# Comprehensive liquid biopsy analysis for monitoring NSCLC patients under second-line osimertinib treatment

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The aim of this study was to perform a comprehensive LB analysis for monitoring NSCLC patients under second-line osimertinib treatment, by combining plasma-cfDNA and CTC analysis to identify molecular alterations at resistance and potential targets for subsequent treatments. Peripheral blood from 30 NSCLC patients was collected before treatment (baseline) and at disease progression (PD). Plasma-cfDNA was analyzed for DNA mutations (*EGFR*, *PIK3CA*, *KRAS*-G12C, *BRAF*-V600E) using digital PCR and for DNA methylation (*RASSF1A*, *BRMS1*, *SLFN11*, *RASSF10*, *APC*, *RARβ*, *FOXA1*, *WIF-1*, *SHISA3)* using methylation specific PCR. CTCs were enriched from identical blood draws using Parsortix (Angle, UK). CTC-derived gDNA was analyzed for the same DNA mutations and methylation markers. CTCs were analyzed for *HER2* and *MET* amplification with FISH. RT-qPCR was performed in CTCs-derived mRNA for *CK-8*, *CK-18*, *CK-19*, *VIM*, *TWIST-1*, *AXL*, *ALDH-1*, *PD-L1*, *PIM-1*, *B2M* genes. PD-L1 was detected in CTCs enriched using ISET (Rare cells, France) using immunofluorescence (IF). *EGFR* mutation analysis in plasma-cfDNA and CTCs have shown complementary information; T790M was detected only in CTC from three patients at PD, but not in paired plasma-cfDNA. *PIK3CA* mutations were detected only in plasma-cfDNA but not in CTCs. *KRAS*-G12C and *BRAF*-V600E were not detected in any sample. *MET* amplification was detected in CTCs of two patients at baseline whereas *HER2* amplification was detected in CTCs of three patients at baseline and in one patient at PD. DNA methylation between CTCs and cfDNA revealed low concordance. Data from IF and RT-qPCR for the presence of PD-L1 positive CTCs in matched samples revealed high detection rates suggesting a theoretical background for immunotherapy in EGFRm NSCLC patients. PD-L1, *PIM-1* and *AXL* expression in CTCs indicate a potential benefit of targeted therapies for NSCLC patients who relapse following osimertinib treatment. Our results indicate the importance of complementary information obtained through parallel analysis of CTC and ctDNA. Comprehensive LB analysis efficiently represents the heterogeneous molecular landscape and provides prominent information on subsequent treatments for NSCLC patients progressing with osimertinib based on different druggable molecular alterations.