Reviewer’s report

Title: Real-time PCR complements immunohistochemistry in the determination of HER-2/neu status in breast cancer

Version: 1 Date: 19 September 2005

Reviewer: Chris De Wolf-Peeters

Reviewer’s report:

General:

The authors evaluated Real Time PCR as an alternative method for the assessment of the HER-2/neu gene status in breast carcinoma with borderline IHC 2+ score. The design of the present study is well described and interesting, since IHC 2+ cases are poorly defined and FISH analysis is an expensive and sophisticated methodology. The abstract reflects the full study.

The authors should consider the following major revisions:

1. The methods used for the present study are all standardized. However, the use of gastrin as the reference housekeeping gene for real-time PCR analysis should be explained better. The latter gene is located at 17q21, a subregion that has been reported frequently as amplified in breast carcinoma. Could this influence the interpretation of the results? In the discussion section, the phenomenon of polysomy 17 was mentioned as it was noticed in 2 FISH negative cases. However, the explanation was poor and unclear (frequency of polysomy 17, detection by real-time PCR?). The data should be better controlled.

2. The manuscript describes in detail the clinical background of HER-2/neu positivity. In contrast, little attention has been paid to the technical advantages and/or shortcomings of the methodologies used. Since the present study aimed at comparing several techniques for accurate assessment of HER-2/neu status, we suggest that the authors focus on this technical aspect (e.g. the advantage of in situ examination for IHC/FISH; degradation of DNA from FFPE tissue; cost-effectiveness…). Also other recently developed techniques such as CISH and real-time RT-PCR could be mentioned.


Minor essential revisions:

1. On page 12, the formulation of the sentence ‘… suggests that real-time PCR is more accurate than IHC for detecting breast cancer (tumors) true positive for HER-2/neu amplification’ is prone to misinterpretation since IHC can only detect aberrations at the protein level.

2. The authors should explain why they use a cut-off value of 2.2 for FISH analysis and 2.0 for real-time PCR?

Discretionary Revisions: /
**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.