Circulating Tumor DNA Analysis in Blood for the Prediction of Treatment Response in Patientswith Advanced Non-Small Cell Lung Cancer

Antonio Russo

Funding: The author(s) received no specific funding for this work.

Potential competing interests: The author(s) declared that no potential competing interests exist.

Abstract

Introduction: Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 85% of lung cancer cases. Molecular profiling NGS-based has become a crucial component of clinical management of NSCLC patients. However, despite the main use of tumor tissue, one of the main medical needs is to develop a non-invasive or minimally invasive dynamic tool that can allow the monitoring of disease. In this evolving scenario, our aim was to assess whether serial ctDNA monitoring of plasma predicts treatment response in metastatic NSCLC patients. Methods: Analyses of PFS and OS were performed using the Kaplan-Meier method and the log-rank test. Multivariate Cox regression analysis was used to adjust for clinical factors. Data were analyzed and visualized using MetaDisc statistical software (version 1.4). The p values were considered significant if $p \leq 0.05$. Results: 50 patients were included. The study population was constituted of males, >65 yo, active smokers, affected by lung adenocarcinoma. Considering the metastatic site distribution, bones and lymph nodes were sites mostly involved. Notably, 16% (8\50) of the cohort had brain disease, while only 7 patients had liver metastases. The molecular profile included 20% of patients with a genomic aberration in EGFR, 6% an ALK translocation, thus 24% of the entire cohort was given treatment with a Tyrosine Kinase Inhibitor (TKI). The patients underwent blood withdrawal and the serial measurements of plasma ctDNA were correlated with the overall response rate (ORR). Delta cfDNA between t0-t1 and t0-t2 were less than 20% different in this timeframe. We observed a fair agreement between the radiological progression of disease and the molecular progression identified by high levels of ctDNA. Responders had significantly lower ctDNA levels at t1 compared with baseline (log-rank 0.045). Conclusions: CtDNA analysis of plasma is a useful tool to monitor response in metastatic NSCLC.

Valerio Gristina 1 MD, Antonio Galvano 1 MD PhD, Maria La Mantia 1 MD, Nadia Barraco 1 PhD, Martina Greco 1 MD, Alessandro Perez 1 PhD, Lavinia Insalaco 1 MD PhD, Luisa Castellana 1 MD, Lorena Incorvaia 1 MD PhD, Viviana Bazan 2 PhD, Antonio Russo 1 MD. 1 Department of Surgical, Oncological and Oral Sciences, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy 2 Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy

Keywords: Non-Small Cell Lung Cancer, Circulating Tumor DNA, Liquid Biopsy, Biomarkers, DiChirOnS 2021.

Introduction: Lung cancer (LC) is the main cause of cancer death worldwide with two million newly diagnosed cases, or 13% of all cancers diagnosed, in 2021. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 85% of lung cancer cases. Molecular profiling NGS-based has become a crucial component of clinical management of advanced NSCLC patients. However, despite the main use of tumor tissue, one of the main medical needs is to develop a non-invasive or minimally invasive dynamic tool that can allow the monitoring of disease. In this evolving and fascinating scenario, liquid biopsy detects and analyzes biological material originated within and from the tumor, significantly outperforming standard diagnostic modalities and
facilitating noninvasive detection, genotyping, and monitoring cancer disease. Its main components are circulating tumor cells (CTCs), circulating nucleic acids (circulating tumor DNA as ctDNA, circulating microRNA, and circulating RNA), and extracellular vesicles (exosomes and microvesicles). Circulating tumor DNA (ctDNA) might be <0.01% of the total circulating free DNA (cfDNA) deriving from the tumor masses, which is DNA highly fragmented mainly derived from a combination of apoptosis, necrosis, and active secretion of cells. Tumors' release of ctDNA into biological fluids can be detected and quantified in liquid biopsies to monitor disease and treatment response. Emerging evidence suggests that ctDNA can be used to monitor treatment response. Therefore, the main aim of our research was to assess whether serial ctDNA monitoring of plasma predicts treatment response in metastatic NSCLC patients. Methods: This study aims to further elucidate the role of ctDNA as a novel and reliable biomarker in treatment- naïve advanced NSCLC patients and in NSCLC patients who have received 1 st -line treatment based on chemotherapy, TKIs, and ICIs. This explorative study, including the systematic assessment of ctDNA level and the monitoring of treatment-induced changes in blood profile, would provide key information on the potential biomarkers of early progression identifying strategies to overcome drug resistance. The primary hypothesis of this observational exploratory study was that changing levels of ctDNA may serve as early predictors of response to oncological treatments in advanced NSCLC. The prespecified primary endpoint was the agreement between a molecular ctDNA response from t0 to t1 and the radiographic tumor response. The secondary aim was to compare OS and PFS in patients who presented a ctDNA response versus those who did not achieve this endpoint. Further prespecified outcome measures included the baseline ctDNA levels and the number and types of mutations in association with survival. Descriptive statistics were used for patient and tumor characteristics. For comparisons of ctDNA levels, response, and concordance between ctDNA and clinical response, nonparametric and parametric tests were used. Analyses of PFS and OS (defined from treatment start until radiographic progression or death, the primary outcome was censored on the date of the last follow-up) were performed using the Kaplan-Meier method and the log-rank test. For this analysis, 50 patients' data were available. Multivariate Cox regression analysis was used to adjust for clinical factors. Data were analyzed and visualized using MetaDisc statistical software (version 1.4). The p values were considered significant if $p \leq 0.05$. Blood samples of the

patients (~5 mL) were collected into K2 EDTA tubes at baseline. They were immediately processed for plasma collection and centrifuged twice (10 minutes at 3000 rpm; 10 minutes at 16,000 x g). Samples processing occurred within 1h to obtain plasma. Collected plasma specimens were stored at -80°C. From 1 to 2 ml of plasma samples were processed to isolate circulating free nucleic acids (cfDNAs) using the QIAamp Circulating Nucleic Acid kit (Qiagen). The quantitative of isolated ctDNA was assessed by QubitTM dsDNA HS Assay Kit. Additionally, a further 2 ml of plasma were used to isolate extracellular vesicles using exoEasy Maxi Kit (Qiagen) with buffer XE for elution. Finally, the extraction of RNA nucleic acid from extracellular vesicles was conducted through exoRNeasy Maxi Kit (Qiagen) using the preliminary purification step of the extracellular vesicles with miRCURY® Exosome Kits (Qiagen). These kits were used according to the manufacturer's instructions. Circulating nucleic acids were analyzed using OncomineTM Lung Cell-Free Total Nucleic Acid Research Assay (ThermoFisher Scientific), covering 168 ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, and TP53 hotspot pathogenetic variants (PVs), in addition to ALK, RET and ROS1 fusions, MET exon 14 skipping PV and MET CNV. All NGS runs findings were compared with positive in-house control as a validation set. Libraries were quantified by Ion Library TaqManTM quantification kit (ThermoFisher Scientific) and were sequenced on Ion Torrent S5 platform. Oncomine TagSeq Lung v2 Liquid Biopsy-w2.4-Single Sample was used as workflow of run. The analysis was considered "pass" whit Total Mapped Reads >3M, Median Read Coverage Avg 40,000 (mIN >25,000), and Median Molecular Coverage >2,500. Pathogenic changes in ctNA were categorized by relevance by Ion Reporter Software applying the default filter chains Variant Matrix summary 5.16. They were described using the HGVS standard nomenclature. Results: Patients with advanced NSCLC were recruited from February 2020 to September 2021 at the University Hospital "Paolo Giaccone" in Palermo, Italy, of which 50 patients were evaluable. Main inclusion criteria were signed written informed consent; Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of <2; patients with histologically or cytologically confirmed NSCLC diagnosis with stage IIIB-C\IV disease (according to version 8 of IASLC TNM) or with recurrent or progressive disease following multimodal treatment (radiation therapy, surgical resection, or definite chemoradiation therapy for locally advanced disease) who are treatment naïve and eligible for oncological treatment (CT, TKIs, ICIs). However, exclusion criteria included the following: patients with ECOG PS 3 and patients who received prior therapy. Blood was taken prospectively before oncological treatment administration (t0), after one cycle and after radiological evaluation (on average 3 months). Tumor response was assessed by diagnostic computed tomography using RECIST, version 1.1, by an observer blinded to the biomarker outcome. All patients provided written informed consent. The clinical trial was approved by the medical ethics committee of the University Hospital "Paolo Giaccone" Palermo, Italy. The majority of the study population was constituted by males, >65 yo, active smokers, affected by lung adenocarcinoma. Considering the metastatic site distribution, bones and lymph nodes were sites mostly involved. Notably, 16% (8\50) of the cohort had brain disease, while only 7 patients had liver metastases. The molecular profile mainly included 20% of patients with a genomic aberration in EGFR, 6% an ALK translocation, thus 24% of the entire cohort was given treatment

with a Tyrosine Kinase Inhibitor (TKI). The patients underwent serial blood withdrawal and the consequent serial measurements of plasma ctDNA were correlated with the overall response rate (ORR). Particularly, delta cfDNA between t0-t1 and t0-t2 were mostly less than 20% different in this notable timeframe. Interestingly, we observed a fair agreement between the radiological progression of disease detected by WB CT scan and the molecular progression identified by high levels of ctDNA. Indeed, Responders had significantly lower ctDNA levels at t1 compared with baseline (log-rank 0.045). The correlation between best response and oncological treatment (IO, CT, TKI) showed that patients under TKIs treatments displayed a better response rate (RR). Our study has some limitations. Firstly, the number of patients included in this study is relatively small (N=50). However, the nature of the study was mainly exploratory. Larger prospective randomized clinical trials are awaited in order to identify the best timing for ctDNA testing and whether the levels of ctDNA can be used in the treatments algorithm of NSCLC patients. Conclusions: Serial measurements of plasma ctDNA can be useful for monitoring the treatment response to different treatments in patients with metastatic NSCLC. However, larger prospective clinical trials are warranted to examine whether treatment modification based on ctDNA analysis leads to improvement in survival.