Breast Cancer Test Suite Physician's Brochure

For In Vitro Diagnostic Use

Caution: US Federal law restricts this device to sale by or on the order of a physician

Introduction

The Agendia Breast Cancer Test Suite consists of three molecular diagnostic tests: MammaPrint®, BluePrint® and TargetPrint®, each providing unique information about breast cancer to help make more informed treatment decisions.

Intended Use

All tests are qualitative, in vitro diagnostic test services and performed in a central laboratory, using the gene expression profile of fresh or formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples.

MammaPrint assesses a patient's risk for distant metastasis (up to 10 years for patients less than 61 years old, up to 5 years for patients ≥ 61 years) when performed with fresh tissue. MammaPrint FFPE assesses a patient's risk for distant metastasis up to 5 years.

BluePrint assesses the molecular subtype of breast cancer and informs if tumors are Basal-type, Luminal-type or HER2-type.

TargetPrint is a single gene read-out expression analysis for ER, PR and HER2.

The Agendia Breast Cancer Test Suite is performed in the US for breast cancer patients, with Stage I or Stage II disease, with a tumor size of ≤ 5.0 cm, independent of estrogen receptor status (ER+/-) and lymph-node negative. Outside the U.S., also to be used for breast cancer patients with up to 3 positive lymph-nodes.
The result is indicated for use by physicians as a prognostic marker only, in conjunction with other clinico-pathological factors.

Summary

The analysis is based on several processes: isolation of RNA from fresh frozen or FFPE breast cancer tissue sections; elimination of gDNA; linear amplification and labeling of DNAse treated RNA (fresh only); cRNA purification; hybridization of the cRNA to the diagnostic microarray (fresh only); reverse transcription of RNA resulting in cDNA; amplification and labeling of the cDNA (FFPE only); hybridization of the amplified and labeled cDNA to the diagnostic microarray; washing and scanning the diagnostic microarray and data acquisition (feature extraction); calculation and determination of the risk of recurrence (MammaPrint) or determination of the molecular subtype (BluePrint) or quantification of gene expression levels (TargetPrint).

The MammaPrint analysis (for both fresh and FFPE tissue) is designed to determine the gene activity of specific genes in a tissue sample. The result is an expression profile, or "fingerprint", of the sample. Using this expression profile, the MammaPrint Index is calculated and the molecular prognosis profile of the sample is determined (Low Risk, High Risk). The genes and scoring algorithm for MammaPrint FFPE are the same as those used for MammaPrint, performed with fresh and fresh-frozen tissue [2-4 & 7-10].

The BluePrint analysis is designed to determine the gene activity of specific genes in a tissue sample. The result is an expression profile, or "fingerprint", of the sample. The correlation of the expression profile to the templates (the average mRNA expression levels of Luminal-, Basal- and HER2-type tumors) is calculated (BluePrint Index) and the molecular subtype of the sample is determined (i.e. Basal-type, Luminal-type, HER2-type).

The TargetPrint analysis is designed to determine the mRNA levels of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in a tissue sample using DNA microarray technology.

The results for ER, PR and HER2 expression are quantitative and will also be presented in a binary mode (positive /negative).
A positive result by TargetPrint for ER and PR is equivalent to a 1% or higher IHC positively stained tumor.
For HER2, a TargetPrint HER2 positive result is equivalent to an IHC score of 3+ or to an IHC score of 2+ that has been confirmed to be HER2 positive by in-situ hybridization (FISH, CISH).

Warnings and Precautions

MammaPrint, BluePrint and TargetPrint are not indicated as stand-alone tests to determine the outcome of disease, nor to suggest or infer an individual patient's likely response to therapy. Results should be taken in the context of other relevant clinico-pathological factors and standard practice of medicine.

Procedure

a) Patient selection

In the US, patients are eligible if they are diagnosed with breast cancer, Stage I or Stage II, lymph node negative, with a tumor size of ≤ 5.0 cm, independent of estrogen receptor status (ER+/-).
Outside the US, patients are eligible also with up to 3 positive lymph-nodes.

b) Sample Collection, Registration and Shipment.

Samples will be collected by providing the customers with sample collection kits. These kits consist of:

- Sturdy outer box or container
- Sample receptacle and transportation tube (Fresh)
- Biopsy Punch for sub-sampling on surgical specimens (Fresh)
- Tissue RNA preservative solution (Fresh)
- Slidemailer box (including 10x glass slides) (FFPE)
- 2 zip-lock bags (FFPE)
- Courier transportation materials
- Test Request Form (optional)
- Identification stickers
- Sampling Instruction Folder
- Physician's Brochure

Registration is initiated by notification from the ordering health care provider. This notification (Test Request Form) can take place by fax, online customer portal or other communication channel. Agendia registers all related sample and patient information. The sample is shipped directly to Agendia's central laboratory by the ordering health care provider, at ambient temperature, using the courier transportation materials provided. For non-US / non-Latin America requests, samples should be shipped to Amsterdam, The Netherlands. For all US, Puerto Rico and Latin America requests, samples should be shipped to Irvine, California, USA.

c) Sample Analysis at Agendia

To assess the gene activity in a fresh sample, frozen tissue sections are made using a freeze microtome, and are collected in a receptacle. For FFPE tissue samples, the provided glass slides with FFPE tissue sections are used or slides are made from the FFPE tumor block using a standard microtome. Total RNA is extracted from the tissue sections using a standard commercially available isolation kit. The RNA sample is purified, amplified, and labeled with a cyanine-CTP/ dUTP fluorescent dye.
The RNA/cDNA sample is hybridized on a specifically designed diagnostic microarray (8-pack, Agilent Technologies).
An Agilent microarray scanner is used for scanning the diagnostic microarray and the result is a scan file (TIFF). This file is used by the Agilent Feature Extraction Software. The Feature Extraction Software analyzes the scan file (TIFF) by determining the relative fluorescent intensities of the individual features against the diagnostic microarray chip design file as a template in order to identify control features, normalization features and reporter gene features. The fluorescent intensities of the features are a measure for the expression of particular genes.

d) Data Analysis and Reporting

MammaPrint and MammaPrint FFPE

Data analysis is performed according to the MammaPrint algorithm, which calculates the MammaPrint Index, and determines the molecular prognosis profile of the sample (Low Risk, High Risk).
Samples with a MammaPrint index value greater than 0 are classified as Low Risk, and samples with a value less than or equal to 0 are classified as High Risk.
The MammaPrint Index of a sample can fall within a pre-defined area around the classification threshold between the High Risk and Low Risk profile in which the MammaPrint result has <90% classification accuracy (i.e., borderline sample). When a sample is considered to be "borderline", it is clearly indicated on the MammaPrint analysis report.

BluePrint

Data analysis is performed according to a specific BluePrint algorithm which calculates the BluePrint Index and determines the molecular subtype of the sample (Basal-type, Luminal-type or HER2-type).

TargetPrint

Data analysis is performed by calculation of scores by measurement of the intensity of ER, PR and HER2 genes. After the TargetPrint ER, PR and HER2 scores are calculated the outcomes can be determined based on these scores. Samples with a TargetPrint score above or equal to 0 are classified as Positive and values below 0 are classified as Negative.

Extensive Quality Controls (>25) are implemented in order to ensure the correct analytical result. QC's together with the result are reported internally to the Laboratory Director.
Agendia issues a final report to the health care provider, including patient's demographic data and test result(s).

Limitations of the Procedure

The Agendia Breast Cancer Test Suite has been validated for use only with human female breast cancer tumor tissue. Testing of other specimen types may result in incorrect results or no results.
Reliable results are dependent on adequate specimen collection and transport procedures.

Expected Values

MammaPrint and MammaPrint FFPE

The MammaPrint result is given as "Low Risk" or "High Risk" for risk of recurrence.
The MammaPrint Index of a sample can fall within a pre-defined area around the classification threshold in which the MammaPrint result has <90% classification accuracy (i.e., borderline sample). When a sample is considered to be "borderline", it is clearly indicated on the MammaPrint analysis report.

BluePrint

Basal-type

Basal-type breast cancers are characterized by gene expression of the basal/myoepithelial cells of origin. The Basal-type cancers are typically triple-negative for ER, PR and HER2 (basal-like) with a specific gene expression profile. Hormone therapy and anti-HER2 therapies, such as trastuzumab and lapatinib, are not believed to be effective against these cancers, although chemotherapy is thought to be helpful. A Basal-type BluePrint result means that the tumor phenotype most closely resembles the Basal-type intrinsic subtype.

Luminal-type

Luminal-type breast cancers are characterized by gene expression of the luminal epithelial cells that line the breast ducts and glands. The Luminal-type cancers are typically hormone receptor positive tumors and are likely responsive to hormonal therapy.

A Luminal-type BluePrint result means that the tumor phenotype most closely resembles the Luminal-type intrinsic subtype.
Patients classified as MammaPrint Low Risk and Luminal-type can be expected to have a clinical course similar to luminal A, usually treated with hormonal therapy, whereas those with a MammaPrint High Risk and Luminal-type, can be expected to have a clinical course similar to luminal B patients who usually benefit from more aggressive treatment which may include chemotherapy.

HER2-type

The HER2-type breast cancers are characterized by amplification or over-expression of the HER2 locus.
The HER2-type cancers are typically HER2-positive tumors by IHC or FISH (HER2/neu positive). These cancers tend to grow more rapidly and may recur, although they can often be treated with targeted therapies such as trastuzumab and lapatinib. A HER2-type BluePrint result means that the tumor phenotype most closely resembles the HER2-type intrinsic subtype.

TargetPrint

ER/PR

TargetPrint provides qualitative results supported by quantitative measurements that give health care providers additional insight into the biology of each individual tumor and assists in treatment decisions. A positive ER measurement predicts a Tamoxifen benefit [5].

HER2

Quantitative data of the HER2 expression level in an individual tumor can affect the choice of treatment. HER2 is an important target of the monoclonal antibody, trastuzumab (Herceptin®). Trastuzumab is only effective in breast cancer where the HER2 receptor is over-expressed. [6]

Performance Characteristics

The Agendia Breast Cancer Test Suite performance characteristics investigated comprise: precision, reproducibility, threshold, sensitivity, specificity and accuracy. Independent matched FFPE and fresh tumor samples were analyzed showing a high concordance for analytical and clinical performance [8].

MammaPrint

Results are based on previously published [1] 70-gene molecular prognosis profile in breast tumors. Good outcome patients are classified as Low Risk (i.e. no distant metastasis within at least 5 years). Poor outcome patients are classified as High Risk, (i.e. distant metastasis within 5 years). The MammaPrint Index numerical value is provided.

As published in the reference group, lymph node negative patients under 61 years old classified as "Low Risk" had a 10% chance to develop distant metastases at 10 years without any adjuvant treatment. Patients classified as "High Risk" had a 29% chance to develop distant metastases at 10 years without adjuvant treatment.
MammaPrint has been validated in over 774 breast cancer patients and shown to provide information independent of traditional clinico-pathological risk assessment [2, 3, 9, 10 and 14].

In a consecutive series[1], 131 Dutch patients were selected according to the following criteria: female, unilateral T1 or T2 primary invasive carcinoma, negative nodal status, age between 55 and 87 years at diagnosis, no adjuvant therapy. In this group, MammaPrint has shown significant prognostic value to predict the development of distant metastasis up to 5 years: "Low Risk" had a 7% chance to develop distant metastases at 5 years without any adjuvant treatment. Patients classified as "High Risk" had a 22% chance to develop distant metastases at 5 years without adjuvant treatment.

MammaPrint was developed using adjuvant-untreated, lymph node negative, mainly European patients to capture the biology of the primary tumor in a gene expression profile. It is generally believed that US and European breast cancer is not dissimilar, in particular not for countries where breast cancer screening programs are implemented. [2, 3]

In January 2013, the 5 year outcome results of the prospective observational RASTER study were published [4]. This impact study was a 'first of its kind' biomarker centric trial in which the MammaPrint Fresh assay was performed and reported on 427 early stage breast cancer patients aged 18-61 years old, pT1 and pT2, Lymph node negative, ER+/-, HER 2 +/- prior to the physician-patient decision for adjuvant therapy. Patients were treated according to standard of practice guidelines taking into account all relevant clinico-pathological factors and the MammaPrint Fresh signature results.

Subsequently MammaPrint FFPE was also performed on FFPE tissue of the RASTER patients. Results from MammaPrint Fresh and MammaPrint FFPE were compared for the 345 paired fresh and FFPE samples with 5 year outcome data from the 427 RASTER patient samples [4]. Not accounting for any covariates other than the patient's MammaPrint FFPE status, patients classified as 'Low Risk' by MammaPrint FFPE (71 adjuvantly treated and 108 adjuvantly not treated), demonstrated a 1.3% (95% CI 0 – 3.1) chance of cancer recurrence within 5 years.

Patients classified as 'High Risk' by MammaPrint FFPE (145 adjuvantly treated and 21 adjuvantly not treated), demonstrated an 11.7% (95% CI 6.6 – 16.8) chance of cancer recurrence within 5 years [1].

Prognostic assessment of MammaPrint® FFPE was further investigated using univariate and multivariate analyses [1]. In the univariate and multivariate analyses, a MammaPrint FFPE High/Low Risk result is significantly associated with high/low risk for recurrence.

Univariate analysis: DRFI (Distant Recurrence Free Interval) at 5 years n=345

Variable		p-value	HR	95% CI	
MammaPrint FFPE	High vs Low	0.002	10.366	2.405	44.680
Age	Age<=50 vs Age>50	0.778	1.135	0.470	2.739
Tumor size		0.003	1.047	1.016	1.079
Grade	1	0.008	1.000		
	2		2.430	0.292	20.182
	3		8.675	1.135	66.325
ER	Pos vs. Neg	0.002	0.244	0.101	0.586
Her2	Pos vs. Neg	0.074	2.718	0.908	8.134
ET	Non vs. ET	0.207	0.540	0.208	1.406

Multivariate analysis: DRFI at 5 years n=345

Variable		p-value	HR	95% CI	
MammaPrint FFPE	High vs Low	0.007	10.582	1.902	58.876
Age	Age<=50 vs Age>50	0.173	1.879	0.759	4.656
Tumor size		0.009	1.072	1.017	1.129
Grade	1	0.663			
	2		1.461	0.160	13.381
	3		2.589	0.206	32.528
ER	Pos vs. Neg	0.280	2.840	0.427	18.887
Her2	Pos vs. Neg	0.459	1.531	0.496	4.722
ET	Non vs. ET	0.069	0.209	0.039	1.128

MammaPrint has been independently validated in studies on over 12,000 breast cancer patients with results published in leading peer reviewed medical and scientific journals internationally and shown to provide information independent of clinico-pathological risk assessment.

MammaPrint precision and repeatability was assessed by an independent inter-laboratory study in Agendia's two Clinical Laboratory Improvement Amendments (CLIA) certified laboratories in The Netherlands and the USA [7, 8].
Based on the analytical performance of MammaPrint, the precision of classifying a sample as High Risk or Low Risk is 99% for fresh and 97.3% for FFPE with a repeatability of the measurement being 99% for fresh and 97.6% for FFPE [7, 8].
Reproducibility was measured in over 300 control samples and shown to be 99%.

BluePrint

The technical validity of BluePrint is determined on multiple individual validation experiments [11] and a comprehensive inter-laboratory comparison study.
Based on the analytical performance of BluePrint, the precision of classifying as Luminal-type, Basal–type or Her2-type is 99.3% for fresh and 98.6% for FFPE, with the repeatability of the measurement being 99.6% for fresh and 99.0% for FFPE.

TargetPrint

TargetPrint fresh has been developed in over 600 breast cancer samples against conventional IHC and FISH classification, allowing validation of the microarray values to IHC and FISH equivalence.[12] For ER and PR a threshold of 1% IHC positively stained tumor cells was used to classify samples as positive; for HER2, an IHC score of 3+ was considered positive. In case of 2+ samples, FISH assessment determined final HER2 amplification status [12, 13].
Concordance was shown to be 98% [95% confidence interval (CI): 96-99%] for ER, 85% [95% CI: 82-88%] for PR, and 96% [95% CI: 95-98%] for HER2 for fresh [15].
Based on the analytical performance of TargetPrint, the median precision of classifying as ER, PR or Her2, is 99.1% for fresh and 97.8% for FFPE, with the median repeatability of the measurement being 99.1% for fresh and 98.4% for FFPE.

The performance characteristics are based on the studies and papers listed below.

References

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Regulatory Disclaimer:

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