FEASIBILITY OF AN AMPLICON-BASED LIQUID BIOPSY FOR *ALK* AND *ROS1* FUSIONS IN ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC) PATIENTS

#4581

L Mezquita[1], Y Hu[2], K Howarth[4], C Jovelet[1], D Planchard[1], L Lacroix[1], A Swalduz[3], S Ortiz-Cuaran[3], V Avrillon[3], V Plagnol[4], K Baker-Neblett[4], G Jones[4], N. Rosenfeld[4], C Morris[4], E Green[4], ES Kim[5], M Perol[3], P Saintigny[3], SV Liu[6], GR Oxnard[2], B Besse[1,7]

[1]Gustave Roussy Cancer Campus, Villejuif, France; [2]Dana Farber Cancer Institute, Boston, MA; [3]Centre Leon Berard, Lyon, France; [4]Inivata Limited, The Portway, Granta Park, Cambridge, United Kingdom; [5]Levine Cancer Institute, Charlotte, NC; [6]Georgetown University Medical Center Washington, DC; [7]University Paris-Sud, Orsay, France

INTRODUCTION

- Circulating tumor DNA (ctDNA) is a surrogate material for somatic mutation detection, such as *EGFR*, *BRAF* or *KRAS* mutations in NSCLC patients
- However the applicability of this technique for the detection of *ALK* and *ROS1* fusions is poorly described
- The aim of this combined analysis was to evaluate an amplicon-based ctDNA technology in a cohort of *ALK* and *ROS1* positive NSCLC patients

METHODS

- *ALK* and *ROS1* positive NSCLC patients were either prospectively enrolled or retrospective specimens selected, to be included in this combined analysis across 6 international centers
- Blood collections were taken at different time points between Jan. 2015 to Aug. 2017. ALK and ROS1 positive status was determined by standard of care practice at each institution (FISH/IHC or NGS)
- The analysis of EML4-ALK fusions (variant 1,2,3) and ROS1 fusions (with partner genes CD74, SLC34A2, SDC4 and EZR) was performed using the InVision® platform (***Figure 1***)

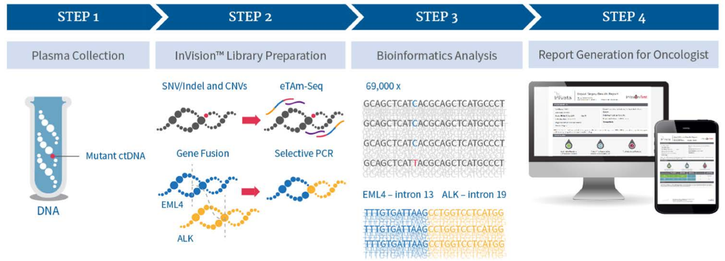


Figure 1. The InVisionFirst™ assay identifies ALK and ROS1 gene fusions using an amplicon-based technology to selectively amplify genomic breakpoints, the sequence of the junctions are then identified using NGS, allowing the genomic breakpoint in ctDNA to be mapped. In a recent analytical validation study[1], the novel methodology detected EML4-ALK and SLC34A2-ROS1 breakpoints in ctDNA reference material at a VAF of 0.0625%, with 100% specificity.

RESULTS

- 66 patients were included; patient characteristics are included in ***Table 1***.
- Samples (n=99) were collected at different time points, including at diagnosis (naïve), on treatment, and at progression (PD) as described in ***Table 2***.

Table 1. *Patient Characteristics*

Characteristics	Overall population (n=66, %)
Age ▪ Median (range)	57 (22-93)
Sex ▪ Male ▪ Female	29 (44%) 37 (56%)
Stage at diagnosis ▪ I-II / III ▪ IV	2 (3%) / 7 (11%) 56 (85%)
Histology ▪ Adenocarcinoma ▪ Squamous ▪ Other	63 (95%) 1 (2%) 1 (2%)
Smoking ▪ Non smoker ▪ Former smoker ▪ Current smoker	40 (61%) 18 (27%) 7 (11%)
Molecular diagnosis ▪ Immunohistochemistry ▪ FISH ▪ Other (NGS)	33 (50%) 45 (68%) 2 (3%)
Type of rearrangement ▪ *ALK* fusion ▪ *ROS1* fusion	59 (89%) 7 (11%)
Therapy at collection ▪ Treatment naïve ▪ 1st generation TKI ▪ 2nd generation TKI ▪ Next generation TKI ▪ Others	20 (30%) 11 (17%) 11 (17%) 18 (27%) 6 (9%)

Table 2. *Sample collection timepoints*

	ctDNA Positive *ALK* fusion N (%)
At diagnosis or treatment-*naïve* (n=21)	15 (71%)
During ongoing *response* (n=57)	7 (12%)
At *PD* (n=15)	8 (53%)

	ctDNA Positive *ROS1* fusion (N, %)
At diagnosis or treatment-*naïve* (n=6)	6 (100%)
During ongoing *response* (n=0)	0 (0%)
At *PD* (n=1)	1 (100%)

- 31 patients tested positive for fusion in liquid biopsy (25 *ALK* fusions and 6 *ROS1* fusions), described in ***Table 3***.
- **Sensitivity for ALK was 71% and 100% for ROS1** in treatment-naïve patients
- In contrast, ALK/ROS1 fusions were detected in minority of samples (7/57) from patients who were undergoing treatment with clinical response

Table 3. Frequency of **A**. ALK variants 1, 2, 3 and **B**. ROS1 variants in chromosome 4 and 5 observed in 31 patients

Fusion	Variant	Frequency observed
EML4-ALK	1	28% (7/25)
EML4-ALK	2	8% (2/25)
EML4-ALK	3	64% (16/25)

Fusion	Frequency observed
CD74-ROS1	67% (4/6)
SLC34A2-ROS1	33% (2/6)

- The ***DNA breakpoints*** observed in *ALK* and *ROS1* in this study, are illustrated ***in figures 2 and 3***. The majority of *ALK* and *ROS1* breaks reported occur in defined exons/introns, however, there is high diversity in the precise breakpoint positions
- Breaks were observed scattered throughout ***intron 19 of ALK, and introns 31-33 of ROS1***, consistent with those reported previously[2]
- ***No two breaks occurred in the exact same position***, although the minimum distance observed between two independent breaks in this investigation was only 2 bp
- The structure of the breakpoint junctions indicated micro-homology of length 2bp or greater in 6 out of 31 fusion-positive patients, which represents 3.1 odds ratio increase over a random model *(p = 0.011)*

Figure 2. Clustering of breakpoints in EML4 and ALK. **A**. Chromosome inversion on chromosome 2 and translocation resulting in the oncogenic fusion *EML4-ALK*. **B.** Distribution of breakpoints in *ALK* and its major partner gene *EML4*.

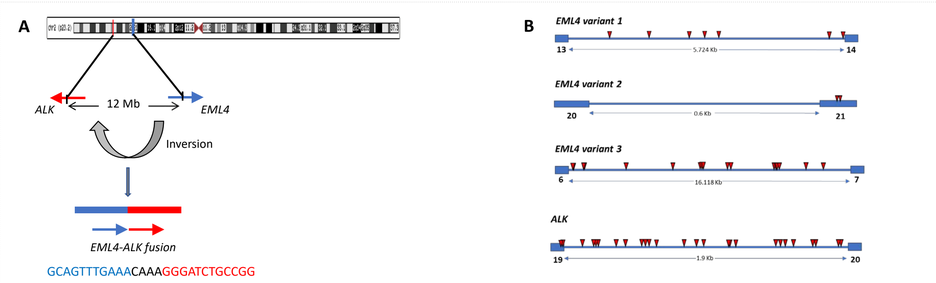
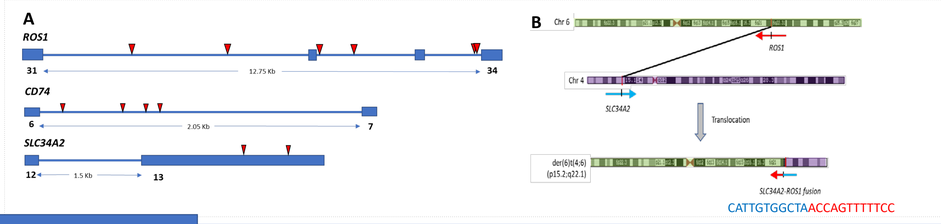


Figure 3. Clustering of breakpoints in SLC34A2, CD74 and ROS1; A. Chromosome translocation between chromosomes 4 and 6 resulting in the oncogenic fusion *SLC34A2-ROS1*; **B.** Distribution of breakpoints in *ROS1* and its major partner genes *CD74 and SLC34A2*.



CONCLUSION

- The detection of *ALK and ROS1* fusion in plasma is feasible in routine clinical practice, with good sensitivity for clinically actionable *ALK* and *ROS1* structural rearrangements in untreated advanced NSCLC patients

References: [1]Plagnol V , Woodhouse S, Howarth K, Lensing S, Smith M, Epstein M, et al. PLoS ONE (2018) 13(3): e0193802 doi.org/10.1371/journal. pone.0193802 [2] Seki *et al, Biomolecules* (2015) *5*(4), 2464-2476; doi:10.3390/biom5042464