High Sensitivity Detection of Rare EGFR Mutations with ctDNA using Target-Selector™ Assays

Vassilios Alexiadis, Tim Watanaskul, Vahid Zarrabi, Veena Singh and Lyle Arnold

Biocept Inc., San Diego, CA

II. Clinical Data

The EGFR Target-Selector™ assays were used to detect presence of mutated T790M, L858R and Del19 sequences in blood plasma from lung cancer patients where the biopsy results were unavailable. Plasma from in-house donors or breast cancer samples were used as negative control. Samples found positive using the Target-Selector™ assay were confirmed by Sanger sequencing. Droplet Digital PCR was used as an orthogonal method to evaluate discrepancies between plasma and tissue results.

Table 2: Comparison of blood plasma results by EGFR Target-Selector™ to tissue biopsies confirmed to contain EGFR mutation by a Reference Lab. Overall concordance was 93%. One possible explanation for discrepancies between blood and tissue is the time between initial diagnosis to time of blood draw and analysis using Target-Selector™.

Conclusions

- Highly sensitive Target-Selector™ assays have been developed for the detection of activating EGFR mutations L858R and Del19 or resistance mutation T790M, which can detect the mutations >0.05% mutant allele frequency in analytical validation experiments.
- Mutations were found in the circulating nucleic acid of plasma from lung cancer patient’s matching tissue biopsy results performed in a reference lab.
- Target-Selector™ assays have the advantage of allowing any mutation in a stretch of DNA (~10mb) to be enriched and specifically identified with Sanger sequencing.
- We identified T790M mutations in patient plasma with as low as 0.0045% mutant allele frequency.
- We identified 4 patients with mutations c.2573 T>C, c.2572, 2573 CT>AG in patient plasma, as well as synonymous mutations in 8G857 c.2571 T>GT and 8L857 c.2572 C>T.
- We identified Del19 mutations in 5 samples, De747-750 ins, De747-750 insD, De747-750 and De747-750 in patient plasma.
- Using EGFR Target-Selector™ in a patient cohort of 74 samples we observed concordance to tissue biopsy results at 93%.

References

- Sundaresan TK et al., Detection of T790M, the acquired resistance EGFR mutation, by biopsy versus noninvasive blood-based analyses. Clin Cancer Res. 2015;21(17):3613-3619.