Association of MammaPrint® with Gene Expression Pathways Predictive of Resistance to Cyclin-Dependent Kinase 4/6 (CDK) Inhibition

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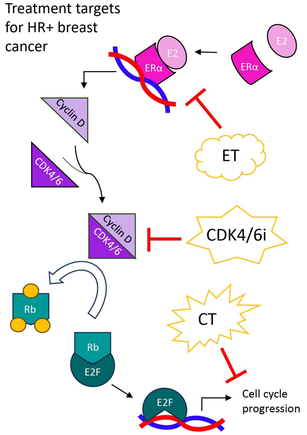
Presentation ID: PS2-08, December 12, 2024

Background

- Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) have become a first-line targeted treatment in combination with endocrine therapy (ET) for patients with recurring HR+HER2- breast cancer (BC) and have recently been approved in the adjuvant setting for the treatment of clinically high-risk early-stage BC.
- Trials have demonstrated that approximately 30% of advanced stage breast cancers are resistant to CDK4/6i, but no biomarkers exist to inform which patients are least likely to respond.[1]
- The commercially available MammaPrint genomic signature has demonstrated utility in predicting treatment response to chemotherapy (CT) as well as targeted immunotherapies (IO). [2,3]
- We investigated the utility of MammaPrint for identifying tumor subtypes that predict resistance to CDK4/6 pathway inhibition by comparing MammaPrint to gene expression patterns indicative of cellular proliferation pathways that bypass CDK4/6 function.
- We hypothesize that MammaPrint Risk categories with higher correlation to gene expression associated to Retinoblastoma (Rb) loss-of-function[4] and high proliferation independent of CDK4[5] are most likely resistant to CDK4/6 inhibition via the CDK-Rb-E2F Axis (Figure 1).

Figure 1. Treatment targets for HR+ breast cancer



MammaPrint Index

UltraLow Risk
- ET benefit < 5 years

Low Risk
- ET benefit 0-10 years

High 1 Risk
- CT benefit
- ET benefit 0-5 years

High 2 Risk
- CT benefit
- ET benefit 0-5 years
- Increased sensitivity to PARPi, IO, Platinum

Table 1. Clinical Characteristics of FLEX Patients with HR+HER2- disease

Clinical Characteristics	No. patients (%) (n=5778)
Age in years – Mean (SD)	60 (±12)
Menopausal Status	
Pre-/Peri-	1181 (20.4)
Post-	4301 (74.4)
Race	
White	4633 (80.2)
Black	496 (8.6)
Latin/Hispanic	198 (3.4)
AAPI	156 (2.7)
Other	27 (0.5)
Nodal Status	
N0	3546 (61.3)
N1	750 (13.0)
N2	42 (0.7)
N3	18 (0.3)
Grade	
G1	1714 (29.6)
G2	2851 (49.3)
G3	939 (16.3)
MammaPrint/BluePrint	
UltraLow/Luminal A	809 (14.0)
UltraLow/Unknown	25 (0.4)
Low/Luminal A	2117 (36.6)
Low/Unknown	84 (1.5)
High 1/Luminal B	2053 (35.5)
High 1/Basal	21 (0.4)
High 1/Unknown	100 (1.7)
High 2/Luminal B	314 (5.4)
High 2/Basal	234 (4.0)
High 2/Unknown	16 (0.3)

BluePrint HER2-Type excluded from analysis due to small sample size (n=5); Unknown values excluded from table.

Figure 2. Association of Rb loss-of-function genes with MammaPrint/BluePrint subtype

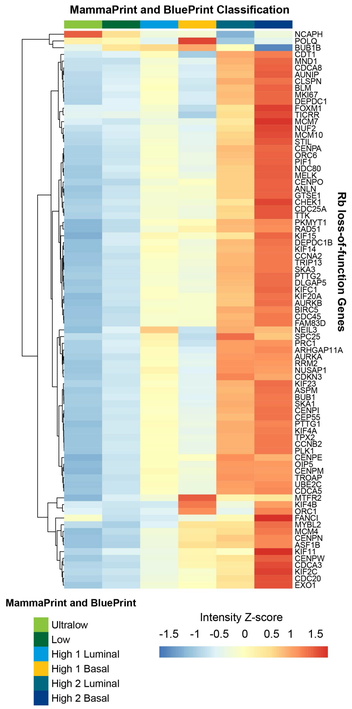


Figure 3. Rb loss-of-function genes among MammaPrint/BluePrint subtypes

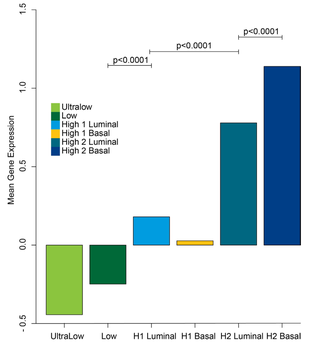


Figure 4. Proportion of tumors with hypothesized resistance to CDK4i by MammaPrint category

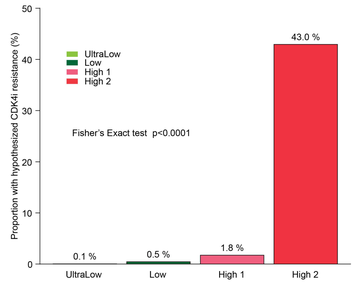
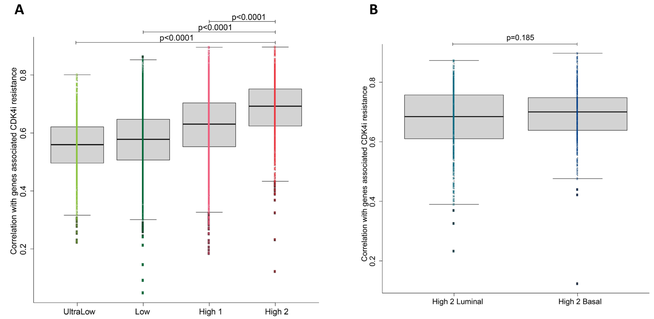


Figure 5. Correlation of **A)** MammaPrint and **B)** High 2 Blueprint subtypes with genes associated with resistance to CDK4i



Methods

Patients: All from FLEX (NCT03053193) with HR+HER2- disease (n=5657).

FLEX Genomic Testing:

MammaPrint	UltraLow	Low	High 1 (H1)		High 2 (H2)	
BluePrint	Lum A		Lum B	Basal	Lum B	Basal

Analysis: Correlation of genes associated with Rb loss-of-function and subsequent E2F-dependent cell proliferation[4] to MammaPrint Risk groups was performed. MammaPrint Risk groups were then compared to genes associated with high proliferation independent of CDK4 and hypothesized resistance to the CDK4i, Palbociclib.[5]

Statistics: The association of MammaPrint and Rb genes was assessed using Spearman's rank correlation. Differences in mean gene expression correlations within MammaPrint/BluePrint subtypes were assessed using 2-way ANOVA. Differences in proportions of tumors with resistance to CDK4i among MammaPrint was measured by Fisher's exact test.

Results

- Among all HR+HER2- tumors, 14.4% (n=834) were classified as UltraLow, 38.1% as (n=2201) Low, 37.6% (n=2175) as High 1, and 9.8% (n=568) as High 2 Risk (**Table 1**).
- A linear correlation was observed with increasing MammaPrint Risk and increasing loss-of-Rb function (p<0.001) (**Figure 2**). Mean gene expression for Rb loss-of-function exhibited highest correlation with High 2 tumors. All MammaPrint/BluePrint subtype comparisons were significant (2-way ANOVA, p<0.0001 for all comparisons) (**Figure 3**).
- MammaPrint High 2 had the highest proportion of tumors with predicted resistance to CDK4i (43.0%) compared to other MammaPrint groups (p<0.001) (**Figure 4**).
- High 2 exhibited the strongest correlation to genes associated with high proliferation independent of CDK4, demonstrating significantly higher correlation compared to all MammaPrint groups (2-way ANOVA, p<0.0001 for all MammaPrint comparisons) (**Figure 5A**). However, no significant difference was observed in the correlation between predicted resistance to CDK4i between Blueprint High 2 Luminal and High 2 Basal subgroup comparisons (2-way ANOVA, p=0.185) (**Figure 5B**).

Conclusions & Future Work

- These data identify High 2 Risk tumors as most likely resistant to CDK 4/6 targeted inhibition compared to other MammaPrint Risk groups.
- Gene expression associated with Rb loss-of-function and high cellular proliferation independent of CDK4 were most closely correlated with MammaPrint High 2 Risk.
- These data are consistent with previous studies demonstrating that increasing MammaPrint index is closely associated with Cyclin E (CCNE1 and CCNE2) and 8q22-24 (CCNE2, MTDH, TSPYL5) genomic expression, associated with tumor proliferation driven by activity downstream CDK4/6 function in HR+ BC.[6,7]
- These data suggest that High 2 tumors might exhibit resistance to CDK4/6i, indicating that these patients could benefit more from immunotherapy rather than CDK4/6i.
- To our knowledge, MammaPrint is the only clinically available genomic signature that may identify patient subgroups potentially resistant to CDK4/6 inhibitors.
- Further research is warranted to understand the role of MammaPrint informed decision making in CDK4/6i treatment decision making for patients with HR+HER2- breast cancer.

References

1. Vinciguerra, et al. Front Oncol. 2022. 2. Whitworth et al., Ann Surg Oncol. 2022. 3. Pusztai et al., Cancer Cell. 2021. 4. Malorni, et al. Oncotarget. 2016. 5. Raspe, et al. EMBO Mol Med. 2017. 6. Mittempergher, et al. SABCS. 2019. 7. Diab et al. ASCO. 2020.