Validation of a highly sensitive TargetSelector™ ctDNA assay for ESR1 resistance mutations using an NGS enrichment strategy

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Background
Acquired resistance to anti-hormonal therapy is a common reason for treatment failure in advanced and metastatic hormone-sensitive breast cancer. Mutations in the ESR1 gene, encoding the estrogen receptor (ER) protein, are often associated with the drug-resistant phenotype and timely ESR1 mutation detection may enable early identification of progression and prompt initiation of treatment. In contrast to using difficult and patient tissue biopsies, “liquid biopsies” offer an non-invasive and supreme approach to identify solid tumor mutations in peripheral blood by analyzing circulating tumor DNA (ctDNA). Biocept has developed the highly sensitive TargetSelector™ assay to detect low frequency mutations alleles in ctDNA using a patented blocker that allows non-amplified tumor DNA, while allowing amplification of tumor derived mutant templates.

ESR1 (Estrogen Receptor-α)
- The ESR1 gene encodes the nuclear hormone receptor stimulated by estrogen. It is mainly commonly associated with breast cancer but also with others.
- Abnormal ER expression occurs in >70% of invasive breast cancers but ESR1 mutations are rare in primary breast cancers at the time of diagnosis.
- Once patients have been treated with anti-hormone therapy however ESR1 mutations have been identified in up to 51% of luminal breast cancers.
- The implication of these mutations have spurred efforts to develop therapies that antagonize ER activity in a different manner; one example being fulvestrant which stimulates ESR1 protein-degradation.

Technology
TargetSelector™ NGS Assay Workflow & Diagram

Methods
We validated the TargetSelector™ assays by spiking the liquid biopsy DNA fragments (500 ng) containing point mutations for each of the target ESR1 sites, into WT human plasma DNA. Mutant sequences were enriched via PCR and mutations were subsequently confirmed on the Illumina MiSeq down to a limit of detection (LOD) of 0.01-0.05% minor allele frequency (MAF). For ctDNA testing, whole blood was collected in Biocept CEE Stat™ Blood Collection tubes, and total nucleic acid extraction from plasma was performed on the QIAxymax (QIAGEN, Inc).

Results

Results - Results continued

Clinical Specificity and Clinical Experience of TargetSelector™ ESR2 Assay

4. Analytical validation of our TargetSelector™ cartridge shows single mutant copy detection and LOD at 0.03% or better in a background of excess WT DNA.
5. Ultra low copy mutation targets were enriched by the TargetSelector™ Assay >3000 fold.
6. The ESR1 TargetSelector™ Assay maintains >95% sensitivity for MAF ranging from >0.01% (300 copies of MT) to 0.03% (10 copies of MT).
7. We measured analytical specificity at >95% and clinical specificity 95% (±20), indicating the test is competent for use in a CLIA/CAP accredited laboratory.
8. In a cohort of 20 mBC patients we detected two mBC mBC patients as a mBC >20%. Studies are underway to evaluate potential clinical applications of Biocept’s highly sensitive TargetSelector™ ctDNA ESR1 mutation assay.

Conclusions

Figure 5: Sensitively discriminated mutant was applied to a 10,000 copy WT background for the analytical sensitivity test. Numbers below the plot indicate detected/expected replicates. The detection rate is 100% from MAF 5% down to 0.01% (300 → 9 copies of MT) of the output signal. The dotted line represents a theoretical perfect assay based on a Poisson distribution of samples including also shown in the table below. The establishment of ESR1 V535 X35% and V538 shows single copy sensitivity at 0.01% MAF for ESR1 V535 assay. The expected range for this ESR1 TargetSelector™ assay is ±5% down to 0.01% MAF or a single copy depending on WT background.

Establishing sensitivity and specificity cut-offs using mutationally enriched data

ESR1 Mutations Associated with Early Drug Resistance and Clinical Outcomes

80% of metastatic breast cancers (mBC) carry ESR1 mutations. The majority (95%) of these mutations are in the 4-amino acid regions of ESR1 Exon 2, D538G (QIAGEN, which is used in the clinical setting as a negative control).

Figure 6: The analytical specificity was determined for the ESR1 TargetSelector™ assay by applying direct sequencing DNA (DNA from patients with mBC) at 100pg, 33 ng and 10 copies (equivalent to 0.03% MAF) or non-extracted templates. Each individual variant of ESR1 was tested. The analytical specificity of the ESR1 TargetSelector™ assay showed 97% or better for each variant tested.

Table: Analytical and Clinical Specificity of TargetSelector™ ESR2 Assay

Condition | Sensitive copies | Specificity | Mutated Detected
--- | --- | --- | ---
Healthy | 20 | none | 100 & 538
Breast cancer, mBC | 20 | none | 100 & 538

Please note: 0 = 0.03% MT

Figure: The clinical specificity was determined based on ctDNA extracted from 40 ml plasma samples from healthy donors. One false positive from 20 healthy samples yielded a clinical specificity of 0.97%

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