CLINICAL RELEVANCE OF *ALK/ROS1* RESISTANCE MUTATIONS AND OTHER ACQUIRED MUTATIONS DETECTED BY LIQUID BIOPSY IN ADVANCED NSCLC PATIENTS

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INTRODUCTION

- Liquid biopsies for circulating tumor *DNA* (*ctDNA*) can be a tool for somatic mutation detection, such as *EGFR*, *BRAF* or *KRAS* mutations
- In *ALK* and *ROS1* populations, *ctDNA* can be used for detecting and monitoring *ALK/ROS1* fusions or resistance mutations, as well as other acquired mutations
- However, the applicability and clinical relevance of *ALK/ROS1* alterations and other acquired mutations detected by liquid biopsy, is poorly described in TKI-treated patients

OBJECTIVE

- We evaluated the feasibility and clinical relevance of *ALK/ROS1* resistance mutations and other acquired mutations detected by *ctDNA* in a large cohort of *ALK/ROS1*+ NSCLC patients

METHODS

- Advanced *ALK/ROS1*+ NSCLC patients were prospectively enrolled from October 2015 to April 2018 across 9 French institutions
- ctDNA molecular analysis was performed using amplicon-based NGS (InVision® liquid biopsy platform, InVisionFirst™-Lung) for *ALK* (*EML4* variants v1, v2, v3), *ROS1* (*CD74, SLC34A2, SDC4 and EZR)* fusions, and other somatic mutations.
- 20ml blood were collected in Streck BCT or EDTA tubes and processed for DNA extraction

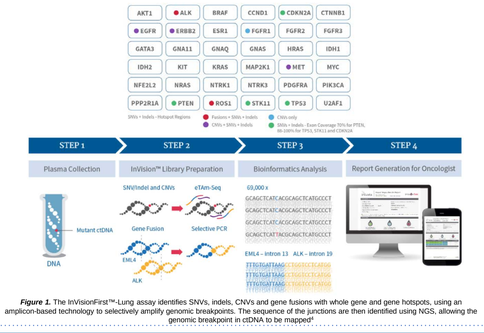


Figure 1. The InVisionFirst™-Lung assay identifies SNVs, indels, CNVs and gene fusions with whole gene and gene hotspots, using an amplicon-based technology to selectively amplify genomic breakpoints. The sequence of the junctions are then identified using NGS, allowing the genomic breakpoint in ctDNA to be mapped[2]

RESULTS

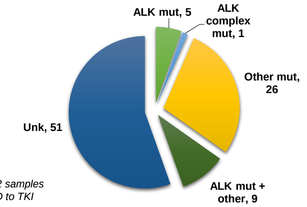
- 128 patients were included: 101 *ALK*, 27 *ROS1*. Blood samples (n=353) were collected at different timepoints: tyrosine kinase inhibitors (TKI)-naive (n=27), during treatment (n=206) or at progression (PD)

Characteristics	*ALK* N=101 (%)	*ROS1* N=27 (%)
Age (mean (SD))	51.9 (14.1)	54.4 (13.7)
Sex		
Male	42 (41.6)	14 (51.9)
Female	59 (58.4)	13 (48.1)
Smoking status		
Never	57 (57.6)	18 (69.2)
Smoker	42 (42.4)	8 (30.8)
Histology		
Adenocarcinoma	97 (96.0)	25 (96.2)
Stage at diagnosis		
I-IIIA	7	1
IIIB-IV	74	21
Brain metastasis at baseline	30 (29.7)	5 (19.2)
Molecular diagnosis		
FISH (+)	63 (62.4)	19 (70.4)
IHC (+)	80 (76.2)	15 (55.6)
N# Tyrosine kinase inhibitors (TKI)		
1	27 (26.7)	15 (55.6)
2	42 (41.6)	5 (18.5)
≥3	27 (26.8)	2 (7.4)
N# Samples at PD to TKI		
Post-crizotinib	43	8
Post-alectinib	6	-
Post-ceritinib	20	-
Post-brigatinib	14	-
Post lorlatinib	12	2

Table 1. Baseline characteristics

ALK RESISTANCE MECHANISMS

- **95 samples** were collected at PD to TKI; 92 were available for analysis



N=92 samples at PD to TKI

Figure 2. Distribution of *ctDNA* molecular alterations in samples at PD: 18% (17/92) demonstrate acquired *ALK* resistance (single *ALK* mutation or *ALK* complex mutation -if ≥ 2 ALK mutations-); in 57% no *ctDNA* mutations were detected

ctDNA RESISTANCE MUTATIONS BY TKI GENERATION

- 42 samples were collected post-crizotinib, 25 post 2nd gen-TKI and 25 post next gen-TKI
- Higher incidence of *ALK* resistance mutation following treatment with 2nd and next-gen TKI vs. crizotinib: 7% vs. 12% (p=0.5) vs 36% (*P*=0.00641)

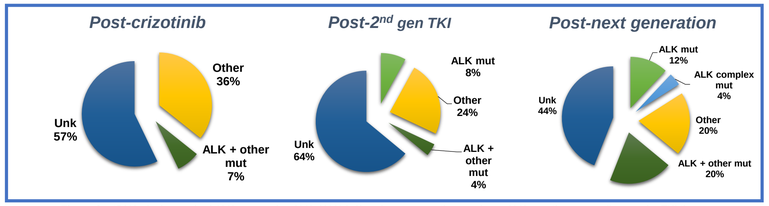
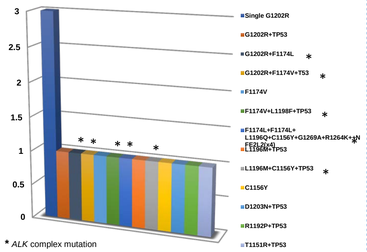


Figure 3: *ctDNA single acquired ALK vs complex ALK (ALK (>1) resistance mutations and non-ALK mutations according to prior TKI therapy*

ALK POSITIVE

ALK resistance mutations

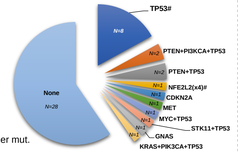


* *ALK* complex mutation

Figure 4. Distribution of *ALK* resistance and concurrent mutations in samples at PD to TKI

Other concurrent mutations

- **48% of all patients** presented other concurrent mutations at PD (26/54)
- **TP53** was the most common (22/54)



concurrent ALK mut. + other mut.

Figure 5. Other concurrent mut. in *ctDNA* at PD to TKI

ROS1 POSITIVE

ROS1 resistance mutation

N= 10 patients with samples at PD to TKI

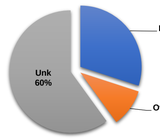
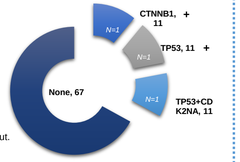


Figure 6. Distribution of *ROS1* resistance and concurrent mutations in samples at PD to TKI

- *4 ROS1 G2032R* mutations were detected (30%): 2 post-crizotinib from the same patient, and 2 post-lorlatinib

Other concurrent mutations

- **33% of all patients** presented other concurrent mutations at PD (26/54)



+ concurrent with ROS1 mut.

Figure 7. Other concurrent mutations in ctDNA at PD to TKI

ctDNA ALK RESISTANCE MUTATIONS AND OUTCOMES

- Median follow-up from 1st liquid biopsy in ALK cohort was 10.0 months [95%CI 1-31] and 8.1 months [95% CI 1-22.5] in the ROS1 cohort. Median overall survival (OS) has not been met in either cohort.

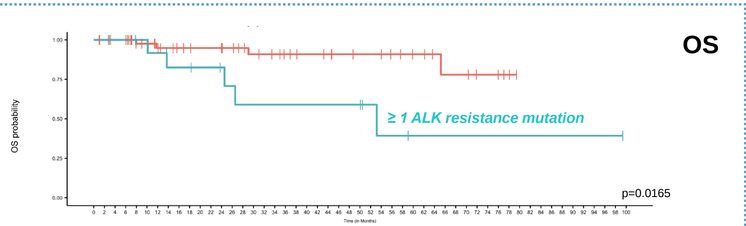


Figure 8. OS according the presence of ALK resistance mutations vs in *ctDNA* in *ALK* positive population

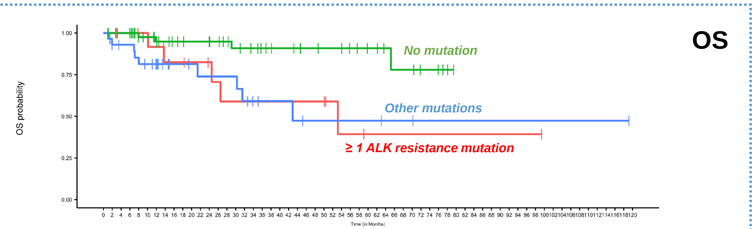


Figure 9. OS according the presence of mutations in *ctDNA* in *ALK* positive population

CONCLUSIONS

- Routine liquid biopsies can assess the heterogeneity of the TKI resistance, detecting *ALK* resistance and other acquired mutations in pretreated advanced *ALK & ROS1* NSCLC patients
- Feasibility of using liquid biopsy with Invision platform in routine clinical practice was demonstrated in this study, across a breadth of *ALK* and *non-ALK* resistance mutations
- In our cohort, next-generation TKIs lead to increase *ALK* resistance mutations than crizotinib
- Patients who develop TKI *ALK* resistance mutations show trend to having poorer outcomes, suggesting that routine *ctDNA* monitoring could benefit patients in supporting treatment decisions,
- This study is currently ongoing to further evaluate *ctDNA* dynamics for correlation to clinical response, patient outcomes and clinical characteristics

Additional data of this study is presented by *A Swalduz*, L Mezquita et al (MA16.09) mini oral communication: 25/09/2018, 14:30h, Room 203 BD**

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