Validation of Highly Sensitive TargetSelector™ ctDNA Assays for EGFR, BRAF, and KRAS Mutations

Shan-Fu Wu, PhD; Timothy T. Lu, PhD; Anh Pham; Jeffrey Chen; Erinn Samuelsz; Manisha Patel; Veena M. Singh, MD; Lyle J. Arnold, PhD; Jason C. Poole, PhD
Biocept, San Diego, CA, USA

Abstract #4534
2018
AACR

Background

Accurate detection of actionable mutations in patients with cancer is vital for targeted therapy. Compared to tissue biopsy, liquid biopsy offers a non-invasive and more accessible approach to identify tumor mutations by assessing circulating tumor DNA (ctDNA) released from tumor cells into peripheral blood. We have developed TargetSelector™ Real-Time PCR based assays to detect low frequency mutant alleles in ctDNA. The TargetSelector™ assays use a patented blocking approach to suppress amplification of excess WT DNA released from normal cells, while allowing specific amplification of mutants. Here we focus on five important targets: EGFR, KRAS, BRAF, BRAF (V600), and KRAS (G12/G13), which are relevant to lung cancer, melanoma, and colorectal cancer.

TargetSelector™ ctDNA Assay Workflow & Diagram

Methods

The TargetSelector™ ctDNA assays apply a specific blocker to cover variants on a short stretch of target DNA (up to 15 bp for nucleotide variants). For example, one KRAS exon 2 blocker covers all variants on both G12 and G13 codon positions. DNA from cancer cell lines carrying the specific target mutations were used for analytical validation of the TargetSelector™ ctDNA assays incorporating the QuantStudio 5 (LifeTech) Real-Time PCR instrument (Thermo Fisher). Sanger or NGS DNA sequencing was subsequently performed to confirm the mutations. Analytical validation was conducted by 3 independent operators using 5 instruments across 5 days in Biocept's CLIA-certified and CAP-accredited laboratory for ctDNA testing, where whole blood samples were collected in CEE-Sure™ Blood Collection tubes and DNA extraction from plasma was performed using the QIAasympoy (QIAGEN). Each Biocept's TargetSelector™ assay was analytically validated first based on experimental data compared to theoretical estimates, such as >99% analytical sensitivity and >99% analytical specificity. Limit of detection (LOD) for each assay was tested in the presence of 1,000 WT copies, and showed sensitivity at 0.02% MAF or better, TN, false negative; TP, true positive; FN, false positive; TN, true negative.

Results

In total, we tested 3086 samples for EGFR, BRAF, and KRAS TargetSelector™ ctDNA assays for analytical validation, with WT DNA as the background reference. The inter-assay and intra-assay analyses showed r2 >0.94, suggesting a consistent performance among variances. Each Biocept's TargetSelector™ ctDNA assay showed >99% analytical sensitivity and >99% analytical specificity. Sensitivity of single mutant copy detection was >99% across all tested samples. The actual data copy numbers between standard levels demonstrated single mutant copy detection sensitivity.

Clinical Specificity of TargetSelector™ Assays

Each Biocept's TargetSelector™ ctDNA assay shows >99% analytical sensitivity and >99% analytical specificity. TargetSelector™ ctDNA assays show single mutant copy detection based on experimental data compared to theoretical estimates, with sensitivity at 0.02% MAF or better in a background of excess WT DNA. Biocept's ctDNA assays detected no false positives from 20 healthy donors, and showed >99% clinical specificity.

Conclusions

• Each Biocept's TargetSelector™ ctDNA assay shows >99% analytical sensitivity and >99% analytical specificity.
• TargetSelector™ ctDNA assays show single mutant copy detection based on experimental data compared to theoretical estimates, with sensitivity at 0.02% MAF or better in a background of excess WT DNA.
• Biocept's ctDNA assays detected no false positives from 20 healthy donors, and showed >99% clinical specificity.
• Implementation of the QuantStudio 5 (LifeTech) platform into Biocept's TargetSelector™ ctDNA assays translated into high clinical sensitivity and fast turnaround time for patients in Biocept's CLIA-certified and CAP-accredited laboratory.