LIQUID BIOPSY: A REAL CLINICAL PRACTICE

1)Iacobelli C. 2)Rofi E. 3)Del re M. 4)Pino MS. 5)Pozzessere D. 6)Belluomini L. 7)Frassoldati A. 8)Iannopollo M. 9)Di lieto M. 10)Danesi R.

University of Pisa

Chemotherapy remains the most validated standard option in the first-line setting for non small cell lung cancer (NSCLC) patients without any known driver mutations (Pignon et al. 2008). Tissue biopsy is still the gold standard for clinical diagnosis and molecular analysis. However, analyses carried out on small and/or insufficient tumoral material do not reflect tumor heterogeneity, leading to misinterpretation of the mutational status of primary NSCLC and difficulty in making precise treatment decision (Hofman et al. 2016). Nowadays, under the label "liquid-biopsy", cell-free circulating tumor DNA (cftDNA) is gradually becoming an attractive tool in the clinical routine practice. cftDNA is less influenced by tumor heterogeneity, it can be easily repeated and, above all, it offers the opportunity a "real time" instrument for dynamically monitoring tumor genomes, as well as drug-resistance, in a non-invasive manner (Ilie et al. 2014). The aim of this study was to demonstrate the impact and utility of liquid biopsy as an alternative to tissue biopsy in the clinical practice of NSCLC patients undergoing first-line chemotherapy. Fifty-eight patients with histologically proven advanced NSCLC, who came from November 2016 to February 2017 at the Clinical Pharmacology and Pharmacogenetic Unit of the University Hospital of Pisa for evaluating EGFR mutation status and/or EML4-ALK rearrangements after first-line chemotherapy, were included in this study. Of 58 patients, 10 had progressed on chemotherapy treatment. Of note, 8 out of 10 patients were EGFR mutations and/or EML4-ALK fusions negative in tumor tissue samples. Molecular tissue analyses were not performed in 2 out of 10 cases due to insufficient tumor biopsy material. For these 10 patients cftDNA was extracted from 6 ml of plasma with the QIAmp Circulating nucleic acid Kit (Qiagen®, Valencia, CA, USA) and analyzed by the Droplet Digital™ PCR (ddPCR, BioRad®, Hercules, CA, USA) for EGFR mutations in exons 19 (p.E746_A750del and p.L747_P753>S), 21 (p.L858R) and 20 (p.T790M). Plasmatic RNA (ctRNA) was isolated from platelets using the RNeasy mini kit (Qiagen®, Valencia, CA, USA) and analyzed by the Droplet Digital™ PCR (ddPCR, BioRad®, Hercules, CA, USA) for the detection of the three most common EML4-ALK variants (v1 [e13:a20], v2 [e20:a20], and v3 [e6:a20], with breakpoints at EML4 exons 13, 20, and 6 and at ALK exon 20, respectively). As described in table 1, data obtained from ddPCR analyses of cftDNA and ctRNA revealed that all of 10 patients with progression disease had mutations in EGFR gene or/and ALK rearrangements. In detail, 5 out of 10 patients harbored EGFR exon 19 deletion mutations without detectable EML4-ALK translocations. Conversely, 2 out of 10 patients had EML-4-ALK-rearranged tumors and wild-type EGFR. Moreover, 2 patients presented both EGFR exon 19 deletions and p.T790M mutations, while one patients harbored EGFR exon 19 deletion mutations and detectable EML4-ALK translocations. To conclude, these preliminary results highlighted the clinical utility of liquid biopsy as an alternative to tissue biopsy when tumor material is insufficient or not adequate for molecular analyses. Moreover, liquid biopsy could assess and detect small, pre-existing intrinsically resistant subclones routinely undetected within the primary tumor that can expand as a result of the stringent pressure induced

by chemotherapy-resistance. However, in order to assess these preliminary data, further analyzes are needed.

Ilie et al. (2014). Ann Transl Med. 2(11):107.

Hofman et al. (2016). Virchows Arch. 469(6):601-609.

Pignon et al. (2008). J Clin Oncol. 26(21):3552-9.

Table 1. Tissue biopsy vs liquid biopsy in a real clinical comparison.

ID PATIENTS	**TISSUE BIOPSY**		**LIQUIDI BIOPSY**	
	EGFR mutations	*EML4-ALK Translocations*	*EGFR mutations*	*EML4-ALK Translocations*
1	Tissue not available	Tissue not available	ex19dels	Absence
2	wt	Absence	wt	variant 2 [E20;A20]
3	wt	Tissue not available	ex19dels p.T790M	Absence
4	wt	Absence	ex19dels p.T790M	Absence
5	Tissue not available	Tissue not available	ex19del	variant 2 [E20;A20]
6	wt	Tissue not available	ex19dels	Absence
7	wt	Absence	ex19dels	Absence
8	wt	wt	wt	variant 2 [E20;A20]
9	wt	Tissue not available	ex19dels	Absence
10	wt	wt	ex19dels	Absence

Legend: wt: wild-type; ex19del: exon 19 deletions.