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Gene and pathway differences between MammaPrint High1/High2 risk classes: results from the I-SPY 2 TRIAL in breast cancer

Background: Further stratification of the 70-gene MammaPrint™ prognostic signature into 'high' and 'ultra-high' risk groups may help predict chemo-sensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/2 classification was added to HR and HER2 to define the subtypes used in the I-SPY 2 adaptive randomization engine. The first two experimental agents/combinations to graduate from I-SPY 2 were veliparib/carboplatin (V/C) in the TN subset, and neratinib (N) in the HR-HER2+ subset. MP2 was found to be a sensitivity marker for V/C but not N, whereas MP1 class appears associated with resistance to N within the HER2- subset. Here, we present exploratory analysis to identify the genes and pathways that distinguish MP1 from MP2.

Methods: 263 patients (V/C: 71, N: 115, and controls: 77) with pre-treatment Agilent 44K microarrays and MP1/2 class assessments were considered in this analysis. To identify signature genes associated with MP1 vs. MP2 class, we (1) apply a Wilcoxon rank sum test and (2) fit a logistic model. P-values are corrected for multiple comparisons using the Benjamini-Hochberg (BH) method, with a significance threshold of BH $p<0.05$ from both tests. We then perform pathway enrichment analysis using DAVID. In addition, we perform multivariate analysis adjusting for receptor subtype. Our study is exploratory and does not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: 63% (165/263) of patients are MP1 class and 37% (98/263) MP2. MP1/2 class is associated with receptor subtype (Fisher's exact test: $p<2E-16$), where 71% of TN patients are MP2 and 96% of HR+HER2+ patients are MP1. Of the 70 signature genes, 86% (60/70) differ in expression between MP1 and MP2, with 70% (42/60) expressed at a higher level in MP2, including CDCA7, MELK and CENPA. In a whole transcriptome analysis, 10,500 genes (of ~30,000) appear differentially expressed. Following adjustment for HR and HER2 status, 4368 genes are significantly differentially expressed between MP1 and MP2. By DAVID enrichment analysis, the biggest pathway-level differences are found in cell cycle, proliferation, and DNA repair, with the MP2 set showing higher expression.

Conclusion: MP2 class appears associated with higher expression of cell cycle genes. Association between MP2 class and response to V/C suggests that higher cell cycle activity may contribute to V/C sensitivity.