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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 (BC) which have led to the evaluation of CDK4/6i in the adjuvant setting for early-stage BC (EBC). To date, 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 ~25% of breast cancer overexpressing *CCNE2* with significant correlation between *CCNE2* and *CCNE1* expression[1,2,3]. Recently the PALOMA-3 trial reported a positive correlation between expression of *CCNE1* and resistance to CDK4/6i (i.e. palbociclib) which was also confirmed in *in vitro* studies[4,5,6] .

The 70-gene MammaPrint® (MP) signature is a recurrence assay that stratifies EBC 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 EBCs with respect to their MP risk profile.

METHODS

The expression of *CCNE2* was measured in a series of 5022 EBC for which FFPE microarray data were available. Intensities were Lowess normalized and log2 transformed. The 80-gene BluePrint (BP) profile[8] was used in combination with MP to stratify patient samples into distinct functional molecular subtypes: Luminal A- (MP Low Risk, BP Luminal), Luminal B- (MP High Risk, BP Luminal), HER2- and Basal-type.

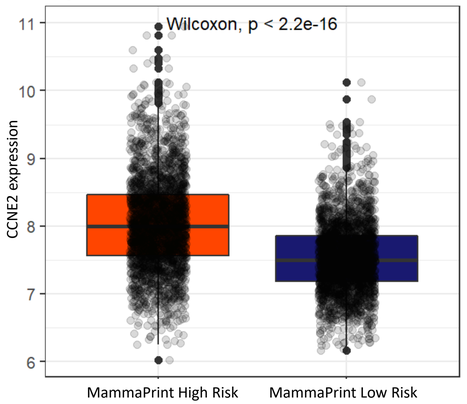
In addition, *CCNE2* expression was assessed in a set of ER+ HER2- EBCs from the NeoPalAna trial[9] (N=108) for which response data to neoadjuvant palbociclib/anastrozole therapy at different timepoints were available (Baseline, N=29; prior start palbociclib N=30; after 15 days palbociclib, N=28; surgery, N=21). As previously reported[10], microarray data were generated from fresh frozen RNA and the 70 genes of the MP signature were selected from the GPL8253 array to calculate a research approximation of the 70-gene MP index (70-GS).

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 (**N=5022**) (**Figure 1**).

Figure 1



When looking at the clinically **ER positive** group (**N=1207**), *CCNE2* is significantly higher expressed in the High Risk Luminal B, HER2 and Basal tumors compared to the Low Risk Luminal A tumors (**Figure 2A**). However, we observed 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. Within the luminal group, Luminal B tumors have a range of *CCNE2* expression with the highest levels present in the highest MP Risk interval (Ultra high, MP index closer to -1.0) (**Figure 2B**).

Figure 2A

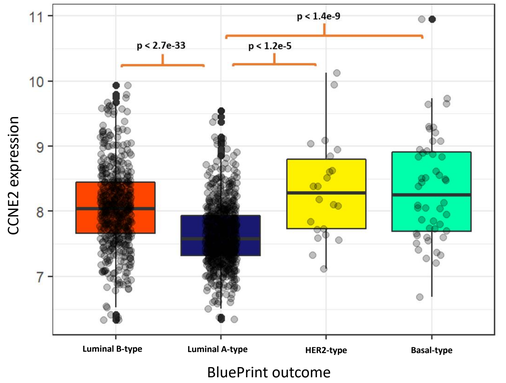
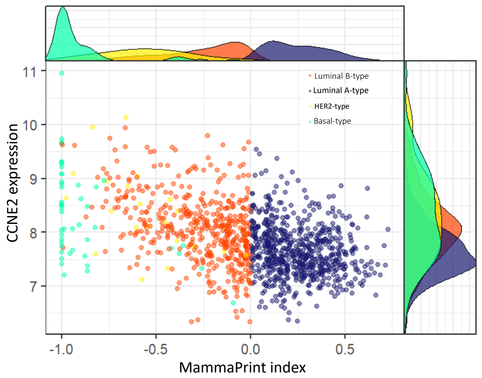


Figure 2B



In the NeoPalAna dataset (N=28), patients resistant to palbociclib/anastrozole therapy show a trend towards higher expression of *CCNE2* compared to palbociclib/anastrozole sensitive patients, after 15 days of palbociclib treatment (**Figure 3A**). Interestingly, when looking at a research 70-GS, resistant patients showed significantly higher risk 70-GS results compared to sensitive patients (**Figure 3B**), highlighting the added value of the 70-GS in identifying patients resistant to palbociclib.

Figure 3A

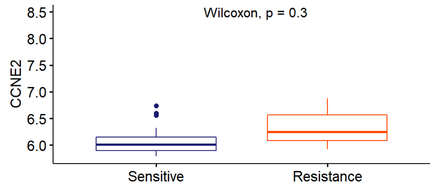
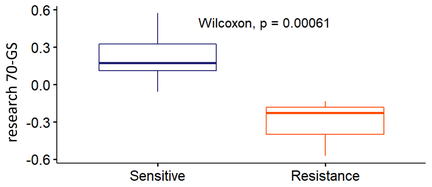


Figure 3B



CONCLUSIONS

MammaPrint has the potential to help stratify ER+ tumors into subgroups with differential *CCNE2* expression and identify patients who are likely resistant to CDK4/6i. Our exploratory analysis highlights the added value of a multi-gene signature profile versus single-gene testing as tool for patient stratification and treatment planning. Additional analyses that further support and investigate these observations are needed.

References

1. Asghar U et al Nat Rev Drug Discov. 2015
2. Caldon C et al Cell Div. 2010
3. Payton M et al Oncogene. 2002
4. Turner N et al. J Clin Oncol. 2019
5. Chandarlapaty S et al J Clin Oncol. 2019
6. Guarducci C et al NPJ Br Cancer. 2018
7. Caldon CE et al Mol Cancer Ther. 2012
8. Krijgsman O et al BRCT 2012
9. Ma CX et al CCR 2017
10. Hoog J et al SABCS 2017