MammaPrint® 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

Intended Use

MammaPrint® is a qualitative in vitro diagnostic test, performed in a central laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess 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).

The test 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 MammaPrint® result is indicated for use by physicians as a prognostic marker only, along with other clinico-pathological factors.

Summary

The analysis is based on several processes: isolation of RNA from fresh frozen breast cancer tissue sections; DNAse treatment of isolated RNA; linear amplification and labeling of DNAse treated RNA; cRNA purification; hybridization of the cRNA to the diagnostic microarray; washing and scanning the diagnostic microarray and data acquisition (feature extraction); calculation and determination of the risk of recurrence

The MammaPrint analysis is designed to determine the expression 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).

Warnings and Precautions

MammaPrint is not indicated as a standalone test 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
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 U.S., also to be used for breast cancer patients with up to 3 positive lymph-nodes.

b) Sample Collection, Registration and Shipment.
Samples will be collected by providing the ordering health care providers with sample collection kits.
These kits consist of:
- Sturdy outer box or container
- Sample receptacle and transportation tube
- Biopsy Punch for sub-sampling on surgical specimens
- Tissue RNA preservative solution
- 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. 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 fluorescent dye and hybridized on a specifically designed diagnostic micro-array (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
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 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.

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

MammaPrint 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.

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Expected Values

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.

Performance Characteristics

MammaPrint analytical performance metrics that have been investigated include: precision, reproducibility, threshold, sensitivity, specificity and accuracy.

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-certified laboratories in The Netherlands and the USA [5, 6].
Based on the analytical performance of MammaPrint, the precision of classifying a sample as High Risk or Low Risk is 99% with a repeatability of the measurement being 99% [5, 6].
Reproducibility was measured in over 300 control samples and shown to be 99%.

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, 7, 8, and 9].

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].

Published in January 2013, the RASTER [4] study was the first prospective impact study with outcome for a breast cancer risk of recurrence test. This study confirmed the prognostic value of MammaPrint and noted an excellent outcome for Low Risk patients and treated without cytotoxic therapy. Use of MammaPrint reduced the proportion of high risk patient as classified by Adjuvant Online by 20% and omission of chemotherapy appeared not to compromise outcome.

References

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(7) Mook S et al., Ann Oncology, 2010; 21: 717–722
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Regulatory Disclaimer:

Clinical Laboratory Improvement Amendments (CLIA)
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