

# HER2 STATUS ASSESSMENT BY CHROMOGENIC IN-SITU HYBRIDISATION (CISH) DEMONSTRATES HIGH SENSITIVITY FOR PREDICTING RESPONSE TO HERCEPTIN®

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## Abstract

**Background:** Accurate determination of HER2 positivity is a prerequisite for treatment with the anti-HER2 monoclonal antibody therapy, trastuzumab (Herceptin®). Fluorescence in-situ hybridisation (FISH) and immunohistochemistry (IHC) are the most common methods used to determine HER2 status in breast cancer; both tests are highly reliable and reproducible. Chromogenic in-situ hybridisation (CISH) has been developed as an alternative technique for detection of HER2 gene amplification and has been shown to be highly concordant with the established HER2 testing methods, FISH and IHC. Samples from a Herceptin® phase II monotherapy trial were retrospectively tested by CISH to assess the utility of this technique for prediction of response to therapy. CISH scores were compared with previous FISH and IHC results for the same samples.

**Methods:** Testing of tumour samples by IHC (HercepTest™), and 2-colour FISH (PathVysion™) was conducted as part of a study investigating Herceptin® administered every 3 weeks in metastatic breast cancer (WO16229). In the current analysis, 86 samples were re-tested for HER2 status by CISH (Spot-LIGHT™). Samples scored as having ≥6 signals per cell by CISH were classified as HER2 positive. Patients with complete or partial responses were defined as responders; all patients were followed until disease progression.

**Results:** CISH scores were highly concordant with previous results from both FISH and IHC. Concordance between FISH and CISH results was seen in 77 of 86 cases (90%), whereas CISH and IHC results were concordant in 75 of 86 cases (87%), which was similar to IHC to FISH concordance in the same cohort of samples (88%). Of the 86 patients included in this analysis, 19 responded to monotherapy treatment with Herceptin®. Seventeen of these 19 patients had HER2-positive disease as determined by CISH (90% sensitivity), compared with 16/19 for FISH (84% sensitivity) and 19/19 for IHC (100% sensitivity). Chromosome 17 polysomy can result in a negative 2-colour FISH result, i.e. a ratio of HER2 to CEP17 signal <2. Five of six cases showing negative FISH, but positive CISH, results exhibited high or intermediate levels of polysomy, indicating that this is a major cause of FISH/CISH discrepancy. Interestingly, two of the three patients responding to Herceptin® therapy that were negative by FISH exhibited high levels of polysomy and were CISH positive.

**Conclusions:** The results of this study show that CISH concordance with FISH and IHC is high, and that CISH has a high sensitivity for predicting response to Herceptin® therapy. These findings support the use of CISH as an alternative to FISH for detecting HER2 gene amplification, and confirm its utility for assessment of eligibility for treatment with Herceptin®. Detailed analyses of concordance, polysomy, sensitivity and specificity will be discussed.

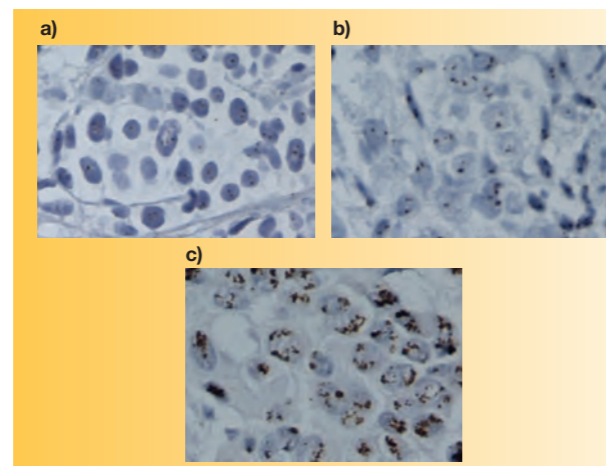
## Introduction

Accurate determination of HER2 status is essential for optimum patient management with Herceptin®, a humanised monoclonal antibody directed against HER2. Patients who are likely to benefit from Herceptin® have tumours with strong HER2 receptor overexpression and/or HER2 gene amplification.

Immunohistochemistry (IHC) and fluorescence in-situ hybridisation (FISH) are both globally accepted and used as standard methods for determining HER2 status in breast cancer.<sup>1</sup> IHC detects HER2 receptor levels on the surface of tumour cells, while FISH is used to detect HER2 gene levels. According to the recommended HER2 testing algorithm, patients with IHC 3+ tumours are eligible for Herceptin®, while those with IHC 0/1+ tumours are not. IHC 2+ is defined as equivocal and such samples should be reassessed by FISH.<sup>1</sup> FISH positivity also indicates eligibility for treatment with Herceptin®.

Both IHC and FISH are reliable, robust and reproducible, but they do have some limitations. For example, IHC results can be dependent upon methodology variables (e.g. tissue fixation, antibody retrieval, etc.) and score interpretation is semi-quantitative. It is therefore essential to apply a standardised and validated IHC staining protocol to ensure high quality and reliable test results. FISH requires the use of a costly fluorescence microscope, extensive user training, and storage of stained slides is difficult as the signals decay relatively quickly at room temperature.

Chromogenic in-situ hybridisation (CISH) is similar to FISH as it also identifies HER2 gene copy levels on a cell-to-cell basis. However, it utilises a chromogenic reaction, similar to that used in IHC, to highlight HER2 copy number. Consequently, CISH signals can be viewed using a standard light microscope, staining remains stable over a long period of time, and specimen histopathology may be viewed simultaneously.<sup>2,3</sup> Examples of CISH assay results are shown in Figure 1.



**Figure 1.** CISH assay results: (a) not amplified; (b) low amplification; (c) high amplification.

## Objectives

Tumour samples from a phase II study of Herceptin® monotherapy were retrospectively assessed by CISH to

- Validate CISH against IHC and FISH as a method of determining HER2 status.
- Assess the utility of CISH as a means of predicting response to Herceptin® therapy.

## Methods

- Study WO16229 investigated q3-weekly Herceptin® monotherapy (8mg/kg loading dose followed by 2mg/kg every 3 weeks until disease progression).<sup>4</sup>

- All patients were followed until disease progression. The primary endpoint was response rate (complete plus partial response), assessed according to WHO criteria.

- Initially, patients with HER2-positive disease eligible for the study were identified solely by local testing with IHC and/or FISH. However, central confirmation of HER2 positivity was subsequently required. In reality, most tumour samples were collected centrally, and, therefore, the HER2 status was determined in a central laboratory either retrospectively or prospectively.

- The HercepTest™ (DakoCytomation) was used for IHC – 3+ cases were considered positive.

- The PathVysion™ (Vysis) 2-colour assay was used for FISH – amplification was defined as a ratio of HER2 gene to centromere 17 (CEP17) signals of ≥2 – polysomy was defined as ≥3 CEP17 signals per nucleus and graded as low (3–4 signals per nucleus), medium (4–5) and high (≥5).

- Samples for which both IHC and FISH results were available were retrospectively reassessed by CISH using the SPoT-LIGHT™ assay (Zymed) – ≥6 signals per cell was defined as positive.

## Results

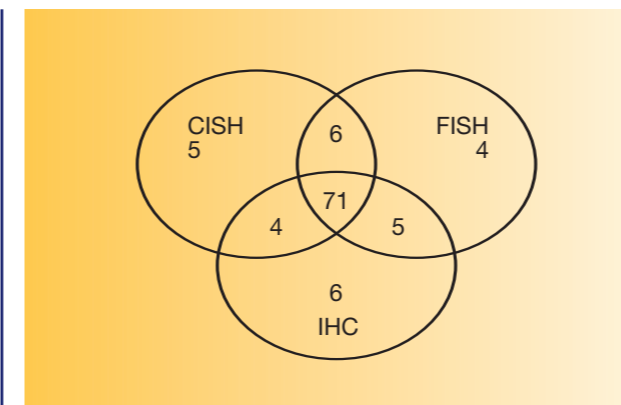
In total, 105 patients were enrolled into the study.

- Both IHC and FISH results (all centrally confirmed) were available for 94 patient samples.

- CISH results were available for 86 of these samples.

### Concordance between IHC, FISH and CISH

- In total, 71 of the 86 samples were concordant for all three technologies (Figure 2).



**Figure 2.** Venn-diagram showing a concordance rate of 81.4% between CISH, FISH and IHC.

- Concordance between CISH and FISH is shown in Table 1 – 90% concordance (kappa = 0.66).

**Table 1.** Concordance between CISH and FISH.

Response	Number of samples	
	CISH–	CISH+
FISH–	12	6
FISH+	3	65

- Concordance between CISH and IHC is shown in Table 2 – 87% concordance (kappa = 0.56).

**Table 2.** Concordance between CISH and IHC.

Response	Number of samples	
	CISH–	CISH+
IHC–	10	6
IHC+	5	65

- The concordance between CISH and IHC is similar to that seen between FISH and IHC (Table 3)

- 88% concordance (kappa = 0.64).

**Table 3.** Concordance between FISH and IHC.

Response	Number of samples	
	FISH–	FISH+
IHC–	12	4
IHC+	6	64

### Concordance between HER2 test result and HER2 polysomy

- Polysomy of chromosome 17 can result in a negative HER2 score by 2-colour FISH, i.e. if the ratio of HER2 to CEP17 is <2.
- In 23 patients for whom IHC, FISH and CISH results were known, polysomy was detected (Table 4)
  - low-level polysomy was predominantly seen in samples where FISH and CISH were concordant
  - five of the six cases showing negative FISH, but positive CISH results (the sixth case was also negative by CISH), exhibited high or intermediate levels of polysomy, indicating that this is a major cause of FISH/CISH discrepancy.

**Table 4.** Correlation of IHC, FISH and CISH results with level of polysomy.

Polysomy	FISH+ IHC+	FISH– IHC–	FISH+ IHC–	FISH– IHC+	FISH– IHC–	FISH– IHC+
Low	9	4	1 (SD)	0	0	1 (PD)
Medium	1	0	1 (PD)	0	1 (PD)	0
High	1	0	0	4*	0	0

A positive result is defined as: IHC 3+, FISH ratio >2, CISH result >6  
SD = stable disease; PD = progressive disease; PR = partial response  
\*SD, 2 PR, PD

### Comparison of HER2 status by IHC, FISH or CISH and response to therapy

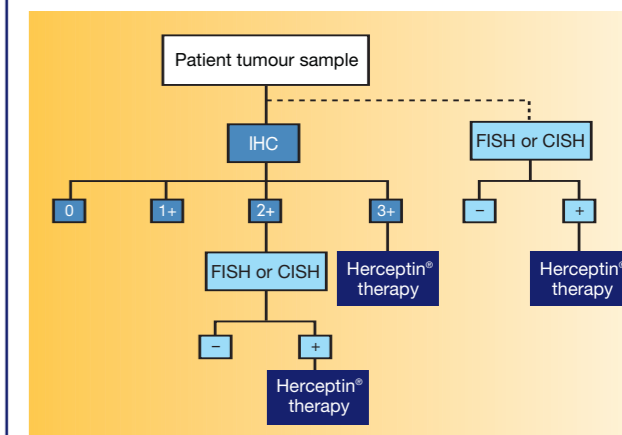
- Of the 86 patients included in this analysis, 19 responded to treatment with Herceptin®.
  - Of these 19 responders
    - all had IHC 3+ disease (100% sensitivity)
    - 16 were FISH positive (84% sensitivity)
    - 17 were CISH positive (90% sensitivity) (Table 5).
  - Of the three patients responding to Herceptin® with IHC 3+ but FISH-negative tumours, two exhibited high level polysomy and were CISH-positive.

**Table 5.** Comparison of IHC, FISH and CISH results and response.

Response	IHC		FISH		CISH	
	3+	–	+	–	+	–
Complete response	2	0	2	0	2	0
Partial response	17	0	14	3	15	2
Stable disease	33	9	34	8	35	7
Progressive disease	18	7	18	7	19	6

## Conclusions

- Herceptin® is indicated for patients whose breast cancer demonstrates strong HER2 receptor overexpression (IHC 3+) or gene amplification.
- In this study, the concordance of CISH with FISH and IHC was high, supporting the use of CISH for assessing HER2 gene amplification
  - this is in-line with results from a recently reported international CISH validation study.<sup>5</sup>
- CISH demonstrated a high sensitivity for predicting the response to Herceptin® therapy.
- These findings support the use of CISH as an alternative to FISH for detecting HER2 gene amplification, particularly in laboratories where FISH is not available, and confirm its suitability for assessing eligibility for treatment with Herceptin®.
- Based on these data the recommended HER2 testing algorithm is shown in Figure 3.



**Figure 3.** Recommended HER2 testing algorithm.

## References

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