MammaPrint® FFPE Physician's Brochure

For In Vitro Diagnostic Use
Caution: Federal law restricts this device to sale by or on the order of a physician

Intended Use

MammaPrint® FFPE is a qualitative in vitro diagnostic test, performed in a central laboratory, using the gene expression profile obtained from formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples to assess a patient's risk for distant metastasis within 5 years.

The test is performed for breast cancer patients, with Stage I or Stage II disease, with tumor size ≤ 5.0 cm and lymph node negative. The MammaPrint FFPE 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 FFPE breast cancer tissue sections; elimination of gDNA, reverse transcription of RNA resulting in cDNA; amplification and labeling of the cDNA; 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.

The MammaPrint FFPE 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 FFPE 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 [2-9].

Warnings and Precautions

MammaPrint FFPE 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+/-).

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
- Slidemailer box including 10x glass slides
- 2 zip-lock bags
- 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 expression, 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 treated with DNAse, after which a reverse transcription takes place resulting in cDNA. cDNA is amplified and labeled with a Cy3-dUTP fluorescent dye and hybridized to 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 FFPE Index, and then determines the molecular prognosis profile of the sample (Low Risk vs. High Risk).

Samples with a MammaPrint FFPE 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 FFPE Index of a sample can fall within a pre-defined area around the classification threshold in which the MammaPrint FFPE result has <90% classification accuracy (i.e., borderline sample).

When a sample is considered to be "borderline", it is clearly indicated on the MammaPrint FFPE 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 FFPE 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

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

Performance Characteristics

MammaPrint FFPE analytical performance metrics that have been investigated include: 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 [(6)].

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 (81 adjuvantly treated and 112 adjuvantly not treated), demonstrated a 2.3% (95% CI 0.1-4.5) chance of cancer recurrence within 5 years. Patients classified as 'High Risk' by MammaPrint FFPE (135 adjuvantly treated and 17 adjuvantly not treated), demonstrated an 11.5% (95% CI 6.0-17.0) 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 analysis, a MammaPrint FFPE High/Low Risk result is significantly associated with high/low risk for recurrence. Multivariate analysis of the 345 samples analyzed did not conclusively demonstrate prognostic significance for MammaPrint FFPE beyond that of other clinico-pathological factors. This is attributable to the RASTER study design [(4)], in which MammaPrint result was included along with all relevant clinico-pathological factors, and treatment decisions were guided by assessed prognostic risk and the standard of practice. In this real–world context, the overall cohort experienced a low event rate which, despite the favorable trend, diminishes independent contribution of MammaPrint. Aggregate outcomes were broadly similar between study patients who received adjuvant chemotherapy and study patients who did not.

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	5.443	1.82	16.28
Age	Age<=50 vs Age>50	0.778	1.135	0.470	2.739
Tumor size		0.003	1.047	1.02	1.08
Grade	1	0.008	1.000		
	2		2.430	0.29	20.18
	3		8.675	1.14	66.33
ER	Pos vs Neg	0.002	0.244	0.10	0.59
HER2	Pos vg Neg	0.074	2.718	0.91	8.13
ET	None vs ET	0.207	0.540	0.21	1.41

Multivariate analysis: DRFI at 5 years n=345

Variable		p-value	HR	95% CI	
MammaPrint FFPE	High vs Low	0.087	3.776	0.827	17.250
Age	Age<=50 vs Age>50	0.250	1.708	0.686	4.253
Tumor size		0.013	1.063	1.013	1.115
Grade	1	0.539	1		
	2		1.867	0.206	16.939
	3		3.623	0.285	46.108
ER	Pos vs Neg	0.463	2.071	0.296	14.465
HER2	Pos vg Neg	0.392	1.649	0.525	5.181
ET	None vs ET	0.133	0.270	0.049	1.488

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

References

(1) FDA label - USFDA Clearance; http://www.accessdata.fda.gov website.
(2) Buyse M et. al., J Natl Cancer Inst 2006; 98(17):1183-1192
(3) Van 't Veer et al; Nature 2002; 415(31): 530-536.
(4) Drukker, C. A. et al. Int. J. Cancer 2013; 133(4): 929-36.
(5) Delahaye LJM et al. Pers. Med 2013; 10:801
(6) Sapino A et al. 2014; 16:190
(7) Glas AM, et al., BMC Genomics 2006; 7: 278
(8) Van de Vijver MJ et al., New Engl J Med 2002; 347(25): 1999-2009.
(9) Mook S et al., Ann Oncol 2010, 21:717–722.

Regulatory Disclaimer:

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