Multiplex crystal digital PCR for detection and quantification of *EGFR* mutations in plasma of advanced non small cell lung cancer patients

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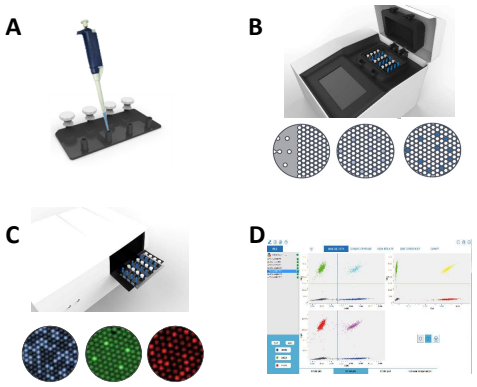
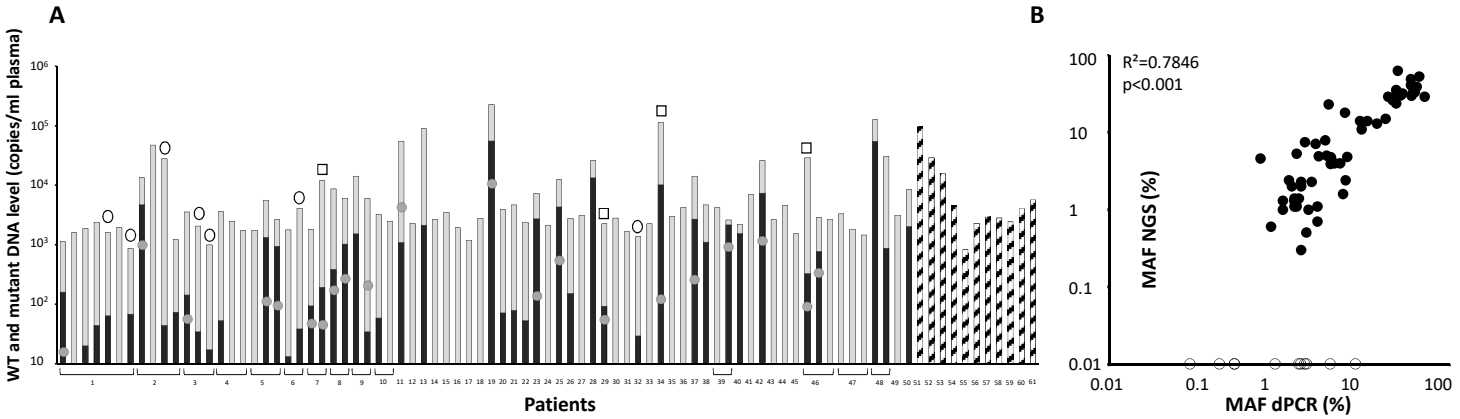
BACKGROUND :

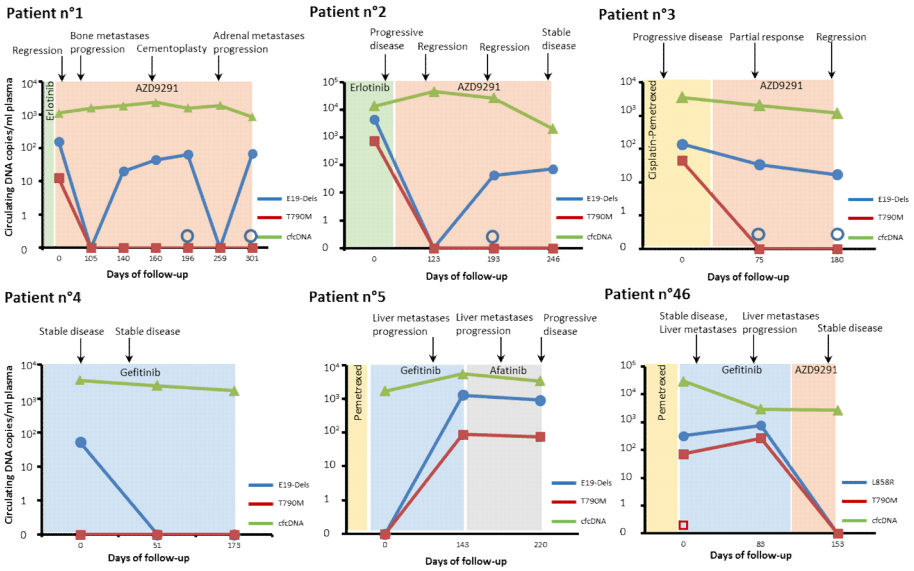
The Naica system from Stilla Technologies, a newly developed droplet digital PCR (dPCR) platform, was evaluated for the detection of *EGFR* sensitizing and resistance mutations in the plasma of non-small cell lung cancer (NSCLC) patients. Taking advantage of the 3 fluorescent channels available, multiplex assays targeting *EGFR* L858R, L861Q, E19-Dels and T790M were designed. A total of 87 plasma samples from 61 patients with metastatic NSCLC under anti-*EGFR* therapy and for whom re-biopsy was not feasible were included in the study. Fourteen patients had at least one follow-up sample and 7 patients had at least 3 samples. Measurements obtained with dPCR were compared to that of next generation sequencing (NGS) using the same samples.



Crystal digital PCR workflow using the Naica system. A. Pipet the samples into the Sapphire chip and seal chip using caps. **B**. Place chips onto the Naica Geode, and start the program that will generate droplets prior to thermal cycling. **C**. Place thermocycled chips in the Naica Prism3, and acquire images for channels Blue, Green and Red. **D**. Analyze data using the Crystal Miner software.



A. Wild-type and mutant DNA levels measured by crystal digital PCR in plasma samples from metastatic NSCLC patients. Greyed bar: total DNA concentration in NSCLC patients with confirmed targetable *EGFR* mutations in tumor tissue, filled bar: sensitizing (E19-Dels, L858R and L861Q) mutations concentration, dashed bar: wild-type DNA concentration in NSCLC patients with WT *EGFR* in tumor tissue. Grey dots: T790M mutation concentration. Empty circles and squares indicates sensitizing and resistance mutations positives by dPCR but not detected by NGS respectively. **B. correlation between mutant allele fraction (MAF) measured for sensitizing and resistance mutations by dPCR and NGS**. Empty circles represent samples not detected by NGS.



Monitoring of circulating sensitizing and resistance *EGFR* mutations and total DNA levels over time in 6 metastatic NSCLC patients using dPCR. The coloured region indicate periods of chemotherapy. Radiological assessment of patient response is indicated above the graphs. Empty circles and squares indicates sensitizing and resistance mutations positive by dPCR but not detected by NGS respectively.

CONCLUSION :

We have characterized 2 new multiplex digital PCR assays to detect major sensitizing and resistance *EGFR* mutations in the plasma of NSCLC patients. These assays were also used in follow-up samples for monitoring *EGFR* mutations, whose levels correlated with the evolution of disease, and response to treatment. Both digital PCR assays enabled detection of mutations with higher sensitivity than NGS. Moreover, Crystal Digital PCR appeared better suited to clinical use than NGS in terms of cost and time to results