6603 *BRCA1* promoter methylation in sporadic breast cancer patients detected by liquid biopsy

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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 65% 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 (ctDNA).

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.

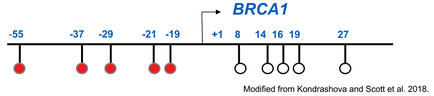
Methods

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

Genomic sequencing of 800+ genes and PM profiling of 398 cancer-related genes was performed by GuardantINFINITY™.

For *BRCA1* analysis, the promoter region covering relevant CpG sites as previously determined[3] was analyzed. For each sample, a methylation score were calculated and used as the basis for making PM calls.

The limit of detection (LoD) was determined through experimental titrations of ctDNA from HCC-38, a cell line with known *BRCA1* PM[4,5], into the plasma of cancer-free donors.



Modified from Kondrashova and Scott et al. 2018.

Figure 1. BRCA1 promoter region. The 10 CpG sites (circles) with promoter activity previously shown to be hypermethylated in breast cancer[1] (red), are covered in the panel BRCA1 promoter definition. The numbers refer to the nucleotide positions relative to the transcription start for BRCA1.

Results

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

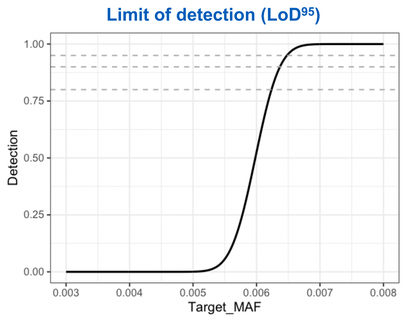


Figure 1: The 95% Limit of Detection (LoD) for *BRCA1* promoter methylation on GuardantINFINITY™. Serial titrations were performed using HCC-38, a breast cancer cell line previously determined to be methylated at the *BRCA1* locus, to varying degrees, by bisulfite sequencing and RT-PCR[4,5]. The limit of detection was defined as the the mutant allele fraction (MAF) at which PM could be detected in 95% of the specimens, as estimated through the titrations and probit analysis. Further analysis is investigating the relationship between plasma PM signal and saturation of methylation sites and BRCA1 copies in the promoter region.

Table 1. Specificity/Limit of Blank (LoB) of BRCA1 promoter methylation. The Specificity/LoB was established as the fraction of healthy donors that had detectable promoter methylation in BRCA1.

Total Assessed No. Patients	BRCA1 promoter methylation detected No. Patients	Specificity (1 - FPR)
80	0	100%

Prevalence Analysis: *BRCA1* promoter methylation frequencies in Guardant plasma and in TCGA tissue patient cohorts

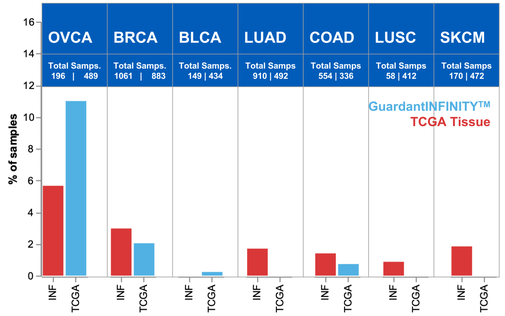


Figure 2: Prevalence of *BRCA1* promoter methylation across cancer types in select patient cohorts. Note that differences in methylation frequencies may be attributed to the unselected, non-random patient subtype composition in the GuardantINFINITY™ cohort, as well stage of cancer (wherein patients may have lost methylation over the course of treatment), and may not be directly comparable to patient cohorts in The Cancer Genome Atals (TCGA). Abbrev: Ovarian (OVCA), Breast (BRCA), Bladder (BLCA), Lung Adenocarcinoma (LUAD), Colorectal Adenocarcinoma (COAD), Lung Squamous Cell Carcinoma (LUSC), Melanoma (SKCM).

Epigenetic and pathogenic genomic occurrence of alterations in HRR genes in patients with advanced breast cancer

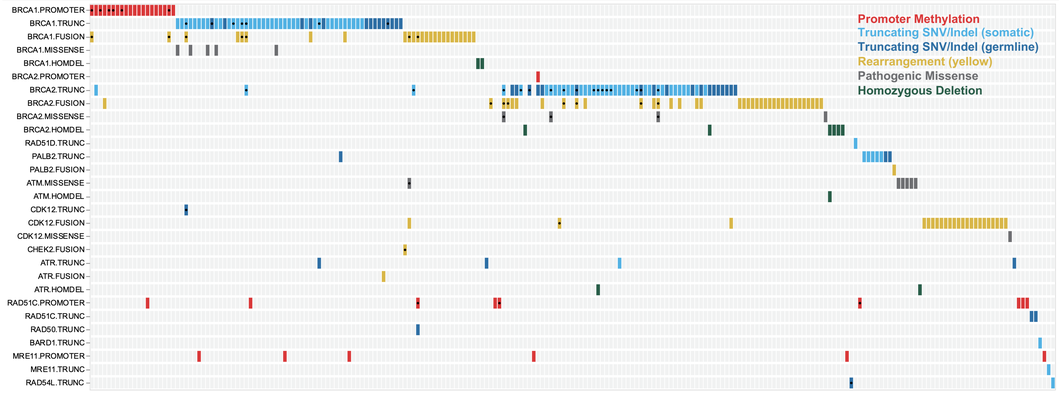


Figure 3: Oncoprint analysis of epigenetic and genomic alterations in HRR genes. Pathogenic genomic was defined as any nonsense, frameshift, rearrangement or pathogenic ClinVar missense mutations in the HRR genes above. Somatic truncating mutations in *ATM* and *CHEK2* were omitted from this analysis due to possible interference from clonal hematopoiesis. Promoter methylation is highlighted in pink - note that these alterations are majority mutually exclusive with other pathogenic genomic alterations in other HRR genes. Co-occurrence of rearrangements and genomic pathogenic variants are frequently reversion events.

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 identify changes 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

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5. **Xu J et al.** CpG island methylation affects accessibility of the proximal BRCA1 promoter to transcription factors. *Breast Cancer Res Treat*. 2010