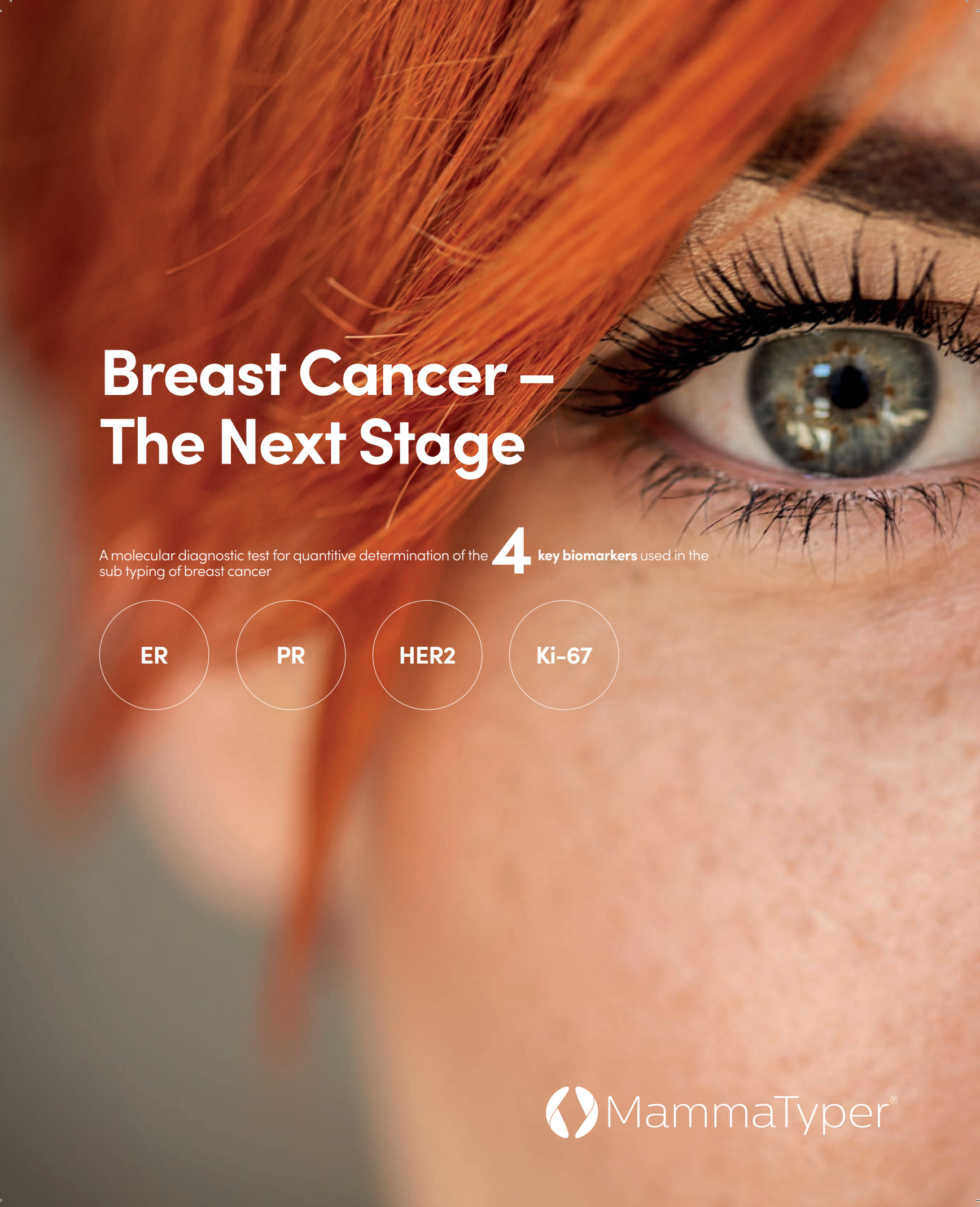


Breast Cancer – The Next Stage

A molecular diagnostic test for quantitive determination of the **4** **key biomarkers** used in the sub typing of breast cancer

- ER
- PR
- HER2
- Ki-67

MammaTyper®

MammaTyper®

Molecular information drives treatment choices in breast cancer

Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and the marker of proliferation Ki-67 are key biomarkers in the evaluation of breast cancer tumours

The combination of the biomarker results allows the assessment of the different St. Gallen Breast Cancer subtypes, which are a key parameter for treatment decisions

Ki-67 is a prognostic and predictive marker. Analytical challenges like high observer variability hinder its standardized and reproducible determination

Definition of Breast Cancer Surrogate Subtypes (St Gallen 2013)

Breast Cancer Subtypes	ER	PR	HER2	Ki-67
Luminal A-like	Pos	Pos	Neg	Neg
Luminal B-like (HER2 negative)	Pos	Pos/Neg*	Neg	Pos/Neg*
Luminal B-like (HER2 positive)	Pos	Pos/Neg	Pos	Pos/Neg
HER2 positive (non-luminal)	Neg	Neg	Pos	Pos/Neg
Triple negative (ductal)	Neg	Neg	Neg	Pos/Neg

*with the exception of the combination PR pos and Ki-67 neg = Luminal A-like

MammaTyper®'s RT-qPCR technology has the following accepted advantages:

- Standardized performance and fast turn-around time
- Minimized inter- and intra-laboratory variability
- Quantitative results with wide dynamic range

Goldhirsch A. et al., Annals of Oncology 2013; 24: 2206-2223
Kreipe H. et al., Der Gynäkologe 2016; 49:737-745
Luporsi E. et al., Breast Cancer Res. Treat 2012; 132(3):895-915
Noske A. et al., Breast Cancer Res. Treat 2011; 109-17

MammaTyper

MammaTyper® is an easy-to-use test delivering precise results within 6 hours

1

Sample preparation

10 µm FFPE tissue section (tumor cell content > 20 %).

2

RNA extraction

Use of RNXtract® or validated commercial RNA extraction systems is recommended.

3

MammaTyper® test set up

Preparation of mastermixes and distribution on 96 well plate.

Analysis of up to 8 patient samples per run.

4

RT-qPCR analysis

Validated on the following qPCR **instruments**:

- Roche cobas z® 480 Analyzer
- Roche LightCycler® 480 II
- Applied Biosystems® 7500 Fast (Dx)
- Siemens Versant® kPCR Cycler
- Bio-Rad CFX96® (IVD, non-deep well)
- Agilent Technologies Mx3000P

5

Data processing and reporting

Export of mRNA expression data.

Calculation and assessment of results.

Results provided within 6 hours.

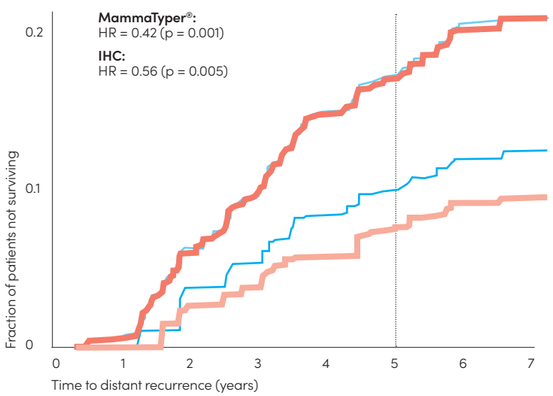
RNXtract® is an easy to perform and robust procedure for the extraction of highly purified total RNA for RT-qPCR applications like MammaTyper®

MammaTyper®

MammaTyper® delivers consistent treatment guidance supported by accurate MKI67 determination

Precise Ki-67 evaluation provides prognostic value for patient outcomes

Comparison of Ki-67 expression determined by MammaTyper® and IHC



Favorable DDFS is independently associated with low MKI67 mRNA expression determined by MammaTyper®

Determination of MKI67 by MammaTyper® delivers extended information on patient's risk of developing distant metastases based on validated cut-off

DDFS = Distant disease free survival

Clinical outcome proves that Ki-67 determination by MammaTyper® is superior to IHC*

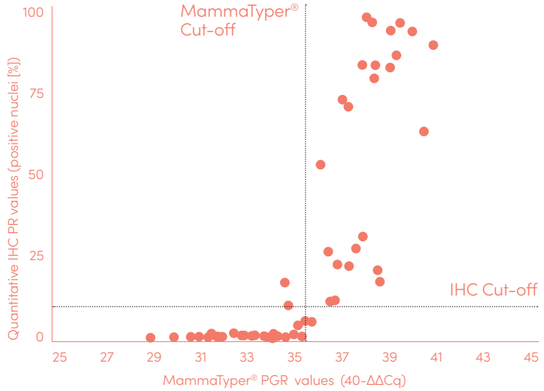
*see page 11

Wirtz RM et al., Breast Cancer Res Treat 2016; 157(3), 437-446

MammaTyper® enhances breast cancer biomarker assessment

MammaTyper® results provide a quantitative measure for each biomarker

Example: Scatter plot shows quantitative MammaTyper® PGR values vs. quantitative IHC PR values



Scatter plot based on in-house data

MammaTyper® mRNA expression results correlate very well to quantitative IHC values.

MammaTyper® cut-offs classify biomarker values into positive or negative results. Dichotomized biomarker results enable stratification into subtypes.

Precise and reproducible biomarker assessment with MammaTyper®

- MammaTyper® cut-offs are validated based on clinical outcome
- MammaTyper® results express a wider dynamic range than IHC
- Precise and highly reproducible biomarker results through standardized assessment

Wirtz RM et al., Poster San Antonio Breast Cancer Symposium December 9-13, 2014
Laible M et al., BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x

MammaTyper® provides guidance for treatment decisions of breast cancer patients

Quantitative gene expression report

Results enable accurate molecular subtyping of tumor tissue according to St Gallen guidelines

MammaTyper® provides quantitative results reflecting the degree of mRNA expression per biomarker

Validated cut-offs are used to determine the positivity or negativity of the biomarker

Values in the ranges colored in light blue/orange have been observed in a cohort of 752 breast cancer samples. Values in the ranges colored in dark blue/orange have not yet been observed but are still valid

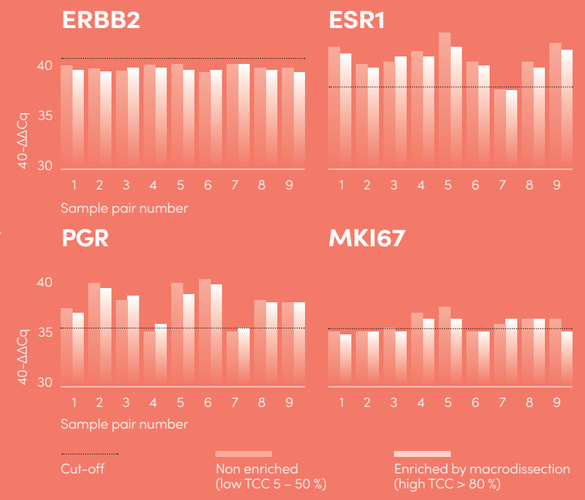
Goldhirsch A et al., Annals of Oncology 2013; 24: 2206-2223

Varying tumor cell content has minimal influence on MammaTyper® performance*

Fluctuating tumor cell content (TCC) could possibly affect the validity of quantitative assessment of ER, PR, HER2 and Ki-67. Therefore, the performance of MammaTyper® was investigated under different scenarios of TCC.

MammaTyper® results of paired tumor samples with high TCC (> 80 %) derived by macrodissection and low TCC (5 – 50 %) including varying DCIS* content (10 – 70 %)

Gene nomenclature ERBB2 = HER2, ESR1 = ER, PGR = PR, MKI67 = Ki-67



Cut-off

Non enriched (low TCC 5 – 50 %)

Enriched by macrodissection (high TCC > 80 %)

Despite varying tumor cell content, MammaTyper® results for HER2, ER, PR and Ki-67 revealed a high level of concordance between low TCC and high TCC samples

Macrodissection of FFPE samples with a tumor cell content > 20 % is not required for gene expression analysis using MammaTyper®

* shown for HER2 negative tissue samples with TCC > 20 %; DCIS = ductal carcinoma in situ

Wirtz RM et al., Poster San Antonio Breast Cancer Symposium December 9-13, 2014
Laible M et al., BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x

MammaTyper®

MammaTyper® International Multicenter Study demonstrates excellent reproducibility

MammaTyper® International Multicenter Reproducibility Study

Evaluated the inter- and intra-site reproducibility of the quantitative detection of ERBB2, ESR1, PGR and MKI67 mRNA expression in clinical samples

- 10 different sites in Europe, North America and Asia
- Standardized application training
- Locally and centrally extracted total RNA of 24 clinical FFPE samples
- Assessment of the precision of the test under different conditions: Laboratories, operators, instruments, days and lots

MammaTyper® Reproducibility Study results

Excellent inter-site agreement of binary single biomarker classification (positive/negative) is represented by high Kappa values:

Binary biomarker results	ERBB2	ESR1	PGR	MKI67
Kappa values	1.00	0.91	0.94	0.94

Low inter-site variance of quantitative single biomarker results is demonstrated by excellent K values ≥ 0.98:

Quantitative biomarker results	ERBB2	ESR1	PGR	MKI67
Kappa values	0.987	0.992	0.998	0.980

Determination of breast cancer subtypes shows a high level of agreement across all sites (Kappa = 0.90)

MammaTyper® is highly reproducible – reducing inter- and intra- laboratory variations and allows e.g. standardization of Ki-67 assessment

Varga Z et al., Breast Cancer Research 2017; 19:55: DOI 10.1186/s13058-017-0848-z

MammaTyper® allows accurate biomarker assessment

Clinical performance evaluation: MammaTyper® FinHer study

Concordance of MammaTyper® results with IHC/CISH based standard diagnostic methods was evaluated using 769 tissue samples obtained within the FinHer trial.

Patients:

Node positive or high risk node negative invasive breast cancer.

FinHer trial design:

The trial evaluated the efficacy of combining FEC with Docetaxel vs. Vinorelbine. Patients with HER2 positive tumors were also assigned to receive or not receive Trastuzumab.

Concordance between MammaTyper® and IHC/CISH-based biomarker assessments

	ESR1 (ER)	PGR (PR)	ERBB2 (HER2)	MKI67 (Ki-67)
Concordance	**91.8%** 660/719	**82.5%** 593/719	**91.8%** 660/719	**75.0%** 516/688
PPA	**95.9%** 490/511	**93.2%** 368/395	**85.9%** 140/163	**89.1%** 369/414
NPA	**81.7%** 170/208	**69.4%** 225/324	**93.5%** 520/556	**53.7 %** 147/274
Kappa statistic	0.80 0.75–0.85	0.64 0.58–0.70	0.77 0.72–0.83	0.45 0.38–0.52
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

PPA Positive percent agreement, NPA Negative percent agreement

Assessment of ER, PR and HER2 by MammaTyper® correlated well with results obtained by IHC and CISH

As expected, Ki-67 shows moderate concordance between the IHC and MammaTyper® results due to known technical limitation in standardization of Ki-67 IHC assessment

MammaTyper® results show a high degree of concordance with IHC/CISH for ER, PR and HER2

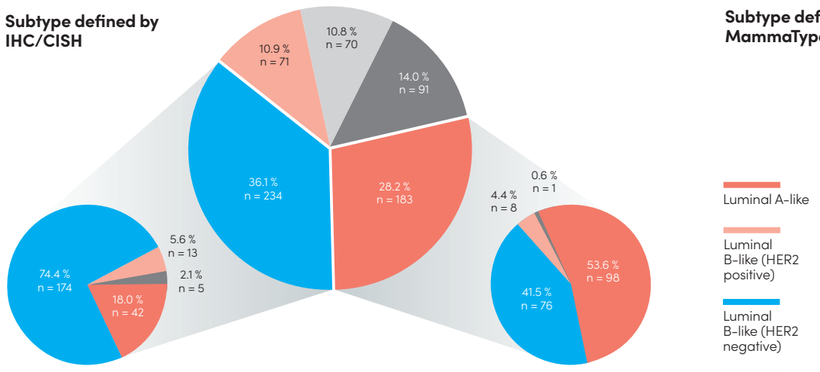
Wirtz RM et al., Breast Cancer Res Treat 2016; 157(3), 437446.
Joensuu H et al., J Clin Oncol 2009; 27:5685–5692.
Polley et al., Modern Pathology 2015; 28, 778–786.

MammaTyper® results ensure precise determination of breast cancer subtypes correlated with clinical outcome

Accurate MKI67 assessment by MammaTyper® has substantial impact on distinction between Luminal A- and B-like breast cancers.

MammaTyper® luminal subtypes correlated with FinHer* clinical outcome data, thus proving the accuracy of MammaTyper® results

Concordance of molecular surrogate subtypes defined by MammaTyper® and IHC (FinHer* study)



By precise subtyping, MammaTyper® results support selection of appropriate treatment strategy for each patient

* FinHer results reclassified according to St Gallen classification

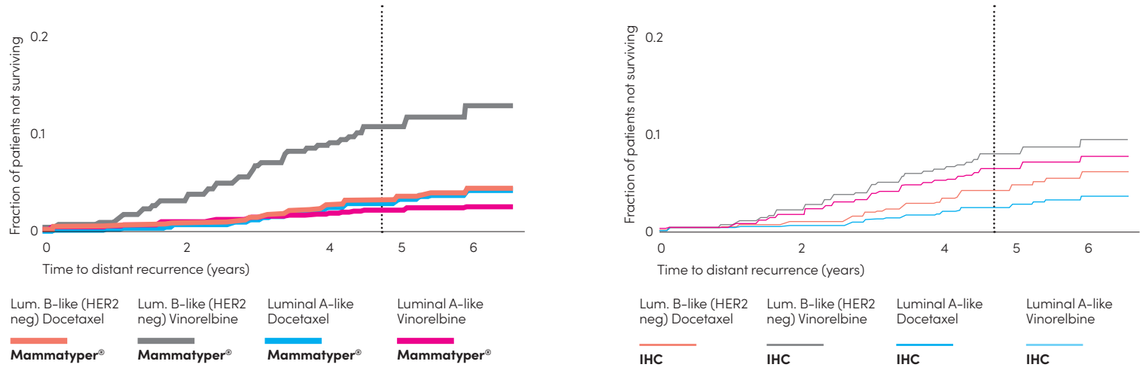
Wirtz RM. et al., Breast Cancer Res Treat 2016; 157(3), 437-446

Hypothesis-generating data:

MammaTyper® predicts benefit from Taxane treatment* by accurate distinction of molecular subtypes

Patients classified as Luminal B-like (HER2 negative) by MammaTyper® show benefit from Taxane-based chemotherapy.

Comparison of OS and different treatment regimens of Luminal A-like and Luminal B-like (HER2 negative) patients stratified by MammaTyper® or IHC



Shown for Luminal B-like (HER2 negative) patients

FinHer results reclassified according to St Gallen classification, OS = Overall survival

Patient stratification based on MammaTyper® shows improved OS of the Luminal B-like (HER2 negative) patients receiving Docetaxel-based treatment compared to those receiving Vinorelbine

By accurate stratification of patients into Luminal A-like and Luminal B-like (HER2 negative) subtypes using MammaTyper®, patient groups that might benefit from Docetaxel-based treatment can be revealed

Classification of the patients in Luminal B-like (HER2 negative) subtype by IHC did not reveal a clear separation in responders and non-responders to a Docetaxel- based treatment

MammaTyper® opens up new opportunities of providing predictive information about the benefit of adjuvant Taxane-based treatment

Wirtz RM et al., Breast Cancer Res Treat 2016; 157(3), 437-446

MammaTyper®

MammaTyper® precisely determines mRNA expression of ER, PR, HER2 and Ki-67

High-performance test

Quantitative RT-qPCR assay (CE marked IVD)

- Highly reproducible biomarker assessment
- Reliable results through standardized biomarker detection
- Accurate stratification of breast cancer tumors into St Gallen subtypes

Promising clinical utility

Clinical value validated in numerous performance evaluation studies

- Outperforms IHC by accurate Ki-67 determination
- Provides information on patient's prognosis
- Accurate subtyping supports treatment decisions

Easy-to-use

- Reliable method for any molecular pathology laboratory
- Validated for multiple qPCR instruments*
- From resection or core needle biopsy FFPE sample to result within 6 hours

FFPE = formalin fixed paraffin embedded

* Validated RT-qPCR instruments: Roche cobas z® 480 Analyzer, Roche LightCycler® 480 II, Applied Biosystems® 7500 Fast (Dx), Siemens Versant® kPCR Cycler, Bio-Rad CFX96®, Agilent Technologies Mx3000P

Laible M. et al., BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x
Wirtz RM. et al., Breast Cancer Res Treat 2016; 157(3), 437-446
Varga Z. et al., Breast Cancer Research 2017; 19:55: DOI 10.1186/s13058-017-0848-z
Sinn HP. et al., BMC Cancer 2017; 17:124

Innovation for your breast cancer diagnostics

High-performance	Precise quantitative results for mRNA expression of HER2, ER, PR and Ki-67 Outperforms IHC by accurate Ki-67 determination Accurate and reliable test to stratify breast cancer into surrogate subtypes acc. to St Gallen
Reproducible	Highly reproducible results with extremely low inter-/intra-laboratory variation
Validated	Clinical value has been validated in numerous performance evaluation studies
Prognostic	Extended information on patient's prognosis
Predictive	Accurate subtyping supports treatment decisions
Optimized	Reliable method for any pathology laboratory
Easy-to-use	Easy-to-use test which allows results within 6 hours

All scientific and technical information in this brochure is based on the MammaTyper® Instructions for Use Rev. 3.1 and Wirtz et al., Breast Cancer Res Treat. 2016; 157(3) 437-446; Laible et al., BMC Cancer 2016; DOI: 10.1186/s12885-016-2476-x; Varga Z et al., Breast Cancer Research 2017; 19:55: DOI 10.1186/s13058-017-0848-z; Sinn et al., BMC Cancer 2017; 17:124

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