Early detection of competing resistance mutations using plasma next-generation sequencing in patients with EGFR-mutant NSCLC treated with osimertinib

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### Background

- In patients with EGF-R-NGS, genotyping of plasma with free DNA (cfDNA) has become a routine option for noninvasive detection of EGFR T790M.
- Loss of T790M is a common resistance mechanism as osimertinib, thought to be due to overgrowth of competing resistance mechanisms.
- We studied the ability of serial amplification-based targeted NGS of cfDNA to early detect resistant resistance mutations during osimertinib treatment.

### Methods

- Patients were identified with advanced EGFR-mutant NSCLC with T790M-acquired resistance mutations detected in the cfDNA.
- ddPCR for EGFR mutations was performed previously in part of the ongoing research (DF/HCC protocol #14-147).
- Of the patients treated with osimertinib, 26 were selected for further study based upon availability of adequate serial plasma samples for analysis.
- For each case, up to 6 samples were assayed, blinded to tumor genetics.
  - Pre osimertinib
  - Post osimertinib
  - Post-doxorubicin
  - Post-epirubicin
- A total of 14 eligible samples were identified for these 26 cases.
- Plasma NGS was performed using the Invata system.
- Assay performance curves were used to combine results of repeated amplification and analysis.
- Plasma NGS results were reported blinded to any existing information on tumor and plasma genotyping.
- The Invata NGS system is approved for use in tumor genotyping (including tumor NGS when available).

### Results

A. Amplicon-based plasma NGS is highly concordant with ddPCR

- All resistance genes detected in plasma NGS were identical to genes detected in plasma samples analyzed with ddPCR.

B. False positives are rare with amplicon-based plasma NGS

- NGS samples for each case were analyzed with ddPCR for EGFR T790M.
- When the NGS amplicon for EGFR T790M was detected both with plasma NGS and plasma ddPCR.

C. Plasma NGS can detect a range of acquired resistance mechanisms, some of which pre-exist with T790M

- The initial activation of ERBB2 amplification (driver AF 2.7%) was identified by qPCR.
- The initial activation of KRAS G12C amplification (driver AF 2.4%) was identified by qPCR.

### Conclusion

- In this blinded retrospective validation, we find amplicon-based plasma NGS have high sensitivity and specificity.
- Quantitative concordance with ddPCR is excellent.
- Plasma NGS can detect emergence of common resistance mutations.
- Acquired KRAS mutation appears to be a newly identified recurring resistance mechanism to osimertinib.
- In a subset of patients, these mutations can be detected in plasma, creating an opportunity for the study of osimertinib-based targeted therapy combinations.

### References

- Schrock K et al. Development of a targeted, rapid next-generation sequencing panel for plasma DNA testing in patients with cancer. JCO Precis Oncol. 2018;2:10534.

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