ORIGINAL ARTICLE

Prospective Multicenter Validation of the Detection of *ALK* Rearrangements of Circulating Tumor Cells for Noninvasive Longitudinal Management of Patients With Advanced NSCLC

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ABSTRACT

Introduction: Patients with advanced-stage NSCLC whose tumors harbor an *ALK* gene rearrangement benefit from treatment with multiple ALK inhibitors (ALKi). Approximately 30% of tumor biopsy samples 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.

Methods: We conducted a multicenter, prospective observational study (NCT02372448) of 203 patients with stage IIIB/IV NSCLC across nine French centers, of whom 81 were *ALK* positive (immunohistochemistry or fluorescence in situ hybridization [FISH]) and 122 *ALK* negative 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 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 the tumor tissue and in CTCs as determined by immunocytochemistry (sensitivity, 94.4%; specificity 89.4%). The performance was lower for the FISH analysis (sensitivity, 35.6%; specificity, 56.9%). No significant association between the baseline levels or the dynamic change of CTCs and overall survival (hazard ratio = 0.59, 95% confidence interval: 0.24–1.5, $p = 0.244$) or progression-free survival (hazard ratio = 0.84, 95% confidence interval: 0.44–1.6, $p = 0.591$) was observed in the patients with *ALK*-positive NSCLC.

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

Keywords: ALK; CTCs; Immunocytochemistry; FISH; Lung adenocarcinoma

Introduction

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 patients with NSCLC to receive ALKi relies on a companion immunohistochemistry (IHC) performed on tumor tissue,[4] for example, IHC or fluorescence in situ hybridization (FISH) that needs 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. In addition, because tumors evolve and adapt to targeted therapies, it is becoming increasingly important to monitor the molecular changes occurring in

patients with NSCLC in real-time, especially when selecting subsequent lines of treatment.[5] Moreover, the degree of benefit from ALKi varies widely between patients with NSCLC, despite sharing molecular drivers. Longitudinal biopsies on 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 and tumor evolution during therapy could have significant impact on the therapeutic strategy, likely to have the greatest impact on patient outcome.[6] Therefore, developing noninvasive 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, noninvasive, 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 on 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 or immunocytochemistry (ICC).[10–12] To confirm these previous retrospective feasibility studies, we performed the first multicenter, 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.

Table 1. Main Clinicopathologic Data of the 203 Patients With NSCLC Included in This Study

Clinicopathologic Features	ALK Negative n (%)	ALK Positive n (%)
Overall	122 (60)	81 (40)
Age (median, range)	64 (33-92)	62 (29-82)
Sex		
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)
Histological subtype		
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[a]	1 (1)	9 (11)
Stage IIIB	17 (14)	10 (12)
Stage IV	104 (85)	62 (77)

NOS, not otherwise specified.
[a]Cases with initially diagnosed stage I to IIIA NSCLC with metastatic relapse and rebiopsy to evaluate the *ALK* status.

Materials and Methods

Patients and Samples

This multicenter, prospective observational study (NCT02372448) was conducted across nine French clinical institutions from January 23, 2015, to 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 (Comité de Protection des Personnes Sud-Méditerranée V) and authorized by the Agence nationale de sécurité du médicament et des produits de santé. Informed written consent was obtained from all the patients. Eligible patients had histologically confirmed stage IIIB/IV NSCLC (Table 1). A few number of patients had initially been diagnosed with having stages I to IIIA NSCLC, enrolled in the study at the time of the metastatic relapse (Table 1). For these patients, a rebiopsy was performed to evaluate the *ALK* status. These patients may have had previous chemotherapy delivered as part of their primary treatment, but they must have completed primary therapy at least 3 months before their entry in the study.

The prespecified primary outcome was sensitivity and specificity of FISH between tumor tissue and CTCs at baseline, follow-up (visit 1) after 6 weeks (V1), and follow-up (visit 2) after 12 weeks (V2). Prespecified secondary outcomes included sensitivity and specificity of ICC at baseline, V1, and V2 and association of ALK-positive CTCs and clinical outcome.

ALK rearrangement was locally tested on the tumor tissue by IHC (anti-ALK antibody, prediluted, clone D5F3, Roche Diagnostics, Basel, Switzerland) and FISH (Vysis ALK Break Apart Rearrangement Probe Kit; Abbott Molecular Inc., Abbott Park, IL), performed according to prespecified standard operating procedures in the International Organization for Standardization 15189–accredited laboratories (accreditation no. 8-3034; https://tools.cofrac.fr/annexes/sect8/8-3034.pdf).

A 30 mL of peripheral blood was collected in ethylenediaminetetraacetic acid or Streck tubes at baseline (V0) and at 6 weeks (V1) and 12 weeks (V2) on ALKi initiation 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, the stained filters were examined by

a senior thoracic cytopathologist using light microscopy in the following two steps: (1) screening at ×20 magnification to perform cell localization and detection, and then, (2) observation at ×100 magnification using an oil immersion for detailed cytomorphological analysis. CTCs were defined as cells presenting all the following criteria: (1) nuclear size equal or larger than one pore (i.e., equal or larger than 8 μm in diameter); (2) irregularity of the nuclear contour; (3) presence of a well-defined and visible cytoplasm; and (4) high nuclear-to-cytoplasmic ratio (>0.8).

Consequently, computed tomography scans or magnetic resonance imagings 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 on the basis of RECIST.

ALK ICC 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 (prediluted, clone D5F3; Ventana) for 32 minutes at room temperature. FISH analysis was carried out using a break-apart probe for the ALK gene (Vysis ALK Break Apart Rearrangement Probe Kit) in accordance with the manufacturer's instructions. Cells revealing 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, and FISH data and tissue genotype. We used the human NSCLC cell line H2228 obtained from the American Type Culture Collection (Manassas, VA) as an *ALK* rearrangement-positive control in every run.[10]

Statistical Analysis

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

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

Progression-free survival was defined as the time between the date of the start of ALKi and the date of clinical or radiologic progression according to RECIST or death. Overall survival 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).

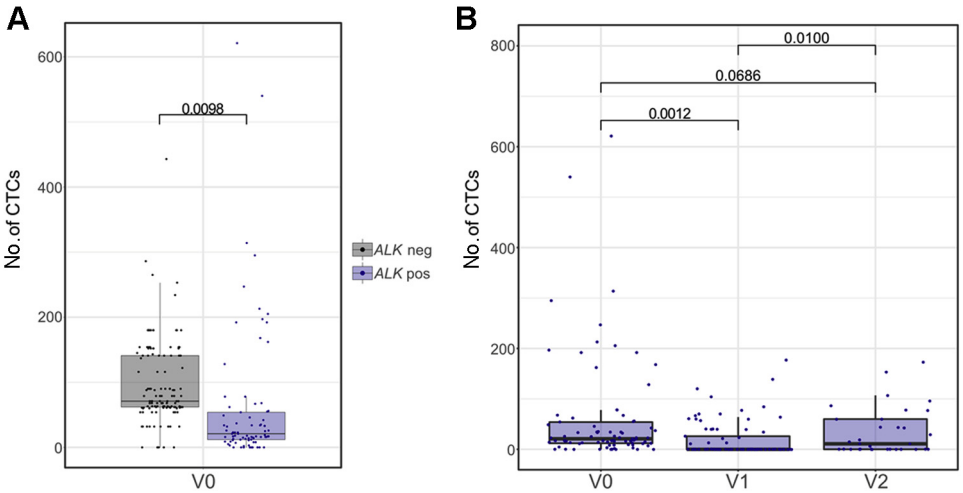

Figure 1. Boxplots revealing the levels of CTCs (*A*) according to the *ALK* status at baseline (visit) 0 (V0) and (*B*) during the monitoring of patients with *ALK*-pos NSCLC at follow-up (visit 1) after 6 weeks (V1) and follow-up (visit 2) after 12 weeks (V2). CTC, circulating tumor cell; neg, negative; pos, positive.

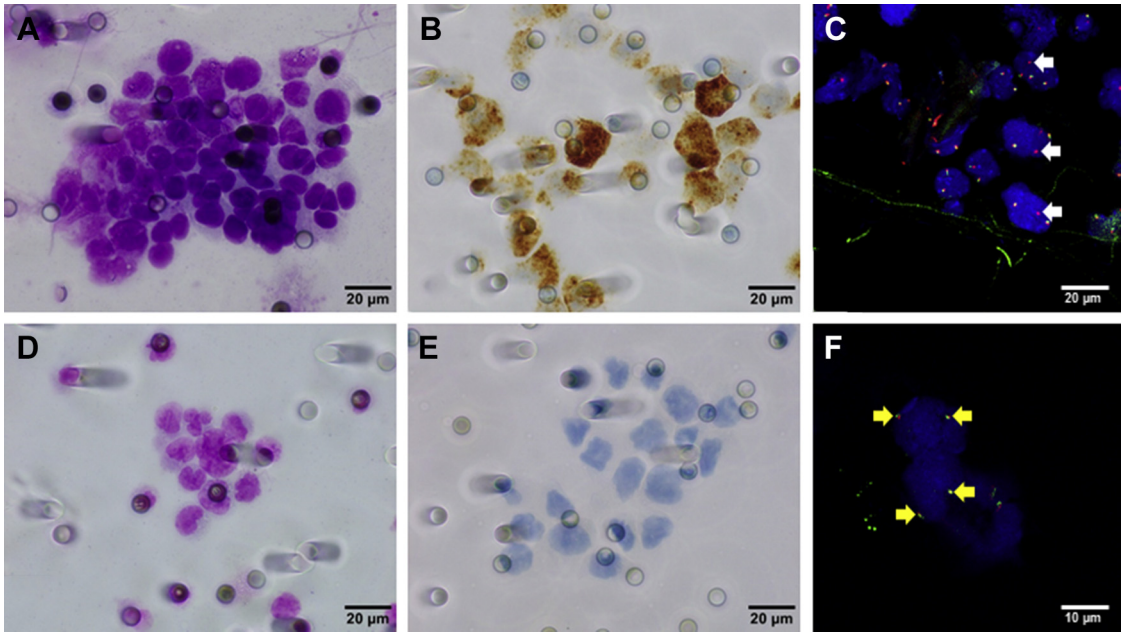

Figure 2. Representative images of (*A*-*C*) *ALK*-positive and (*D*-*F*) *ALK*-negative NSCLC cases. (*A*) CTCs revealing malignant cytomorphologic criteria isolated by the ISET method (original magnification ×200; MGG staining; bar: 20 μm). (*B*) CTCs revealing an intense and cytoplasmic staining (score 3+) (*ALK* immunostaining using D5F3 mAb, immunoperoxidase; original magnification ×200; bar: 20 μm). (*C*) CTC nuclei hybridized with the Vysis ALK Break Apart Rearrangement Probe Kit. The two probes (3′ red; 5′ green) reveal a distinct separation of the red and green signals (arrows) indicating a rearrangement in the 2p23 *ALK* gene locus (original magnification ×1000; bar: 20 μm). (*D*) Circulating cells revealing malignant cytomorphologic criteria isolated by the ISET method (original magnification ×200; MGG staining; bar: 20 μm). (*E*) CTCs revealing lack of staining (score 0) (D5F3 mAb, immunoperoxidase; original magnification ×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 the nuclei without the rearrangement (arrows; original magnification ×1000; bar: 20 μm). CTC, circulating tumor cell; mAb, monoclonal antibody; MGG, May-Grünwald-Giemsa.

Results

Patient Characteristics

Peripheral blood was collected from 193 patients with stage IIIB/IV NSCLC and 10 patients initially diagnosed with having stages I to 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 patients with *ALK*-positive NSCLC were never or former smokers (97%) with adenocarcinoma tumor histology (99%).

At baseline (V0), CTCs were detected in 187 patients (92%) (range: 2–621 per 4 mL; median, 62 CTCs per 4 mL) (Fig. 1*A*). A significantly higher number of CTCs were detected in the patients with *ALK*-negative NSCLC than in the patients with *ALK*-positive NSCLC (median CTCs = 71 versus 21; $p = 0.0098$) (Fig. 1*A*). CTCs were not detected in 16 of 203 patients (8%); 10 of 203 patients with *ALK*-positive NSCLC (5%) and 6 of 203 patients with *ALK*-negative NSCLC (3%).

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

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*A*–*F*). 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. 1*B* and Supplementary Fig. 1).

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.

Table 2. Correlation Analysis Between Patients With *ALK*-Positive NSCLC With Detectable CTCs and Patients With ALK-Positive NSCLC Without Detectable CTCs

Clinicopathologic Features	ALK Positive CTCs < Median n (%)	ALK Positive 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
Sex			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
I/II	2 (3.2)	4 (22.2)	
III/IV	61 (96.8)	14 (77.8)	

CTC, circulating tumor cell; NOS, not otherwise specified.

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 nonsmoking status. *ALK*-positive CTCs were present more often in younger patients (mean, 60 versus 64 y, $p = 0.038$) and nonsmokers ($p < 0.0001$). All the other clinical parameters were not significantly associated with *ALK*-rearranged CTC counts at baseline. In addition, in the patients with *ALK*-positive NSCLC, the levels of CTCs significantly correlated to age and stage (Table 2).

At the first follow-up visit, 25 patients (31%) 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 the patients with *ALK*-positive NSCLC. We did not observe a significant association between baseline CTC levels and overall survival (hazard ratio = 0.59, 95% confidence interval: 0.24–1.5, $p = 0.244$, Fig. 3*A*) or progression-free survival (hazard ratio = 0.84, 95% confidence interval: 0.44–1.6, $p = 0.591$) (Fig. 3*B*).

In addition, we considered the dynamic change in the CTC counts under treatment as a possible parameter to evaluate. The patients were assigned into the following 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*A* and *B*, Supplementary Fig. 2, and Supplementary Fig. 3).

Discussion

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

Currently, the eligibility of patients with NSCLC 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 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 patients with NSCLC can be deprived of potentially more efficient therapies. Finding alternative and more effective means of diagnosing and monitoring *ALK* rearrangements is a critical issue 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), reverse transcriptase polymerase chain reaction, or RNA sequencing with variable sensitivities (70%–80%) for

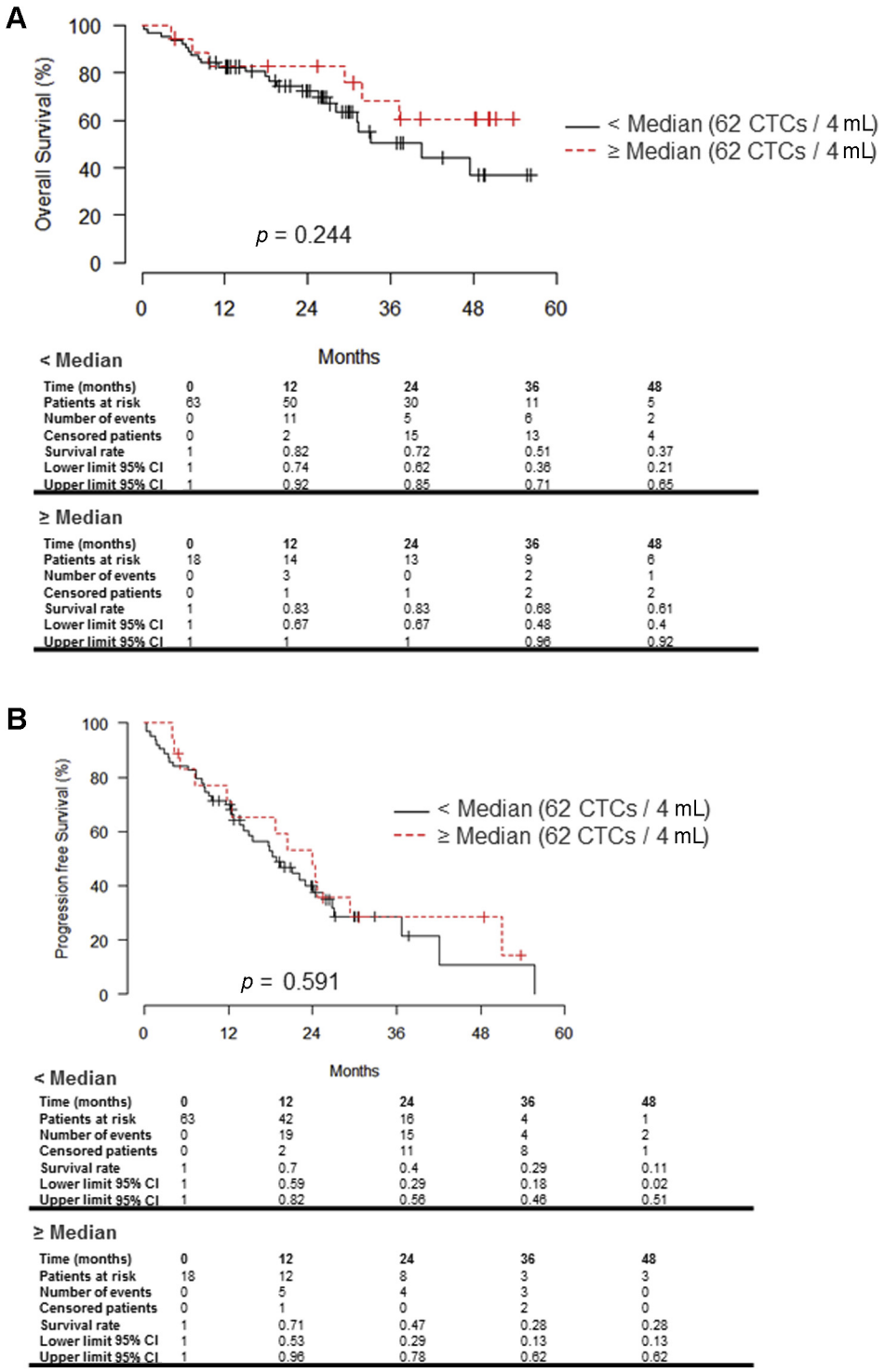

Figure 3. Kaplan-Meier survival curves according to the median of CTC level (n = 62 CTCs per 4 mL) at baseline from the 81 patients with *ALK*-positive NSCLC. (*A*) Overall survival and (*B*) progression-free survival curves. The *p* values were calculated using the log-rank test. CI, confidence interval; CTC, circulating tumor cell.

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, multicenter NILE study revealed that ctDNA can rescue biomarker-positive patients with nondiagnostic tissue results, suggesting the clinical use of ctDNA in newly diagnosed metastatic NSCLC.[19] However, the LB test used was compared with a standard-of-care tissue genotyping test and not the tissue-based NGS, and the results were only applicable to

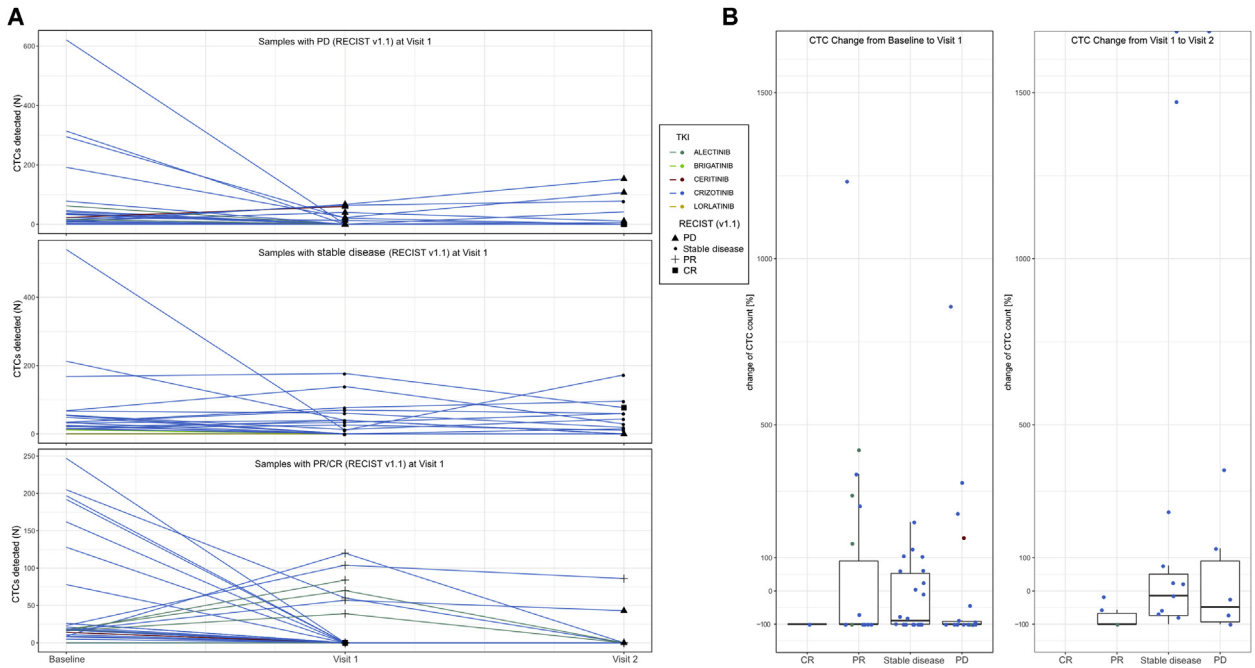

Figure 4. Plots of ALK-CTC counts per 3 mL of blood on the basis of ICC detection in patients who were serially sampled (e.g., paired time points for the same patient) during treatment with *ALK* inhibitors. (*A*) Spaghetti plots depicting the ALK-CTC counts in relation to PD, stable disease, and PR/CR on separate panels. (*B*) Boxplots depicting ALK-CTC changes in relation to CR, PR, stable disease, 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. CR, complete response; CTC, circulating tumor cell; ICC, immunocytochemistry; N, number; PD, progressive disease; PR, partial response; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; TKI, tyrosine kinase inhibitor.

the Guardant360 test (Guardant Health, Inc., Redwood City, CA) and not to other LB tests.[19] In addition, the FoundationOneLiquid CDx assay (Roche Foundation Medicine, Cambridge, MA), a pan-cancer ctDNA-based comprehensive genomic profiling assay, was recently approved by the Food and Drug Administration.[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 are appealing alternatives that meet 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 found to be detectable by ISET in 80% to 100% of patients with late stages of lung cancer represent a noninvasive 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 patients with NSCLC.[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 owing to (1) the pores of 8 μm during the procedure of filtration or (2) 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 multicenter project.10 In our first feasibility monocentric study, we found 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 five patients harboring an *ALK* rearrangement.[10]

In this multicenter, prospective observational study across nine centers, there was perfect concordance (100%) between the tissue and CTC *ALK* analyses, 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 of 5000 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 patients with *ALK*-rearranged NSCLC.

It is worth noting that other noninvasive biological components that can be isolated from peripheral blood, such as ctDNA, circulating exosomes, platelet RNA, or circulating tumor RNA, are currently being investigated for the detection of gene rearrangements,[18,27,28] although the sensitivity and precision of these strategies need to be further evaluated and compared with those of CTC assays.

Although 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 LB in clinical practice for patients with NSCLC, 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 polymerase chain reaction, BEAMing, and NGS have been reported to have a promising sensitivity for the detection of *ALK* gene fusions, but they are more variable than those observed for point mutations and indels.[32] Further studies should focus on orthogonal comparisons using CTCs, ctDNA, and circulating tumor RNA to better characterize the presence of *ALK* rearrangements and to validate the clinical use of detecting these genomic variants in plasma, notably in naïve-treated patients with no tumor tissue accessibility.

In conclusion, this multicenter study is unique as no CTC-based *ALK* rearrangement assay has yet been independently validated with clinical samples. The development of a noninvasive predictive test through the genomic analysis of CTCs is a clinically relevant goal for noninvasive stratification of patients with cancer, 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 patients with NSCLC can often jeopardize optimal treatment management; CTC-based *ALK* testing can be useful to guide the choice of anti-*ALK*–targeted therapy.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2021.01.1617.

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