

## BACKGROUND

- Circulating tumor DNA (ctDNA) can be used as a non-invasive liquid biopsy for the detection and quantification of molecular abnormalities.
- Sensitivity and specificity of ctDNA varies across the different methods and its evolution during chemotherapy is unknown.

## OBJECTIVES

A prospective study was performed:

- To assess the molecular alterations in the ctDNA of advanced NSCLC patients in whom the initial molecular profile was unknown due to insufficient cellularity in the biopsy.
- To assess correlation of dynamic ctDNA with radiological response by RECIST 1.1 criteria after 2 cycles of chemotherapy.
- To assess the concordance of molecular alteration in ctDNA with those in the tissue (if available) in this lung cancer population.
- To assess ctDNA as an early surrogate dynamic marker of chemotherapy efficacy.

## METHODS

- 10 ml of 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 validated enhanced TAM-Seq<sup>TM</sup> (eTAM-Seq) assay covering regions from 34 cancer-related genes (Figure 1).

## METHODS cont.

- Blood tests were performed day 1 before chemotherapy initiation, and then on day 2, 21 and 42 at the time of first radiological evaluation.
- Base-line tumor biopsy samples: tumor DNA extracted using DNeasy FFPE kit. Targeted gene panel sequencing was performed using Ion Torrent approach with 5 to 74 gene panel amplified by multiplex PCR according to the Ion AmpliSeq<sup>TM</sup> Custom Panel protocol. Data were analyzed with the Torrent Suite Variant Caller software.

AKT1	CTNNB1	FOXL2	IDH1	MYC	PTEN
ALK	EGFR	GATA3	IDH2	NFE2L2	RET
BRAF	ERBB2	GNA11	KIT	NRAS	STK11
CCND1	ESR1	GNAQ	KRAS	PDGFRA	TP53
CDKN2A	FGFR2	GNAS	MED12	PIK3CA	
U2AF1	FGFR3	HRAS	MET	PPP2R1A	

Figure 1. Inivata eTAM-Seq Panel with hotspot region (dark blue) and exon tiling (light blue, 88-100%) gene coverage.

## Clinical Characteristics

- 30 advanced NSCLC patients, all adenocarcinoma subtype and treated with platinum-based chemotherapy or concomitant chemotherapy-radiotherapy were recruited. Main patients' clinical characteristics are reported in Table 1.
- Molecular profile on tissue was performed in 27 patients. Among 4 of the 27 (15%) there was insufficient cellularity in biopsy. In 9 out of 23 (39%), a molecular alteration was reported.

	N=30 (%)
<b>Median age-years (range)</b>	<b>59.5 (37-80)</b>
<b>Gender</b>	
-Male	17 (57)
-Female	13 (43)
<b>Stage:</b>	
IV	19 (63)
IIIB	2 (7)
IIIA	9 (30)
<b>Smoking habit:</b>	
Never	7 (23)
Current	17 (57)
Former	6 (20)

Table 1. Patients' characteristics

## ctDNA Molecular Profiling

- Liquid biopsy detected at least one molecular alteration in 70% of patients (21/30).
- Liquid biopsy detected genomic alterations reported in tissue in 8 out of 9 patients (89% concordance).
- eTAM-Seq analysis identified alterations in 10 genes (*KRAS*, *EGFR*, *ERBB2*, *MET*, *BRAF*, *TP53*, *PTEN*, *STK11*, *PIK3CA*, *U2AF1*)

## Early ctDNA changes

- Early evolution (D2) of ctDNA by responder cohorts (RECIST 1.1) was analyzed (Figure 2).

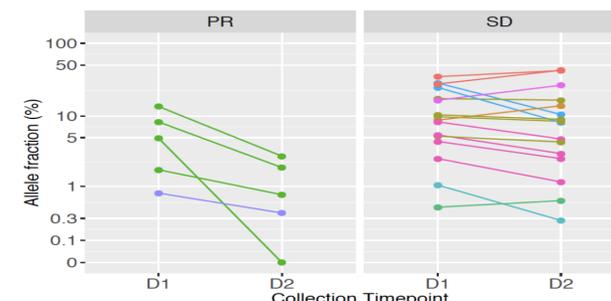


Figure 2. Change in ctDNA allele fraction between D1 and D2 (colors represent individual patients).

## CONCLUSIONS

This prospective study in advanced NSCLC patients demonstrates the dynamic nature of ctDNA and its significant correlation with radiological response. Moreover, these preliminary data suggest a high correlation between tissue and liquid biopsies (89%). While further clinical validation is ongoing, these data support the use of liquid biopsy for patient stratification as well as patient monitoring.

## RESULTS

## Patient ctDNA monitoring and RECIST

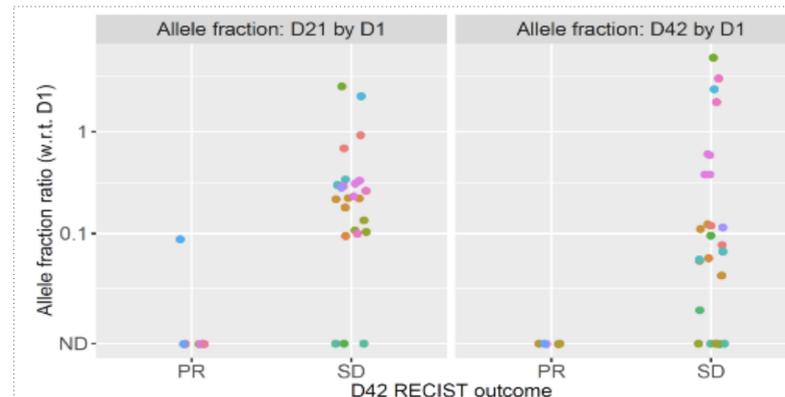


Figure 3. Distribution of the ctDNA mutant allele fraction (at D21 and D42) grouped by response (D42, partial response (PR) and stable disease (SD) by RECIST v1.1. Colors represent individual NSCLC patients.

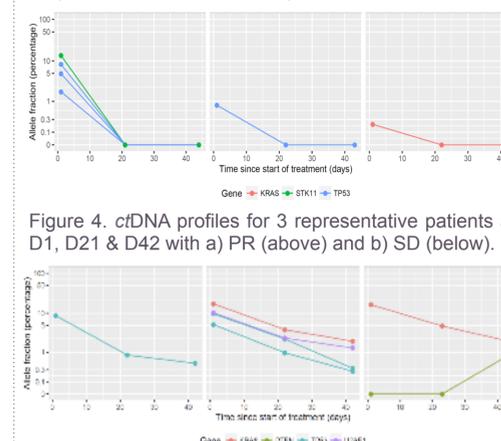


Figure 4. ctDNA profiles for 3 representative patients at D1, D21 & D42 with a) PR (above) and b) SD (below).

Of 17 patients with repeated liquid biopsy testing (D1, D21, D42) and at least one molecular alteration, the dynamic ctDNA profiles at D21 and D42 correlate with radiological response by RECIST 1.1 at D42 ( $p < 0.01$ )