Prevalence and characterization of *ESR1* alterations detected with an ultra-sensitive, liquid-only CGP assay in a large breast cancer cohort

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Lenny Hong[1], Matthew G. Varga[1], Patrick Cherry[1], Ali Aboosaiedi[1], Jeff Gaudet[1], Tiffany Farmer[1], Atri Choksi[1], Jason J Lukas[2], Michael Castro[3], and Gary Palmer[1]

[1] BillionToOne, Inc., Menlo Park, CA
[*2] Fred Hutch Cancer Center, Issaquah, WA
[3.] Beverly Hills Cancer Center and Personalized Cancer Medicine PLLC, Beverly Hills CA

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BACKGROUND

Early detection of emergent *ESR1* alterations is a clinical unmet need

- Breast cancer is the 2nd leading cause of death among women in the USA
- While prevalence of *ESR1* alterations is low in treatment naïve, primary tumors (<5%), **tumors often progress due to acquired endocrine therapy resistance, which can be driven by alterations in Estrogen Receptor alpha (*ESR1*)**
- Genomic profiling using tissue acquired at the time of diagnosis does not account for molecular mediators of disease progression or therapy resistance
- Prevalence of *ESR1* alterations can approach 20-40% in advanced ER+ disease, especially after multiple lines of therapy
- Because *ESR1* alterations more commonly occur over the course of treatment, liquid biopsies are an ideal tool for monitoring both treatment efficacy and tumor evolution

METHODS

Tissue-free, ctDNA based comprehensive genomic profiling and treatment response monitoring

Northstar Select® is a ctDNA based 84-gene comprehensive genomic profiling panel covering SNVs, indels, CNAs, fusions, and MSI-H status. With industry leading LOD95 at 0.15% VAF for SNV/indels, it is up to 5x more sensitive than other liquid biopsy assays (Fig. 1A). Northstar Select was used for identification of somatic variants in this study.

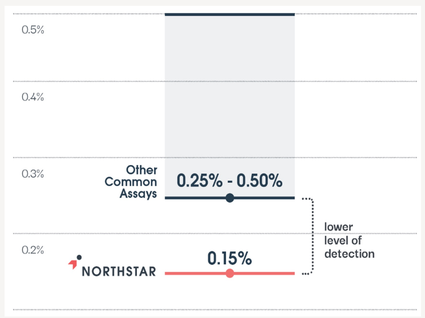


Figure 1A: Publicly listed 95% limit of detection (LOD95) thresholds for SNV/indels of common assays in the market range from 0.25% to 0.50% VAF. Northstar Select LOD95 for SNV/indels =0.15% VAF[1] (VAF= variant allele fraction)

Northstar Response® is a tumor-naïve, ctDNA methylation-based therapy response monitoring assay that quantifies cancer-specific methylated DNA molecules[2] from over 2,200 targeted loci in paired plasma and buffy coat samples. Northstar Response is used for longitudinal tracking of tumor burden changes giving real-time epigenomic insight into treatment response to help optimize treatment strategy.

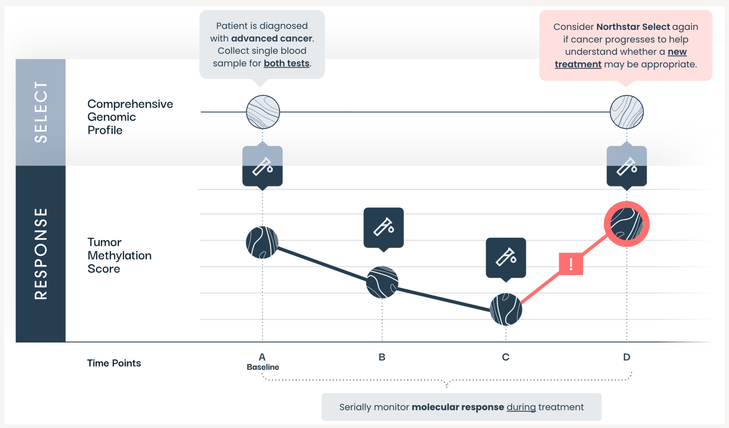
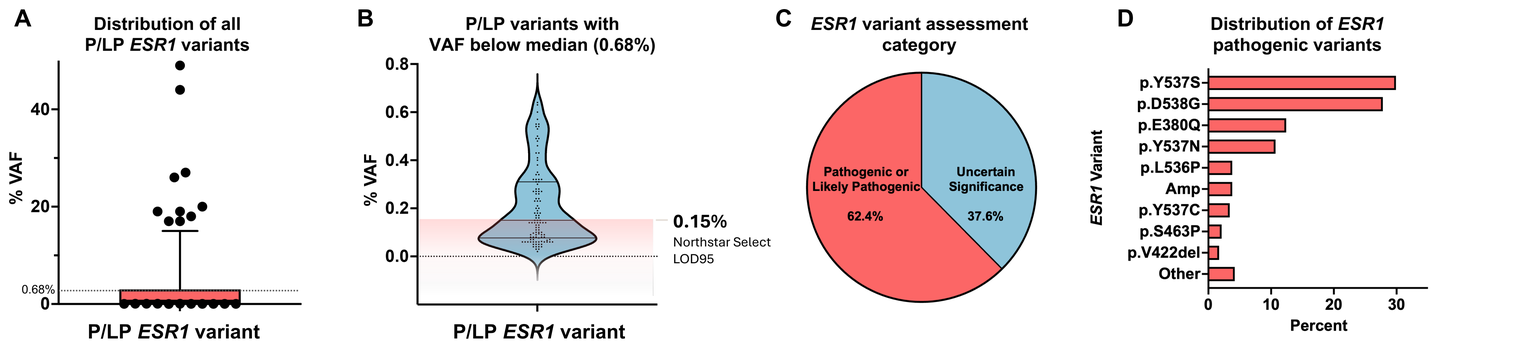


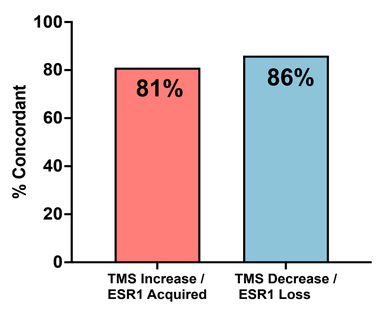
Figure 1B: Schematic demonstrating the paired utility Select and Response

RESULTS

Emergent, actionable *ESR1* alterations frequently occur at low VAF and their dynamics correlate with quantified changes in Tumor Methylation Scores™ (TMS™) using Northstar Response

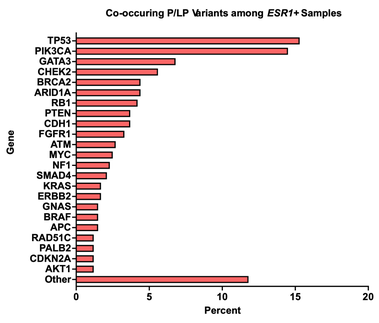


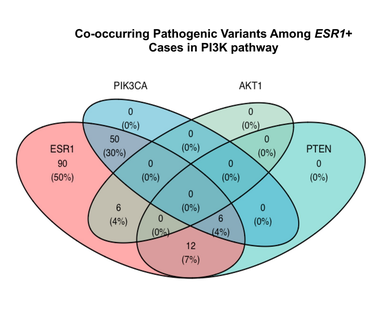
Prevalence, Pathogenicity, and VAF Distribution of *ESR1* Alterations in a Real-World Cohort
In a retrospective analysis, **1,044 unique breast cancer patients** were identified who underwent Northstar Select testing from BillionToOne. *ESR1* alterations were detected in 112 patients (**10.7%**), with **62.4%** classified as pathogenic or likely pathogenic (P/LP). The most frequent P/LP variants occurred at Y537S (29.9%) and D538G (27.8%), consistent with known hotspots in endocrine-resistant metastatic breast cancer. (A) Box-and-whisker plot showing the VAF distribution of all P/LP *ESR1* variants; (B) violin plot highlighting low-frequency variants below the median VAF **(0.68%);** (C) pie chart depicting the proportion of P/LP versus variants of unknown significance (VUS); and (D) bar chart showing prevalence of individual *ESR1* variants within the cohort.



- Of the 112 *ESR1*-positive patients (11% of the total), 23 patients were identified who had at least 2 paired, longitudinal Select and Response testing performed that were either negative for *ESR1* at baseline or acquired an *ESR1* P/LP variant as part of their care
- Sixteen were *ESR1*-negative at Select baseline but later **developed emergent *ESR1* variants** with a median VAF of **0.22%** (range: 0.06%–3.6%)
  - **13/16 (81%) of patients exhibited a concomitant increase in TMS** from the previous Response assessment, **a potential early signal to re-profile for new alterations**
- Seven patients were *ESR1*-positive at Select baseline with a median VAF of 1.23% (range 0.16-49%), but later became undetectable through longitudinal monitoring
  - **6/7 (86%) of those patients demonstrated a concomitant TMS decrease from the previous Response assessment**

Concurrent Genomic Alterations in ESR1-mutant mBC: ESR1 mutations, while a major contributor to endocrine resistance in metastatic breast cancer, are frequently accompanied by co-occurring alterations in PI3K–AKT–mTORC1 signaling pathway. These concurrent genomic events collectively promote ligand-independent ER activation and reduced sensitivity to endocrine therapy, contributing to worse clinical outcomes. The figure illustrates the frequency and distribution of such co-alterations in the PI3K/AKT pathway, including *PIK3CA* (34%), *PTEN* (11%), and *AKT1* (4%).

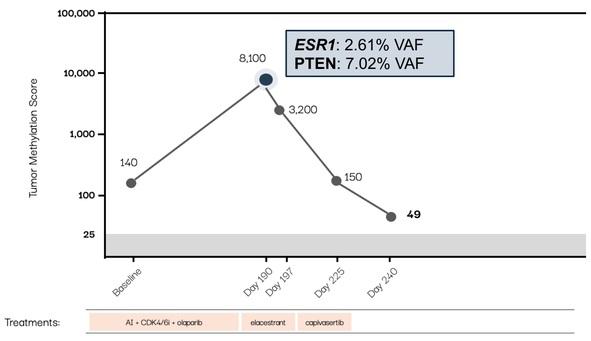




CASE STUDIES

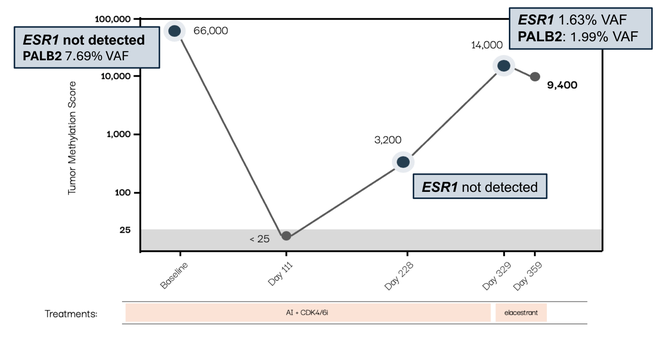
Real-world case-reports of ctDNA-based detection of *ESR1* dynamics

Case Study 1: 40yo F | Relapsed IDC | ER+/HER2- | Germline BRCA1 | Bone & Liver Mets



The patient had a baseline TMS of 140. Longitudinal monitoring with Northstar Response at day 190 revealed a **57-fold increase (TMS 8,100)**, coinciding with the emergence of an ESR1 mutation detected by Northstar Select. Initial treatment with elacestrant in combination with capivasertib led to a molecular response, with TMS **decreasing to <50 by day 240**.

Case Study 2: 42yo F | ER+/HER2- | Mets to Bone & CNS



The patient's baseline TMS of 66,000 dropped to <25 by day 111 on initial therapy. Serial Northstar Response testing at day 226 showed an **11-fold increase (TMS 3,200)** without new actionable alterations. Treatment was continued, but a subsequent **52-fold rise at day 329 (TMS 14,000)** coincided with the emergence of an ESR1 mutation detected by Northstar Select.

CONCLUSIONS

Longitudinal monitoring with Northstar Response, coupled with comprehensive re-profiling using Northstar Select (Auto-Select), provides an effective strategy to elucidate evolving *ESR1* mutation dynamics

- **~50% of *ESR1* alterations** were detected below 0.68% VAF, **with half of those detected below 0.15% VAF**, highlighting the need for a highly sensitive profiling assay to uncover low frequency alterations in blood.
- Paired analysis of Northstar Select with Northstar Response **enabled dynamic assessment of emergent and cleared *ESR1* variants**, **supporting the clinical utility of combining both assays to guide therapy planning and disease monitoring in advanced breast cancer.**