**Immunodetection**

# Tissue sections underwent microwave antigen retrieval (5 min 750W, 15 min 350W) in 10 mM citrate buffer (pH 6.5) and were incubated overnight with a 1:300 dilution of U407 or a 1:1000 dilution of AR-N20 in blocking buffer (5% normal goat serum in PBS) at 4°C in a humidified chamber. Visualization of immunoreactivity was achieved using biotinylated anti-rabbit immunoglobulins (Dako), streptavidin–peroxidase conjugate (Dako) and diaminobenzidine tetrahydrochloride (DAB) to yield an insoluble brown deposit as described previously ([1](#_ENREF_1)). A whole tissue section from a breast cancer paraffin block known to be immunoreactive for AR was utilized as a positive control in both instances, and the primary antibody was omitted for the negative control. Positive immunostaining for AR was predominantly seen in the nucleus of tumor cells with use of either antibody. The percentage of AR positive tumor nuclei in each TMA core was assessed within 100-200 tumor cells by independent scorers blinded to clinical outcome (CR and AS for training cohort, SJ for validation cohort) in 2-4 40x high power fields. A high concordance was observed between the two observers for the training cohort (ρc=0.849, Lin's Concordance test) and an audited subset of cases in the validation cohort (ρc=0.998, Lin's Concordance test). The average AR positivity was calculated between the two independent observers for both the training and validation cohorts. The highest AR value of the replicate cores were used for statistical analysis ([2](#_ENREF_2)). ERα, PR, HER2/*neu* and Ki67 were stained and scored as described previously for the training cohort ([3](#_ENREF_3), [4](#_ENREF_4)). For the validation cohort, ERα, PR and HER2/*neu* status had already been determined by the Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada ([2](#_ENREF_2)).

**Breast Cancer Subtype Classification**

Luminal A was classified as ERα+ or PR+ HER2- and Ki67 <14%; luminal B (HER2-) classified as ERα+ or PR+ HER2-, Ki67 ≥14% or luminal B (HER2+) classified as ERα+ or PR+, HER2+ and any Ki67, HER2+ classified as ERα- and PR- and HER2+; triple negative breast cancer (TNBC) classified as ERα-, PR- and HER2-.

**Statistical Analyses**

All analyses were performed using PAWS statistics 17 Windows software (SPSS Inc., Chicago, IL). To evaluate the relationship with clinical outcome, AR levels were analyzed initially as continuous variables using univariate Cox regression analysis. Variables which were significant as continuous variables were then analyzed as dichotomized values using univariate and multivariate Cox regression analysis. Only variables significant by univariate analyses were included in multivariate analyses. ROC analysis was used to dichotomize AR and Ki67 positivity. The optimal cut-points were determined using Youden index (J) which was calculated using the formula J= max [sensitivity + specificity-1] ([5](#_ENREF_5)). In addition to ROC analysis, recursive partitioning was also applied to the training cohort to select the most appropriate AR cut-point ([6](#_ENREF_6)). In Cox regression and Kaplan-Meier analyses, relapse or death due to breast cancer was used as the endpoint to determine whether AR levels or the AR to ERα ratio are associated with relapse-free survival (RFS, only available for the training cohort) or breast cancer specific overall survival (OS). Patients who died from other causes were censored on their date of death. The AR to ERα ratio was analyzed as tertile groups to compare outcome for patients with tumors containing comparable levels of AR and ERα to tumors with a predominance of either receptor (AR > ERα or AR < ERα). The training and validation cohorts were combined to increase the sample size of patient groups. AR to ERα ratio tertiles were calculated to be <0.87, 0.87-1.05 and >1.05. For the calculation of the AR:ERα ratio, tumors that were negative (<1%) for ERα, AR or both receptors, were excluded. Six patients with AR and ERα measurements were not included in this analysis due to loss of clinical follow-up.

**References**

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