Circulating tumor DNA-based analyses of solid tumors

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Background: Circulating tumor DNA (ctDNA) are extracellular fragments of tumor DNA released to the pool of other cell-free DNA (cfDNA) fragments present in the bloodstream. CtDNA-based analysis is a dynamically developing and complex method of liquid biopsy (LB). LB is a minimally invasive approach with the potential to facilitate diagnosis, monitor response to treatment or predict prognosis. LB has the advantage of bridging the pitfalls of limited availability of tumor tissue samples or the riskiness of collection through repeatable examination of tumor biomolecules circulating in peripheral blood.

Methods: The optimization of hLINE-1 sequence-based quantification of cfDNA by RT-PCR as well as specific sequence-based quantification of ctDNAby digital droplet PCR (ddPCR) in different types of biological sampleswith the focus on plasma samples and the detection of clinically relevant mutations in ctDNA.

Results: The combination of cfDNA quantification by hLINE-1-based RT-PCR and ctDNA quantification using ddPCR, corresponding to tumor characteristics and clonal heterogeneity over time, was optimized using samples of various biological samples - peritoneal and pleural effusions, plasma, and culture medium. The highest cfDNA concentrations were observed in effusions, lower in plasma and lowest in culture medium which was associated with a dynamic occurrence of longer cfDNA fragments. We observed a decrease of plasma cfDNA as well as H1047R mutation in ctDNA with persistent levels of E545K mutation in breast cancer patient over time. Moreover, ddPCR-based EGFR T790M testing in the non-small-cell lung cancer patient plasma samples was implemented with the limit of detection of about 0.1%.

Conclusion: Combined cfDNA and ctDNA quantification was optimized and the detection of various clinically relevantctDNA-derived mutations implemented in cancer patients.

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