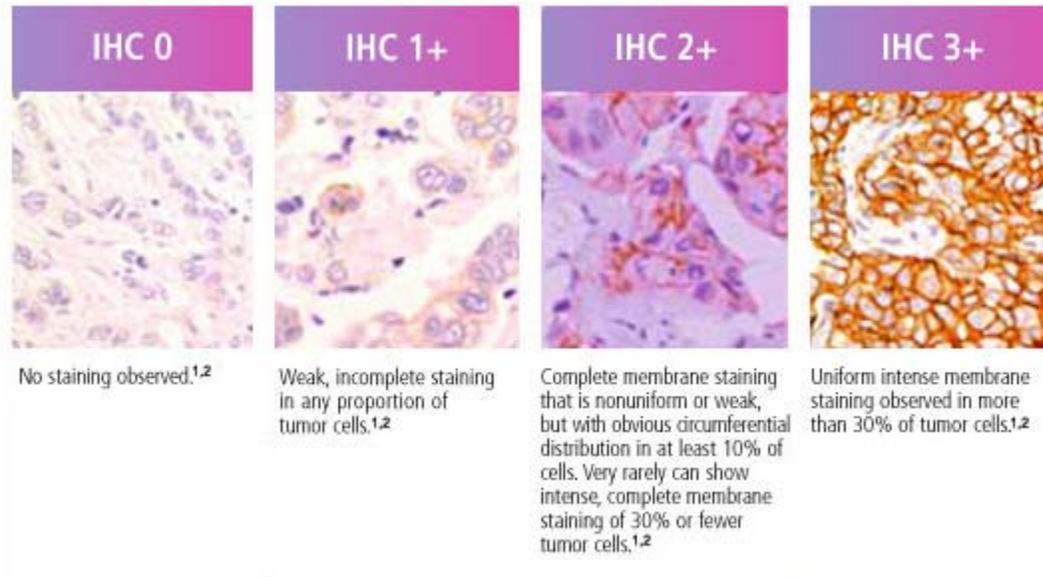


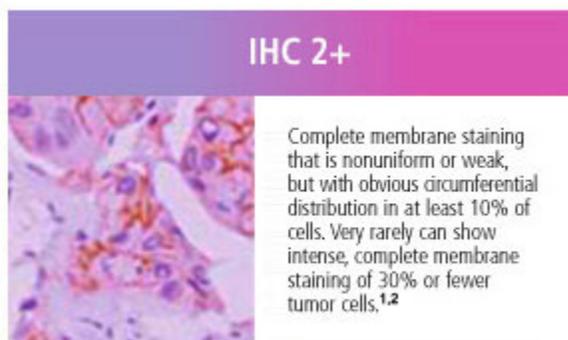
# How Results are Interpreted

Interpretation of IHC relies on a qualitative scoring system on a scale of 0 to 3+. A tumor biopsy is scored as 0 (negative), 1+ (negative), 2+ (borderline), or 3+ (positive) on an IHC test based on the reviewer's interpretation of staining intensity and completeness of membrane staining.<sup>5</sup> With FISH testing, the results are quantitative instead of qualitative; tumors are interpreted as HER2 "negative" or "positive" by enumerating the HER2/neu gene copy number.<sup>3</sup>



## What does it mean if a patient is weakly positive by IHC?

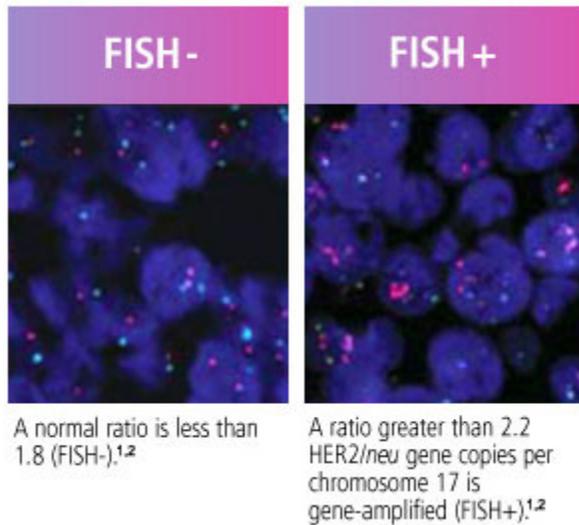
Patients whose tumors are weakly positive by IHC have weak or moderate intensity staining (see IHC 2+ slide below).<sup>5</sup>



This borderline group is the most difficult to score consistently by IHC and has a high rate of interobserver variability among pathologists.<sup>9</sup> Analysis by FISH may be useful for accurate

determination of HER2 status in this group. NCCN guidelines recommend confirming an IHC result of 2+ with FISH. <sup>4</sup>

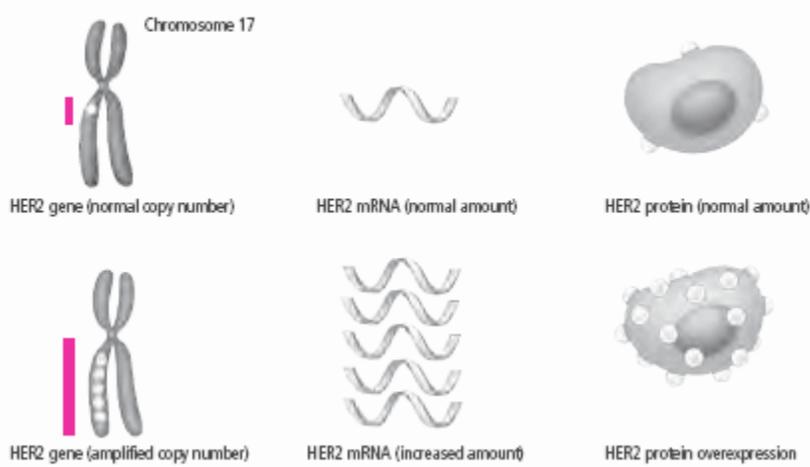
FISH\* results are presented as a quantitative score of the level of gene amplification. FISH testing measures the HER2/neu gene copy number against a standard internal chromosomal control (CEP 17). Results are expressed as a ratio of the number of HER2 gene copies (orange) per number of chromosome 17 copies (green). <sup>3</sup>



A normal ratio is less than 2 (FISH-). <sup>3</sup>

A ratio greater than or equal to 2 HER2/neu gene copies per chromosome 17 is gene-amplified (FISH+). <sup>3</sup>

## HER2 gene amplification and protein overexpression



HER2 gene amplification is a permanent genetic change. The excess copies of the HER2 gene result in the continuous overexpression of the HER2 protein on the cell surface.<sup>6</sup>

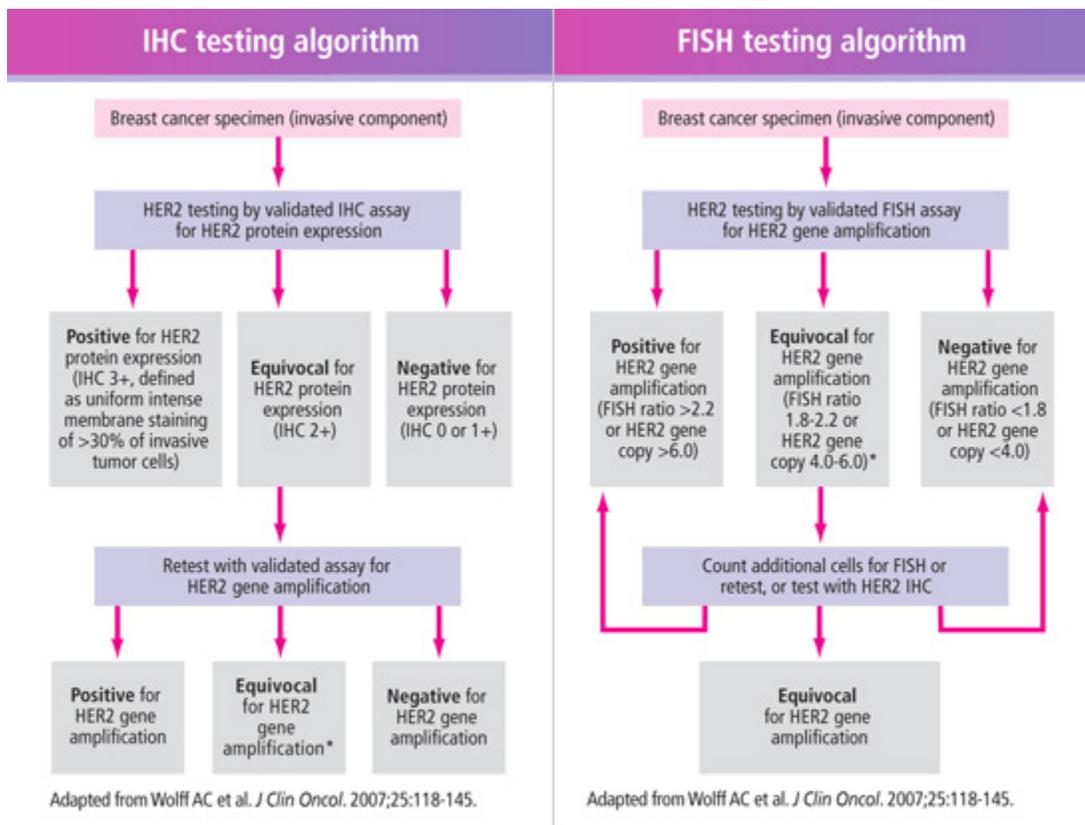
\*Vysis PathVysion®

## If A Patient is IHC 2+ or 3+ and FISH-, are they HER2+?

Overexpression of the HER2 protein rarely occurs in the absence of gene amplification.<sup>10</sup> FISH analysis reveals that some patients with apparent protein overexpression (IHC 2+ or 3+) do not have gene amplification (FISH-), suggesting that these patients may be "false positives." Approximately 2%-4% of patients who demonstrated HER2 protein overexpression by molecular techniques did not have gene amplification.<sup>10,11</sup> In current laboratory testing, variability in pre-analytical tissue processing, reagent variability, antigen retrieval, and scoring may result in IHC false-positives.<sup>7,8</sup>

## HER2 testing algorithms<sup>1,2</sup>

ASCO/CAP consensus guidelines for HER2 testing recommend that a final positive or negative HER2 result be achieved using the testing algorithms for IHC and FISH



**\*Patients with HER2/CEP17 ratio  $\geq 2.0$  were eligible for the adjuvant trastuzumab trials.**

\*Patients with strong complete membrane staining in more than 10% of tumor cells were eligible for the adjuvant trastuzumab trials. Users should refer to the package inserts of specific assay kits for information on the validation and performance of each assay.