TT12. A Quantitative Real-Time PCR Based Approach for Resolution of *HER2* Amplification/Over-Expression Status in FISH and IHC "Double Equivocal" Breast Cancer

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Introduction: This study assesses the feasibility of using Quantitative Real-Time PCR (qRT-PCR) to resolve *HER2* amplification/over-expression status in invasive breast cancer cases that fail resolution via immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) testing following ASCO/CAP guidelines. The IHC and FISH equivocal patient population (Double Equivocal) represent a particularly problematic breast cancer sub-group that currently lacks standardized clinical management guidelines. **Methods:** Cases were selected from the Cleveland Clinic electronic records from 1/2008 to 12/2010. RNA extraction was performed following macro-dissection using High Pure RNA Paraffin Kit (Roche Applied Biosciences, Indianapolis, IN). qRT-PCR was carried out using TaqMan® RNA-to-CT™ 1-Step Kit with primers and probes (*HER2*, B2M, GAPDH, ACTB, TFRC, Applied Biosystems, Foster City, CA). qRT-PCR was performed on a LightCycler 480 II (Roche Applied Biosciences, Penzberg, Germany) according to the manufacturer's instructions. Results were expressed as the ratio of *HER2* to reference gene copies, all normalized against calibrator RNA from MCF7. **Results: q**RT-PCR performed on two breast cancer control groups, *HER2* amplified (AMP) and non-amplified (Non-AMP) as defined by FISH and IHC, demonstrated 2 non-overlapping populations. ROC curve analysis, using a cut off of 7.0, showed the qRT-PCR assay separates AMP from Non-AMP cases with 100% sensitivity and specificity. Applying the 7.0 RT-PCR cut off to a group of double equivocal cases resulted in resolution of *HER2* amplification/expression status for all cases (10 AMP and 40 Non-AMP). Cases with heterogeneity of *HER2* expression did not alter sensitivity of the RT-PCR assay. **Conclusions: q**RT-PCR analysis of *HER2* gene expression represents a viable approach to resolve cases with double equivocal *HER2* status at the time of diagnostic biopsy. This molecular approach accurately determined *HER2* status in a population that failed classification by both FISH and IHC. qRT-PCR combines the precision and high sensitivity of real-time PCR with the morphological specificity of histological evaluation and ultimately allows definitive *HER2* classification at the time of initial diagnosis. Further studies correlating response to anti-*HER2* therapy and *HER2* status by qRT-PCR are warranted.