ASCO GUIDELINES

Data Supplement

TITLE: USE OF BIOMARKERS TO GUIDE DECISIONS ON ADJUVANT SYSTEMIC THERAPY FOR WOMEN WITH EARLY-STAGE INVASIVE BREAST CANCER: FOCUSED UPDATE OF 2016 AMERICAN SOCIETY OF CLINICAL ONCOLOGY CLINICAL PRACTICE GUIDELINE

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Data Supplement 1

Table 1: Key Definitions and Evidentiary Requirements

Term	Definition	Evidentiary Requirements
analytic validity	The ability to accurately and reliably measure the analyte of interest (e.g., metabolites, proteins, nucleic acids, specific mutations, antigens, etc.) in the clinical laboratory, and in tissue or fluid specimens obtained from patients with the same disease & stage as those who will be tested	• analytic sensitivity & range (detection rate) • analytic specificity (1-false positive rate) • Reliability of test results • assay robustness (analyte stability to pre-analytic & analytic methods) • QA, QC, & proficiency testing data • for new assays and test platforms, comparison to "gold standard"
clinical validity	The ability to accurately and reliably predict the clinically defined disorder, phenotype, or outcome of interest.	• clinical sensitivity & specificity • positive and negative predictive values using valid estimates of prevalence • ROC curve (if appropriate)

clinical utility	The use of test results to guide clinical decisions improves measurable clinical outcomes of patient management, compared with outcomes of management decisions independent of test results	• Direct comparison of outcomes from management decisions consistent with test results versus outcomes of decisions independent of test results
		• Hierarchy of study designs:
		  ○ systematic reviews or meta-analyses
		  ○ RCTs
		  ○ prospective-retrospective studies
		  ○ comparative observational studies

Adapted from Teutsch, et al. 2009[1]

Table 2: Classification of patients according to clinical risk assessment by the modified version of Adjuvant!Online as reported by Cardoso et al, 2016[2]

ER status	HER2 status	Grade	Nodal status	Tumor Size	Clinical Risk in Mindact
ER positive	HER2 negative	well differentiated	N-	≤ 3 cm	**C-low**
				3.1-5 cm	**C-high**
			1-3 positive nodes	≤ 2 cm	**C-low**
				2.1-5 cm	**C-high**
		moderately differentiated	N-	≤ 2 cm	**C-low**
				2.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
		poorly differentiated or undifferentiated	N-	≤ 1 cm	**C-low**
				1.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
	HER2 positive	well differentiated OR moderately differentiated	N-	≤ 2 cm	**C-low**
				2.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
		poorly differentiated or undifferentiated	N-	≤ 1 cm	**C-low**
				1.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
ER negative	HER2 negative	well differentiated	N-	≤ 2 cm	**C-low**
				2.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
		moderately differentiated OR poorly differentiated or undifferentiated	N-	≤ 1 cm	**C-low**
				1.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
	HER2 positive	well differentiated OR moderately differentiated	N-	≤ 1 cm	**C-low**
				1.1-5 cm	**C-high**
			1-3 positive nodes	Any size	**C-high**
		poorly differentiated or undifferentiated	Any	Any size	**C-high**

Table 3: Evidentiary requirement of studies for MammaPrint

Use Context	Marker test	Study	Category of evidence[1]	Level of evidence[1]	Analytical validity[2]	Clinical validity	Clinical utility
Patient Group: Node negative ER Positive HER2 negative **Use:** With-hold adjuvant chemotherapy (prognosis)	70 gene assay (MammaPrint®)	Cardoso et al, (NEJM 2016)[2]	A	1	+	+	+
		Buyse et al , (J Natl Cancer Inst 2006)[4]	D				
		Mook et al, (Breast Cancer Res Treat 2009)[5]	D				
		Bueno-de-Mesquita et al, (Breast Cancer Res Treat 2009)[6]	D				
		Knauer et al, (B J Cancer 2010)[7]	D				
		Knauer et al, (Breast Cancer Res Treat 2010)[8]	D				
		Drukker et al, (Breast Cancer Res Treat 2014)[9]	C				

[1]per Simon RM, Paik S, Hayes DF: Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 101:1446-52, 2009[3]

[2]+ = meets criteria for the column; - = does not meet criteria for the column; N/E = not evaluated

Table 4: Recommendation Table

Marker	Specimen used	Test type	Recommendations	Evidence rating
70 gene assay (MammaPrint®)	Formalin-fixed paraffin-embedded tissues Fresh frozen tissue	Gene expression profile	If a patient has **ER/PgR-positive, HER2-negative, node-negative,** breast cancer, the MammaPrint assay may be used in those with **high clinical risk** per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit.	**Type:** Evidence based **Quality of evidence:** High **Category of evidence:** A **Strength of recommendation:** Strong **Clinical Utility:** No
			If a patient has **ER/PgR-positive, HER2-negative, node-negative,** breast cancer, the MammaPrint assay **should not** be used in those with **low clinical risk** per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy as women in the low clinical risk category had excellent outcomes and did not appear to benefit from chemotherapy even with a genomic high risk cancer.	**Type: Evidence based** **Quality of evidence: High** **Category of evidence: A** **Strength of recommendation: Strong** **Clinical Utility: No**
			If a patient has **ER/PgR-positive, HER2-negative, node-positive,** breast cancer, the MammaPrint assay may be used in patients with 1-3 positive nodes and at **high clinical risk** per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit.	**Type: Evidence based** **Quality of evidence: High** **Category of evidence: A** **Strength of recommendation: Moderate** **Clinical Utility: No**
			If a patient has **ER/PgR-positive, HER2-negative, node-positive,** breast cancer, the MammaPrint assay **should not** be used in patients with 1-3 positive nodes and at **low clinical risk** per MINDACT	**Type:** Informal consensus **Quality of evidence:** Low **Category of evidence:** D **Strength of recommendation:** Moderate **Clinical Utility:** No

			categorization to inform decisions on withholding adjuvant systemic chemotherapy. There are insufficient data on the clinical utility of MammaPrint in this specific patient population.	
			If a patient has **HER2-positive** breast cancer, the clinician should not use the MammaPrint assay to guide decisions regarding adjuvant systemic therapy. Additional studies are required to address the role of MammaPrint in patients with this tumor subtype who are also receiving HER-2-targeted therapy.	**Type:** Informal consensus **Quality of evidence:** Low **Category of evidence:** D **Strength of recommendation:** Moderate **Clinical Utility:** No
			If a patient has ER/PgR negative and HER2-negative breast cancer **(triple negative),** the clinician should not use the MammaPrint assay to guide decisions about adjuvant systemic chemotherapy.	**Type:** Informal consensus **Quality of evidence:** Insufficient **Strength of recommendation:** Strong **Clinical Utility:** No

Data Supplement 2

Table 5. Characteristics of Studies with Evidence on Clinical Utility of Biomarkers to Guide Decisions on Treatment versus No Treatment for Patients Considering Adjuvant Therapy for Early-Stage Breast Cancer

Author Year Reference	Assigned Treatments (N in each arm)	Marker Study Objective (& Tx)	Biomarker Subsets	# and % of Pts.	Patient Characteristics					Disease Characteristics			
					Median Age	Menopausal Status (%)		Distribution by Race	Prior Neo-adjuvant Therapy (%)	Stage	Tumor Size	Nodal Status	ER, PR, & HER2 status
						pre	post						
Mammaprint: Randomized Controlled Trials													
Cardoso et al, (NEJM 2016)[2]	NR/NA	Mammaprint	Low Risk High Risk	6693	55	NR	NR	NR	NR	Grade 1: 1447 Grade 2: 3287 Grade 3: 1927 Missing: 32	<1cm: 920 1 to 2 cm: 3875 >2 to 5 cm: 1819 >5cm: 20	Node Negative: 5288 1 positive: 942 2 positive: 300 3 positive: 154 ≥4 positive: 8	ER+, PR+, or both: 5914 HER2+: 638
Mammaprint: Prospective Studies													
Drukker et al, (Breast Cancer Res Treat 2014)[9]	CT (110) ET (40)	Mammaprint	Low Risk High Risk	295	All patients were less than 53yrs	NR	NR	NR	NR	Grade 1: 75 Grade 2: 101 Grade 3: 119	≤20mm: 155 >20mm: 140	0: 151 1-3: 106 ≥4: 38	ER+: 226
Mammaprint: Retrospective Studies													
Knauer et al, (Breast Cancer Res Treat 2010)[8] Pooled data from 6 studies	ET (315) ET + CT (226)	Mammaprint	Low risk High risk	541 (100%)	NR	NR	NR	NR	0	Grade 1: 134 Grade 2: 233 Grade 3:163 n.a: 11	T1: 279 T2: 254 T3: 7 n.a: 1	N0: 265 N1: 276	ER: 484 PR: 371 HER2: 59
Buyse et al , (J Natl Cancer Inst 2006)[4]	NR/NA	Mammaprint	Low Risk High Risk	307 (100%)	NR	NR	NR	NR	0	Grade 1: 47 Grade 2: 125 Grade 3: 124 Unknown: 6	T1ab: 11 T1c: 99 T2: 192	NR	ER+: 212 ER-: 90
Mook et al, (Breast Cancer Res Treat 2009)[5]	Mastectomy or breast-conserving	Mammaprint	Good Signature	241 (100%)	NR	NR	NR	NR	NR	Grade 1: 57 Grade 2: 99	pT1: 117 pT2: 121 pT3: 3	1 positive node: 123 2 positive nodes: 77	ER+: 191 PR+: 151 HER2+: 37

Author Year Reference	Assigned Treatments (N in each arm)	Marker Study Objective (& Tx)	Biomarker Subsets	# and % of Pts.	Patient Characteristics					Disease Characteristics			
					Median Age	Menopausal Status (%)		Distribution by Race	Prior Neo-adjuvant Therapy (%)	Stage	Tumor Size	Nodal Status	ER, PR, & HER2 status
						pre	post						
	surgery followed by radiotherapy and adjuvant systemic therapy if indicated		Poor Signature							Grade 3: 83 Unknown: 2		3 positive nodes: 41	
Bueno-de-Mesquita et al, (Breast Cancer Res Treat 2009)[6]	Adjuvant Systemic Treatment None (78) CT (18) ET (14) Both (13)	Mammaprint	Good signature Poor signature	123 (100%)	NR	NR	NR	NR	NR	Grade 1: 20 Grade 2: 53 Grade 3: 50	pT1: 76 pT2: 47	NR	ER+: 94 PR+: 84 HER2+: 9
Knauer et al, (B J Cancer 2010)[7]	No Chemotherapy or trastuzumab	Mammaprint	Good signature Poor Signature	168	49	NR	NR	NR	0	Grade ½: 65 Grade 3: 104	T1: 89 T2/3: 79	N0: 96 N1: 73	ER+: 103 PR+: 70 HER2+: 168

Abbreviations: NR, not reported; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CT=Chemotherapy and Tamoxifen; ET=Endocrine Therapy; n.a=Not Available; NR=Not Reported

Table 6. Quality of Studies Reporting Evidence of Clinical Utility of Biomarkers to Guide Decisions on Treatment versus No Treatment for Patients Considering Adjuvant Therapy for Early-Stage Breast Cancer

Author Year Reference	Provided details on randomization	Provided details on blinding	Provided details on any planned analysis	Expected effect size calculation and power calculation	Reported on length of follow-up	Reported on any differences in patient characteristics	Funding source	Overall Potential Risk of Bias [a]
Mammaprint: Randomized Controlled Trials								
Cardoso et al, (NEJM 2016)[2]	√	N/A	√	√	√	√	√	Low

Author Year Reference	Appropriate Study Design	Appropriate Study Population	Adequate Sample Size	Reproducible & Valid Assay Methods	Reliable & Valid Outcomes Measures	Adequate Follow-up	Appropriate Data Analysis Methods	Adequate Reporting of Results	Insignificant COI	Overall Potential Risk of Bias [a]
Mammaprint: Prospective Studies										
Drukker et al, (Breast Cancer Res Treat 2014)[9]	√	√	?	√	√	√	√	√	2 of the authors are named inventors on the patent for the biomarker and 2 are also shareholders of the company that markets the biomarker	Intermediate
Mammaprint: Retrospective Studies										
Knauer et al, (Breast Cancer Res Treat 2010)[8] Pooled data from 6 studies	√	√	?	√	√	√	√	√	2 of the authors work for the company that manufactures the test	Intermediate
Buyse et al , (J Natl Cancer Inst 2006)[4]	√	√	√	√	√	√	√	√	1 of the authors are named inventors on the patent for the biomarker and 3 are also shareholders of the company that markets the biomarker	Intermediate

Mook et al, (Breast Cancer Res Treat 2009)[5]	√	√	?	√	√	√	√	√	1 of the authors is named inventor on the patent for the biomarker and also a shareholder and 2 employees of the company that markets the biomarker	Intermediate
Bueno-de-Mesquita et al, (Breast Cancer Res Treat 2009)[6]	√	√	?	√	√	√	√	√	3 of the authors are named inventors on the patent for the biomarker and one is a shareholder and 2 are employees of the company that markets the biomarker	Intermediate
Knauer et al, (B J Cancer 2010)[7]	√	√	?	√	√	√	√	√	study was supported by the Breast Cancer Research Foundation and unrestricted educational grants from the Austrian Society of Surgery and Agendia BV, Amsterdam	Intermediate

Note: √ indicates criterion was met; - indicates criterion was not met; ? indicates insufficient detail, not reported, and/or uncertain whether the criterion was met; NA, indicates not applicable.

[a] Ratings are based on assessment of whether the criterion was met and the extent of potential bias, not simply on reporting.

Table 7. Outcomes of Interest from Studies with Evidence on Clinical Utility of Biomarkers to Guide Decisions on Treatment versus No Treatment for Patients Considering Adjuvant Therapy for Early-Stage Breast Cancer

Author Year Reference	Treatments Compared	Biomarker(s) Investigated	# and % of Pts.	Survival									
				Median (months)				% at X years					
				Relapse Free	Event Free	Distant Mets-free	Overall	Relapse Free	Event Free	Distant Mets-free		Overall	
Mammaprint: Randomized Controlled Trials													
Cardoso et al, (NEJM 2016)[2]	CT No CT	Mammaprint	High Risk (and treated with CT) 224 Low Risk (and not treated with CT) 636	NR	NR	NR	NR	High Risk 5 year 92.7% (95%CI, 87.9–95.7) HR 0.74 (95%CI, 0.40–1.39) p=0.36	Low Risk 97.3 (95% CI, 95.6–98.4)	NR	High Risk 5-year 96.1% (95% CI, 92.4-98.1) HR 0.90 (95% CI, 0.40-2.01) p=0.80	Low Risk 94.8% (95% CI, 92.6-96.3)	High Risk 5 year 98.1% (95%CI 94.9–99.3) HR 0.72 (95%CI 0.23–2.24) p=0.57	Low Risk 97.3% (95%CI 95.6–98.4)
Mammaprint: Prospective Studies													
Drukker et al, (Breast Cancer Res Treat 2014)[9]	CT ET	Mammaprint	Low Risk: 115 High Risk: 180	NR	NR	NR	NR	NR	NR	5 years High Risk 58.5% (95%CI, 51.6–66.4) Low Risk 94.7% (95%CI, 90.7–98.9) HR 9.6 (95 % CI 4.2–22.1)		5 years High Risk 74.0% (95%CI, 67.8–80.7) Low Risk 97.4% (95%CI, 94.5–100) HR 11.3 (95 % CI 3.5–36.4)	
Mammaprint: Retropsective Studies													
Knauer et al, (Breast	ET	MammaPrint	315 (58%)	NR	NR	NR	NR	NR	NR	**Low risk:**	**High risk**:	**Low risk:**	**High risk**:

Author Year Reference	Treatments Compared	Biomarker(s) Investigated	# and % of Pts.	Survival									
				Median (months)				% at X years					
				Relapse Free	Event Free	Distant Mets-free	Overall	Relapse Free	Event Free	Distant Mets-free		Overall	
Cancer Res Treat 2010)[8] Pooled data from 6 studies	ET + CT		226 (42%)							93% 99% HR: 0.26 (95%CI, 0.03-2.02; p=0.20)	76% 88% HR: 0.35 (95% CI, 0.17-0.71; p<0.01)	97% 99% HR: 0.58 (95%CI, 0.07-4.98; p=0.62)	81% 94% HR: 0.21 (95%CI, 0.07-0.59; p<0.01)
Buyse et al , (J Natl Cancer Inst 2006)[4]	NR	Mammaprint	307	NR	NR	NR	NR	HR 1.50 (95% CI 1.04 to 2.16)	NR	NR		HR 2.79 (95% CI 1.60 to 4.87)	
Mook et al, (Breast Cancer Res Treat 2009)[5]	ET CT	Mammaprint	166 128	NR	NR	NR	NR	NR	NR	ET: HR 0.31 (95% CI, 0.12-0.80), p=0.02 CT: HR 0.64 (95%CI, 0.25-1.69) p=0.37		ET: HR 0.36 (95% CI, 0.13–0.96) p = 0.04 CT: HR 0.80 (95% CI, 0.32–2.04) p = 0.64	
Bueno-de-Mesquita et al, (Breast Cancer Res Treat 2009)[6]	None Any systemic treatment	Mammaprint	78 (63%) 45 (37%)	NR	NR	NR	NR	NR	NR	HR 1.2 (95% CI, 0.72-1.9) p=0.55		HR 1.2 (95% CI, 0.78-2.0) p=0.36	
Knauer et al, (B J Cancer 2010)[7]	No chemotherapy or trastuzumab	Mammaprint	89 (53%)	NR	NR	NR	NR	NR	NR	HR 5.78 (95%CI, 1.25-26.66) p=0.025		HR 4.70 (95% CI, 1.01-21.75) p=0.048	

Abbreviations: HR, hazard ratio; CI, confidence interval; OS, overall survival; HER2, human epidermal growth factor receptor 2; CT=Chemotherapy and Tamoxifen; ET=Endocrine Therapy; n.a=Not Available; NR=Not Reported; ITT= intention-to-treat;

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