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Prospective multi-center validation of the detection of *ALK* rearrangements of circulating tumor cells for non-invasive longitudinal management of patients with advanced non-small cell lung cancer

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ABSTRACT

INTRODUCTION: Patients with advanced-stage non-small cell lung cancer (NSCLC) whose tumors harbor an anaplastic lymphoma kinase (*ALK*) gene rearrangement benefit from treatment with multiple *ALK* inhibitors (*ALKi*). Approximately 30% of tumor biopsies contain insufficient tissue for successful *ALK* molecular characterization. This study evaluated the added value of analyzing circulating tumor cells (CTCs) as a surrogate to *ALK* tissue analysis and as a function of the response to *ALKi*.

PATIENTS AND METHODS: We conducted a multi-center prospective observational study (NCT02372448) of 203 patients with stage IIIB/IV NSCLC across 9 French centers, 81 were *ALK*⁺ (IHC or FISH) and 122 *ALK*⁻ on paraffin embedded tissue specimens. Blood samples were collected at baseline and at 6 and 12 weeks after *ALKi* initiation or at disease progression. *ALK* gene rearrangement was evaluated with CTCs using immunocytochemistry (ICC) and FISH analysis after enrichment using a filtration method.

RESULTS: At baseline, there was a high concordance between the detection of an *ALK* rearrangement in tumor tissue and in CTCs as determined by ICC (sensitivity, 94.4%; specificity 89.4%). The performance was lower for the FISH analysis (sensitivity, 35.6%; specificity, 56.9%). No significant association between baseline levels or the dynamic change of CTCs and survival was observed in *ALK*-positive patients.

CONCLUSIONS: CTCs can be used as a complementary tool to a tissue biopsy for the detection of *ALK* rearrangements. Longitudinal analysis of CTCs showed promise for real-time patient monitoring and improved delivery of molecularly guided therapy in this population.

Keywords: *ALK*, CTCs, immunocytochemistry, FISH, lung adenocarcinoma.

INTRODUCTION

Non-small cell lung cancer (NSCLC) harboring *ALK* receptor tyrosine kinase (*ALK*)–rearrangements is a distinct subset of lung cancers, occurring in approximately 4% of patients at an advanced stage.¹ Several *ALK* inhibitors (*ALKi*) have been approved by the U.S. Food and Drug Administration and the European Medicines Agency for the treatment of patients with *ALK*-positive NSCLC at first or latter lines of treatment.^{2, 3}

The eligibility of NSCLC patients to receive *ALKi* relies on a companion immunohistochemistry (IHC) performed on tumor tissue⁴, e.g. IHC or FISH that need a sufficient number of tumor cells to be conclusive. However, procedures providing small biopsy samples (e.g. fine needle aspiration, transthoracic needle biopsy, endobronchial ultrasound, bronchoscopy, bronchial biopsies) may lack a sufficient number of tumor cells. Furthermore, invasive procedures can be related to a relative risk of morbidity. Moreover, because tumors evolve and adapt to targeted therapies, it is becoming increasingly important to monitor the molecular changes occurring in NSCLC patients in “real-time” especially when selecting subsequent lines of treatment.⁵ In addition, the degree of benefit from *ALKi* varies widely between NSCLC patients, despite sharing molecular drivers. Longitudinal biopsies upon disease progression have been useful in identifying the molecular mechanisms driving resistance to *ALKi*. Yet, repeated tissue biopsies are not always feasible and sampling of a single tumor site may not reflect the spatial heterogeneity of resistance mechanisms.⁶

In daily practice, accurate identification of *ALK* fusions as well as tumor evolution during therapy could have significant impact on the therapeutic strategy likely to have the greatest impact on patient outcome.⁶ Therefore, developing non-invasive surrogate methods suitable to detect *ALK* rearrangements at baseline and during tumor evolution can be pivotal to improving the success rate of *ALKi*.

It is now well admitted that liquid biopsy (LB) is a simple, non-invasive and easily repeatable alternative to tumor biopsies to assess the tumor’s molecular status and to monitor genetic changes in “real-time”.⁷ Among the LB components, circulating tumor cells (CTCs) contain tumorigenic cell clones with high relevance for metastatic dissemination.⁸ CTCs are likely released from distinct metastatic sites and may inform on the genomic heterogenic picture of the metastatic disease. In contrast to tumor biopsies, CTCs could allow longitudinal monitoring at different time-points

during treatment, thereby having the potential to guide therapeutic decisions in “real-time”.⁹

We and other groups have reported the feasibility of *ALK*-rearrangement detection with CTCs collected from patients with *ALK*-positive NSCLC at baseline by using a filter-based technique for CTC enrichment and *ALK* testing by FISH and/or immunocytochemistry (ICC).¹⁰⁻¹² To confirm these previous retrospective feasibility studies, we performed the first multi-center prospective clinical validation of the use of CTCs as a surrogate for tumor tissue for *ALK* testing, thus aiding in prognostication and therapy selection at baseline and during treatment with ALKi of patients with stage IIIB/IV NSCLC.

MATERIALS AND METHODS

Patients and samples

This multi-center prospective observational study (NCT02372448) was conducted across 9 French clinical institutions from January 23, 2015 through November 21, 2019 in accordance with the Declaration of Helsinki and the European Directive and Good Clinical Practices. The study was approved by the Ethics Committee (CPP Sud-Méditerranée V) and authorized by the National Security Agency for Medicines and Health Products (ANSM). Informed-written consent was obtained from all patients. Eligible patients had histologically confirmed stage IIIB/IV NSCLC (**Table 1**). A few number of patients had initially diagnosed stage I-IIIa NSCLC, enrolled in the study at the time of the metastatic relapse (**Table 1**). For these patients, a re-biopsy was performed to evaluate the *ALK* status. These patients may have had prior chemotherapy delivered as part of their primary treatment but must have completed primary therapy at least 3 months before their entry in the study.

The pre-specified primary outcome was sensitivity and specificity of FISH between tumor tissue and CTCs at baseline as well as a follow-up « visit 1 » after six weeks (V1) and follow-up « visit 2 » after 12 weeks (V2). Pre-specified secondary outcomes included sensitivity and specificity of ICC at Baseline, V1 and V2 as well as association of *ALK* positive CTCs and clinical outcome.

ALK rearrangement was locally tested on tumor tissue by IHC (anti-*ALK* antibody, pre-diluted, clone D5F3, Roche Diagnostics, Basel, Switzerland) and FISH (Vysis *ALK* Break Apart Rearrangement Probe Kit; Abbott Molecular Inc., Des Plaines, USA),

performed according to pre-specified standard operating procedures in ISO 15189 accredited laboratories (Accreditation n° 8-3034; <https://tools.cofrac.fr/annexes/sect8/8-3034.pdf>).

Thirty ml of peripheral blood was collected in EDTA or Streck tubes at baseline (V0) and at 6 (V1) and 12 (V2) weeks on ALKi initiation and/or at disease progression.¹³ CTCs were enumerated on ISET® filters (Isolation by Size of Tumor Cells, Rarecells, Paris, France), as previously reported.^{10, 14} Briefly, stained filters were examined by a senior thoracic cytopathologist using light microscopy in two steps: (i) screening at × 20 magnification to perform cells localization and detection, then, (ii) observation at × 100 magnification using an oil immersion for detailed cytomorphological analysis. CTCs were defined as cells presenting all the following criteria: (i) nuclear size equal or larger than one pore (i.e., equal or larger than 8 µm in diameter); (ii) irregularity of the nuclear contour; (iii) presence of a well defined and visible cytoplasm; and, (iv) high nuclear-to-cytoplasmic ratio (>0.8).

Consequently, CT scans and/or MRIs were performed at baseline and during follow-up. The response to ALKi (**Supplementary Table 1**) was evaluated according to the “Response Evaluation Criteria in Solid Tumors” (RECIST) version 1.1. The disease control rate was defined as a complete or partial response or stable disease based on RECIST.

ALK immunocytochemistry and FISH on ISET-enriched CTCs

All ISET filters were sent to the coordinating team at the CHU Nice for centralized ALK analysis. ICC and FISH were performed on CTCs isolated using the ISET method on unstained spots of the corresponding filters containing CTCs detected on four spots colored with May-Grünwald-Giemsa [15]. Three spots were used for ICC and three spots were used for FISH per filter. For ICC, the spots were incubated with a primary antibody against the ALK protein (pre-diluted, clone D5F3; Ventana) for 32 min at room temperature. FISH analysis was carried out using a break-apart probe for the ALK gene (Vysis ALK Break Apart Rearrangement Probe Kit, Abbott Molecular Inc.) in accordance with the manufacturer’s instructions. Cells showing at least one split signal or single 3’ signals were considered positive for an ALK rearrangement. ISET filters were examined independently and blinded to clinical, IHC, ICC, FISH data and tissue genotype. We used the human NSCLC cell line

H2228 obtained from ATCC (Manassas, VA, USA) as an *ALK* rearrangement positive control in every run.¹⁰

Statistical analysis

The performance of *ALK* assessment on ICC or FISH was compared to the paired tumor biopsy and equivalent method using sensitivity and specificity analysis. The same analysis was carried out first on the population with available CTCs (n=185 patients and n=97 analyzed for ICC and FISH respectively) and second on the entire population including patients with no available CTCs (n=203 patients analyzed). In this latter case, *ALK* assessment was not available by using ICC or FISH methods, we used the worst scenario by considering all *ALK* positive cases on the biopsy as false negative with ICC and FISH and all *ALK* negative on the biopsy as false positive with ICC and FISH.

The association to clinical variables was assessed using χ^2 , Student-*t* test and Fisher's exact test analyses.

Progression-free survival (PFS) was defined as the time between the date of the start of ALKi and the date of clinical or radiological progression according to RECIST, or death. Overall survival (OS) was defined as the time between the date of the start of ALKi and the date of death. Patients who were progression-free and alive at the time of analysis were censored.

All statistical analyses were performed using R (version 3.5.0, R Foundation for Statistical Computing) or Python (version 3.7.2, Python Software Foundation).

RESULTS

Patient characteristics

Peripheral blood was collected from 193 patients with stage IIIB/IV NSCLC and 10 patients with initially diagnosed stage I-IIIa NSCLC enrolled in the study at the time of the metastatic relapse (**Table 1**). In total, 81 (40%) patients with *ALK*-positive NSCLC and 122 (60%) patients with *ALK*-negative NSCLC were included in the study cohort. Most *ALK*-positive patients were never or former smokers (97%) with adenocarcinoma tumor histology (99%).

At baseline (V0), CTCs were detected in 187 (92%) patients (range, 2–621/4 ml; median, 62 CTCs/4 ml; **Fig. 1A**). A significantly higher number of CTCs were detected in *ALK*-negative patients than in *ALK*-positive patients (median CTCs = 71

vs. 21; $P = 0.0098$) (**Fig. 1A**). CTCs were not detected in 16/203 (8%) patients, 10/203 (5%) *ALK*-positive patients and 6/203 (3%) *ALK*-negative patients.

At first visit (V1) during monitoring of *ALK*-positive patients, 27/81 (33%) patients had CTCs (range, 6–177/ 4 ml; median, 6 CTCs/ 4 ml; **Fig. 1B**). At the second follow-up visit (V2), we detected CTCs (range, 11–173/ 4 ml; median, 19 CTCs/ 4 ml) in 17/81 (21%) *ALK*-positive patients (**Fig. 1B**).

Performance of CTCs for detection of *ALK* rearrangements

At baseline, there was substantial concordance between the detection of an *ALK* rearrangement in the tumor tissue (IHC and FISH) and CTCs (**Fig. 2**). The sensitivity and the specificity for ICC were 94.36% and 89.39%, across all samples, and 100%, respectively, for patients with detectable CTCs at baseline. Lower concordance was observed for FISH with 35.63% sensitivity and 56.89% specificity, across all samples, and 100%, respectively, for patients with detectable CTCs at baseline.

Moreover, the levels of CTCs or *ALK*-positive CTCs significantly decreased with ALKi treatment (**Fig. 1B**; **Supplemental Fig. S1**).

In all positive samples by FISH and IHC, 100% of detected CTCs were positive in ICC with an intensity score of 3. All but one FISH positive sample were 100% positive for rearranged *ALK* with one sample at baseline having only 80% FISH positive cells).

Association between *ALK*-CTCs and clinical parameters or outcome

An exploratory analysis revealed a significant association between the status of *ALK*-CTCs, younger age and non-smoking status. *ALK*-positive CTCs were present more often in younger patients (mean, 60 vs. 64 years, $P=0.038$) and non-smokers ($P<0.0001$). All the other clinical parameters were not significantly associated with *ALK*-rearranged CTC counts at baseline. In addition, in *ALK*-positive patients, the levels of CTCs significantly correlated to age and stage (**Table 2**).

At the first follow-up visit, 25 (31%) patients had disease progression according to RECIST.

For the univariate analysis, the level of CTCs at baseline was assigned into low and high groups according to their respective median in *ALK*-positive patients. We did not

observe a significant association between baseline CTCs levels and OS ($P=0.244$, HR=0.59, 95% CI, 0.24–1.5) or PFS ($P=0.591$, HR=0.84, 95% CI, 0.44–1.6; **Fig. 3**). In addition, we considered the dynamic change in the CTC counts under treatment as a possible parameter to evaluate. Patients were assigned into two groups: 13 patients presented stable or increased numbers of *ALK*-CTCs (median increase, 36; range, 2–290), whereas 37 patients presented a decrease in *ALK*-CTCs (median decrease, –7; range, –250 to –280). However, we did not observe a statistically significant association between the dynamic change in the CTC number on *ALKi* and response to treatment with a univariate analysis ($P=0.268$; **Fig. 4, Supplemental Fig. S2, and Supplemental Fig. S3**).

DISCUSSION

In this study, we demonstrated that CTCs could be a reliable surrogate to a tissue biopsy for the detection of *ALK* rearrangements in advanced-stage or recurrent NSCLC patients. Moreover, the decrease in the levels of *ALK*-positive CTCs could be highly informative of the predictive efficacy of *ALKi* in *ALK*-rearranged NSCLC patients. Our study offers a promising perspective into the use of CTCs for real-time monitoring of this population.

Currently, the eligibility of NSCLC patients for *ALKi* relies on an assay performed on a tumor sample collected by biopsy.¹⁵ This invasive procedure is associated with a non-negligible risk of morbidity.¹⁶ The detection of an *ALK* rearrangement is currently performed on small biopsies or fine-needle aspirates and can be hindered by the limited quantity of tissue and/or the number of tumor cells. Tumor tissue is difficult to obtain from patients with advanced/metastatic lung cancer for whom surgery is rarely a component of the treatment care. Thus, a subset of NSCLC patients can be deprived of potentially more efficient therapies. Finding alternative and more effective means of diagnosing and monitoring *ALK* rearrangements are critical issues to identify the patients who may benefit from treatment with *ALKi*. For instance, detection of *ALK* fusions in circulating tumor DNA (ctDNA) has been previously tested using next-generation sequencing (NGS-, RT-PCR or RNA-Seq with variable sensitivities (70%-80%) for the different assays.^{17, 18} Thus, in contrast to *EGFR* mutations, detection of *ALK* rearrangements with ctDNA is rarely implemented in daily practice.

Recently, the prospective multi-center NILE study demonstrated that ctDNA can rescue biomarker-positive patients with non-diagnostic tissue results, suggesting the

clinical utility of ctDNA in newly diagnosed metastatic NSCLC.¹⁹ However, the LB test used was compared to a standard-of-care tissue genotyping test and not the tissue-based NGS, and the results were only applicable to the Guardant360 test and not to other LB tests.¹⁹ In addition, the FoundationOne®Liquid CDx assay, a pan-cancer ctDNA-based comprehensive genomic profiling assay, was recently approved by the FDA.²⁰ However, the number of cases with an *ALK* rearrangement used for the analytic validation was quite low.²⁰

A "LB" of CTCs present in the blood and the characterization of their *ALK* status is an appealing alternative that meets the urgent needs of these patients. In addition, no CTC-based molecular test is currently available in routine clinical practice.²¹

In this setting, CTCs which have been shown to be detectable by ISET in 80% to 100 % of late stages lung cancer patients represent a non-invasive and easily accessible source of tumor material for assessing *ALK* rearrangement in a dynamic manner.^{14, 22} In our study, we did not isolate CTCs in only 8% of patients, which is in the range of sensitivity described for the detection of CTCs by ISET approach in NSCLC patients.^{14, 22-24} The lack of CTC detection in these patients could be related to either technical or biological hurdles. It may be that patients truly present negative for CTCs, or CTCs may be lost at different times during the filtration and staining process, (i) through the pores of 8 µm during the procedure of filtration, and/or (ii) during the sequential washes used during the staining procedure performed after filtration.^{23, 25} The high sensitivity and specificity of ISET were two essential starting points for the feasibility of the present independent and multi-center project.¹⁰ In our first feasibility mono-centric study, we showed excellent concordance for the *ALK* status between CTCs and the matched tumor tissue sample in a limited series of 87 patients with advanced NSCLC, with only 5 patients harboring an *ALK* rearrangement.¹⁰

In this multi-center prospective observational study across 9 centers there was perfect concordance (100%) between the tissue and CTC *ALK* analysis, in all cases with CTCs isolated on filters (92%). In the small proportion of cases without CTCs, the *ALK* status could not be determined on the filters, suggesting technical challenges rather than biological heterogeneity, as described previously.²³

Our study presents some limitations. One ISET spot contains on average 5,000 white blood cells, mainly leukocytes, and only a small number of CTCs.²⁶

Consequently, the manual analysis of one ISET spot to identify *ALK*-rearranged CTCs by FISH is laborious, time consuming and operator-dependent.

In this setting, the *ALK*-ICC or FISH analysis increases the feasibility, precision and reliability of *ALK* testing of filtration-enriched CTCs and may contribute to the validation and qualification of CTC assays for *ALK*-rearranged NSCLC patients.

It is worth noting that other non-invasive biological components that can be isolated from peripheral blood, such as ctDNA, circulating exosomes, platelet RNA or ctRNA, are currently being investigated for the detection of gene rearrangements.^{18, 27, 28}, although the sensitivity and precision of these strategies needs to be further evaluated and compared with those of CTC assays.

Whereas each of these approaches has the potential to provide novel diagnostic information and their exploration is highly encouraged, ctDNA certainly represents the most mature example of the investigation of the LB in clinical practice for NSCLC patients, in particular for gene mutation detection.^{5, 6, 29, 30}

However, the analysis of ctDNA requires extensive deep sequencing of genomic DNA for reliable detection of the chromosomal break-point.³¹ Moreover, digital-droplet PCR, BEAMing and NGS have been reported to have a promising sensitivity for the detection of *ALK* gene fusions, but are more variable than that observed for point mutations and indels.³² Further studies should focus on orthogonal comparisons using CTCs, ctDNA and ctRNA to better characterize the presence of *ALK* rearrangements and to validate the clinical utility of detecting these genomic variants in plasma, notably in naïve treated patients with no tumor tissue accessibility.

In conclusion, this multi-center study is unique as no CTC-based *ALK* rearrangement assay has yet been independently validated with clinical samples. The development of a non-invasive predictive test through the genomic analysis of CTCs is a clinically relevant goal for non-invasive stratification of cancer patients, avoiding morbidity related to lung biopsy and surgery. Analysis with a blood sample should allow patient eligibility to targeted therapies to be determined. Small-sized samples with a low percentage of tumor cells and poor biopsy quality from NSCLC patients can often jeopardize optimal treatment management; CTC-based *ALK* testing can be useful to guide the choice of anti-*ALK* targeted therapy.

388

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393

394 **Conflicts of Interest:**

395 M. Ilié reports speaker’s bureau honoraria from Roche, Merck & Co, AstraZeneca,
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420 All other authors have no conflict of interest to declare.

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Legend to Tables

Table 1. Main clinicopathological data of the 203 NSCLC patients included in this study.

Table 2. Correlation analysis between *ALK*-positive patients with detectable CTCs and *ALK*-positive patients without detectable CTCs.

Legend to Figures

Figure 1. Boxplots showing the levels of CTCs according to (A) the *ALK* status at baseline « visit » 0 (V0), and (B) during the monitoring of *ALK*-positive patients at follow-up « visit 1 » after six weeks (V1) and follow-up « visit 2 » after 12 weeks (V2).

Figure 2. Representative images of *ALK*-positive (A-C) and *ALK*-negative (D-F) NSCLC cases.

A. Circulating tumor cells showing malignant cyto-morphological criteria isolated by the ISET method (original magnification $\times 200$; MGG staining; bar: 20 μm).

B. Circulating tumor cells showing an intense and cytoplasmic staining (score 3+) (*ALK* immunostaining using D5F3 mAb, immunoperoxidase; original magnification $\times 200$; bar: 20 μm).

C. Circulating tumor cell nuclei hybridized with the Vysis *ALK* Break Apart Rearrangement Probe Kit. The two probes (3' red; 5' green) show a distinct separation of the red and green signals (arrows) indicating a rearrangement in the 2p23 *ALK*-gene locus (original magnification $\times 1000$; bar: 20 μm).

D. Circulating cells showing malignant cytomorphological criteria isolated by the ISET method (original magnification $\times 200$; MGG staining; bar: 20 μm).

E. Circulating tumor cells showing lack of staining (score 0) (D5F3 mAb, immunoperoxidase; original magnification $\times 200$; bar: 20 μm).

F. Circulating tumor cell nuclei hybridized with the Vysis *ALK* Break Apart Rearrangement Probe Kit. The probes give overlapping signals in nuclei without the rearrangement (arrows; original magnification $\times 1000$; bar: 20 μm).

Figure 3. Kaplan-Meier survival curves according to the median of CTCs level (n=62 CTCs/4 ml) at baseline from the 81 NSCLC ALK-positive patients. **A)** Overall survival, and **B)** Progression-free survival curves. The *P*-values were calculated using the Log-rank test.

Figure 4. Plots of ALK-CTC counts per 3 ml of blood based on ICC detection in patients who were serially sampled (e.g., paired time points for the same patient) during treatment with ALK inhibitors. **A)** “Spaghetti” plots depicting the ALK-CTC counts in relation to PD, SD, and PR/CR on separate panels. **B)** Box-plots depicting ALK-CTC changes in relation to CR, PR, SD, PD from baseline to visit 1, and from visit 1 to visit 2.

The color of the lines is highlighting the respective TKI used at baseline for the treatment of each patient. The dots highlight the response according to RECIST v1.1 at the two respective follow up visits (Visit 1 and Visit 2). Only patients with an ALK-positive tumor are included in the analysis.

Figure 1

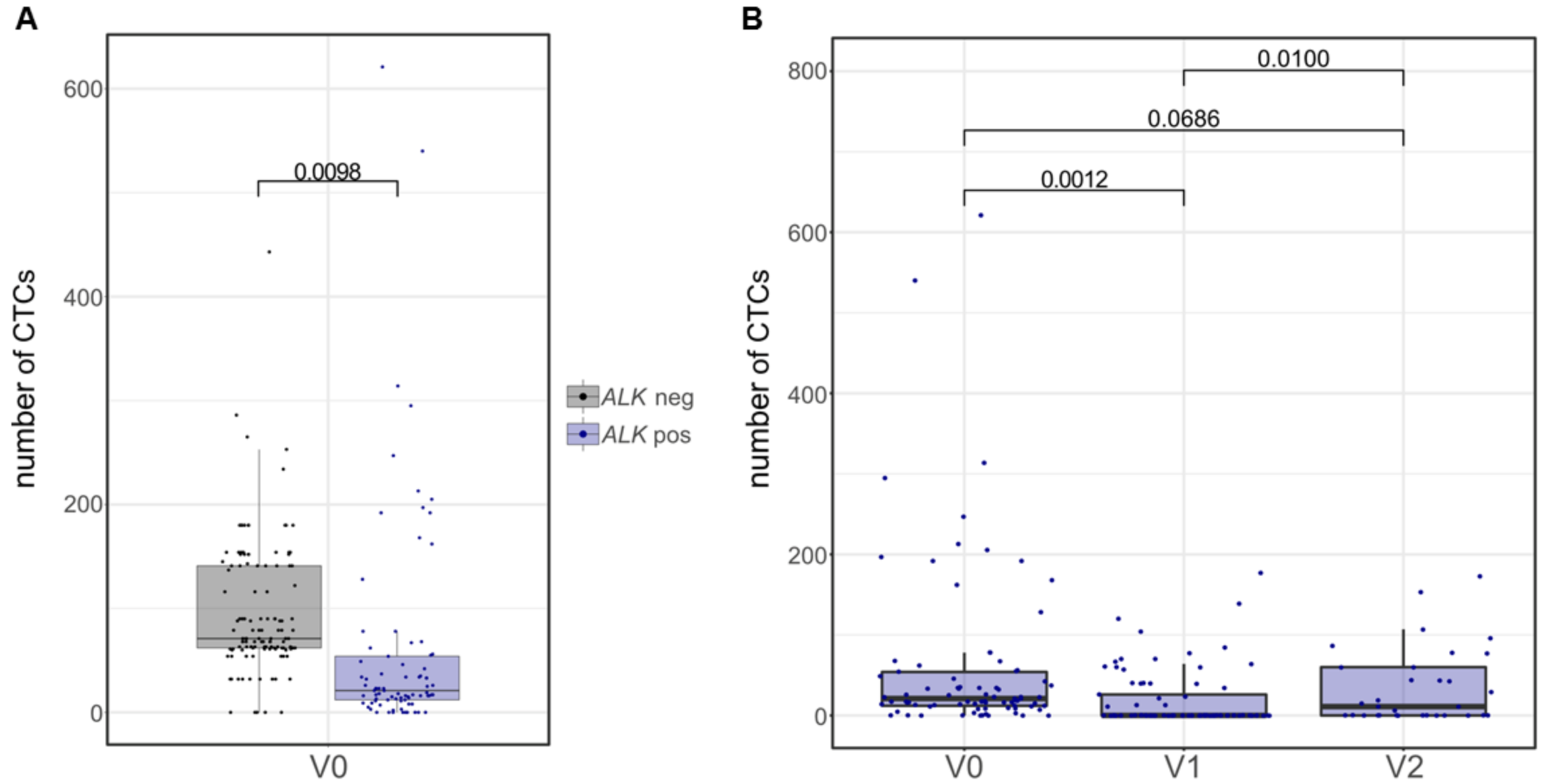


Figure 2

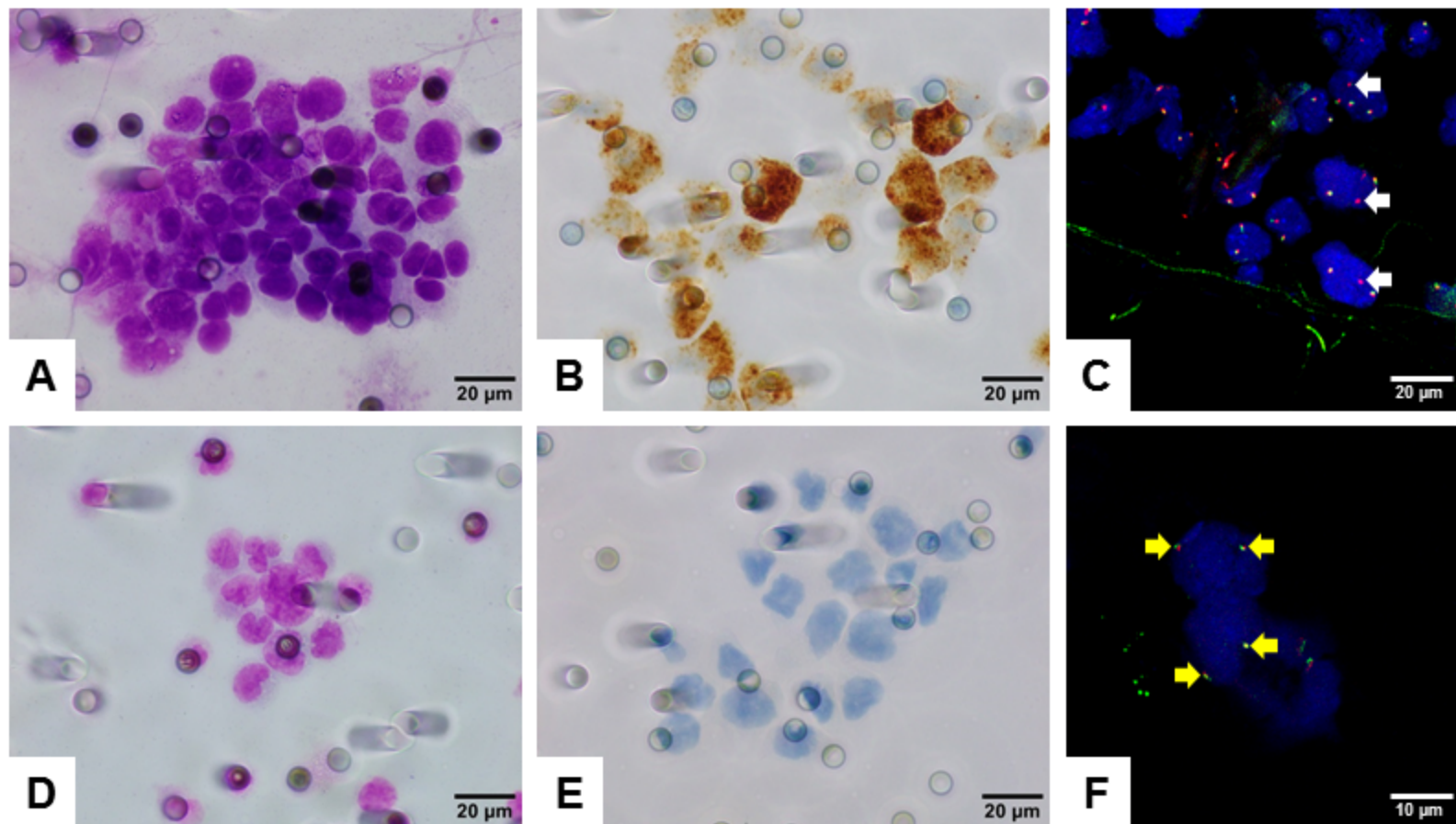
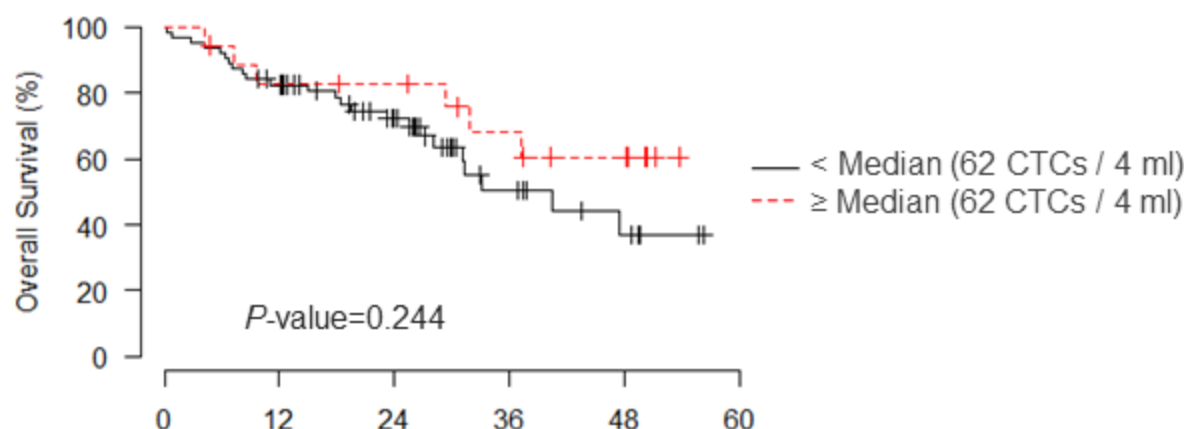


Figure 3

A



< Median

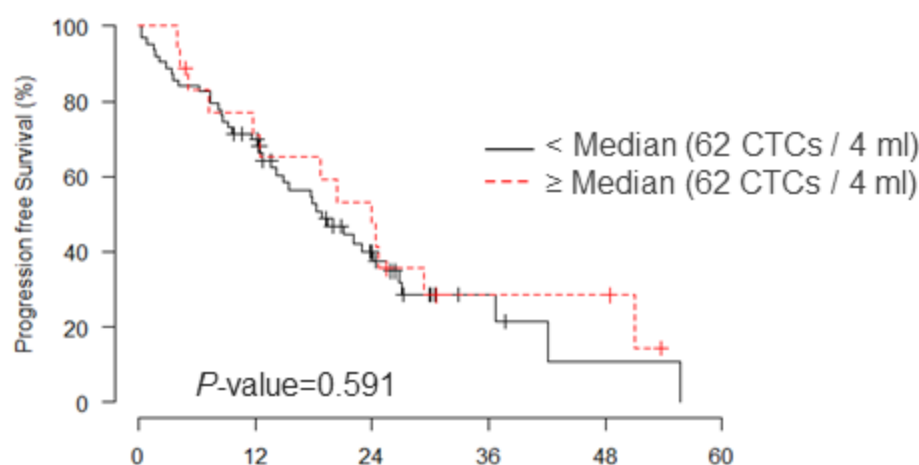
Months

Time (months)	0	12	24	36	48
Patients at risk	63	50	30	11	5
Number of events	0	11	5	6	2
Censored patients	0	2	15	13	4
Survival rate	1	0.82	0.72	0.51	0.37
Lower limit CI 95%	1	0.74	0.62	0.36	0.21
Upper limit CI 95%	1	0.92	0.85	0.71	0.65

≥ Median

Time (months)	0	12	24	36	48
Patients at risk	18	14	13	9	6
Number of events	0	3	0	2	1
Censored patients	0	1	1	2	2
Survival rate	1	0.83	0.83	0.68	0.61
Lower limit CI 95%	1	0.67	0.67	0.48	0.4
Upper limit CI 95%	1	1	1	0.96	0.92

B



< Median

Months

Time (months)	0	12	24	36	48
Patients at risk	63	42	16	4	1
Number of events	0	19	15	4	2
Censored patients	0	2	11	8	1
Survival rate	1	0.7	0.4	0.29	0.11
Lower limit CI 95%	1	0.59	0.29	0.18	0.02
Upper limit CI 95%	1	0.82	0.56	0.46	0.51

≥ Median

Time (months)	0	12	24	36	48
Patients at risk	18	12	8	3	3
Number of events	0	5	4	3	0
Censored patients	0	1	0	2	0
Survival rate	1	0.71	0.47	0.28	0.28
Lower limit CI 95%	1	0.53	0.29	0.13	0.13
Upper limit CI 95%	1	0.96	0.78	0.62	0.62

Figure 4

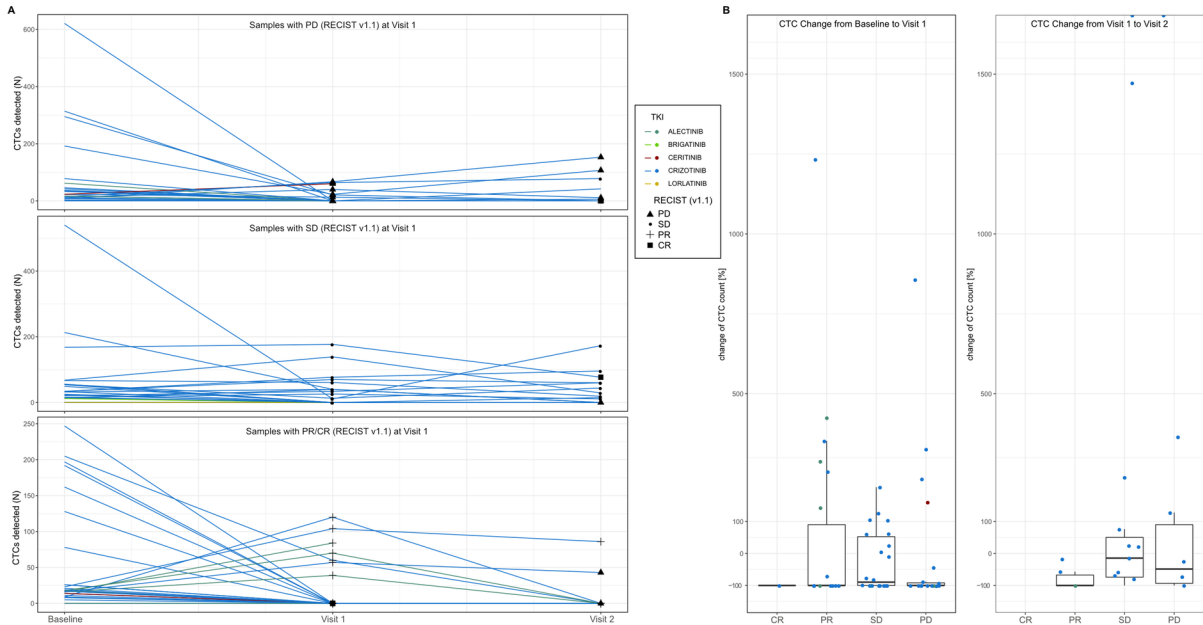


Table 1

	ALK – n (%)	ALK + n (%)
Overall	122 (60%)	81 (40%)
Age (median, range)	64 (33-92)	62 (29-82)
Gender		
Male	85 (70%)	39 (48%)
Female	37 (30%)	42 (52%)
Smoking status		
Current smoker	49 (40%)	6 (7%)
Former smoker	65 (53%)	30 (37%)
Never smoker	8 (7%)	45 (56%)
Histology		
Adenocarcinoma	98 (80%)	80 (99%)
Squamous cell carcinoma	15 (12%)	0 (0%)
NSCLC NOS	9 (8%)	1 (1%)
Disease status at study inclusion		
Metastatic relapse [#]	1 (1%)	9 (11%)
Stage IIIB	17 (14%)	10 (12%)
Stage IV	104 (85%)	62 (77%)
[#] Cases with initially diagnosed stage I-IIIa NSCLC with metastatic relapse and rebiopsy to evaluate the <i>ALK</i> status.		

Table 2

	ALK + CTCs < median n (%)	ALK + CTCs ≥ median n (%)	P-value
Overall	63 (77.7%)	18 (22.3%)	
Age (mean, SD)	61.6 (13.3)	52.3 (12.2)	0.009
Gender			0.4305
Male	32 (50.8%)	7 (38.9%)	
Female	31 (49.2%)	11 (61.1%)	
Smoking status			0.749
Current smoker	4 (6.4%)	2 (11.2%)	
Former smoker	25 (39.7%)	7 (38.8%)	
Never smoker	34 (53.9%)	9 (50%)	
Histology			1
Adenocarcinoma	61 (96.8%)	18 (100%)	
Squamous cell carcinoma	1 (1.6%)	0 (0%)	
NSCLC NOS	1 (1.6%)	0 (0%)	
Stage			0.02
Stage I / II	2 (3.2%)	4 (22.2%)	
III / IV	61 (96.8%)	14 (77.8%)	