Department of Pathology
Herlev and Gentofte University Hospital
Denmark

Herlev and Gentofte Hospital
Department of Pathology

# Implementation of a Real-Time PCR based plasma ctDNA analysis for detection of progressive disease acquired by EGFR T790M mediated TKI resistance in NSCLC patients.

Steffen Grann Jensen[1], Jon Alexander Lykkegaard Andersen[2] Svetlana Borissova[2], Lotte Engell-Nørregård[2], Anders Mellemgaard[2], Estrid Høgdall[1]

[1]Department of Pathology, the Molecular Unit, Herlev-Gentofte Hospital
[2]Department of Oncology, Herlev-Gentofte Hospital

## INTRODUCTION

In spite of initial response in the majority of patients with epidermal growth factor receptor (EGFR) mutated non-small cell lung cancer (NSCLC), all will eventually develop progressive disease while receiving tyrosine kinase inhibitors (TKIs). The most frequent resistance mechanism is development of the T790M mutation. EGFR T790M circulating tumor DNA (ctDNA) in plasma from NSCLC patients with acquired TKI resistance has been reported to range from 30-50% using qualitative PCR-based assays(1). With the primary aim of clinical patient care we implemented T790M analysis within existing laboratory facilities.

## MATERIALS AND METHODS

Between 9-36 ml of blood were collected from 27 NSCLC patients and centrifuged (2500g/10min/4°C) within an hour from collection. Plasma was either stored at -80°C or immediately used for extraction of cell free DNA (cfDNA) (Qiagen 55114). Absolute and/or relative quantification of EGFR mutations in priority, T790M or Exon 19 deletions and L858R was done using a ctEGFR PCR kit (Entrogen ctEGFR-48). Samples were measured in triplicate. Fifty cycles of PCR were performed using ABI7500xl with manual setting of threshold, baseline and cutoff.

## RESULTS

| Pt ID | Previous EGFR status (Tissue/Pleura/Bronchus) | ctEGFR status (Blood) |
|---|---|---|
| 1 | L858R | L858R |
| 2 | Exon 19 del | Exon 19 del |
| 3 | Exon 19 del | Exon 19 del |
| 4 | L858R | T790M & L858R |
| 5 | L858R | T790M & L858R |
| 6 | Exon 19 del | Exon 19 del |
| 7 | G719C | G719C & T790M |
| 8 | L858R | WT |
| 9 | L858R | T790M & L858R |
| 10 | L858R | T790M & L858R |
| 11 | L858R | L858R |
| 12 | Exon 19 del (2013) | WT |
| 13 | Exon 19 del (2015) | WT |
| 14 | WT | WT |
| 15 | Exon 19 del (2015) | WT |
| 16 | L858R | T790M & L858R |
| 17 | L858R | L858R |
| 18 | Exon 19 del | Exon 19 del |
| 19 | Exon 19 del | T790M & Exon 19 del |
| 20 | L858R | T790M & L858R |
| 21 | E829Q (Not detected by our assay) | WT |
| 22 | Exon 19 del | T790M & Exon 19 del |
| 23 | Exon 19 del | T790M & Exon 19 del |
| 24 | Exon 19 del | Exon 19 del |
| 25 | Exon 19 del | T790M & Exon 19 del |
| 26 | L861Q | L861Q |
| 27 | Exon 19 del | T790M & Exon 19 del |

Table 1: The EGFR T790M resistance mutation was detected in 44% of the patients. For those patients and in 85% of the patients in total, previously detected EGFR mutations were confirmed, e.g. L858R or an exon 19 deletion, original found in tissue, pleura or bronchial sample material.

## CONCLUSION

Using Real-Time PCR, we detected EGFR T790M mutation in 44% of the patients. Additionally, in 85% of cases, we were able to recover previous reported EGFR mutations using plasma ctDNA.

## PATIENT CASE

Illustration of the importance of careful extraction of minute amounts of ctDNA among total cfDNA and the relationship to the input amount of cfDNA for relative Real - Time PCR quantification of the presence of few copies of T790M mutant alleles among the total amount of cfDNA.

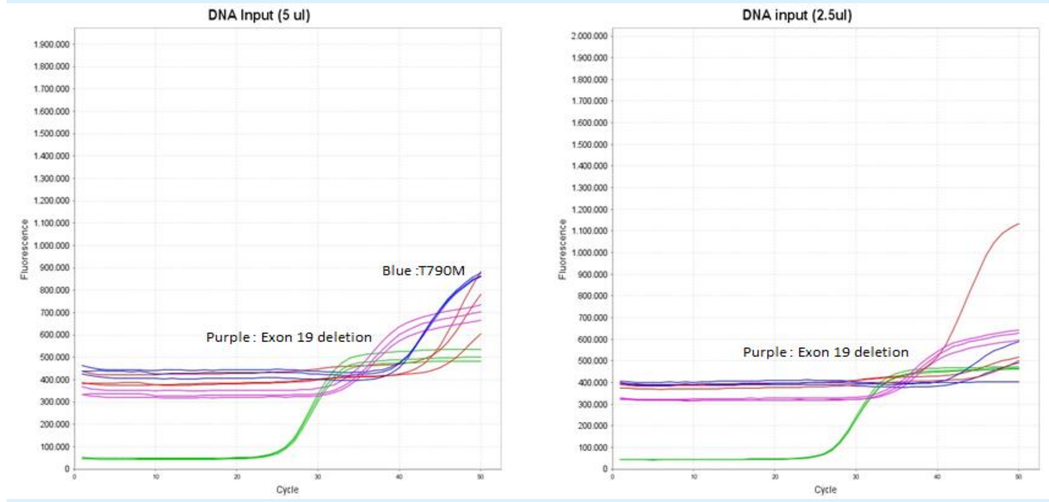


Table 2: Patient case (Pt ID 27) illustrating low copy numbers of EGFR T790M in triplicate measures (Blue). Comparison of DNA sample input amounts, 5ul (Left) vs. 2,5ul (Right).

Comparison of DNA sample input amounts, 5ul (Left) vs. 2,5ul (Right) shows the importance of sufficient cfDNA sample input for detection of low copy alleles of the EGFR T790M TKI resistance mutation.

A standard curve was used to estimate the approximate number of EGFR T790M mutant alleles in the patient case sample (Pt ID 27).

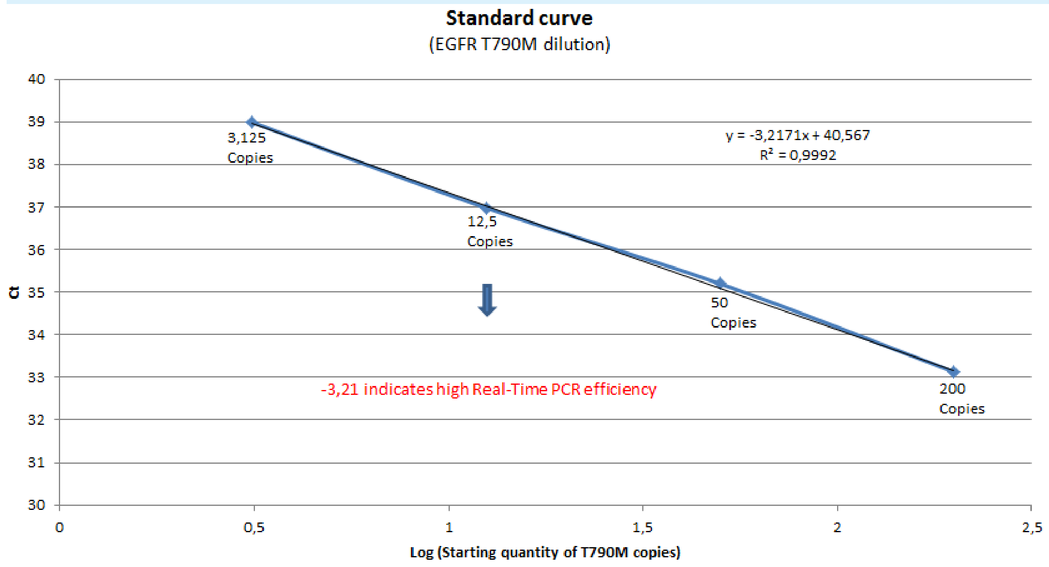


Table 3: Standard curve for EGFR T790M made from a control with known copy numbers of EGFR T790M, Exon19 deletions, and L858R.

As compared to the relative expression of EGFR T790M in table 2 the absolute number of EGFR T790M alleles were estimated to be around 1-2 copies in 5µl of cfDNA.

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## REFERENCES

(1) Zheng D, et al, Plasma EGFR T790M ctDNA status is associated with clinical outcome in advanced NSCLC patients with acquired EGFR-TKI resistance, *Sci Rep. 2016 , Feb 12;6:20913.*