Liquid biopsies for molecular profiling of mutations in non-small cell lung cancer (NSCLC) patients lacking tissue samples

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BACKGROUND

- Approximately 30% of patients with an adenocarcinoma have a druggable driver mutation[1].
- In our experience, 22% of patients are lacking a molecular profile mostly due to insufficient tumor cells in the specimen or poor quality DNA[2]
- Circulating tumor DNA (*ct*DNA) can be used for detection and quantification of molecular abnormalities as a minimally-invasive tool.
- Moreover, *ct*DNA analysis may provide a molecular profile when tumor tissue is not available or of poor quality enabling treatment stratification. It can also be used to monitor treatment efficacy and detect resistance mutations

OBJECTIVES

A prospective study was performed:
- To assess the molecular alterations in the *ct*DNA of NSCLC patients in whom the initial molecular profile or profile at acquired resistance was unknown due to lack of tumor tissue biopsy or insufficient cellularity in the biopsy.
- To assess resistance mutations on treatment
- To assess the proportion of patients who receive personalised treatment based on these results.

PATIENTS

- We enrolled 116 pre-treated advanced NSCLC patients (1 patient treatment-naïve) with progressive disease described as 2 cohorts:
  - Patients with unknown molecular profile
  - Patients with *EGFR* mutant NSCLC tested in tissue.

METHODS

- 10 ml. blood were collected in EDTA-K2 tubes and processed at the Gustave Roussy Cancer Campus to obtain plasma samples.
- DNA was extracted from < 5 ml of plasma and analysed using Inivata's enhanced TAm-Seq™ assay covering regions from 35 cancer-related genes (Figure 1). Sequences were generated using Illumina sequencing.

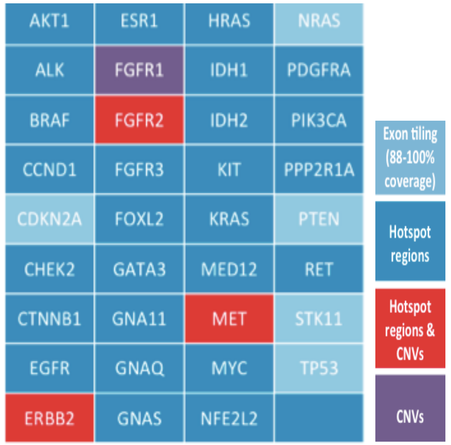

Figure 1. Inivata Tam-Seq Panel

RESULTS

Clinical Characteristics

- From July 2015 to January 2016, 116 patients were enrolled (66% female, 53% never-smoker, 92% diagnosed with an adenocarcinoma subtype, 97% with stage IV disease, and 55 patients (47%) had known *EGFR* mutant tumors of which 30% had mutations in *exon 19* and 15% had mutations in exon 21). (Table 1)

Characteristics	Population (N=116)
Sex:	
Male / Female	40 (34%) / 76 (66%)
Median Age (years)	63 (22-89)
Histology	
Adenocarcinoma	107 (92%)
Squamous	7 (6%)
Neuroendocrine	2 (2%)
Smoking status:	
Never-smoker	62 (53%)
Former-smokers	42 (36%)
Smokers	12 (11%)
Initial *EGFR* mutation (%)	
Del19 / *Exon 21* / Exon18 / Not reported	35 (30%) / 17 (15%) / 1 (1%) / 2 (2%)

Table1. Patients' characteristics

*ct*DNA results

- ctDNA profiling was successfully performed for all patients, and mutations were detected in 83 of 116 patients (72%). (Figure 2)
- 50% of the mutations detected were <1% allele fraction, with 32 % between 0.1-0.5% (Figure 3). TP53 was the most frequent mutation reported in the population (46 out of 116, 40%)
- Personalised treatment based on *ct*DNA molecular profile was performed in 22% of patients (Figure 4).
- Among *EGFR* mutant NSCLC patients (n=55) progressing on EGFR TKI at the moment of liquid biopsy (n=41), *T790M* mutation was reported in 51% (21 out of 41. 1 patient treated with osimertinib had *C797S* mutation). These 20 patients were subsequently treated with osimertinib (AZD9291).
- Among those patients with unknown initial molecular profile clinically relevant molecular alterations found and treatments initiated based on these results were:
  - 8 patients with *KRAS* mutation (3 *G12D*, 2 *G12C*, 1 *G12F*, 1 *G12S*, 1 *G12V*)
  - 3 patients with common *EGFR* mutation, 1 treated with Erlotinib. Double *EGFR* mutation exon 18 and 20 (G719A + S768I) was reported in 1 patients who was treated with afatinib.
  - 3 patients with ERBB2 exon 20 insertions, 1 treated with afatinib
  - 2 samples with *MET* exon 14 mutation and treated with crizotinib
  - 1 sample with *BRAF G469A* mutation.

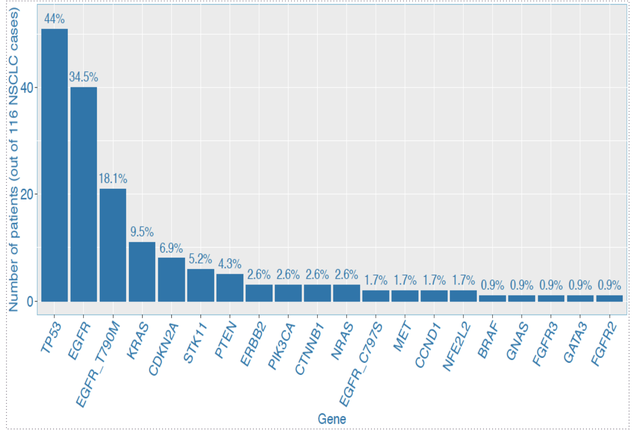

Figure 2. Mutations detected in *ct*DNA

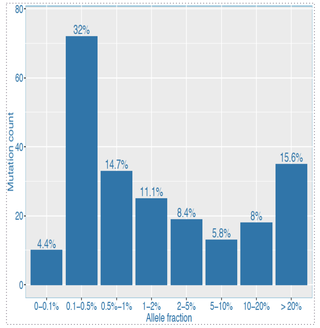

Figure 3. Allelic fraction of mutations detected

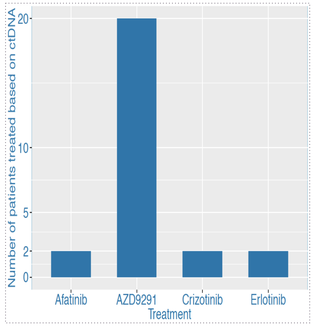

Figure 4. Treatment prescribed according ctDNA profile (n=26)

CONCLUSIONS

In the absence of an invasive biopsy, *ct*DNA can be used as a 'liquid biopsy' for molecular profiling of mutations in NSCLC patients. Liquid biopsy using enhanced TAm-Seq analysis identified cancer mutations in 72% of the study population. Moreover, 22% of the study population subsequently received treatment tailored to the plasma *ct*DNA detected mutations.

Bibliography: (1) Kris – JAMA 2014 * (2) Planchard – WCLC 2013