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


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Background
After initial responses to tyrosine kinase inhibitors (TKIs), NSCLC patients harboring EGFR mutations inevitably relapse, with the “gainerase” EGFR T790M resistance mutation accounting for approximately 50% 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 (cfDNA). ctDNA is released by dying tumor-exosome RNA is acquired by many tumor cells (Schwaab et al. Nat Rev Clin Oncol 2014. Jain et al. Nat Rev Clin Oncol 2014. Thiery et al. Nat Rev Cancer 2009). Here we present rescued tumor and plasma data from a subset of patients in TIGER-X (NCT01935846), a PI3K study of rescued tumor EGFR patients with acquired NSCLC. We demonstrate the detection of EGFR mutations in plasma using a non-column-based method (Exclusion Plus) for combined isolation of exosomal RNA (exoRNA) and cfDNA in a single step. This approach improves sensitivity and demonstrates the ability to detect mutations using exosomal RNA in patients previously described as negative by an external high-sensitivity qPCR testing on cfDNA alone.

Fig. 1 - Two distinct sources of cell-free nucleic acids in plasma

1. **Exosomes**
   - Approximately 50-200 nm diameter
   - Contain intact tumor cell RNA, DNA, and proteins
   - Released by tumor cells
   - Circulating free RNA (cfRNA)

2. **cfDNA**
   - Released by dying tumor cells
   - Smaller DNA fragments
   - RNA is acquired by many tumor cells

Fig. 2 - A simple two-step isolation platform for exoRNA and cfDNA from patient plasma samples

1. **Whole plasma and RNA**
   - RNA isolation and DNase treatment
   - Additional RNA quality check

2. **Gene type and release**
   - RNA isolation and DNase treatment
   - Additional RNA quality check

3. **Exosome and cfDNA**
   - RNA isolation and DNase treatment

Workflow for cell-free RNA isolation and cfDNA from blood using the Exclusion Plus technology platform. The Exclusion Plus platform employs a proprietary capture mechanism in a disposable spin-column format to enable tumor-specific co-isolation of exosomal and cfDNA from whole blood.

Fig. 3 - Overview of study cohort and sub-cohorts of EXO1000 liquid biopsies on tissue-matched plasma of NSCLC patients

**A.** Overall EGFR patients enrolled in TIGER-X. Phase II/III trial of resistance mutations and their sensitivity to second-generation EGFR-TKIs.

- **Advanced or recurrent NSCLC with a driving EGFR mutation in a baseline sample**
- **Plasma sample collected within 48 hours of biopsy**

**B.** Clinical characteristics of NSCLC patients enrolled in TIGER-X. Phase II/III trial of resistance mutations and their sensitivity to second-generation EGFR-TKIs.

- **Age (years)**
  - Median: 67.5
  - Range: 43-83

- **Sex**
  - Male: 65%
  - Female: 35%

- **Histology**
  - Non-squamous cell carcinoma: 90%
  - Squamous cell carcinoma: 10%

- **EGFR mutation status**
  - Somatic mutation: 90%
  - Deleterious mutation: 80%

- **Sub-cohort size**
  - T790M negative patients: 20/38
  - T790M positive patients: 33/69

**C.** Liquid biopsy sensitivity

- **EGFR TKI**
  - OS: 15/19
  - CR: 15/19

**D.** Liquid biopsy resistance

- **EGFR TKI**
  - OS: 15/19
  - CR: 15/19

**E.** Liquid biopsy sensitivity

- **OS**
  - 15/19
  - CR: 15/19

**F.** Liquid biopsy resistance

- **OS**
  - 15/19
  - CR: 15/19

**G.** Liquid biopsy sensitivity

- **OS**
  - 15/19
  - CR: 15/19

**H.** Liquid biopsy resistance

- **OS**
  - 15/19
  - CR: 15/19

Fig. 4 - Experimental Setup

**A.** Overview of NSCLC samples enrolled in EXO1000. Among the 64 patients in total, 48 patients had the sample collected after biopsy, while the low copy biopsy, tissue was defined according to criteria specified in Table 8.

**B.** Circulating tumor DNA (ctDNA) enrichment in plasma.

- **Exclusion Plus**
  - Plasma DNA (ppDNA)
  - plasma free DNA (pFDNA)

**C.** Third-party cDNA enrichment.

- **Enrichment protocol**
  - cfDNA enrichment
  - Exosome enrichment

Fig. 5 - Patient baseline characteristics

**A.** Demographics of NSCLC patients enrolled in TIGER-X. Phase II/III trial of resistance mutations and their sensitivity to second-generation EGFR-TKIs.

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  - CR: 15/19

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  - CR: 15/19

Conclusions

- **Detection of both acquiring and acquired resistance mutations to EGFR therapy** in plasma offers a promising alternative to tissue-based biopsy.

- **Combined plasma exoRNA + ctDNA isolation** increases the number of gene copies available for low abundant somatic mutation detection, compared to ctDNA-only, an improvement in the clinical sensitivity of liquid biopsies.

- **In challenging cases, such as M0/M1, the combined exoRNA + ctDNA isolation offers improved sensitivity for EGFR mutations.**

- **Tissue-adjacent/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.**