
<tr>
<td>Age related risk</td>
<td>HR 0.64, P=0.075</td>
<td>HR 0.61 (0.4 - 1.0), -</td>
</tr>
<tr>
<td>pT stage</td>
<td>HR 1.99, P=0.006</td>
<td>HR 1.48 (0.9 - 2.5), -</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td>HR 1.77, P=0.060</td>
<td>HR 1.09 (0.6 - 2.0), -</td>
</tr>
</tbody>
</table>

Significant prognostic factors, including uPA/PAI-1 measured using the FEMTELLE test, in univariate and multivariate proportional hazard model for DFS in Chemo-N0 Trial. Patients with no adjuvant systemic therapy included. Hormone receptor status was not significant. Model AIC was 335.8 (best value).

Better prognosis compared to traditional tumor factors

Combined uPA and PAI-1 testing yields the highest prognostic value amongst various tumor-related markers

<table>
<thead>
<tr>
<th>All patients</th>
<th>Relative risk of recurrence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPA/PAI-1(*)</td>
<td>11.0 (3.2-37.4)</td>
</tr>
<tr>
<td>PAI-1 (≤ vs &gt; 14 ng/mg protein)</td>
<td>6.2 (2.7-14.6)</td>
</tr>
<tr>
<td>SPF (≤ vs &gt; 6%)</td>
<td>2.9 (1.1-7.6)</td>
</tr>
<tr>
<td>Cathepsin D (≤ vs &gt; 41 pmol/mg protein)</td>
<td>4.0 (1.91-10.8)</td>
</tr>
<tr>
<td>uPA (≤ vs &gt; 3 ng/mg protein)</td>
<td>2.9 (1.3-6.6)</td>
</tr>
<tr>
<td>Tumor size (≤ vs &gt; 2 cm)</td>
<td>1.8 (0.8-4.2)</td>
</tr>
<tr>
<td>Ploidy (diploid vs aneuploid)</td>
<td>2.1 (0.8-5.5)</td>
</tr>
<tr>
<td>Grading (G1/2 vs G3/4)</td>
<td>2.0 (0.8-4.8)</td>
</tr>
<tr>
<td>p53 (negative vs positive)</td>
<td>2.6 (0.8-9.0)</td>
</tr>
<tr>
<td>MIB1 (≤ vs &gt; 25%)</td>
<td>2.7 (1.1-6.6)</td>
</tr>
<tr>
<td>HER-2/neu (≤ vs &gt; 2.5%)</td>
<td>1.5 (0.6-3.7)</td>
</tr>
<tr>
<td>Steroid hormone receptor status (negative vs positive)</td>
<td>1.1 (0.4-3.0)</td>
</tr>
</tbody>
</table>

*uPA/PAI-1: both factors low vs either or both factors high
Combined uPA and PAI-1 levels from breast tumor extracts are prognostic for disease-free survival and overall survival in patients with lymph node-negative breast cancer.

High levels of uPA and PAI-1 from tumor extracts help to establish risk stratification of various subgroups of patients with breast cancer.

The NNBC-3 trial confirms that 42% of the N0 patients at an intermediate risk (G2 grading) can be clearly classified as low risk by uPA and PAI-1.

Many node-negative patients can be spared the burden of chemotherapy.
Verified in multiple independent studies by pooled analysis

**uPA/PAI-1 Stratification**
Verified in Independent Clinical Trials

<table>
<thead>
<tr>
<th>Study</th>
<th># LN0</th>
<th>Patients</th>
<th>Stratification</th>
<th>Low Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo-N0</td>
<td>282</td>
<td>uPA/PAI-1</td>
<td>37.40%</td>
<td>62.60%</td>
<td></td>
</tr>
<tr>
<td>JCO2002</td>
<td>745</td>
<td>uPA/PAI-1</td>
<td>45.30%</td>
<td>54.60%</td>
<td></td>
</tr>
<tr>
<td>NNBC-3</td>
<td>2500c</td>
<td>uPA/PAI-1</td>
<td>39%</td>
<td>61%</td>
<td></td>
</tr>
</tbody>
</table>

FEMTELLE for Selecting Patients for Post-Surgical Adjuvant Chemotherapy

uPA and PAI-1 predict benefit of treatment with adjuvant therapy for patients with high- and low-risk of recurrence of breast cancer following surgical removal of primary tumor

Adjuvant chemotherapy reduces the probability of disease recurrence in patients identified as high risk (high tumor levels of uPA and PAI-1)

CMF = patients treated with cyclophosphamid, methotrexate, and 5-fluorouracil;

* HT = hormonal therapy; CT = chemotherapy; Low = low uPA/PAI-1 levels; High = high uPA/PAI-1 levels.
Advantages of FEMTELLE to other breast cancer prognostic tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Advantage</th>
<th>Superior to Genomic Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>uPA and PAI-1 validated proteomic biomarkers</td>
<td>🔄</td>
</tr>
<tr>
<td>Technology</td>
<td>ELISA “gold standard” proteomic assay</td>
<td>🔄</td>
</tr>
<tr>
<td>Readout</td>
<td>low risk/high risk no zone of uncertainty</td>
<td>🔄</td>
</tr>
<tr>
<td>Clinical Studies</td>
<td>prospective, randomized, pooled analysis highest level of evidence</td>
<td>🔄</td>
</tr>
<tr>
<td>Tumor Stage</td>
<td>I and II most difficult stage to make therapy decisions after surgery</td>
<td>🔄</td>
</tr>
<tr>
<td>ER/PR</td>
<td>positive or negative any steroid receptor status</td>
<td>🔄</td>
</tr>
<tr>
<td>Lymph Nodes Status</td>
<td>LN0, LN1-10 helps decision making with LN+ patients</td>
<td>🔄</td>
</tr>
<tr>
<td>Tumor Size</td>
<td>&lt; 5 cm effective results with larger tumors</td>
<td>🔄</td>
</tr>
<tr>
<td>Menopausal Status</td>
<td>pre, post younger and older patients</td>
<td>🔄</td>
</tr>
<tr>
<td>Recommendations</td>
<td>ASCO, AGO, NABC, EORTC international recognition</td>
<td>🔄</td>
</tr>
</tbody>
</table>
Breast cancer is personal…
Personalize Prognosis and Risk Assessment with

FEMTELLE®

FEMTELLE for Prognosis and Risk Assessment

Benefits of FEMTELLE to patients and clinicians

• Accurately predicts risk of breast cancer relapse.
• Guides post-operative adjuvant chemotherapy decision making in node-negative breast cancer.
• Prevents over-treatment with adjuvant chemotherapy.
• Avoids under-treatment of patients that require chemotherapy.
• Validated in independent prospective multicenter trials.
• Recommended at highest Level of Evidence (LOE-1) from American and German guidelines.
• Currently in routine clinical use in Europe.

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