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%). Hence, the ability to accurately identify mutations in patients with NSCLC, who would potentially benefit from targeted therapy as well as for monitoring both emergence of resistance, recurrence and tumor burden, require assays to consistently and reliably detect low level mutations. ctDNA collected tubes should maximally stabilize cells to minimize breakdown of white blood cells (WBCs) that would significantly increase the amount of ctDNA in plasma fraction leading to potential false negative results for mutations analyzed in patients with NSCLC.

Methods

Peripheral blood was collected from 8,797 patient samples in CEE-Sure™ 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 (ctDNA) 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 sequence (ctDNA). Nager sequencing of the amplified product is used to confirm the presence of the mutation.

Conclusion

- 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%).

References