Rapid fluorescence in situ hybridisation (FISH) for HER2 (ERBB2) assessment in breast and gastro-oesophageal cancer

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ABSTRACT

Evaluation of HER2 (ERBB2) gene amplification or protein expression is standard of care in breast (BR) and advanced stage gastro-oesophageal cancers to identify patients eligible for anti-HER2 therapies. Here, we evaluate a rapid fluorescence in situ hybridisation (FISH) technology (HER2 instant quality (IQ) FISH pharmDx Kit) for detection of HER2 in patients with BR and gastro-oesophageal cancer using 30 FFPE samples that had been previously evaluated with the PathVysion HER2 DNA Probe Kit. Cases were scored as positive (HER2:CEN-17 ≥2.0), negative (HER2:CEN-17 <2.0) or equivocal according to the ASCO/CAP 2013 BR cancer guidelines. Ten samples were positive for HER2 amplification while 20 were negative; none were equivocal. The IQ FISH was able to detect low level amplification (HER2:CEN-17 ratio 2.4). The HER2 IQ FISH pharmDx Kit is a FDA approved kit that offers a rapid turnaround time (approximately 3.5 h) and in our laboratory was 100% concordant with prior PathVysion results.

INTRODUCTION

Routine testing for HER2 (ERBB2) gene amplification or protein expression in breast (BR) and advanced stage gastro-oesophageal cancers is standard of care to identify patients eligible for anti-HER2 therapies. Typical testing methodologies include fluorescence in situ hybridisation (FISH) to detect HER2 gene amplification and immunohistochemistry (IHC) for detection of membranous protein expression.1 While some laboratories perform IHC followed by FISH for equivocal cases, our laboratory performs primary FISH testing. IHC results can be obtained in 1 day and the most commonly used FISH assays require 2 days for slide processing, potentially delaying the reporting of results. A rapid turnaround time may be of utmost importance to patient care when neoadjuvant therapy is indicated or enrolment in a clinical trial is being considered. Therefore, it is desirable to decrease this wait time so patients have quicker access to targeted therapeutics. Here, we evaluate a rapid, instant quality (IQ) FISH technology for detection of HER2 amplification in patients with BR and gastro-oesophageal cancer.

METHODS

Thirty FFPE samples (13 BR needle biopsies, five BR excisions, nine gastro-oesophageal biopsies and three biopsies of metastatic BR cancer to the liver, brain tissue and lung) that had been previously evaluated with the PathVysion HER2 DNA Probe Kit (Abbott, Abbott Park, Illinois) were studied with the HER2 IQ FISH pharmDx Kit (Dako, Glostrup, Denmark) (figure 1). After deparaffinisation and rehydration, a 10 min microwave oven pretreatment step is performed followed by pepsin digestion and dehydration. Slides are then probed and sealed; denatured for 10 min and hybridised for 60–120 min in the hybridisation oven. The IQ FISH assay uses a novel hybridisation buffer in which formamide is replaced with the less toxic solvent ethylene carbonate and thus, the hybridisation time is dramatically reduced. In addition, this ethylene carbonate buffer does not require blocking against repetitive sequences and can be used at a lower denaturation temperature (67°C) which reduces background staining.2 The probe chemistry is based on a combination of peptide nucleic acid (PNA) and DNA technology and contains Texas Red-labeled DNA probes for the HER2 gene and green (FTIC) fluorescein-labeled PNA probes targeted at the centromeric region of chromosome 17 (CEN-17). After hybridisation, the slides undergo a stringent wash and are mounted with a fluorescence mounting medium containing DAPI. Twenty nuclei were enumerated by each of two technologists with a fluorescent microscope using a DAPI filter in combination with a Texas Red/FITC double filter and, the HER2:CEN-17 ratio was calculated. Cases were scored as positive (HER2:CEN-17 ≥2.0), negative (HER2:CEN-17 <2.0) or equivocal according to the ASCO/CAP 2013 BR cancer guidelines.3

RESULTS

All 30 cases were successfully evaluated with the IQ FISH method. The HER2 IQ FISH correctly identified 10 samples as amplified and 20 as non-amplified; no samples were equivocal. The IQ FISH was able to detect low level amplification (HER2:CEN-17 ratio 2.4). There was 100% concordance between the IQ FISH and the PathVysion results (table 1). The HER2 IQ FISH signals were clear, bright and easy to interpret (figure 2). The turnaround time for IQ FISH was compared with traditional FISH which requires an overnight hybridisation step; IQ FISH can be performed in 1 day in approximately 3.5 h.

DISCUSSION

The HER2 IQ FISH pharmDx Kit is a recently available, FDA approved kit that offers a rapid turnaround time and in our laboratory was 100% concordant with prior PathVysion results. To date,
only a few studies have examined the concordance between the IQ FISH assay with conventional HER2 testing assays (FISH, SISH, CISH and IHC) in BR and gastro-oesophageal cancers. None of these studies are from a population in the USA and none have compared IQ FISH with the performance of the PathVysion HER2 FISH kit.4–7

The assay can be performed in approximately 3.5 h with about 1 h of hands-on tech time. By using ethylene carbonate instead of formamide in the hybridisation reagent, it is less toxic, the hybridisation time is dramatically reduced and results are available in 1 day. One potential drawback is that the slides, which can be stored in a refrigerator, can only be evaluated for 7 days. Also, a DAPI filter in combination with a high quality Texas Red/FITC double filter is required, which might not be standard in all laboratories.

In summary, the IQ HER2 FISH assay is an alternative to conventional FISH assays; it offers a decreased turnaround time, less toxic reagents, bright, crisp signals and a comparable performance with the PathVysion assay.

**Table 1** HER2 IQ FISH pharmDx versus PathVysion HER2 results; 100% concordance was observed

<table>
<thead>
<tr>
<th>PathVysion HER2</th>
<th>Amplified</th>
<th>Non-amplified</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Breast</td>
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</tr>
<tr>
<td></td>
<td>Non-amplified</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Gastro-oesophageal</td>
<td>Amplified</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-amplified</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Other (metastases)</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-amplified</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridisation; IQ, instant quality.

**Figure 1** HER2 instant quality fluorescence in situ hybridisation pharmDx workflow.

**Figure 2** HER2 instant quality fluorescence in situ hybridisation pharmDx in breast (BR) and gastro-oesophageal tissue. (A) Non-amplified BR; (B) amplified BR; (C) non-amplified gastro-oesophageal; (D) amplified gastro-oesophageal.
Take home message

Rapid HER2 FISH assessment decreases turnaround time and has a comparable performance as the PathVysion kit.

Handling editor Runjan A Chetty

Acknowledgements The authors thank the staff of the Dartmouth-Hitchcock Medical Center’s Department of Pathology Molecular Pathology Laboratory for technical support.

Contributors LJT was responsible for the overall content as guarantor which includes the study design, obtaining and interpreting the data and composing the manuscript. HBS, SFA and BJD were responsible for generating and interpreting the data. GJT was involved with the study design, interpreting the data and reviewing the content of the manuscript.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

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doi: 10.1136/jclinpath-2014-202787