# # 557 Noninvasive analysis of acquired resistance to EGFR-TKI

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## Abstract

Epidermal growth factor receptor (EGFR) T790M mutation is associated with EGFR tyrosine kinase inhibitors (EGFR-TKIs) resistance in non-small cell lung cancer (NSCLC). However, tissue availability limits the genotyping of EGFR T790M mutation in a clinical setting. The aims of this study are to develop a blood-based, non-invasive approach to detecting the EGFRT790M mutation in advanced NSCLC patients. Additionally, the plasma concentrations of HGF and MET, which are also factors associated with EGFR-TKIs resistance, will be examined.

***Methods*:** Blood samples were collected from NSCLC patients with activating EGFR mutations, who were resistant to EGFR-TKIs treatment. EGFR T790M mutations were detected using a droplet-digital PCR (ddPCR) system and plasma DNA. The plasma concentrations of HGF and soluble MET (sMET) were determined using enzyme-linked immunosorbent assays. The relationships between the EGFR T790M mutation status and the concentrations of HGF and sMET, the progression-free survival (PFS), and the overall survival (OS) were analyzed.

***Results*:** Twenty-one (35%) of the patients had EGFR T790M mutations in their plasma DNA as detected using the ddPCR system. No correlations between the post-T790M status and the post-HGF and sMET levels were seen. The PFS and OS of patients with the EGFR T790M mutation were significantly shorter than those of patients without the EGFR T790M mutation (P = 0.03 and 0.04, respectively).

***Conclusions*:** The ddPCR system is a useful method for determining the plasma EGFR T790M mutation status. The combination of EGFR T790M mutation status, as detected using ddPCR, and the amphiregulin levels may be useful for monitoring drug resistance and for making a prognosis.

## Methods

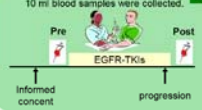
60 lung adenocarcinoma patients with EGFR mutations from tumor specimen, who received EGFR-TKIs at Cancer Institute Hospital, Japanese Foundation for Cancer Research,

**Patients' Records**

Age Sex Histologycal type
EGFR mutation status in tumor cells
Response to EGFR-TKIs
Progression free survival time (PFS)
Survival time from EGFR-TKIs

**Blood collection and DNA extraction**

10 ml blood samples were collected.




| Sampling points | | |
|---|---|---|
| Pre | Post | Enrolled Pts. |
| ✓ | ✓ | 30 |
| | ✓ | 30 |

5 ml in native tube
5 ml in EDTA tube
250 g for 10 min
Serum
Plasma
Serum and plasma were stored at –80C °

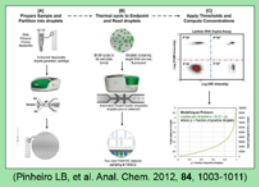
**HGF and sMET levels in serum**

Human HGF Quantikine ELISA Kit (R&D Systems)

c-Met (Soluble) Human ELISA Kit (Invitrogen™)

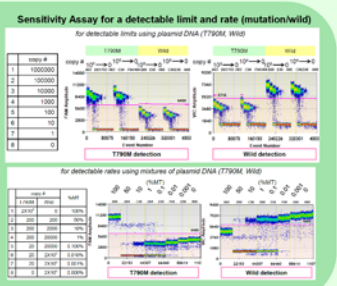
**Detection of T790M mutation in plasma DNA using droplet digital PCR**

DNA extraction
QIAamp Circulating Nucleic Acid kit (Qiagen)



(Pinheiro LB, et al. Anal. Chem. 2012, **84**, 1003-1011)

**Sensitivity Assay for a detectable limit and rate (mutation/wild)**







## Objectives

- ✓ To detect the T790M mutation in EGFR plasma DNA using ddPCR.
- ✓ To clarify the associations between the T790M mutation status and serum protein levels (HGF and sMET).
- ✓ To analyze the associations between measurements (T790M, HGF and sMET) and the prognoses of EGFR-TKIs treatment.

## Results

**T790M status in plasma DNA**

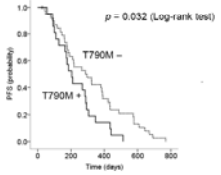
| Pt. # | Pre | Post | Pt. # | Pre | Post | Pt. # | Pre | Post | Pt. # | Pre | Post |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | 16 | | | 31 | | | 46 | | |
| 2 | + | + | 17 | | | 32 | | | 47 | | |
| 3 | | | 18 | + | + | 33 | | | 48 | | |
| 4 | | + | 19 | + | | 34 | | + | 49 | | |
| 5 | | | 20 | + | + | 35 | | | 50 | | + |
| 6 | | | 21 | + | | 36 | | | 51 | | |
| 7 | | + | 22 | | | 37 | | + | 52 | | |
| 8 | | + | 23 | | | 38 | | | 53 | | |
| 9 | | | 24 | | | 39 | | | 54 | | |
| 10 | | | 25 | | | 40 | | | 55 | | |
| 11 | | | 26 | + | | 41 | | | 56 | | + |
| 12 | | + | 27 | | | 42 | | + | 57 | | |
| 13 | | | 28 | | | 43 | | | 58 | | |
| 14 | + | + | 29 | | + | 44 | | + | 59 | | + |
| 15 | | | 30 | | | 45 | | | 60 | + | + |

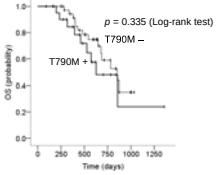
+, T790M positive; , not done

**Serum concentration levels**




**Survival Analysis**




## Conclusions

- ✓ T790M mutations were detected in 21 (35.0%) of the 60 patients.
- ✓ No correlations between the post-T790M status and the post-HGF and sMET levels were seen.
- ✓ The PFS in patients with the T790M mutation was shorter than that in those without the T790M mutation ($P = 0.03$). The ddPCR system is a useful method for determining the plasma EGFR T790M mutation status. The combination of the EGFR T790M mutation status, as detected using ddPCR, and the amphiregulin levels may be useful for monitoring drug resistance and for making a prognosis.