2019 World Conference in Lung Cancer
Session P1.14 Targeted Therapy
Poster #: 1930

The Dynamic Range of Mutant Allele Fraction Detected in Patients with NSCLC: Clinical Experience Data and Clinical Implications

Smitha Boorgula, Julie Ann Mayer PhD, Robbie D. Schultz PhD, Deanna Fisher, Lyle J. Arnold PhD and Veena M. Singh MD
Biocept, San Diego CA

Biocept
Completing the Answer™

Background

The detection of mutations in the circulating tumor DNA (ctDNA) fraction of overall cell-free DNA (cfDNA) isolated from plasma in patients with non-small cell lung cancer (NSCLC), to identify both activating mutations and the emergence of resistance, has gained widespread adoption and clinical practice guideline recommendations. However, the vast majority of cfDNA in blood samples is derived from non-cancerous tissue and white blood cells. ctDNA must be distinguished from cfDNA. A recent study suggested that technical factors may be the major cause of discordance among plasma based next generation sequencing (NGS) testing especially at low mutation allelic frequency (MAF < 1%)[1]. Hence, the ability to accurately identify mutations in patients with NSCLC, who would potentially benefit from targeted therapy as well as for monitoring for both emergence of resistance, recurrence and tumor burden, require assays to consistently and reliably detect low level mutations. Additionally blood collection tubes should maximally stabilize cells to minimize breakdown of white blood cells (WBCs) that would significantly increase the amount of cfDNA in the plasma fraction leading to potential false negative results for mutations analyzed in patients with NSCLC.

Methods

Peripheral blood was collected from 3,797 patient samples in CEE-Sure™ blood collection tubes to minimize the non-tumor cfDNA content and preserve circulating tumor cells (CTC). Plasma fraction was removed from the blood samples; circulating total nucleic acids (cfTNA) was extracted from plasma and used in Target Selector™ Switch-Blocker™ assays that have demonstrated high level of sensitivity in the detection of mutations in *EGFR*, *BRAF* and *KRAS* genes. The Target Selector™ Switch-Blocker™ assays utilize forward and reverse primers and a Switch-Blocker™ probe to specifically block cfDNA wild-type amplification, and selectively enrich for mutant sequences (ctDNA). Sanger sequencing of the amplified product is used to confirm presence of the mutation.

Figure 1: Workflow of the Target Selector™ Switch-Blocker™ Assays

CEE-Sure™ Blood Collection Tubes

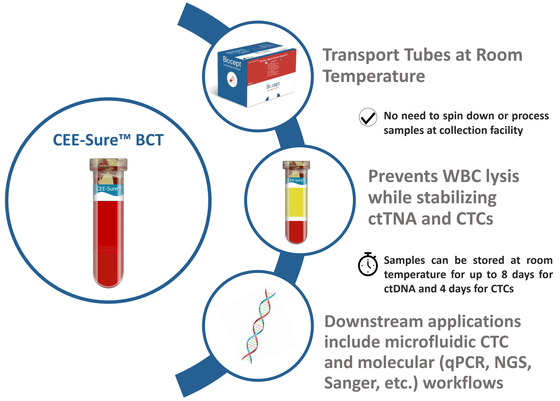

Figure 2: Schematic Illustrating the benefits of the CEE--Sure™ Blood Collection Tubes

Target Selector™ Switch-Blocker™

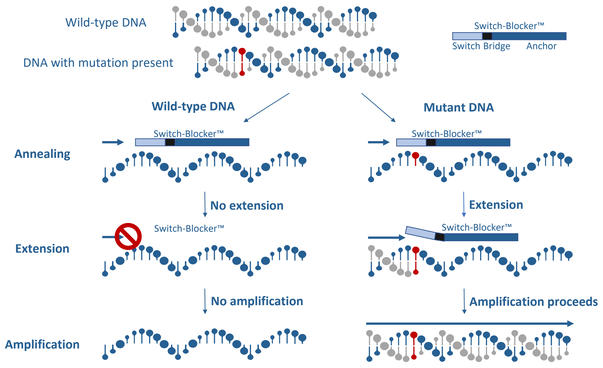

Figure 3: Diagram Illustrating the Target Selector™ Switch-Blocker™ Technology

Target Selector™ *EGFR* Assays

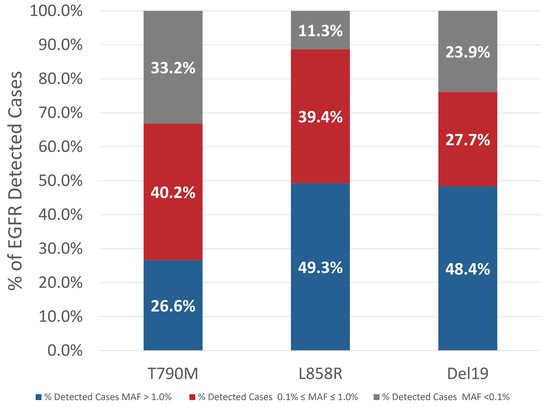

Figure 4: Graph Depicting the % of *EGFR* detected cases broken down by MAF

Target Selector™ *BRAF* & *KRAS* Assays

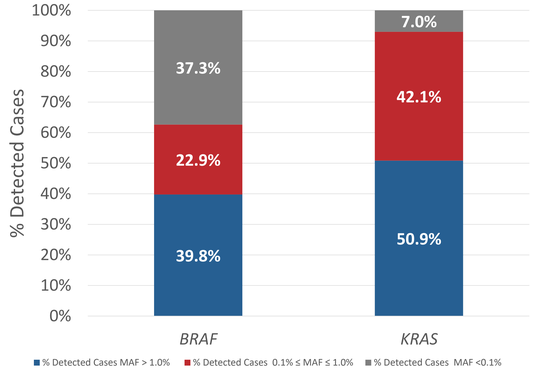

Figure 5: Graph Depicting the % of *BRAF* and *KRAS* detected cases broken down by MAF

Target Selector™ Overall Detection

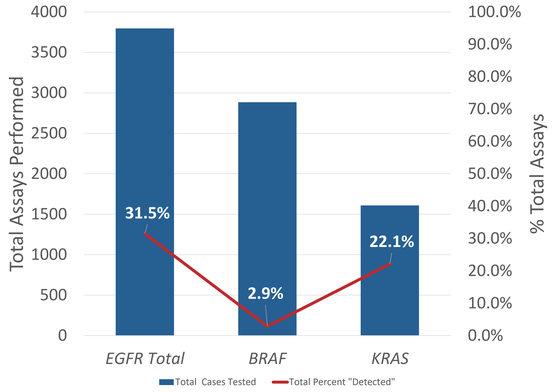

Figure 6: Graph Depicting the overall detection of cases broken down by gene

Conclusions

- Target Selector™ Switch-Blocker™ has demonstrated consistent performance for detecting actionable mutations in the ctDNA of patients with NSCLC as low as 0.01% mutant allele frequency.
- Overall detection rates are on par or slightly above those reported in literature (EGFR 26%, BRAF 1%, KRAS, 14%).[2-3]

References

1. Stetson D, Ahmed A, Nuttall BRB, et al: Orthogonal comparison of four plasma NGS tests with tumor suggests technical factors are a major source of assay discordance. https://doi.org/10.1200/PO.18.00191
2. Arrieta O, Andrés C, Claudio M, et al: Updated Frequency of EGFR and KRAS Mutations in Non Small-Cell Lung Cancer in Latin America: The Latin-American Consortium for the Investigation of Lung Cancer (CLICaP). Journal of Thoracic Oncology 10, no. 5 (May 2015): 838–43. https://doi.org/10.1097/JTO.0000000000000481
3. Herbreteau, G, Vallée A, Charpentier S, et al: Circulating free tumor DNA in non-small cell lung cancer (NSCLC): clinical application and future perspectives. Journal of thoracic disease, 11(Suppl 1), S113–S126. https://dx.doi.org/10.21037%2Fjtd.2018.12.18