Cyclin E overexpression is associated with High Risk 70 Gene Signature, and may indicate intrinsic resistance to CDK4/6 inhibitors

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Background: The use of CDK4/6 inhibitors (CDK4/6i) is a promising therapeutic strategy for recurrent ER+, HER2- breast cancers that have escaped previous treatment targeting the endocrine pathway. A number of adjuvant and neoadjuvant trials with CDK4/6i in early breast cancer are also underway. However, no clear predictive biomarkers for resistance are available other than loss of the *RB1* tumor suppressor gene. Cyclin E genes (*CCNE1*, *CCNE2*) play a critical role in cell cycle control with approximately 25% of breast cancer overexpressing *CCNE2*, and there is a significant correlation between *CCNE2* and *CCNE1* expression[1,2,3]. Furthermore, a positive correlation between expression of *CCNE1* and resistance to CDK4/6i (i.e. palbociclib)[4,5,6] has been reported *in vitro* and in a gene expression analysis from the PALOMA-3 trial. The 70-gene MammaPrint® (MP) signature is a prognostic assay that stratifies early-stage breast cancer (ESBC) patients into Low and High-risk of distant relapse. One of the 70 genes is *CCNE2*, shown to be associated with resistance to both endocrine therapy and CDK4 inhibition[7]. Considering the potential role of the Cyclin E genes as biomarker of resistance for CDK4/6i, we assessed the expression of *CCNE2* in a large series of ESBCs with respect to their MP risk profile.

Methods: The mRNA expression of *CCNE2* (and *CCNE1*) was measured in a series of 5022 breast cancer samples for which FFPE microarray full-transcriptome data were available for testing. Intensities were Lowess normalized and log2 transformed. The 80-gene BluePrint (BP) profile[8] was used in combination with MP to stratify patient samples based on their molecular subtype: Luminal A- (MP Low Risk, BP Luminal), Luminal B- (MP High Risk, BP Luminal), HER2- and Basal-type. Wilcoxon rank sum test was used to examine expression differences.

Results: *CCNE2* is significantly higher expressed in MP High risk compared to MP Low risk tumors when all samples are included ($p < 0.0001$). When looking at the clinically ER positive BP Luminal group, *CCNE2* is significantly higher expressed in the Luminal B compared to the Luminal A tumors ($p < 0.0001$). However, we observe a broad distribution of *CCNE2* expression within the Luminal group, indicating a biological diversity in both Luminal A and B tumors, which *CCNE2* may help to further define. Specifically, Luminal B tumors have a range of *CCNE2* expression with the highest levels being in the highest MP risk interval (Ultra High). Of importance, *CCNE1* showed similar expression patterns as *CCNE2*.

Conclusions: Our preliminary results show that MP could help in stratifying BP Luminal-type B tumors in subgroups with differential *CCNE2* expression. Since increased *CCNE1* expression is correlated with CDK4/6i resistance, and *CCNE2* is a functionally-related gene whose expression correlates to some extent with *CCNE1*, it is reasonable to speculate that high *CCNE2* expression and MP High Risk Luminal B may also correlate with CDK4/6i resistance, a testable hypothesis.

This warrants additional analyses that integrate treatment response data to support and further investigate these observations. Our exploratory analysis also highlights the added value of a multi-gene signature profile *versus* single-gene testing as tool for patient stratification and treatment recommendation.

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