



## Prosigna®(PAM50) (*Breast cancer Gene Signature Assay*) a prognostic gene expression assay for breast cancer

Reminder: prognostic and predictive factors in breast cancer

In breast cancer, the currently validated prognostic factors are as follows:

- patient age
- lymph node status
- tumour size
- histological grade and type of tumour
- presence of vascular and lymphatic emboli
- hormone receptor status
- HER2 status
- Ki67 tumour proliferation index

In 2015, only two factors are recognized as predictive of therapeutic response:

- **hormone receptor status (hormone receptors:ER – PR):** positive or negative
- **HER2 status:** positive (amplified) or negative (non-amplified)

These two factors are validated following immunohistochemical analyses (hormone receptors and HER2) and in situ hybridization (HER2). With regard to hormone receptor status, the results can

be used to initiate hormone therapy, and in the event of overexpression of HER2, the results can be used to initiate targeted anti-HER2 treatment.

### Role of molecular signature testing

Over recent years, innovative tests in the field of molecular biology have enabled a concrete approach to the theranostic treatment of cancer patients. The concept of "**personalized medicine**" is based on the principle that all patients with the same disease should not all receive the same treatment regimen. In breast cancer, the level of expression of proteins specific to the tumour cell should make it possible to define, beyond molecular types, the concept of aggressive tumours with metastatic potential, or to suggest the likelihood of response to treatment or late relapse.

Gene expression analyses have thus enabled breast cancers to be classified into **four molecular subtypes**. Each of these intrinsic subtypes or classes has its own prognosis and specific recommended treatment regimen: **Luminal A** (30 to 40% of breast cancers), **Luminal B** (20%),

**HER2 positive** (or amplified) (20%) and **Basal like** (20%) (or triple negative: absence of hormone receptors and no overexpression of HER2). Luminal breast cancers express hormone receptors and thus respond to hormone therapy. They are divided into two classes: Luminal A and Luminal B, with the Luminal B subtype having a more unfavourable prognosis. The addition of chemotherapy should therefore be discussed in a multidisciplinary meeting. Breast cancers that are HER2 positive (i.e., with HER2 amplification) are responsive to antiHER2 treatment and chemotherapy. For the basal type, which has a poor prognosis, only chemotherapy is available. These assays are called tests of "**gene expression**" or "**gene signature**" or "**GEP**" (for Gene Expression Profiling). They are based on the identification and quantification of messenger RNA (mRNA) or complementary DNA (cDNA) of the genes primarily involved in tumour proliferation.

The main advantage of these tests is to provide the clinician with an indication of the benefit of adding or not adding chemotherapy to the standard treatment of women with breast cancer diagnosed at an early stage, particularly for breast cancers that are ER/PR positive but HER2 negative.

The purpose of these tests is to permit a more detailed analysis for each patient than that provided by the immunohistochemical methods of risk stratification (Mammostrat, IHC4, uPA/PAI1, etc.). Access to this innovation is part of the 2014-2019 cancer plan. Biomnis offers access to these innovative assays both in France and Internationally.

## What molecular tests are available in 2015?

Name	Method	Number of genes studied
Oncotype DX® (Genomic Health)	RT-PCR	21
Prosigna®(PAM50) (NanoString)	RT-PCR (nCounter®)	50
Endopredict® (Myriad)	RT-PCR	11
Mammaprint®/ BluePrint®(Agendia)	Micro-array	70 / 80

These various tests are performed on samples of tumour tissue (biopsy or surgical specimen) obtained at the time of diagnosis. It is therefore not necessary to obtain a new sample from the patient.

## The Prosigna® assay (PAM50) in breast cancer - principles and advantages of the test

### The inclusion criteria for this molecular test are:

- post-menopausal female patients with a diagnosis of early-stage breast cancer who have undergone mastectomy or breast-conserving surgery and who have lymph node involvement (N1 to N3) or no lymph node involvement (N0),
- hormone receptor positive (ER/PR positive) tumours,
- tumours with no overexpression (or amplification) of HER2 (HER2 negative),
- patient eligible for adjuvant chemotherapy treatment.

In order to perform the Prosigna® assay (PAM50), it is necessary to know two clinicopathological variables: **tumour size** ( $\leq 2$  cm or  $> 2$  cm) and **lymph node involvement** (0 to 3). These

two parameters are integrated into the Prosigna® algorithm.

Tumour infiltration is checked in advance by a pathologist using the tissue block technique. The Prosigna® assay is performed on RNA obtained from breast tumour samples fixed in formalin and embedded in paraffin. Prosigna® uses RT-PCR to simultaneously measure the expression of 50 genes used by the algorithm for the classification of intrinsic subtypes. The assay is conducted on the nCounter® DX analysis system from Nanostring. The algorithm provides a ROR score (Risk of Recurrence), which reflects the risk of distant recurrence at 10 years.

**The results of the Prosigna® assay (PAM50) are as follows:**

Result	Values
Intrinsic tumour subtype	Luminal A Luminal B HER2 “enriched” Basal-like
Individual estimate of the likelihood of distant recurrence at 10 years	0 – 100 %
ROR score: Risk of Recurrence	Value on a scale of 0 to 100
Risk classification	Low Intermediate High

The Prosigna® assay (PAM50) has been validated on an analytical level by several studies. The reproducibility and precision of Prosigna® have been demonstrated after analysis at multiple study sites, using different nCounter® platforms, different operators and different batches of reagents.

Prosigna® (PAM50) has also been clinically validated (in more than 2400 patients). The results of two studies (*the TransATAC clinical validation study and the ABCSG-8 validation study*) have made it possible to validate the model that links the ROR score from the PAM50 test to the likelihood of distant recurrence in the population tested with a confidence interval of 95%.

In conclusion, the Prosigna® assay (PAM50) provides a rapid, reliable and individualized assessment of risk, in particular with regard to:

- the prognostic indicator of disease-free survival at 10 years for the aforementioned target population,
- the identification of intrinsic subtype: in particular differentiation between the luminal subtypes Luminal A and Luminal B, the latter of which has a more unfavourable prognosis and may therefore benefit from the a combination of hormone therapy and chemotherapy.

Finally, the Prosigna® assay (PAM50) has both the CE mark and the 510(k) approval from the FDA in the above indication for the analysis of breast tumour tissue (FFPE).

## References

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