ddPCR, qPCR, and NGS performance to detect KRAS and EGFR mutations in plasma cell-free DNA of stage IIIB/IV lung adenocarcinoma patients, and comparison with the tissue molecular profile

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Background & objectives

Multiple platforms are available for circulating cell free tumor DNA (ctDNA) detection from liquid biopsies. The aim of this study was to examine the performance of ddPCR, qPCR (Cobas, Idylla) and NGS (Avenio) to detect pathogenic KRAS and EGFR mutations in liquid biopsies of patients with stage IIIB/IV lung adenocarcinomas. Analysis of tumor tissue DNA by targeted NGS served as reference.

Methods

A prospective observational study was performed in patients with stage IIIb/IV lung adenocarcinomas in Maastricht University Medical Centre and Zuyderland Medical Centre (Sittard/Heerlen) from 2018 until 2022. Liquid biopsies (blood draws) were collected in Streck tubes from each patient and plasma was isolated and stored at -80^{o}C until use. ctDNA isolation and ddPCR, qPCR and NGS were performed according to the manufacturers' instructions. Sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) were calculated for each technique, and results were compared with targeted NGS results of the patients' tumor tissue.

Results

ctDNA plasma testing was conducted in 193 samples of patients with stage IIIB/IV lung adenocarcinomas. Median patient age was 68 (range 31-88) and 54% were female. Targeted NGS on tumor tissue identified in 33% KRAS mutations, in 29% EGFR mutations, in 6% BRAF mutations and in 32% other/no driver mutations. Platform comparisons revealed that ddPCR and NGS detected more KRAS and EGFR ctDNA mutations than qPCR (sensitivity 65-80% and 57-70%, respectively; specificity 99-100% and 97-100%, respectively). In addition, our results show that low cfDNA input negatively impacts limit of detection, and that ctDNA is more often detected in patients with stage IVB disease compared to stage IVA.

Conclusion

In comparison with KRAS and EGFR mutations detected in stage IIIB/IV lung adenocarcinoma tissue DNA with targeted NGS, ddPCR, NGS and qPCR technologies show sensitivities ranging from 57-80% for detection of these mutations in cfDNA of the same patients. cfDNA input appears to be an essential factor for optimal sensitivity, and a higher disease stage is associated with a higher sensitivity for ctDNA detection.