Detection of HER2 and Topo 2 in breast cancers: comparison between MLPA and FISH approaches

Sara Bravaccini,1 Claudia Rengucci,1 Laura Medri,2 Wainer Zoli,1 Rosella Silvestrini,1 Dino Amadori1

ABSTRACT
A significant proportion of breast cancers with HER2 amplification show simultaneous amplification or deletion of Topo 2. Amplification of Topo 2 may lead to the overexpression of the Topo 2 protein and ultimately to hypersensitivity to Topo 2 inhibitors. HER2 and Topo 2 gene status in breast cancer patients has been determined in several studies using immunohistochemistry, florescence in situ hybridisation (FISH) and multiplex ligation-dependent probe amplification (MLPA). Although comparisons of FISH and MLPA have been reported for HER2, it is believed that there are no similar studies for Topo 2. In this study, HER2 and Topo 2 were analysed by MLPA and FISH. There was a high agreement between the two approaches, although MLPA was easier to perform and cheaper than FISH. In conclusion, MLPA is a fast and accurate quantitative method to detect HER2 and Topo 2 amplification, and could be considered a good alternative to FISH.

INTRODUCTION
HER2 is a cell membrane surface-bound receptor tyrosine kinase that is normally involved in the signal transduction pathways leading to cell growth and differentiation. It is encoded by HER2/neu, a known proto-oncogene. Reports suggest that approximately 20–30% of breast cancers have amplified HER2/neu genes or overexpression of its protein product. Because of its ability to predict response to trastuzumab (Herceptin) as well as its prognostic role, breast tumours are routinely tested for overexpression of HER2/2/neu.

Topoisomerase inhibitors block the ligation step of the cell cycle, generating single- and double-stranded breaks that affect genome integrity, leading to apoptosis and cell death. In vitro studies have indicated that sensitivity to Topo 2 inhibitors is dependent on its expression level in the target cancer cells. Amplification of Topo 2 may lead to the overexpression of the Topo 2 protein and ultimately to hypersensitivity to Topo 2 inhibitors.

Most HER2 studies have used immunohistochemistry (IHC) to determine HER2 gene amplification; more recently, in situ hybridisation, which allows for accurate localisation of a specific segment of nucleic acid within a histological section, has been used. One of the most common in situ hybridisation techniques is dual or tricolour fluorescence in situ hybridisation (FISH), used to detect one or two genes together with the chromosome enumeration probe (CEP 17) as control. A number of reports have shown higher accuracy of FISH and apparent superiority over IHC in predicting response to trastuzumab in metastatic breast carcinoma. However, the main difficulty in adopting FISH in the clinical setting is its high cost and the need for specialised personnel for its interpretation. Another technique is chromogenic in situ hybridisation (CISH), which permits greater histological detail and is not subject to rapid fading, but again requires specialised personnel. Recently, a new, high resolution method to detect copy number variation in genomic sequences, multiplex ligation-dependent probe amplification (MLPA).

Figure 1 HER2 detection by florescence in situ hybridisation with Her 2 gene in red and centromere 17 control signals in green; (A) not amplified, and (B) amplified samples.
has been developed. MLPA has rapidly gained acceptance in genetic diagnostic laboratories due to its simplicity and similar execution time compared to other methods, and its relatively low cost, capacity for reasonably high throughput and perceived robustness. A large number of validation studies have either been published or are ongoing.

In this report, we compared the results of determination of HER2 and Topo 2 status by MLPA and FISH on a series of 20 breast cancers for HER2, and 15 for Topo 2.

METHODS
Fluorescence in situ hybridisation
FISH was performed using the FDA approved PathVysion kits (Vysis, Abbott Park, Illinois, USA) for HER2, and the TOP 2A/CEP17 FISH Probe Kit (Vysis, Abbott Park, Illinois, USA) for Topo 2. PCR products were analysed by 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, California, USA). HER2 and Topo 2 gene copy number was calculated by 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, California, USA). HER2 and Topo 2 gene copy number was normalised against the mean of the three probes for each marker. HER2 kit (MRC Holland, Amsterdam), which contains three probes each for HER2 and Topo 2. PCR products were analysed by 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, California, USA). HER2 and Topo 2 gene copy number was normalised against the mean of the three probes for each marker.

Multiplex ligation-dependent probe amplification
MLPA was performed as previously described,11 using the p004 HER2 kit (MRC Holland, Amsterdam), which contains three probes each for HER2 and Topo 2. PCR products were analysed by 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, California, USA). HER2 and Topo 2 gene copy number was determined by considering either the first probe (1st) or by calculating the mean of the three probes for each marker. HER2 and Topo 2 gene copy number were normalised against the control probes.

RESULTS
Table 1 presents the HER2 and Topo 2 results obtained by MLPA and FISH.

Table 1 Evaluation of HER2 and Topo 2 by fluorescence in situ hybridisation (FISH) and multiplex ligation-dependent probe amplification (MLPA)

<table>
<thead>
<tr>
<th></th>
<th>FISH</th>
<th>MLPA</th>
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<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>p Value</td>
<td>Median (range)</td>
<td>p Value</td>
</tr>
<tr>
<td>HER2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplified</td>
<td>2.90 (0.61)</td>
<td>0.003</td>
<td>1.80 (0.18–12.32)</td>
<td>0.025</td>
</tr>
<tr>
<td>Not amplified</td>
<td>1.14 (0.07)</td>
<td>0.069</td>
<td>1.06 (0.39–2.17)</td>
<td></td>
</tr>
<tr>
<td>Topo 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplified</td>
<td>3.27 (0.79)</td>
<td>0.007</td>
<td>2.23 (0.50–8.11)</td>
<td>0.003</td>
</tr>
<tr>
<td>Not amplified</td>
<td>1.13 (0.06)</td>
<td>0.108</td>
<td>1.10 (0.57–2.01)</td>
<td></td>
</tr>
<tr>
<td>Polysomic</td>
<td>1.55 (0.16)</td>
<td>0.48 (0.46–2.49)</td>
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DISCUSSION
HER2 and Topo 2 gene status in breast cancer patients has been determined in several studies using various methodologies, IHC, FISH and MLPA. However, analyses of results obtained with Mean values of HER2 by MLPA were about three times higher in FISH amplified than not amplified samples. Similarly, the mean values of Topo 2 by MLPA were three times higher in FISH amplified than not amplified cases. Moreover, the Topo 2 polysomic cases, as defined by FISH, had an intermediate MLPA value.

A more detailed analysis of individual HER2 values showed a high level of agreement with FISH results at an MLPA cut-off value of 2.0 using only the first probe (1st) (table 2). The three-probe combination gave quite similar results (data not shown). Therefore, 100% of cases were FISH positives for MLPA cut-off values higher than 2.0.

For Topo 2 detection, considering only the first MLPA probe (1st), there was total agreement (100%) between FISH negative results and MLPA cut-off values <1.0 (table 3). Conversely, using three MLPA probes, there was a 100% accuracy between FISH positive cases and an MLPA cut-off >1.4 (table 4).
In conclusion, MLPA is a fast, accurate, quantitative and cheap method to detect HER2 and Topo 2 amplification and could be considered a good alternative to FISH.

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Competing interests None declared.

Ethics approval Ethics approval was provided by the local ethical committee.

Contributors All authors have met the criteria for authorship as established by the International Committee of Medical Journal Editors, believe that the paper represents honest work, and are able to verify the results reported.

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REFERENCES


Table 4  Topo 2 determination by multiplex ligation-dependent probe amplification (MLPA) and florescence in situ hybridisation (FISH) (3 probes)

<table>
<thead>
<tr>
<th>MLPA (cut-off)</th>
<th>Positives n (%)</th>
<th>Negatives n (%)</th>
</tr>
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<tbody>
<tr>
<td>1.0 150 euro</td>
<td>7 (78)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>1.3 150 euro</td>
<td>7 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1.4 150 euro</td>
<td>7 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2.0 150 euro</td>
<td>3 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

FISH and MLPA have been reported for HER2 but not, at least at our knowledge, for Topo 2.

In our study, we analysed HER2 and Topo 2 by MLPA and FISH, observing a high agreement between the two approaches. The MLPA test was found to be accurate, even for the detection of polysomic cases, and was also easier to perform and much cheaper than FISH (about 15 euro/sample for MLPA compared to 150 euro/sample for the most widely used FISH kit commercialised by Vysis/Abbott, or 50 euro using the kit available from other suppliers). Moreover, MLPA is quantitative, not observer-dependent, and provides in a single step all the information required to decide on the best therapeutic approach.

Our MLPA results for HER2 status were similar for the first probe (1st) and the three-probe combination, in contrast to that reported by Moelans et al., with an overall agreement in positivity between FISH and an MLPA cut-off value of 2.0. Conversely, for Topo 2 determination, 100% agreement between the two methods was only observed at an MLPA cut-off of 1.4 using the three probes.

Take-home messages

- Multiplex ligation-dependent probe amplification (MLPA) and florescence in situ hybridisation (FISH) show high concordance in detecting HER2 and Topo 2.
- MLPA is easier to perform and less expensive than FISH.
- It is also a fast and accurate quantitative method to detect HER2 and Topo 2 amplification, and could be considered a good alternative to FISH.
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