**BRCA1 promoter methylation in sporadic breast cancer patients detected by liquid biopsy**

**Introduction**

**BRCA1** promoter methylation (PM) is an early initiating event in cancer, occurring in 3 to 65.2% of all breast tumors depending on subtype, and 30 to 85% of triple negative tumors. **BRCA1** promoter methylation has been associated with defective homologous recombination repair (HRR), early onset of breast and ovarian cancer, and improved clinical response to adjuvant chemotherapy.1,2,3 Historically, there has been no diagnostic assay that comprehensively evaluates both **BRCA1** promoter methylation and genomic alterations in cell-free circulating tumor DNA (cfDNA).

We describe the novel detection of **BRCA1** PM and genomic alterations in a cohort of patients with breast cancer using GuardantINFINITY™, a liquid biopsy assay interrogating 800+ genes and genome-wide methylation detection.

**Methods**

We assessed for **BRCA1** PM in cfDNA from 396 patients with late-stage breast cancer.

Genomic sequencing of 800+ genes and PM profiling of 398 related genes was performed by GuardantINFINITY™. For **BRCA1** analysis, the promoter region covering relevant CpG sites as previously determined was analyzed. For each sample, a methylation score was calculated and used as the basis for making PM calls.

The limit of detection (LoD) was determined through experimental titrations of cfDNA from HCC-38, a cell line with known **BRCA1** PM, into the plasma of cancer-free donors.

**Results**

**Analytical validation:** **BRCA1** promoter methylation is detected with high sensitivity and specificity in cell lines and healthy donors, respectively (Figure 1).

Figure 1: The 95% Limit of Detection (LoD) for **BRCA1** promoter methylation on GuardantINFINITY™. Serial titrations were performed using HCC-38, a breast cancer cell line previously determined to be methylated at the **BRCA1** locus, to varying degrees, by bisulfite sequencing and RT-PCR. The limit of detection was defined as the third quantile detection (i.e., PM) which could be detected in 95% of the specimens, as estimated through the titrations and proficiency analysis. Further analysis is investigating the relationship between plasma PM signal and saturation of methylation sites and **BRCA1** copies in the promoter region.

**Prevalence analysis:** **BRCA1** promoter methylation frequencies in Guardant plasma and in TCGA tissue patient cohorts (Figure 2).

Figure 2: Prevalence of **BRCA1** promoter methylation across cancer types in select cancer EMBL-EBI data. *Note:* References 1 and 2. **BRCA1** methylation frequencies may be attributed to the unannotated, non-random patient subtypes composition in the GuardantINFINITY™ cohort, as well as stage of cancer (where patients may have lost methylation over the course of treatment), and may not be directly comparable to patient cohorts in The Cancer Genome Atlas (TCGA), Abrevo Ovarian (OVAR), Breast (BRCA), Bladder (BLC), Lung Adenocarcinoma (LUAD), Lung Squamous Cell Carcinoma (LUSC), Melanoma (SKCM), and Other (OTH).

**Conclusions**

**BRCA1** PM has important prognostic and therapeutic implications for the management of breast and other cancers. GuardantINFINITY™, a plasma-based diagnostic assay, detected both **BRCA1** promoter methylation and genomic alterations in this unselected advanced breast cancer cohort.

Liquid biopsy is a method to non-invasively change in cancer-related genomics and epigenomics. Additional ongoing studies are investigating the extent of methylation across the **BRCA1** regulatory region, how these PM patterns vary across breast cancer subtypes, and how they both influence and are influenced by disease evolution and therapeutic response.

**References**

3. van der Reijden E, et al. Detection of all **BRCA1** copy numbers and SNVs is the Paninarin solution to ovarian cancers. Nat Commun. 2019

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