AN AMPLICON-BASED LIQUID BIOPSY FOR DETECTING ALK AND ROS1 FUSIONS AND RESISTANCE MUTATIONS IN ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC) PATIENTS

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BACKGROUND

- Circulating tumor DNA (ctDNA), is a surrogate material for somatic mutation (mut.) detection, such as *EGFR* in NSCLC patients
- However, the applicability for the detection of *ALK* and *ROS1* fusions and resistance mutations is poorly described

OBJECTIVE

- To evaluate an amplicon-based *ctDNA* assay for detecting *ALK*/*ROS1* fusions and mut. in a cohort of *ALK*/*ROS1*+ NSCLC patients

PATIENTS AND METHODS

- *ALK* and *ROS1* rearranged NSCLC patients were prospectively enrolled from Dec. 2016 to Dec. 2017 in our institution
- The analysis of *ALK* & *ROS1* mutations, *EML4-ALK* (variant 1,2,3) and *ROS1* fusions (with partner genes *CD74*, *SLC34A2*, *SDC4* and *EZR*) in ctDNA were performed using InVisionFirst Lung™

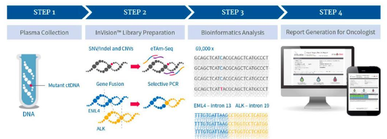


Figure 1. The InVisionFirst Lung™ assay identifies ALK and ROS1 gene fusions using an amplicon-based technology to selectively amplify genomic breakpoints, the sequence of the junctions are then identified using NGS, allowing the genomic breakpoint in ctDNA to be mapped. In a recent analytical validation study (Plagnol V. *et al*, PLoS ONE 2008), this methodology detected EML4-ALK and SLC34A2-ROS1 breakpoints in ctDNA reference material at a VAF of 0.0625%, with 100% specificity

RESULTS

- **51 patients were included**. Patient characteristics are included in ***Table 1***
- *ALK & ROS1* status was confirmed by *ALK* immunohistochemistry (IHC) (n=26) and FISH (n=40)
- The median prior tyrosine kinase inhibitors (TKI) received was 2 (0-4)
- **Blood samples (n=102) were collected at different time points:**

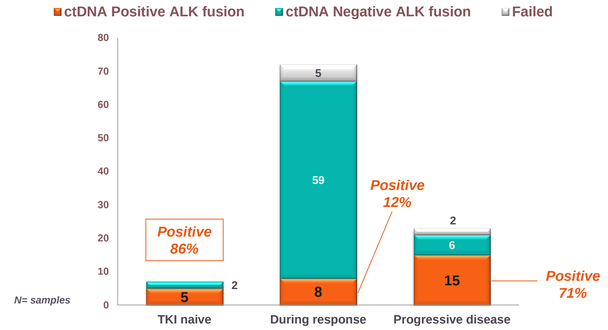
- *treatment-naive (n=7)*
- *on treatment (n=95)*
- *at progressive disease (PD) (n=23), particularly at PD to tyrosine kinase inhibitors (n=17)*

	Characteristics	Overall population (n=51, %)
Age	▪ Median (range)	52 (22-73)
Sex	▪ Male ▪ Female	24 (47%) 27 (53%)
Stage at diagnosis	▪ I-II ▪ IIIA-IIIB ▪ IV	2 (4%) 7 (14%) 56 (82%)
Histology	▪ Adenocarcinoma ▪ Large cells carcinoma	50 (98%) 1 (2%)
Smoking	▪ Non smoker ▪ Former smoker ▪ Current smoker	27 (53%) 19 (37%) 4 (8%)
Molecular diagnosis	▪ IHC ▪ FISH	26 (51%) 40 (78%)
Type of fusion	▪ ALK fusion ▪ ROS1 fusion	43 (84%) 8 (16%)
Therapy at collection	▪ Treatment-naïve ▪ Crizotinib ▪ Next-gen TKI ▪ Others	7 (14%) 9 (18%) 29 (57%) 6 (12%)

Table 1: *Baseline characteristics*

ALK & ROS1 fusion

ctDNA fusion detection at different time points



- **In treatment-naïve patients, *ALK* & *ROS1* ctDNA fusions were detected in 86% of patients** *(5/6 ALK fusion; 1/1 ROS fusion)*
- ***Sensitivity for ALK was 83% and 100% for ROS1,*** *in treatment naïve patients*

EML4-ALK variant

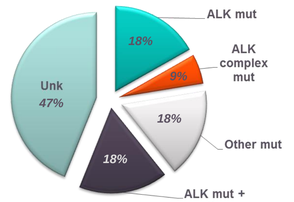
Fusion	Variant	Frequency observed N (%)
EML4-ALK	1	1 (14%)
EML4-ALK	2	-
EML4-ALK	3	5 (83%)

ROS1 gen partner

Fusion	Gen Partner	Frequency observed N (%)
ROS1	*CD74*	1 (100%)

ALK resistance mutations

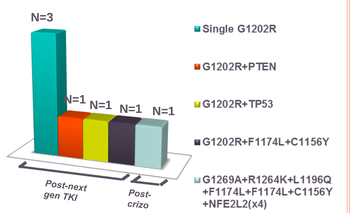
Resistance mechanisms (%)



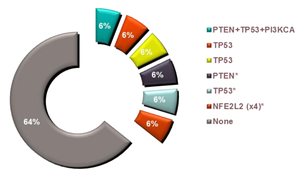
N=17 samples at PD to TKI

- **41% presented *ALK* mut.** at PD to TKI (7/17)
- ***ALK* G1202R** was the most common mutation (71%)
  - **A single *ALK* mut.** *(n=3)*
  - ***ALK* complex mut.** *(n=1)*
  - ***ALK* + other mut.** *(n=3)*

***ALK* resistance mutations**



Other concurrent mutations

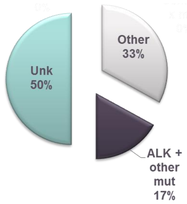


N=1 sample (6%) **concurrent with ALK mutation*

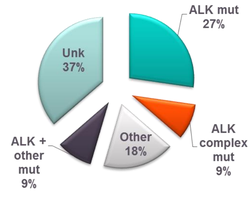
- **35% of all patients presented other concurrent mutations** at PD (6/17)
- **TP53 and PTEN were the most common** mutations (4/6 and 2/6 respectively)

Crizotinib vs. Next-generation TKI

PD to crizotinib, n=6



PD to next-gen TKI, n=11



- **Higher incidence of *ALK* mut. at PD to next-gen TKI vs. crizotinib (45% vs. 17%)**

CONCLUSION

- **Routine liquid biopsy detects *ALK/ROS1* fusions in untreated NSCLC pts and reflects treatment sensitivity in TKI exposed pts.**
- **It is also an accurate tool to assess TKI resistance, detecting ALK and other potential resistance mutations, that reflect the tumor heterogeneity.**

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