Prosigna™ Annotated Patient Report

Prognostic information to enhance treatment decisions

Facilitating an integrated approach to the diagnosis and treatment of breast cancer

This guide provides an explanation of the Patient Report generated by the Prosigna Breast Cancer Gene Signature Assay. These annotations provide context surrounding the customized report content, which may be helpful when discussing the results with your patient.

CE

510(k) pending. Not available for sale in the US.

Prosigna®
BREAST CANCER ASSAY

The Prosigna™ Breast Cancer Gene Signature Assay was developed based on a proprietary algorithm and the independently validated PAM50 gene signature. The Prosigna assay outputs information about a patient's Risk of Recurrence (ROR) based on the size, molecular intrinsic subtype, and proliferation status of the tumor, as well as the patient's nodal status, in the context of a validation data set of more than 2400 postmenopausal women with early-stage breast cancer.[1]

The Prosigna Patient Report provides relevant information to patients and clinicians, and each report is **customized** to contain test results and interpretive information specific to your patient. Page 1 of the Patient Report informs your patient of her intrinsic subtype, risk category, and ROR score, which is interpreted through a correlation to probability of distant recurrence over a 10-year period.

The patient's specific tumor size and nodal status are required to determine ROR and risk group classification.

Probability of distant recurrence is determined for each nodal status in the validation data set. This result is reported as a percentage of the total number of patients with a similar nodal status from the robust validation studies.

ROR is derived from a proprietary algorithm and is reported on a scale from 0 to 100, which is adjusted based on nodal status. Risk groups and the ROR scale are impacted by the number of positive nodes entered into the patient's profile.

Prosigna
BREAST CANCER ASSAY

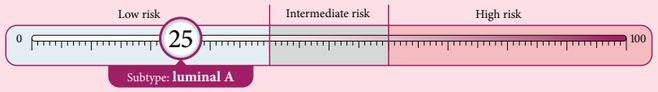
Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm** Lymph Nodes: **node-negative**	ID #: **123-45-6789** Date Reported: **September 13, 2012**	**Enter comments here**

Assay Description:

The Prosigna™ breast cancer gene signature assay measures the expression of 50 different genes to identify subtype and report a Risk of Recurrence Score (ROR), which is used to assign the patient to a predefined risk group. These results are derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, and clinical variables including tumor size and nodal status.

Risk of Recurrence*:

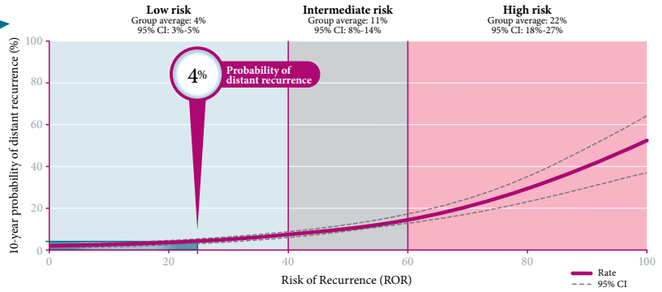


* The ROR ranges from 0 through 100 and correlates with the probability of distant recurrence (DR) in the tested patient population. The risk classification is provided to guide the interpretation of the ROR using cutoffs related to clinical outcome.

Probability of Distant Recurrence:

In the clinical validation studies, patients who were node-negative, luminal A subtype with an ROR score of 25 were in the low-risk group. This group averaged a 4% probability of distant recurrence at 10 years.

The Prosigna algorithm has been validated by 2 randomized clinical trials including more than 2400 patients with varying rates of distant recurrence. An analysis of these 2 clinical validation studies shows that the probability of distant recurrence for the low-risk population is 4%, while the high-risk population has a significantly greater probability of distant recurrence.†



† Data apply to patients being treated with hormone therapy for 5 years as in the tested patient population. See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

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Prosigna assay results are based on a large validation data set of postmenopausal women with early-stage breast cancer, including both node-negative and node-positive patients. The Prosigna report is **customized** and includes only those results relevant to your patient's nodal status.[1]

Number of patients	Nodal status
1786	Node-negative
688	Node-positive

The data on page 2 of the Prosigna Patient Report provide additional context for a patient's reported ROR and intrinsic subtype, allowing the patient's individualized risk of distant recurrence to be evaluated within the context of similar patients from the large validation data set.

Prosigna has been validated in 2 clinical studies consisting of more than 2400 patient samples. Clinical validation studies include the TransATAC and ABCSG-8 trials, with similar study designs and patient populations. Page 2 of the report includes a combined analysis of rate of distant recurrence by subtype, as well as the probability of distant recurrence based on the results of each study individually.

The data and table in the *Clinical Validation Studies* section reflect the patient's comparative intrinsic subtype and nodal status population from the broader validation study population.

These curves depict the probability of distant recurrence for both the node-negative and node-positive populations from the TransATAC and ABCSG-8 studies.

Additional contextual information is provided regarding the patient's specific subtype. This dynamic content reports important clinical information for each of the 4 intrinsic subtypes.

Prosigna
BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm** Lymph Nodes: **node-negative**	ID #: **123-45-6789** Date Reported: **September 13, 2012**	**Enter comments here**

Clinical Validation Studies:

Prognosis for node-negative, luminal A, low-risk breast cancer patients was determined based on the rate of distant recurrence (DR) of this population in 2 prospective-retrospective clinical studies. These studies analyzed more than 2400 samples from postmenopausal women with early stage, hormone receptor-positive breast cancer, using a prospectively defined analysis plan. The data shown are for postmenopausal women with early stage, hormone receptor-positive breast cancer who received 5 years of endocrine therapy after surgical resection of the primary tumor.

Rate of Distant Recurrence (DR) for node-negative patients

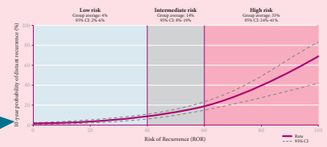
Subtype	Luminal A [95% CI]	Luminal B [95% CI]	HER2-enriched	Basal-like
Rate of DR	**5%** [4%-7%]	**18%** [15%-22%]	*	*

*There were insufficient numbers of basal-like and HER2-enriched patients in these studies to produce data.

Subtype and Prognosis:

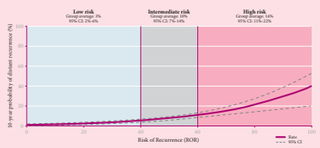
Intrinsic subtype is related to prognosis in the tested patient population. The most common subtypes of breast cancer are the luminal subtypes: luminal A and luminal B. In the combined analysis of 2 clinical validation studies of hormone receptor-positive patients, 68% of the tested patient population was found to be luminal A, and 27% was luminal B.[1] The gene expression pattern of these subtypes resembles the luminal epithelial component of the breast. These tumors are characterized by high expression of estrogen receptor (ER), progesterone receptor (PR), and genes associated with ER activation.[2] Luminal A breast cancers exhibit low expression of genes associated with cell cycle activation and generally have a better prognosis than luminal B.

TransATAC clinical validation study[1]:



The TransATAC study analyzed 1007 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

ABCSG-8 clinical validation study[3]:



The ABCSG-8 study analyzed 1478 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

*See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

REFERENCES: 1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5. **2.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167. **3.** Gnant M, Filipits M, Mlineritsch B, et al. Clinical validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study. Presented at: San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P2-10-02.

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The validation data set included patients with each of the 4 subtypes: luminal A, luminal B, HER2-enriched, and basal-like. Despite the large total sample size of more than 2400 patients, there were insufficient numbers of basal-like and HER2-enriched patients to customize data for those subtypes.[1]

Number of patients	Subtype
1691	Luminal A
682	Luminal B
89	HER-2 enriched
17	Basal-like

Breast cancer is a heterogenous disease that can be subclassified into 4 intrinsic subtypes with distinct genotypes[2] and responses to therapies.[3]

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BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

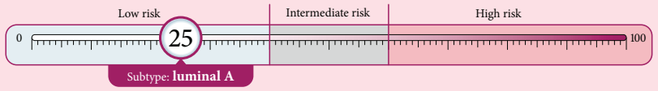
A

Assay Description:

The Prosigna™ breast cancer gene signature assay measures the expression of 50 different genes to identify subtype and report a Risk of Recurrence Score (ROR), which is used to assign the patient to a predefined risk group. These results are derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, and clinical variables including tumor size and nodal status.

B

Risk of Recurrence*:



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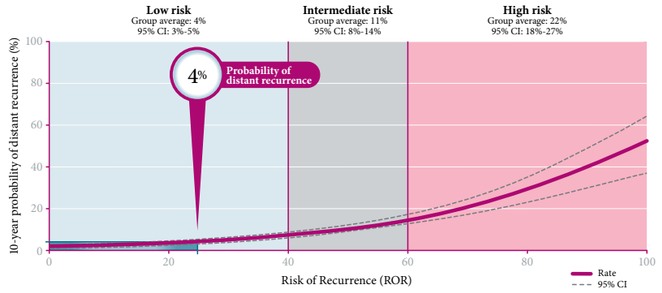
Probability of Distant Recurrence:

In the clinical validation studies, patients who were node-negative, luminal A subtype with an ROR score of 25 were in the low-risk group. This group averaged a 4% probability of distant recurrence at 10 years.

The Prosigna algorithm has been validated by 2 randomized clinical trials including more than 2400 patients with varying rates of distant recurrence. An analysis of these 2 clinical validation studies shows that the probability of distant recurrence for the low-risk population is 4%, while the high-risk population has a significantly greater probability of distant recurrence.†



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Genomic testing augments information obtained from clinicopathological variables. Prosigna™ provides a personalized molecular profile of your patient's tumor biology, enabling a risk-adapted treatment approach.[1]

Assay Description

A Subtype-based gene expression analysis

More than a decade of research into the gene expression patterns of invasive breast cancer has revealed 4 biologically and clinically distinct subtypes: luminal A, luminal B, HER2-enriched, and basal-like.[4] Subtypes are characterized by distinct patterns of gene expression.[2] Prosigna was developed based on the PAM50 gene signature, which measures the expression of 50 genes to classify tumors based on subtype.[4] An independent study conducted by The Cancer Genome Atlas (TCGA) supports PAM50 as a powerful tool for subtype classification, so you can be confident in your patient's tumor subtype classification.[5]

B The Prosigna algorithm[1]

The outputs provided on the patient report are generated using the Prosigna algorithm, which combines genomic data with clinical covariates to give you a comprehensive analysis of your patient's tumor. The PAM50 gene signature is weighted with intrinsic subtype, tumor size, and proliferation score including Ki-67. Each of these factors correlates with prognosis. Prosigna consolidates this information into a numerical risk score, or ROR, that is independently associated with outcome in postmenopausal women with hormone receptor-positive, early-stage breast cancer.

ROR score is calculated from a subset of patients within the 2400-patient analysis pool; patients in this subset have similar clinical features and tumor characteristics. This approach produces an individualized ROR score that is specific to your patient's nodal status, tumor size, and proliferation score.[1]

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BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

Assay Description:

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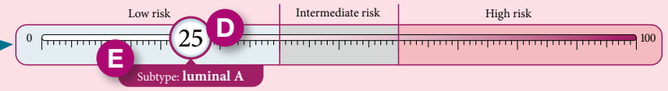
C

Risk of Recurrence*:



D E

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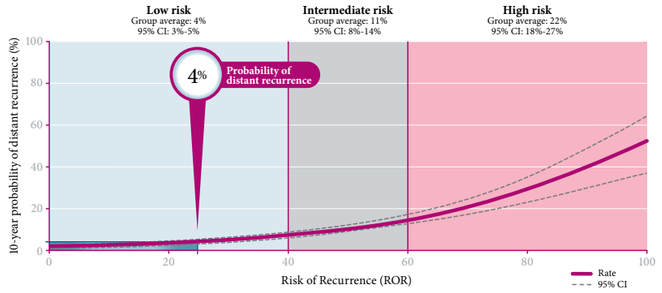

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Based on the 2011 St. Gallen guidelines, the selection of systemic therapy should follow intrinsic subtypes.[3]

C Risk of Recurrence

The ROR section contains 3 key pieces of information specific to the patient:

1. ROR provided as an integer score of 0 to 100 on a sliding scale
2. Subtype classification: luminal A, luminal B, HER2-enriched, or basal-like
3. Risk classification (low, intermediate, or high) based on cutoffs related to clinical outcome

In the clinical validation studies, the ROR score, risk classification, and differentiation between luminal A and luminal B subtypes added statistically significant prognostic information beyond the clinical treatment score ($P<0.0001$). This information can be used in combination with other patient risk factors to determine whether additional chemotherapy beyond endocrine therapy may be required.[1]

D ROR[1]

ROR is derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, tumor size, and proliferation score. The integer value of 0 to 100 correlates with the 10-year probability of distant recurrence.

E Subtype classification

Perou et al first recognized that breast cancer can be classified into 4 intrinsic subtypes.[2,4] Subtypes have different prognoses and sensitivity to systemic therapy.[4,6] The St. Gallen guidelines recommend adjuvant endocrine therapy for patients with luminal A tumors and the addition of chemotherapy for patients with luminal B, HER2-enriched, and basal-like tumors.[3]

Prosigna
BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

Assay Description:

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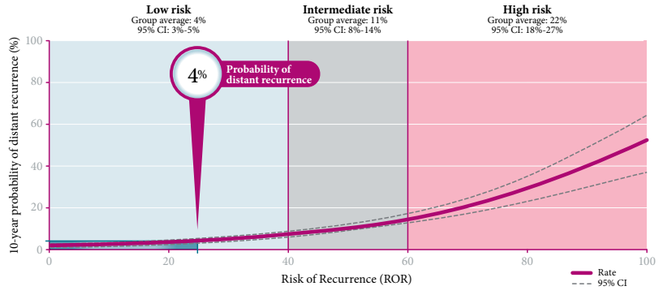

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Prosigna
BREAST CANCER ASSAY

Patient Report:

Patient | Specimen | Comments
Tumor Size (cm): <=2cm | ID #: 123-45-6789 | Enter comments here
Lymph Nodes: node-negative | Date Reported: September 13, 2012

Assay Description:
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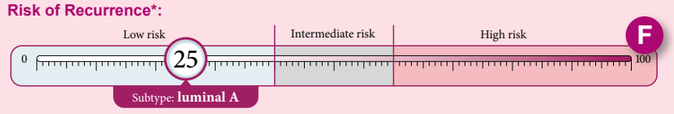

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G

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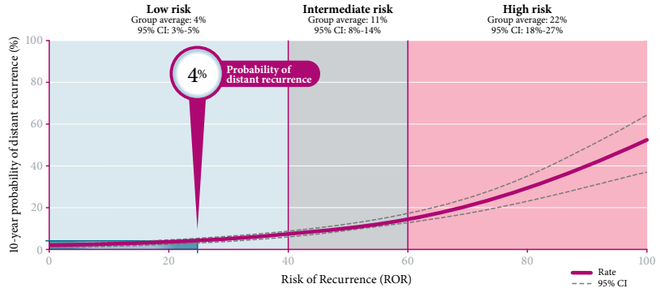

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The continuum of risk is an estimation derived from the broader population of patients with similar nodal status.[1]

F Risk classification[1]

ROR and nodal status are used to assign the patient to a predefined risk group that correlates with the 10-year probability of distant recurrence.

- Low risk: <10% predicted risk
- Intermediate risk: 10% to 20% predicted risk
- High risk: >20% predicted risk

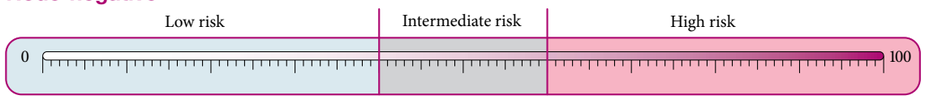
The risk classification cutoffs differ for node-negative and node-positive patients. Consistent with the TNM staging system that is used to define prognosis, ROR is a genomic form of T stage that contains tumor size and expression characteristics but can only be interpreted in the context of a patient's nodal status, or N stage. Therefore, a score of 20 would be classified as low risk for a node-negative patient, whereas the same score would be considered intermediate risk in a patient with 1 to 3 positive nodes because the node-positive patient has a higher probability of 10-year distant recurrence.

Patients with 4 or more positive nodes are classified as high risk; however, there were insufficient numbers of these patients to produce data. Given the limited size of this patient population, the report has been adapted to focus on risk of distant recurrence (see page 11).

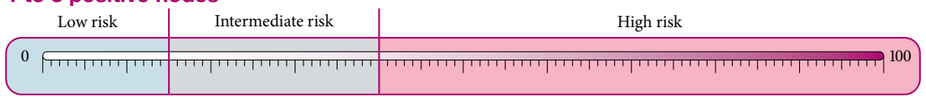
F ROR Scale Variations[1]

The ROR scale includes the risk-adjusted cutoffs for risk group classification, which are different for node-positive and node-negative patients. An ROR of 25 for a node-negative patient has a different risk of distant recurrence at 10 years than does the same score for a node-positive patient.

Node-negative



1 to 3 positive nodes



G Probability of Distant Recurrence[1]

Estimation of risk is derived from the composite patient population across both clinical studies to provide a point estimate of your patient's 10-year probability of distant recurrence. The validation data set is derived from >2400 patients across 2 clinical studies, and the graph is based on data from the N=1786 node-negative patients or N=688 node-positive patients to match your patient's nodal status. All of the patients in the clinical validation studies were postmenopausal to match the intended use population. Using data from a large number of patients across multiple clinical validation studies minimizes the variability of the estimation and reinforces the validity of the data.

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Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

Clinical Validation Studies:

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Rate of Distant Recurrence (DR) for node-negative patients

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Rate of DR	**5%** [4%-7%]	**18%** [15%-22%]	*	*

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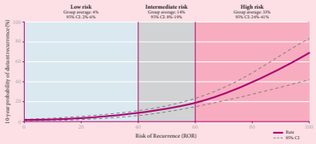

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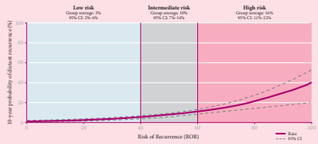

The ABCSG-8 study analyzed 1478 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

*See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

REFERENCES: 1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5. **2.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167. **3.** Gnant M, Filipits M, Mlineritsch B, et al. Clinical validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study. Presented at: San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P2-10-02.

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The contextual information provided is tailored to each subtype and may be helpful when considering treatment.

This table is used to demonstrate the risk of distant recurrence for all subtypes within a specific nodal status group. In many cases, HER2-enriched and basal-like subtypes do not include data, since too few patients with these subtypes exist in the study population.[1]

Prosigna
BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

Clinical Validation Studies:

Prognosis for node-negative, luminal A, low-risk breast cancer patients was determined based on the rate of distant recurrence (DR) of this population in 2 prospective-retrospective clinical studies. These studies analyzed more than 2400 samples from postmenopausal women with early stage, hormone receptor-positive breast cancer, using a prospectively defined analysis plan. The data shown are for postmenopausal women with early stage, hormone receptor-positive breast cancer who received 5 years of endocrine therapy after surgical resection of the primary tumor.

Rate of Distant Recurrence (DR) for node-negative patients

Subtype	Luminal A [95% CI]	Luminal B [95% CI]	HER2-enriched	Basal-like
Rate of DR	**5%** [4%-7%]	**18%** [15%-22%]	*	*

*There were insufficient numbers of basal-like and HER2-enriched patients in these studies to produce data.

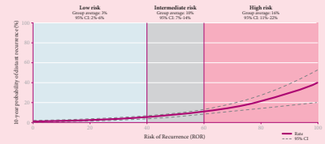
Subtype and Prognosis:

Intrinsic subtype is related to prognosis in the tested patient population. The most common subtypes of breast cancer are the luminal subtypes: luminal A and luminal B. In the combined analysis of 2 clinical validation studies of hormone receptor-positive patients, 68% of the tested patient population was found to be luminal A, and 27% was luminal B.[1] The gene expression pattern of these subtypes resembles the luminal epithelial component of the breast. These tumors are characterized by high expression of estrogen receptor (ER), progesterone receptor (PR), and genes associated with ER activation.[2] Luminal A breast cancers exhibit low expression of genes associated with cell cycle activation and generally have a better prognosis than luminal B.

TransATAC clinical validation study[1]:

The TransATAC study analyzed 1007 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

ABCSG-8 clinical validation study[3]:



The ABCSG-8 study analyzed 1478 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

*See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

REFERENCES: 1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5. **2.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167. **3.** Gnant M, Filipits M, Mlineritsch B, et al. Clinical validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study. Presented at: San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P2-10-02.

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Clinical Validation Studies

This table in the *Clinical Validation Studies* section provides the predicted likelihood of 10-year distant recurrence as a function of nodal status and subtype.

H Nodal status[1]

The clinical validation studies included robust numbers of both node-negative (N=1786) and node-positive (N=688) patients (for a total of >2400). The data are derived solely from the subset of the validation cohort that matches the nodal status of your patient, providing a customized risk assessment in the context of comparable patient populations.

I Subtype

The most common subtypes of breast cancer are the luminal subtypes: luminal A and luminal B. In the combined analysis of 2 clinical validation studies of patients with hormone receptor-positive breast cancer, 68% of the tested patient population was found to be luminal A (N=1691) and 28% was luminal B (N=682). The total number of HER2-enriched and basal-like patients were 89 and 17, respectively.[1] The limited numbers of basal-like and HER2-enriched patients are consistent with findings in the broader population of patients with breast cancer.[4] Given the limited size of these populations, their report has been adapted to focus on risk of distant recurrence.[1]

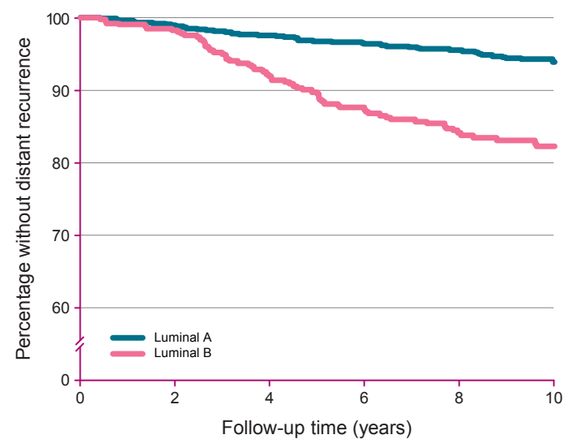
J Subtype and Prognosis

Subtypes provide valuable prognostic information to guide treatment decisions. Intrinsic subtype is related to prognosis in the tested patient population.[1] Luminal A and luminal B subtypes have different gene expression profiles and significantly different rates of DRFS.[1,7-9]

According to the St. Gallen guidelines, systemic therapy recommendations should follow intrinsic subtype classification.

The guidelines recommend endocrine therapy alone for patients with luminal A tumors, endocrine therapy plus chemotherapy for luminal B, the addition of anti-HER2 therapy for HER2-positive, and chemotherapy alone for basal-like tumors.[3]

DRFS in luminal A vs luminal B breast cancer[1]



Prosigna
BREAST CANCER ASSAY

Patient Report:

Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-negative**	Date Reported: **September 13, 2012**	

Clinical Validation Studies:

Prognosis for node-negative, luminal A, low-risk breast cancer patients was determined based on the rate of distant recurrence (DR) of this population in 2 prospective-retrospective clinical studies. These studies analyzed more than 2400 samples from postmenopausal women with early stage, hormone receptor-positive breast cancer, using a prospectively defined analysis plan. The data shown are for postmenopausal women with early stage, hormone receptor-positive breast cancer who received 5 years of endocrine therapy after surgical resection of the primary tumor.

Rate of Distant Recurrence (DR) for node-negative patients

Subtype	**Luminal A** [95% CI]	**Luminal B** [95% CI]	HER2-enriched	Basal-like
Rate of DR	**5%** [4%-7%]	**18%** [15%-22%]	*	*

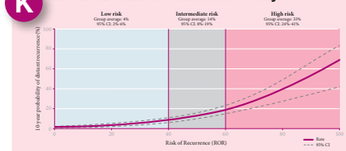
*There were insufficient numbers of basal-like and HER2-enriched patients in these studies to produce data.

Subtype and Prognosis:

Intrinsic subtype is related to prognosis in the tested patient population. The most common subtypes of breast cancer are the luminal subtypes: luminal A and luminal B. In the combined analysis of 2 clinical validation studies of hormone receptor-positive patients, 68% of the tested patient population was found to be luminal A, and 27% was luminal B.[1] The gene expression pattern of these subtypes resembles the luminal epithelial component of the breast. These tumors are characterized by high expression of estrogen receptor (ER), progesterone receptor (PR), and genes associated with ER activation.[2] Luminal A breast cancers exhibit low expression of genes associated with cell cycle activation and generally have a better prognosis than luminal B.

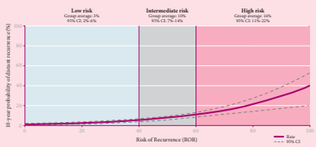
K

TransATAC clinical validation study[1]:



The TransATAC study analyzed 1007 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

ABCSG-8 clinical validation study[3]:



The ABCSG-8 study analyzed 1478 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

*See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

Two clinical validation trials were conducted in similar patient groups, which provided the ability to combine data across trials for a robust validation data set. Curves from the individual validation trials are included for your reference, and demonstrate the consistency of the data across 2 large studies.[1]

REFERENCES: 1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5. **2.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160-1167. **3.** Gnant M, Filipits M, Mlineritsch B, et al. Clinical validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study. Presented at: San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P2-10-02.

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Prosigna
BREAST CANCER ASSAY

Patient Report:

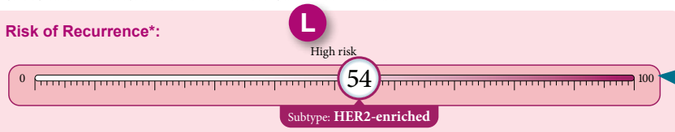
Patient	Specimen	Comments
Tumor Size (cm): **<=2cm**	ID #: **123-45-6789**	**Enter comments here**
Lymph Nodes: **node-positive (≥4 nodes)**	Date Reported: **September 13, 2012**	

Assay Description:

The Prosigna™ breast cancer gene signature assay measures the expression of 50 different genes to identify subtype and report a Risk of Recurrence Score (ROR), which is used to assign the patient to a predefined risk group. These results are derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, and clinical variables including tumor size and nodal status.

Risk of Recurrence*:

L



* The ROR ranges from 0 through 100 and correlates with the probability of distant recurrence (DR) in the tested patient population. The risk classification is provided to guide the interpretation of the ROR using cutoffs related to clinical outcome.

All patients with 4 or more positive nodes are considered high risk regardless of ROR score or intrinsic subtype.[1]

Probability of Distant Recurrence:

In the clinical validation studies, patients who were nodepositive (≥4 nodes), HER2enriched subtype, with an ROR score of 54 were at high-risk of distant recurrence. This group averaged a 43% probability of distant recurrence at 10 years.

The Prosigna algorithm has been validated by 2 randomized clinical trials including more than 2400 patients with varying rates of distant recurrence. An analysis of these 2 clinical validation studies shows that the probability of distant recurrence for the highrisk population ranged from 3152% (95% CI).†

Clinical Validation Studies:

Prognosis for nodepositive (≥4 nodes), HER2enriched, highrisk breast cancer patients was determined based on the rate of distant recurrence (DR) ofthis population in 2 prospective-retrospective clinical studies. These studies analyzed more than 2400 samples from postmenopausal women with earlystage, hormone receptor-positive breast cancer, using a prospectively defined analysis plan. The data shown are for postmenopausal women with earlystage, hormone receptor-positive breast cancer who received 5 years of endocrine therapy after surgical resection of the primary tumor.

Rate of Distant Recurrence (DR) for node-positive (≥4 nodes) patients

Subtype	**Luminal A** [95% CI]	**Luminal B** [95% CI]	HER2-enriched	Basal-like
Rate of DR	**32%** [21%-46%]	**32%** [45%-79%]	*	*

*There were insufficient numbers of basal-like and HER2-enriched patients in these studies to produce data.

Subtype and Prognosis:

Intrinsic subtype is related to prognosis in the tested population. Prior studies suggest that the HER2-enriched subtype comprises approximately 20% of breast cancers.3 However, HER2-enriched subtype tumors are generally hormone receptor-negative,4 so only 4% of the tested hormone receptorpositive patient population was found to have HER2-enriched breast cancer. Regardless of hormone receptor status, HER2-enriched tumors are HER2-positive in the majority of cases with high expression of the ERBB2 cluster, including ERBB2 and GRB7. Genes associated with cell cycle activation are also highly expressed. Patients with a HER2-enriched tumor generally have poor prognosis compared to the luminal A subtypes.

REFERENCES: 1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5. **2.** Gnant M, et al., P2-10-02. Clinical Validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study, SABCS 2012. **3.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160-1167 **4.** Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res.* 2010;16(21):5222–5232.

†Data apply to patients being treated with hormone therapy for 5 years as in the tested patient population. See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

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K Clinical Validation Studies

These graphs are analogous to the *Probability of Distant Recurrence* graph on page 1, limited to those patients from either the TransATAC or ABCSG-8 study.[1] Data were analyzed using a prospectively defined analysis plan to assess the prognostic information provided beyond that given by a Clinical Treatment Score (CTS).[8,9]

Summary of TransATAC study

- Samples: 1007 FFPE breast tumor samples from postmenopausal women with hormone receptor-positive breast cancer in the monotherapy arms of the ATAC (Arimidex or Tamoxifen Alone or Combined) trial[1]
- Study population: Postmenopausal women with hormone receptor-positive breast cancer treated with 5 years of anastrozole or tamoxifen in the ATAC trial[1]
- Conclusion: Prosigna™ ROR is significantly related to 10-year distant recurrence (P<0.0001) and provides prognostic information beyond CTS[1,8]

Summary of ABCSG-8 study

- Samples: 1478 FFPE breast tumor samples from postmenopausal women with hormone receptor-positive breast cancer who were randomized prior to treatment to 2 years of adjuvant tamoxifen, followed by either 3 years of Arimidex or 3 years of adjuvant tamoxifen[1]
- Study population: Postmenopausal women with hormone receptor-positive breast cancer treated with 2 years of adjuvant tamoxifen, followed by either 3 years of Arimidex or 3 years of adjuvant tamoxifen[1]
- Conclusion: ROR score, ROR-based risk classification, and differentiation between luminal A and luminal B add statistically significant prognostic information beyond CTS (P<0.0001)[1,9]

L High-Risk Patient Report[1]

Patients with 4 or more positive nodes are classified as high risk; however, there were insufficient numbers of these patients to produce data. Given the limited size of this patient population, the report has been adapted to focus on risk of distant recurrence. Patients with involvement of 4 or more lymph nodes have a risk of 10-year distant recurrence >20%.

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References:1. Prosigna [CE-IVD Package Insert] South San Francisco, CA: Veracyte, Inc; 2022-05 LB-0032-01. **2.** Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature.* 2000;406(6797):747-752. **3.** Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol.* 2011;22(8):1736-1747. **4.** Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160-1167. **5.** The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature.* 2012;490(7418):61-70. **6.** Hayes DF. Targeting adjuvant chemotherapy: a good idea that needs to be proven! *J Clin Oncol.* 2012;30(12):1264-1267. **7.** Creighton CJ. The molecular profile of luminal B breast cancer. *Biologics.* 2012;6:289-297. **8.** Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with Oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy [published online July 1, 2013]. *J Clin Oncol.* doi:10.1200/JCO.2012.46.1558. **9.** Gnant M, Filipits M, Mlineritsch B, et al. Clinical validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study. Presented at: San Antonio Breast Cancer Symposium; December 4-8, 2012; San Antonio, TX. Abstract P2-10-02.

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