An RNA based method to determine HER2 expression status in Breast Cancer Patients

Shawn Baldacchino[1], Sharon Falzon[2], Christian Saliba[3], Keith Sacco[1], James DeGaetano[2], Christian Scerri[4], Godfrey Grech[1a]

[1] Department of Pathology, University of Malta, Malta.
[2] Pathology Department, Mater Dei Hospital, Malta.
[3] Centre for Molecular Medicine and Biobanking, University of Malta, Malta.
[4] Department of Physiology and Biochemistry, University of Malta, Malta.
[a] Corresponding Author: Dr. Godfrey Grech, PhD, E-mail: godfrey.grech@um.edu.mt, Tel: +356 2340 3047

Introduction & Aim

Guidelines for the diagnostic evaluation of Human Epidermal Growth Factor Receptor 2 (HER2) have been recently revised in the attempt to reduce inter-laboratory variability and accuracy of the currently approved methods: *In situ hybridisation* (ISH) and Immunohistochemistry (IHC) (Wolff et al., 2013). Technical and subjective limitations persist in the determination of HER2 overexpression (Ballinger, Sanders, & Abramson, 2014). This pilot study determined HER2 expression in cell lines and Formalin Fixed Paraffin Embedded (FFPE) tissue lysates using an RNA-based method developed on Luminex technology using the Affymetrix Quantigene 2.0 system.

Background

HER2 overexpression is found in 15-20% of breast cancer (Wolff et al., 2013). These patients are eligible for HER2 targeted therapy. This subtype is associated with aggressive phenotype and without targeted therapy a poor prognosis is expected. Gene amplification accounts for around 90% of increased HER2 expression. In the local setting, IHC is used as a first-line HER2 screening method. The HER2 receptor antigen is sensitive to inadequate fixation of the specimen and often shows a heterogeneous staining pattern. Any ambiguous cases (scored as 2+) are analysed by Fluorescent ISH (FISH) or more recently, Dual ISH (DISH) to measure HER2 gene amplification. In cases of breast tumours with polysomy of chromosome 17 (≥3 CEP17), FISH/DISH results may still be inconclusive (Hanna et al., 2013).

Methods

Patient Selection

42 retrospective (2009-2011) breast tumours were selected based on Immunohistochemistry (IHC) HER2 receptor scoring (Table 1) and availability of HER2 Fluorescence in situ hybridisation (FISH) results in the 2+ (IHC score) ambiguous cases.

Table 1: Cases recruited for pilot study with HER2 receptor status by IHC

HER2 IHC score				Total
Negative	**1+**	**2+**	**3+**	
10	12	14	6	**42**

Sample Preparation

- Haematoxylin and Eosin (H&E) images were examined and their respective Formalin Fixed Paraffin Embedded (FFPE) tissue blocks were selected for macrodissection or Laser Microdissection (LMD).
- Sections were H&E stained followed by: (1) confirmation of tumour material in macro dissected tissue and (2) LMD was used to isolate specific morphological foci such as *in situ* carcinoma or a different tumour clone from heterogeneous samples.
- 2.5mm^3 of FFPE tissue was lysed in homogenizing solution and assayed using a 40 gene multiplex RNA based method (Affymetrix Quantigene 2.0).
- The target RNA in the sample lysate is hybridised onto magnetic beads with specific probes. Signal amplification branching is added onto captured RNA to allow concentration dependant signal detection.

Results

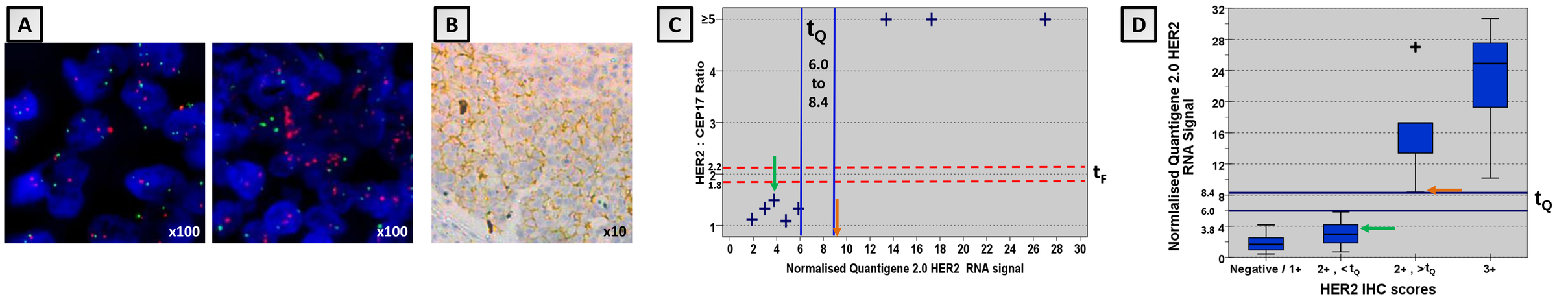

Figure 1: **A** FISH images using control probe CEP17 (Green) and the test probe HER2/neu (Red) on a case with normal HER2 (Left) and a case with amplified HER2 gene (Right) [Magnification x100]

B HER2 IHC 2+ ambiguous membranous staining [Magnification x10].

C FISH results using HER2:CEP17 ratio against the Quantigene 2.0 normalised signals for the HER2 2+ (IHC score) ambiguous cases.

[The Quantigene 2.0 assay threshold (t_Q) is defined by the dark blue lines and is set at 6.0 - 8.4; The FISH HER2:CEP17 ratio threshold (t_F) is set as 1.8 - 2.2 and is indicated by the dotted red line; The orange arrow is indicating a polysomic case with a FISH HER2 Amplification factor of 1.49 while the green arrow indicates the HER2 RNA expression of a difficult polysomic case regarded as HER2 positive by the pathologist following assessment].

D Boxplot illustrating the distribution of HER2 signal detected by the RNA-based method (Quantigene 2.0) across the HER2 IHC classes.

Table 2: Comparison of HER2 results using IHC, the Quantigene 2.0 and FISH. The Quantigene 2.0 results are consistent with the FISH results and also classified all the IHC ambiguous cases. All IHC negative cases had low normalised HER2 RNA signal, while all the IHC 3+ positive cases had high HER2 RNA levels using the Quantigene 2.0 RNA based method, as expected.

IHC		Quantigene 2.0		HER2 FISH	
Score	**Count**	**< t_Q**	**> t_Q**	**Negative**	**Positive**
Negative / 1+	22	22	0	Not Available	
2+	14	9	5	5	3
3+	6	0	6	Not Available	

Quality Control

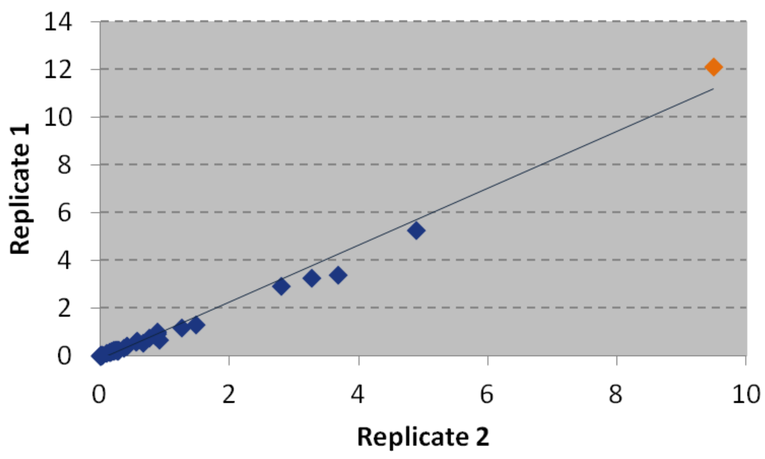

Figure 2: Graph shows RNA signal intensities of 40-genes between replicates. A HER2 positive sample lysate was used in this inter-run correlation resulting in an R^2 of 0.98. [HER2 gene signal is denoted by an orange diamond mark].

- The correlation across the 40 genes between inter-run cell line RNA and patient lysate and intra-run replicates ranged from R^2 of 0.95 to 0.998.
- Expression data was normalised to the geomean of Housekeeping genes.

Conclusions

The Quantigene 2.0 RNA based assay has accurately classified HER2 breast cancer patients, including the IHC ambiguous cases. This assay detects HER2 overexpression with high reproducibility and specificity, also in cases of polysomy chromosome 17. The Quantigene 2.0 assay can be multiplexed up to 100 targets per reaction providing extensive RNA expression profiles for tumours. The RNA based method accompanied with LMD provides a useful tool towards more accurate targeted and personalised therapy.

References

- Ballinger, T. J., Sanders, M. E., & Abramson, V. G. (2014). Clinical Breast Cancer.
- Hanna, W. M., Rüschoff, J., Bilous, M., Coudry, R. A., Dowsett, M., Osamura, R. Y., . . . Viale, G. (2013). *Modern Pathology, 27*(1), 4-18.
- Kao, J., Salari, K., Bocanegra, M., Choi, Y.-L., Girard, L., Gandhi, J., . . . Pollack, J. R. (2009). *PLoS ONE, 4*(7), e6146.
- Wolff, A. C., Hammond, M. E. H., Hicks, D. G., Dowsett, M., McShane, L. M., Allison, K. H., . . . Hayes, D. F. (2013). 31(31), 3997-4013. doi: 10.1200/jco.2013.50.9984

ALIVE charity foundation
IMaGenX
Italy-Malta: a sea of opportunity for your future
www.italiamalta.eu
Italia-Malta Programme - Cohesion Policy 2007-2013
A Sea of Opportunities for the Future
This position is part-financed by the European Union European Regional Development Fund (ERDF)
Co-financing rate: 85% EU Funds; 15% National Funds
Investing in your future