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**Methodology**

The test is based on the reported 50-gene classifier algorithm originally named PAM50 and is performed on the nCounter® De Analysis System using RNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples. The nCounter®-based platform uses gene-specific probe pairs that hybridize directly to the mRNA sample in a single tube reaction without amplification. Digital bar code technology then directly measures gene expression. This digital technology offers a high level of reproducibility, precision, and sensitivity.

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**References**

1. Package Insert: Prosigna Breast Cancer Prognostic Gene Signature Assay; Version 01, created 2013-09; REF LBL-C0223-01
**What is Prosigna?**

Prosigna® Breast Cancer Prognostic Gene Signature Assay is an FDA-approved assay which provides a risk category (low, intermediate, high) and a numerical score (0 -100) for the assessment of distant recurrence in years 0-5 and years 5-10 for post-menopausal women with hormone receptor-positive invasive breast cancer.1

**Indications**

Per the FDA approval/package insert, the following are indications for use of Prosigna in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either:

- A prognostic indicator for distant recurrence-free survival (DRFS) at 10 years in postmenopausal women with hormone receptor-positive lymph node-negative. Stage I/II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors.
- A prog nostic indicator for DF RS at 10 years in postmenopausal women with hormone receptor-positive lymph node-negative (1-3 nodes). Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. The assay is not intended for patients with 4 or more positive nodes.
- DRFS by Risk Group for Node-Positive (1-3): Post-surgical early stage breast cancer (Stage I/II) with standard of care, either:
  - Cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either:
  - Both the ATAC and ABCSG-8 trials assessed the benefit of adjuvant treatment (tamoxifen and anastrozole alone or in combinations for 5 years) in post-menopausal women with hormone receptor-positive early stage breast cancer.1-3
  - A subset of tumor samples from the TransATAC (translational arm of the ATAC trial) and ABCSG-8 trials were analyzed by Prosigna to assess the assay’s ability to predict risk of distant recurrence.1-3
  - Both studies demonstrated that the Prosigna Score provided substantial prognostic information over and above the standard clinical variables (nodal status, tumor size and grade, age, and treatment) in predicting risk of distant recurrence.1-3

**HEAD-TO-HEAD COMPARISON**

**Comparison of Prosigna with Other Risk Recurrent Breast Cancer Assays**

**Prediction of Late Recurrence**

A retrospective analysis of more than 1,000 patient samples from the TransATAC study evaluated the relationship between clinical variables, immunohistochemistry markers (IHC4), Oncotype DX® Recurrence Score (RS), and Prosigna Score (ROR) for distant recurrence in years 5-10 in postmenopausal women with hormone receptor-positive breast cancer. Findings included:

- IHC4, RS, and ROR scored each clinical overall prognostic information beyond established clinicopathological factors in 5-10-year follow-up for distant recurrence.4
- However, IHC4 and RS lost most of their prognostic value after 5 years of follow-up.5
- Prosigna's ROR score showed the highest differential between patients in low-risk and high-risk groups in the 5-10 year follow-up period (15.1% difference), and was noted as the best discriminator between the two groups.5

**Validated Clinical Performance**

Prosigna’s clinical performance has been validated in two large independent studies using retrospective tissue samples from over 2,400 patients within the intended use population.6

Both the ATAC and ABCSG-8 trials assessed the benefit of adjuvant treatment (tamoxifen and anastrozole alone or in combinations for 5 years) in post-menopausal women with hormone receptor-positive early stage breast cancer.1-3

A subset of tumor samples from the TransATAC (translational arm of the ATAC trial) and ABCSG-8 trials were analyzed by Prosigna to assess the assay’s ability to predict risk of distant recurrence.1-3

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**Prosigna Validated in Two Clinical Studies**
Indications
Per the FDA approval/package insert, the following are indications for use of Prosigna in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either:

- A prognostic indicator for distant recurrence-free survival (DRFS) at 10 years in post-menopausal women with hormone receptor-positive, lymph node-negative, Stage II breast cancer
- A prognostic indicator for distant recurrence at 10 years in post-menopausal women with hormone receptor-positive/lymph node-positive (1-3 nodal) Stage II breast cancer
- A prognostic indicator for distant recurrence at 10 years in post-menopausal women with hormone receptor-positive/lymph node-negative, Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors
- A prognostic indicator for distant recurrence at 10 years in post-menopausal women with hormone receptor-positive/lymph node-negative (1-3 nodal), Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. The assay is not intended for patients with 4 or more positive nodes.

Fewer Intermediate Risk Scores
In assessing the patient distribution by risk categories in the TransATAC node-negative patient subset, the Prosigna assay was found to provide more prognostic information for the assessment of disease of 10 years, with fewer patients categorized as intermediate risk and more as high risk when compared to the Oncotype DX.1

Comparison of Prosigna with Other Risk Recurrent Breast Cancer Assays

Prediction of Late Recurrence
A retrospective analysis of more than 1,000 patient samples from the TransATAC study evaluated the relationship between clinical variables, immunohistochemistry markers (IHC4), Oncotype DX® Recurrence Score (RS), and Prosigna Score (ROR) for distant recurrence in years 5-10 in post-menopausal women with hormone receptor-positive breast cancer. Findings include:

- IHC4, RS, and ROR scores each added distinct and independent prognostic information beyond established clinicopathological factors in 5-9-year follow-up for distant recurrence.2
- However, IHC4 and RS lost most of their prognostic value after 5 years of follow-up.3
- Prosigna’s ROR score showed the highest differential between patients in low-risk and high-risk groups in the 5-10-year follow-up period (15.1% difference), and was noted as the best discriminator between the two groups.4

Kaplan-Meier estimates for distant recurrence according to immunohistochemical markers (IHC4), recurrence score (RS), and risk of recurrence (ROR) score group split at the median value.2

VALIDATED CLINICAL PERFORMANCE
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Both studies demonstrated that the Prosigna Score provided substantial prognostic information over and above the standard clinical variables (nodal status, tumor size and grade, age, and treatment) in predicting risk of distant recurrence.1,5,6

Kaplan-Meier curves and data tables showing the percent of patients without distant recurrence by risk group through 10 years and by node status.
**Head-To-Head Comparison**

**Comparison of Prosigna with Other Risk Recurrent Breast Cancer Assays**

**Prediction of Late Recurrence**

A retrospective analysis of more than 1,000 patient samples from the TransATAC study evaluated the relationship between clinical variables, immunohistochemistry markers (IHC4), Oncotype DX® Recurrence Score (RS), and Prosigna Score (ROR) for distant recurrence in women 5-10 years in post-menopausal women with hormone receptor-positive breast cancer. Findings include:

- **Prosigna**’s ROR score showed the highest differential between patients in low-risk and high-risk groups in the 5-10 year follow-up period (15.1% difference), and was noted as the best discriminator between the two groups.

**Fewer Intermediate Risk Scores**

In assessing the patient distribution by risk categories in the TransATAC node-negative patient subset, Prosigna assay was found to provide more prognostic information for the assessment of disease at 10 years, with a better risk classification.

**Kaplan-Meier estimates for distant recurrence according to immunohistochemical markers (IHC4), recurrence score (RS), and risk of recurrence (ROR) score group split at the median value.**

**Indications**

Per the FDA approval/package insert, the following are indications for use of Prosigna in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either:

- A prognostic indicator for distant recurrence-free survival (DRFS) at 10 years in post-menopausal women with hormone receptor-positive lymph node-negative, Stage II breast cancer to be treated with adjuvant hormone therapy alone, when used in conjunction with other clinicopathological factors.

- A prognostic indicator for DRFS at 10 years in post-menopausal women with hormone receptor-positive lymph node-negative (1-3 nodules), Stage II breast cancer to be treated with adjuvant hormone therapy alone, when used in conjunction with other clinicopathological factors. The assay is not intended for patients with Stage III breast cancer.

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