# Detection of *EGFR* Mutations in Plasma cfDNA and Paired CTCs of NSCLC Patients before and after Osimertinib Therapy Using Crystal Digital PCR


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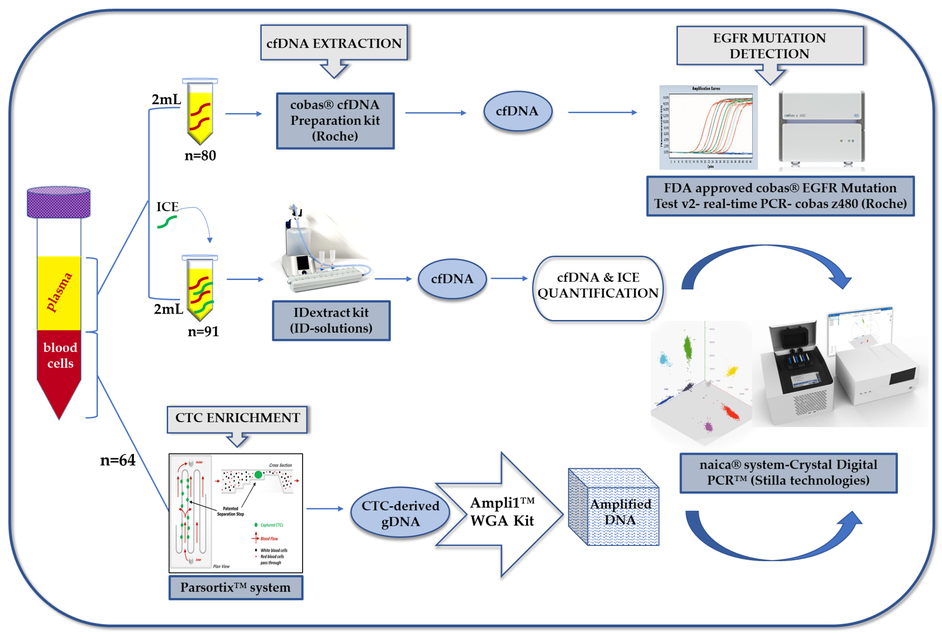
## Introduction

- Osimertinib is administered as 2nd line treatment in NSCLC patients resistant to 1st and 2nd generation EGFR TKIs.
- Liquid biopsy as a minimally invasive method consists a more feasible approach to track tumor evolution during therapy
- Digital PCR consists a breeding ground for molecular analysis in the field of oncology by providing improved precision, increased dynamic range and analytical sensitivity while detecting rare events.
- Our aim was to use crystal digital PCR technology to detect the presence and %MAF of *EGFR* mutations in plasma cfDNA and matched CTC fractions of 48 NSCLC patients before and after osimertinib therapy in the context of a Phase II multicenter clinical study (NCT02771314)

## Experimental Flowchart




## Results

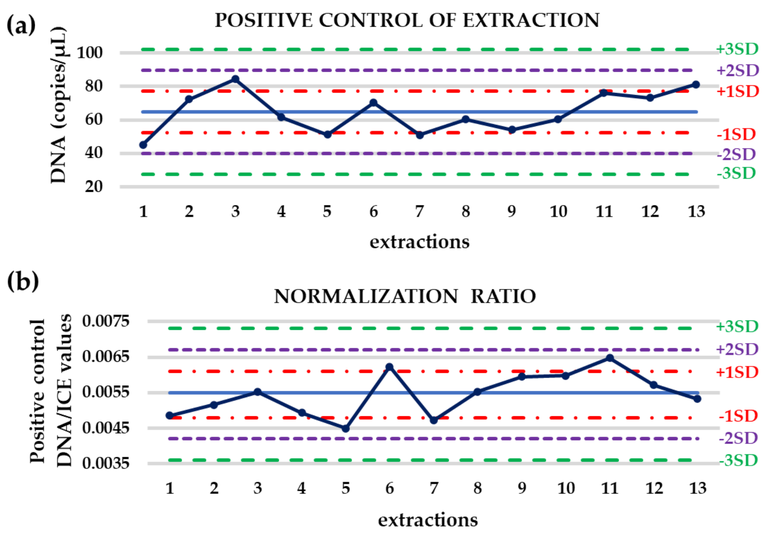



Figure 1. Levey-Jennings graphs for the evaluation of extraction process based on a) DNA con-centration (copies/µL) of the target positive control of extraction and on b) normalized positive control DNA/ICE values

- During cfDNA extraction, internal extraction control (ICE) was added in every sample to assess the efficiency of the extraction
- Levey-Jennings graphs showed that most of the positive control values either presented as concentrations or as normalized DNA values were within ±1SD (Fig.1)
- In some cases, plasma cfDNA levels were significantly increased at PD compared to baseline (Fig.2)

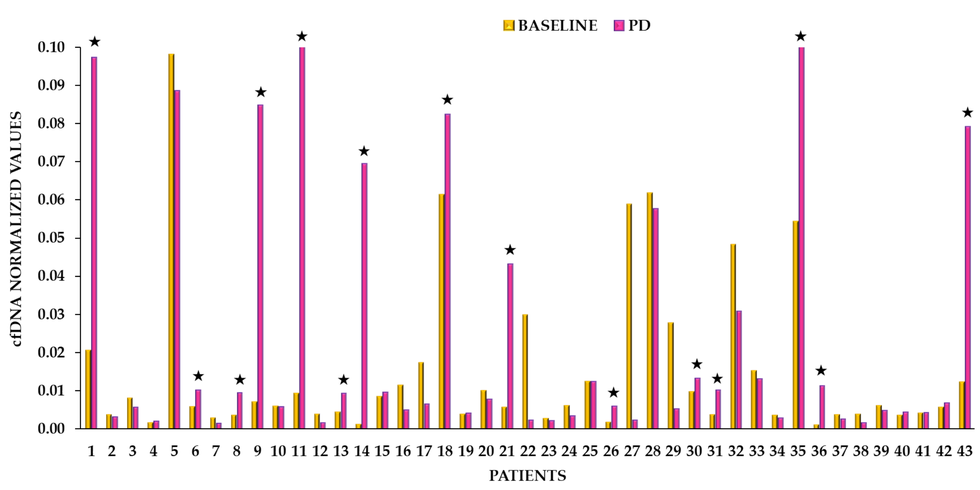



Figure 2. cfDNA fluctuations in plasma samples between baseline and PD for 43 patients based on normalized cfDNA values, ★: cases where normalized cfDNA values are higher in PD than at the baseline

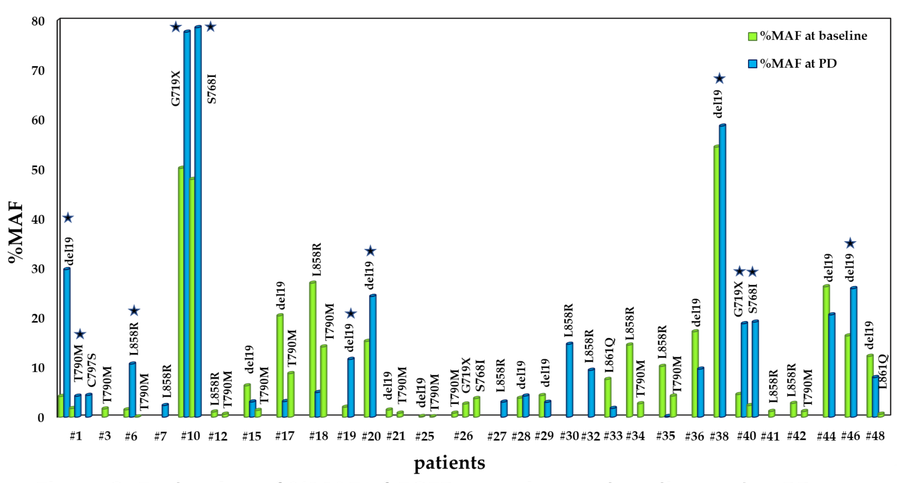



Figure 3. Evaluation of %MAF of *EGFR* mutations at baseline and at PD, ★: cases where %MAF are higher in PD than at the baseline

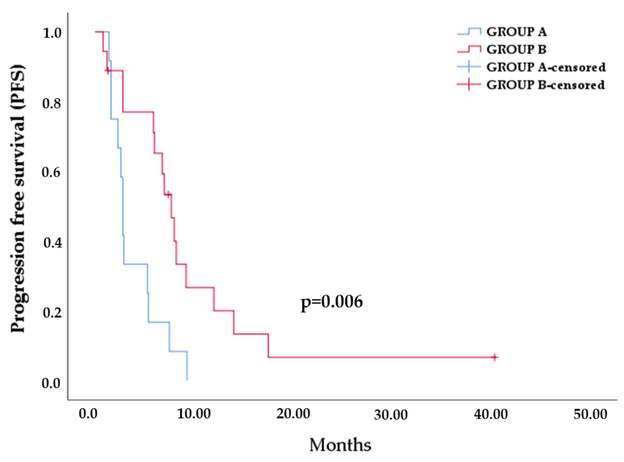



Figure 4: Difference in PFS depending on %MAFs before treatment with osimertinib and at PD between group A and group B

- We compared the % MAFs of each type of *EGFR* mutation in plasma between baseline and PD. In some patients, mutations disappear at PD and in others are maintained or reemerge. In patients #1, #6, #10, #19, #20, #38, #40, #46, %MAFs for various EGFR mutations were significantly increased at PD (Fig.3).
- Patients with higher %MAFs at PD (Group A) presented significantly lower PFS compared to those that lost *EGFR* mutations after osimertinib therapy (Group B) (Fig.4).

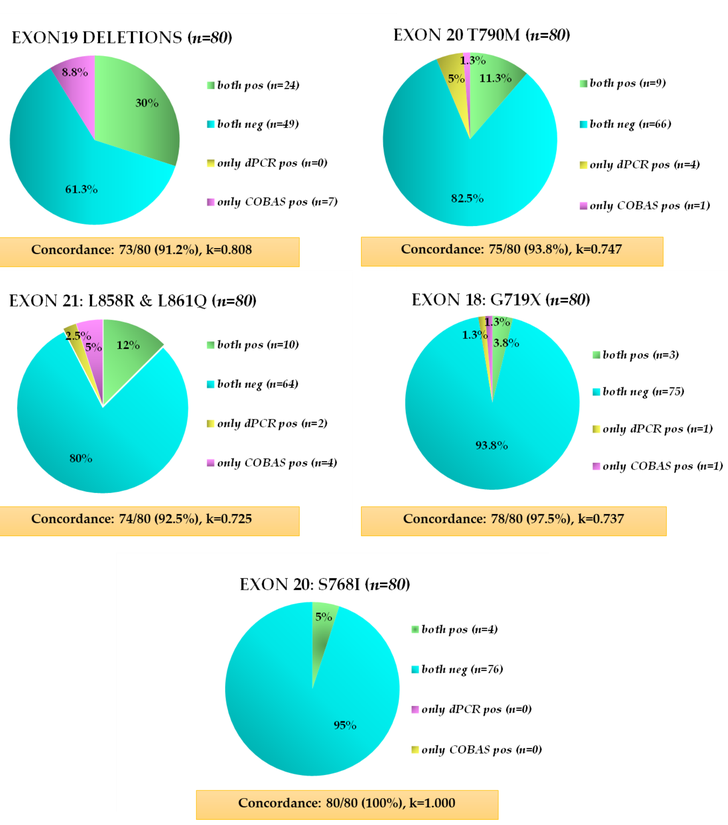
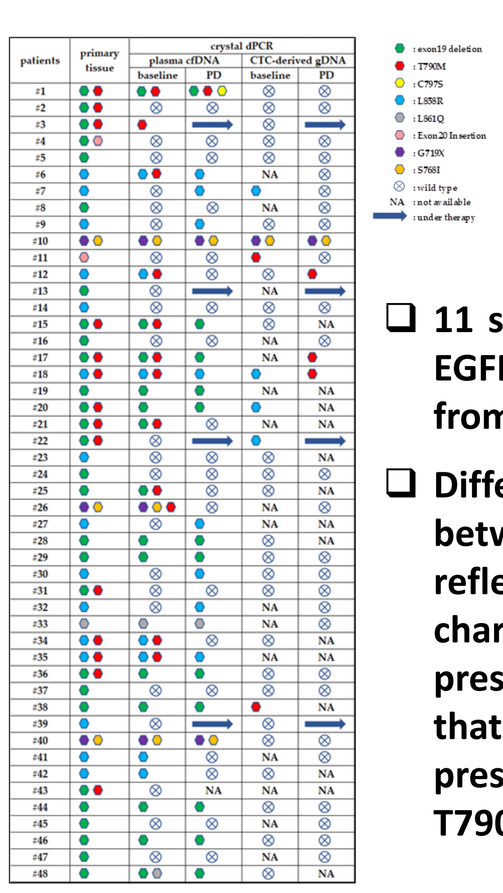



Figure 5. Direct comparison of *EGFR* mutations detected with crystal dPCR and FDA-approved cobas® EGFR mutation test v2

- Direct comparison of crystal dPCR and the FDA approved cobas® EGFR Mutation test in 80 identical plasma samples from both time points revealed high concordance rates for all EGFR mutations (Fig.5)
- In some cases, crystal dPCR was more sensitive in detecting the key resistance *EGFR* mutation, T790M.
- Direct comparison of *EGFR* genotyping between primary tissue and plasma cfDNA samples revealed high rates of concordance for the EGFR the mutations.




- 64 matched CTC-derived gDNA samples were analyzed for the detection of *EGFR* mutations using crystal dPCR.
- 11 samples were found positive for EGFR mutations with %MAF ranging from 0.2 to 2.25%.
- Differences in *EGFR* genotyping between plasma and CTCs might reflect tumor heterogeneity that characterizes NSCLC and also the presence of resistance mechanisms that occur under selective therapy pressure due to dominance of T790M wild type clones.

## Conclusions

- Crystal dPCR, a highly sensitive technology, allowed to track tumor evolution through the detection of low abundance *EGFR* mutations in cfDNA and CTCs predictive for the treatment outcomes of NSCLC patients under osimertinib treatment.
- Crystal dPCR exhibited high concordance rates in correlation with the FDA-approved cobas technology.
- In some cases, crystal dPCR was more sensitive in detecting the T790M mutation.
- For the first time, crystal dPCR was applied to detect *EGFR* mutations in CTC-derived gDNA samples of NSCLC patients under osimertinib.
- Discrepancies found between CTCs and tumor or cfDNA genotyping, confirmed previous evidence about tumor heterogeneity and clonal evolution in NSCLC.

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## Reference


❖ Ntzifa A. et al, Cancers (Basel). 2021 May 31;13(11):2736