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

Cécile Jovelet, Jordan Madic, Aurélie Honoré, Jordi Remon-Masip, Benjamin Besse, Barbara André, Romain Girard, Magali Droniou, Ludovic Lacroix

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.

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.