MammaTyper®

HER2-low breast cancer: the case for molecular diagnostics

Summary

- Recognition of HER2-low and -ultralow breast cancer categories has expanded the need for precise classification, to ensure that patients receive the most appropriate therapies.
- Current immunohistochemistry (IHC) and *in situ* hybridisation (ISH) methods remain central to HER2 testing, but the subjective nature of IHC – combined with variability and poor concordance between pathologists – limits consistency, particularly in distinguishing cases at the lower end of expression.
- Quantitative molecular assays, such as MammaTyper®, provide high accuracy and sensitivity validated across multi-centre clinical studies, offering greater confidence in HER2 classification.
- MammaTyper® can be readily integrated into existing workflows to refine HER2 classification and more precisely identify patients who may benefit from anti-HER2 therapies.

Introduction

Accurate biomarker classification plays a central role in the diagnosis and treatment of breast cancer. One of the most important markers for stratifying patients and determining therapy is human epidermal growth factor receptor 2 (HER2).[1] Historically, HER2 status was categorised as either positive or negative, guiding the use of HER2-targeted therapies such as trastuzumab. However, in recent years, the DESTINY-Breast04 trial revealed the therapeutic relevance of a third group; HER2-low breast cancer, defined by IHC scores of 1+ or 2+ without HER2 gene amplification.[2] More recently still, researchers recognised a fourth category – HER2-ultralow – comprising tumours that fall just above the IHC 0 threshold. The potential clinical implications of this new category are currently being explored in trials such as the DESTINY-Breast06 study.[3,4]

This white paper examines the growing clinical relevance of HER2-low and HER2-ultralow breast cancer, the limitations of current diagnostic approaches, and the emerging role of quantitative molecular methods – such as reverse transcription quantitative PCR (RT-qPCR) – in improving diagnostic accuracy and clinical decision-making.

"RT-qPCR-based assessment of the mRNA expression of *ESR1*, *PGR*, *ERBB2* and *MKI67* showed high concordance with IHC, suggesting that the MammaTyper test on core needle biopsies represents a reliable, efficient, and reproducible alternative for breast cancer classification and refining HER2-low categorisation."

- **Leading UK Pathologist, Badr *et al*.** [5]

HER2 classification and its role in guiding treatment

HER2 is a transmembrane receptor tyrosine kinase that plays a key role in cell growth and survival, and was first identified as a breast cancer marker in 2005.[6,7] HER2 status has long been used to guide treatment decisions for breast cancer, particularly the use of HER2-targeted agents such as trastuzumab, pertuzumab and trastuzumab emtansine (T-DM1).[8–10] Historically, patients have been classed as either HER2-positive or HER2-negative, however this classification is now being challenged.

Current testing protocols

The standard workflow for assessing HER2 status begins with IHC, which detects HER2 protein expression on tumour cells. IHC results are scored from 0 to 3+ based on the intensity and completeness of membrane staining.[11]

- **0 or 1+ is considered HER2-negative**
- **2+ is equivocal, requiring confirmatory testing**
- **3+ is HER2-positive**

If a tumour scores IHC 2+, reflex testing is performed using ISH – such as fluorescence (FISH) or chromogenic (CISH) techniques – to determine whether the HER2 gene is amplified.[11] HER2 amplification confirms eligibility for HER2-targeted therapies. HER2 classification is summarised in **table 1.**

Emergence of HER2-low and HER2-ultralow

The approval of trastuzumab deruxtecan (T-DXd), an antibody drug conjugate (ADC), has expanded the relevance of HER2 classification. T-DXd has shown clinical benefit in HER2-low tumours – a group previously considered HER2-negative, and therefore ineligible for targeted therapy.[2] There is also growing interest in the concept of HER2-ultralow, describing tumours with minimal focal staining just above the threshold of IHC 0, often involving incomplete membrane staining in fewer than 10 % of cells.[3] HER2-ultralow tumours are not yet formally defined in clinical guidelines, but may respond to emerging ADCs.[12] As such, accurate and consistent classification across the full HER2 expression spectrum is becoming increasingly important.

Implications for treatment stratification

The stream of emerging therapies means that HER2 status now directly determines patient access to specific targeted therapies as outlined in **table 1**. This evolution underscores the need for reliable testing methods that can resolve subtle distinctions – especially between IHC 0, 1+ and 2+ – to ensure patients are appropriately matched with available therapies.

HER2 classification	IHC score	ISH status	Treatment eligibility	Notes
HER2-positive	3+	Not required	Trastuzumab, pertuzumab, T-DM1, T-DXd	Clear eligibility; strong, complete membrane staining
	2+	Amplified (ISH+)	Trastuzumab, pertuzumab, T-DM1, T-DXd	Confirmatory ISH testing required
HER2-low	1+ or 2+	Non-amplified (ISH-)	T-DXd	Supported by DESTINY-Breast04 and -Breast06 trials[2,12]
HER2-ultralow (emerging)	0 (with faint staining <10 %)	Not applicable	Not currently eligible. T-DXd under investigation[12]	Subject of ongoing research; potential for ADC response in DESTINY-Breast06 trial[12]
HER2-negative	0	Not applicable	Not eligible for HER2-targeted therapies	No detectable membrane staining

Table 1: HER2 classification categories with associated IHC/ISH results and treatment options.

Diagnostic challenges in the HER2-low space

HER2 testing has become more clinically complex with the recognition of HER2-low and -ultralow categories, yet the diagnostic tools used to determine HER2 status were only designed to detect HER2 overexpression, not to identify subtle differences at the lower end of the expression range. As a result, these methods struggle to provide the level of precision now needed to guide modern treatment decisions.

Limitations of IHC

IHC is a semi-quantitative, subjective technique that introduces two major sources of variability: human interpretation and technical inconsistency. There is no globally standardised protocol for HER2 IHC, and results can vary depending on fixation quality, staining procedure, antibody clone, detection system and scoring threshold. These issues are most pronounced in the low expression range, where distinguishing IHC 0 from 1+ or 2+ is particularly challenging. A study by Zaakouk *et al.* in the UK and Republic of Ireland examined classification by 16 expert pathologists. Results showed absolute agreement in just 6 % of cases, all of which were HER2 3+. For HER2-low cases, inter-observer agreement was only fair to moderate, highlighting the difficulties that even experienced pathologists face in making consistent low-range classifications.[3]

This issue is not unique to the UK and Ireland. In a multi-centre European study by Baez-Navarro *et al.*, pathologists scored 105 HER2-negative breast cancer cases and achieved complete agreement in only 4.7 % of them. Even when using clustered scoring (e.g. combining 1+ and 2+), concordance improved only modestly, and Fleiss' kappa remained in the fair-to-moderate range.[13] US-based data also supports these findings; a study by Robbins *et al.* across 18 specialist pathologists from 15 institutions showed substantial discordance in HER2 IHC scoring. The report showed substantial discordance within the intermediate categories (<1 % agreement for 1+ and 3.6 % agreement for 2+). The discordance within the IHC 0 cases was also substantial, with an overall percent agreement of only 25 % and poor inter-rater reliability metrics.[14] Similar findings were reported by Fernandez *et al.,* who analysed datasets from both the College of American Pathologists and Yale University, and found poor agreement between pathologists, especially in 0 and 1+ cases. When 18 pathologists read the scanned slides from a selected set of breast cancer biopsies, there was only 26 % concordance between 0 and 1+, compared with 58 % concordance between 2+ and 3+.[15] These studies demonstrate that legacy IHC methods fail to deliver reproducibility at the thresholds that now determine access to treatment.

Study	0	1+	2+	3+
Zaakouk *et al.* (2023)[3] UK and Ireland expert pathologists	0.43 κ; 0 % absolute agreement	0.29 κ; 0 % absolute agreement	0.43 κ; 0 % absolute agreement	0.80 κ; 6 % absolute agreement
Baez-Navarro *et al.* (2023)[13] International	Poor concordance	Moderate (clustered scoring improved concordance)	Moderate (some improvement with clustering)	Not separately reported; described as more reproducible
Fernandez *et al.* (2022)[15] Yale cohort	Only 26 % of cases read as 0 by at least one pathologist reached ≥90 % agreement	Not separately quantified	Not separately quantified	58 % of cases read as 3+ by at least one pathologist achieved ≥90 % agreement. The difference in concordance between 0/1+ and 2+/3+ scores was statistically significant (χ^2 = 12.07, P < .001).
Robbins *et al.* (2023)[14] US, multi-institutional	0.49 κ; 25 % overall percent agreement	0.35 κ; 0.98 % overall percent agreement	0.46 k; 3.57 % overall percent agreement	0.63 κ; 50 % overall percent agreement

Table 2: Summary from studies looking at IHC inter-observer agreement.*

* Direct comparison between studies is challenging because they use different reporting and statistical approaches. Zaakouk and Robbins both report k (Fleiss/Cohen), Baez-Navarro reports full-agreement rates and Krippendorff's alpha, and Fernandez reports percent agreement thresholds (≥90 %). Despite these differences, all studies show the same overall trend: reproducibility is consistently higher for strong positive (3+) cases, and challenging in the HER2-low categories (1+ and 2+), highlighting the inherent difficulty in reliably distinguishing low-level expression.

Limitations of *in situ* hybridisation

ISH methods – such as FISH and CISH – can offer more objective assessment by detecting HER2 gene amplification, but their use is also limited in several ways. Most notably, they are only routinely used as confirmatory tests for IHC 2+ cases, not to clarify HER2 status in IHC 0 or 1+ cases. Aside from this, they are challenging and time-consuming techniques. FISH requires fluorescence microscopy, specialist training and high-quality equipment, making it technically demanding and relatively expensive. Interpretation of equivocal ISH groups can be challenging, as borderline cases are prone to interobserver variability in signal counting and tumour area selection, leading to inconsistent results.[16] CISH is more accessible than FISH – as it uses standard brightfield microscopy and produces a permanent, stainable signal – but still requires careful technique and standardisation. It is easier to integrate into routine pathology labs but remains a manual, labour-intensive process with limited scalability for higher throughputs. Neither method is well-suited to distinguishing between HER2-low and -ultralow cases, and neither offers a practical solution for improving reproducibility in the low-expression range on a routine basis. For these reasons, ISH techniques have not been widely applied to address the most pressing diagnostic challenge in the current HER2 landscape.

Why classification accuracy matters

With HER2 status now determining access to specific therapies, accurate classification is critical.

- **Under-treatment: misclassifying HER2-low or -ultralow tumours as HER2 0 can deny patients access to ADCs like T-DXd.**
- **Over-treatment: incorrectly assigning HER2-low status may lead to unnecessary exposure to costly and potentially toxic therapies.**

As treatment decisions increasingly hinge on subtle distinctions in HER2 expression, a method that provides reproducibility at the lower end of the scoring scale is essential to ensure patients receive the most appropriate care.

"MammaTyper® provides a standardised, automated, reproducible test which is easy to implement in any lab that can improve patient selection for targeted therapies."

– **Dr. Laia Bernet, pathologist and co-ordinator of breast pathology laboratories, Ribera health group, Spain**

The potential role of quantitative methods

Quantitative molecular assays – such as RT-qPCR – offer an objective, reproducible alternative that may support more confident HER2 classification. MammaTyper is an RT-qPCR-based assay that quantifies the mRNA levels of key breast cancer biomarkers, including ERBB2 – the gene encoding HER2. The result allows classification into distinct categories as highlighted in **figure 1.**

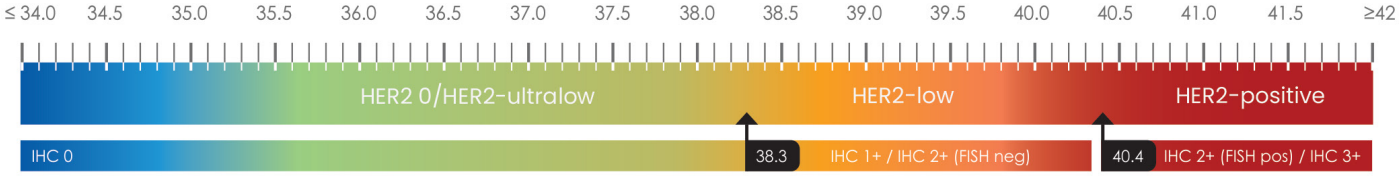

Figure 1: MammaTyper provides quantitative data on mRNA expression, allowing distinction between HER2-negative, HER2-ultralow, HER2-low and HER2-positive.

MammaTyper's analytical robustness was demonstrated in a large multi-centre validation study by Varga *et al.*, which showed near-perfect inter- and intra-laboratory reproducibility across 10 pathology labs in Europe, Canada and China.[17] It delivers standardised, numerical results, avoiding many of the technical and interpretive pitfalls of IHC. Another recent study from Atallah *et al.* looked at the clinical benefits of this approach based on a well-characterised HER2-positive cohort, concluding that ERBB2 mRNA levels measured by MammaTyper were more predictive of treatment benefit from trastuzumab than IHC scores alone.[18] This study also revealed cases that were falsely classified as HER2-positive by IHC, particularly in borderline or heterogeneous tumours. Together, these findings support the integration of RT-qPCR into the HER2 testing workflow.

> **"Compared to the semi-quantitative IHC approach, the MammaTyper assay is more accurate in defining and identifying HER2-positive breast cancer patients that would benefit from anti-HER2 therapy."**
>
> – **Atallah *et al.*[19]**

Implications and implementation

Quantitative methods such as MammaTyper can help to bridge the HER2 diagnostic gap, particularly in borderline HER2-low, HER2-ultralow or equivocal cases, where IHC alone may not provide sufficient clarity. Crucially, these molecular assays are not intended to replace IHC, but to complement it. Implementation is feasible within existing workflows, since MammaTyper can be run on commonly available RT-qPCR instruments without the need for specialised or proprietary equipment (**figure 2**). The process uses standard formalin-fixed, paraffin-embedded (FFPE) tissue samples and requires minimal additional training or infrastructure. Compared to techniques like FISH, it is faster, less labour intensive and more scalable across laboratories. Furthermore, the broader trend in breast cancer diagnostics is already moving towards genomic testing of other biomarkers – such as BRCA1, BRCA2 and PIK3CA – for treatment selection. There is a growing logic in applying the same approach to HER2, particularly as HER2 expression is no longer seen as simply positive or negative, but as a spectrum where different levels can influence treatment choices. In this evolving clinical framework, quantitative methods offer a practical way to increase diagnostic accuracy, reduce misclassification, and help to ensure that patients are matched with the treatments most likely to benefit them – without introducing complexity or cost that would limit adoption. Although MammaTyper is an additional assay to current diagnostic workflows, it has the potential to optimise healthcare resources by enabling more precise patient stratification. This could help to avoid unnecessary treatments, optimise therapeutic regimens and minimise the need for multigene testing.[20]

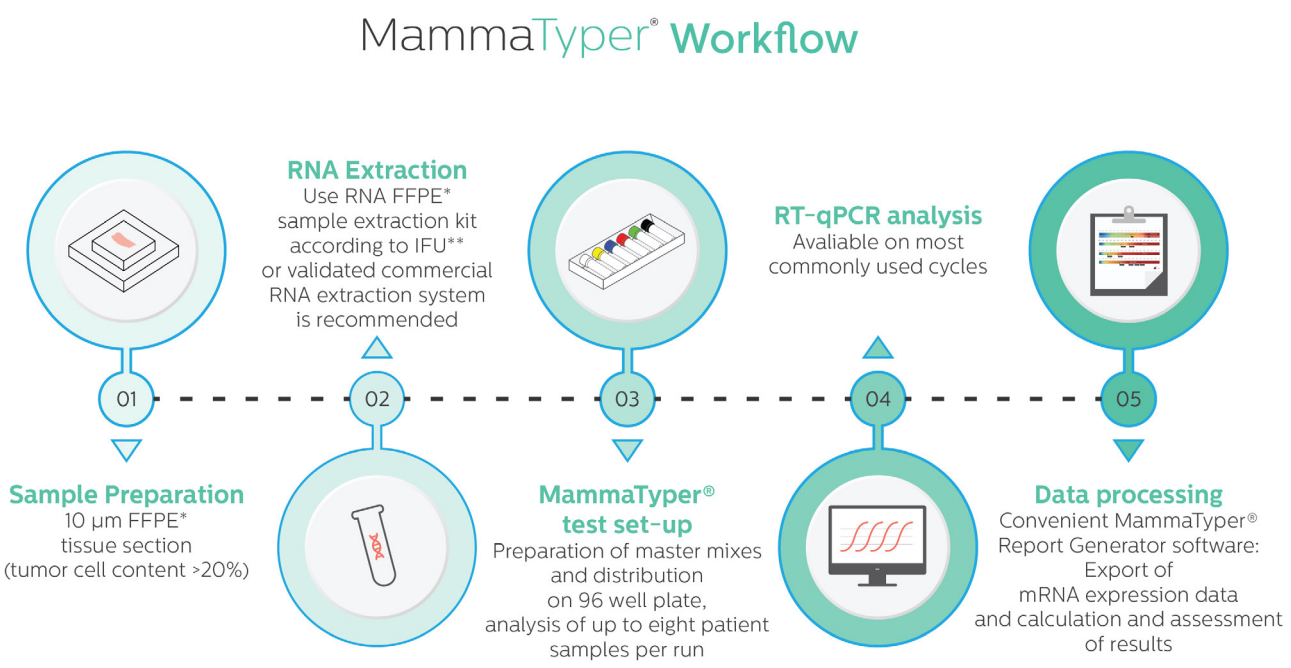

Figure 2: The MammaTyper workflow can be carried out in under six hours using FFPE tissue samples and a standard RT-qPCR machine.

Conclusion

The emergence of HER2-low and HER2-ultralow breast cancers as clinically relevant subtypes marks a significant shift in the diagnostic and therapeutic landscape. These classifications offer new treatment opportunities for patients who were previously considered HER2-negative, but they also introduce complexity into existing testing workflows. Current HER2 assessment methods are no longer sufficient for accurate stratification across the full range of expression. Variability in interpretation – especially between IHC 0, 1+, and 2+ – can lead to inconsistent results that directly impact treatment guidance. As eligibility for targeted therapies increasingly depends on subtle distinctions in HER2 expression, the limitations of subjective, semi-quantitative testing become more apparent. Quantitative molecular tools – such as MammaTyper – offer a practical way to enhance diagnostic accuracy. By supporting IHC, these assays can help to resolve ambiguity in borderline and low expression cases, increasing confidence in HER2 classification to make the most of new treatment options and, ultimately, improve outcomes.

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