Plasma EGFR mutation detection using a combined exosomal RNA and circulating tumor DNA approach in patients with acquired resistance to first-generation EGFR-TKIs

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Introduction

After initial responses to tyrosine kinase inhibitors (TKIs), NSCLC patients lacking EGFR mutations initially respond to the “gatekeeper” EGFR T790M mutation accounting for approximately 60% of cases of acquired resistance (AR) to TKIs. EGFR activating and T790M resistance mutations can be found in plasma in both RNA contained within exosomes and in circulating free tumor DNA (ctDNA). ctDNA is released in tumor lysates and data from a subset of patients in TGEX X (NCT01639881), a PILOT study of resected in previously treated EGFR patients with advanced NSCLC. We demonstrated the detection of EGFR mutations in plasma using a quantification-based method (Exclusivity Plus) for combined isolation of exosomal RNA (exRNA) and ctDNA in a single step. This approach improves sensitivity and demonstrates the ability to detect mutations using exosomal RNA in patients previously described as negative for external high-sensitivity qPCR method on ctDNA alone.

Materials and Methods

Patients with locally advanced NSCLC or NSCLC metastases were enrolled ina multicenter pre­treatment liquid biopsy study. Baseline liquid biopsy testing was performed via two different liquid biopsies: either extracellular vesicle isolation and ctDNA isolation (TIGER; Clovis Oncology, Boulder, CO) and/or (1) plasma and (2) resected tumor tissue. Tumor samples were released in tumor and normal tissue including circulating tumor DNA (ctDNA) (4/16). No therapeutic dose was comprised.

Results

Overview of NSCLC samples evaluated with EXO1000: Among the 84 patients (41 treated by Dec 31, 2014), 72 patients had available samples. The low copy cohort, defined according to criteria specified at the closure of the Venti study, is comprised.

Extracellular DNA and RNA in plasma. Exosomal and necrotic cells may release cell-free DNA (cfDNA) in extracellular vesicles (EV) or as circulating nucleosomes (CN). EVs are actively released by living cells during physiological membrane (EV) or on the extracellularly stabilized (CN), cmsically RNA the homeology of cfDNA

Conclusion

Liquid biopsy results of challenging samples. Patients with non-small cell lung cancer (NSCLC) using a combined approach to detect EGFR mutations in plasma and tumor tissue. Two independent liquid biopsy approaches in patients that have been treated previously by TKIs, assessed in this study. In the liquid biopsy approach, nega­tive patients carry circulating mutations detected by two different liquid biopsies.

Conclusions

- Detection of both activating and acquired resistance mutations to EGFR therapy in plasma offers a promising alternative to tissue-based biopsy.
- Combined plasma exRNA + ctDNA isolation increases the number of samples available for low aberrant somatic mutation detection, compared to ctDNA-only, an improvement in the clinical sensitivity of liquid biopsies.
- In challenging cases, such as M0/M1a, the combined exRNA + ctDNA isolation offers improved sensitivity for EGFR mutations.
- Tissue-­negative/plasma-positive cases identified by EXO1000 are likely “false positives” because they were identified as plasma-positive by a second test methodology.
- EXO1000 provides excellent analytical performance for detection of actionable mutations in plasma of NSCLC with immediate potential for clinical application.