Development of a 6-gene qPCR RUO-validated assay as a predictive biomarker for response of vantictumab (OMP-18R5; anti-frizzled) in HER2- breast cancer patients

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Abstract

Background: We have developed a monoclonal antibody, vantictumab that blocks cytosolic WNT/β-catenin signaling through binding of five FZD receptors (1, 2, 5, 7, 8). This antibody inhibits the growth of several tumor types, including breast. Vantictumab reduces tumor cell frequency and exhibits synergistic activity with standard-of-care (SOC) agents (Gurney et al., 2012). To target breast cancer patients most likely to respond to vantictumab, we undertook a biomarker study.

Methods: We have identified a 6-gene post-pathway-related signature, FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1, based on microarray gene expression data from 8 breast cancer patient derived xenograft (PDX) models established in vivo response to vantictumab plus paclitaxel. This signature successfully predicted the response of 8 additional and independent PDX breast tumors. We further developed a qPCR Research Use Only (RUO) assay for the 6 genes to be used on FFPE human breast tumor samples. Multiple assays targeting different regions spanning each mRNA transcript were tested and selected based on PCR efficiency, specificity and sensitivity. We compared assay sensitivity under different cDNA synthesis and pre-amplification conditions: random vs. gene-specific priming, number of pre-amplification cycles, pre-amplification reaction volumes, and cDNA synthesis kits. A repeatability study was performed to test assay performance. The pre-amplification and PCR were repeated over three separate days and across two independent labs.

Results: Our results showed that cDNA synthesis by gene-specific priming followed by 18 cycles of pre-amplification performed the best and the assay is robust with minimal starting FFPE RNA input. The results of the repeatability study were consistent among the different days and the different kits (-5% CV). Using the 6-gene qPCR RUO assay, the signature score from the microarray data was further refined using 12 PDX HER2- breast tumors known in vivo response to vantictumab with SOC. The prevalence of the 6-gene signature was estimated to be ~100% HER2- breast cancer samples.

Conclusions: A robust 6-gene RUO-validated assay was developed as a predictive biomarker for vantictumab in HER2- breast cancer. The assay is currently being evaluated in a Phase 1b study of vantictumab with paclitaxel in HER2- breast cancer.

Identification of the 6-gene signature

Microarray gene expression data from 8 PDX breast tumors with established in vivo efficacy to vantictumab plus paclitaxel were used to identify the 6-gene signature. The 6-gene signature achieved the best performance and was successfully predicted the response of 8 additional and independent PDX breast tumors. We further developed a qPCR Research Use Only (RUO) assay for the 6 genes to be used on FFPE human breast tumor samples. Multiple assays targeting different regions spanning each mRNA transcript were tested and selected based on PCR efficiency, specificity, and sensitivity. We compared assay sensitivity under different cDNA synthesis and pre-amplification conditions: random vs. gene-specific priming, number of pre-amplification cycles, pre-amplification reaction volumes, and cDNA synthesis kits. A repeatability study was performed to test assay performance. The pre-amplification and PCR were repeated over three separate days and across two independent labs.

Conclusion:

10 FFPE samples were run 3 times each in a separate day by two different labs. In each run, pre-ampl was performed in duplicates, PCR was done in triplicates. Human reference RNA was used as a positive control. Standard curves were used to evaluate assay efficiencies. *NCV was calculated across the three runs for each gene in each sample. Consistent data observed across different days and different labs tested the stability of NCV values are below 15%.

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Summary

6-gene signature was identified as a potential predictive biomarker for the response to vantictumab plus paclitaxel in breast tumors using microarray gene expression data. • Biomarker was tested in 8 PDX models total including validation in 8 independent models.

qPCR based RUO-validated assay was developed for the 6-gene biomarker. • A 16-gene post-pathway-specific priming followed by 18 cycles of pre-amplification with SuperScript II performed the best. Potassium results were obtained with minimal RNA concentrations, which maximized the number of samples that passed concentration QC.

Repeatability study demonstrated consistent data for the 6-gene assay run across different days and different labs tested the stability of the 6-gene signature.

6-gene biomarker was refined by using the qPCR assay results in 12 PDX breast tumors.

6-gene signature was not significantly associated with any clinical or technical factors tested in the 113 HER2- breast cancer cohort. Including TNBC vs. HER2- patients, primary vs. metastatic, resection vs. biopsy, TNBC vs. HER2- patients, and 50% difference between tumor age, % tumor, disease stage, or tumor age.

Using the refined 6-gene signature score, prevalence was estimated to be 43% in HER2- breast cancer FFPE samples (n=113), 42% in TNBC- (HER2- or PR-), and 42% in HER2- hormone receptor positive (HER2-ER+PR+), n=52.

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