

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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1	Prospective multi-center validation of the detection of ALK rearrangements of
2	circulating tumor cells for non-invasive longitudinal management of patients
3	with advanced non-small cell lung cancer
4	
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92 ABSTRACT

93 INTRODUCTION: Patients with advanced-stage non-small cell lung cancer (NSCLC) 94 whose tumors harbor an anaplastic lymphoma kinase (*ALK*) gene rearrangement 95 benefit from treatment with multiple ALK inhibitors (ALKi). Approximately 30% of 96 tumor biopsies contain insufficient tissue for successful *ALK* molecular 97 characterization. This study evaluated the added value of analyzing circulating tumor 98 cells (CTCs) as a surrogate to *ALK* tissue analysis and as a function of the response 99 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.

113 **CONCLUSIONS:** CTCs can be used as a complementary tool to a tissue biopsy for 114 the detection of *ALK* rearrangements. Longitudinal analysis of CTCs showed promise 115 for real-time patient monitoring and improved delivery of molecularly guided therapy 116 in this population.

117

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

120 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}

127 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 128 129 a sufficient number of tumor cells to be conclusive. However, procedures providing 130 small biopsy samples (e.g. fine needle aspiration, transthoracic needle biopsy, 131 endobronchial ultrasound, bronchoscopy, bronchial biopsies) may lack a sufficient 132 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, 133 134 it is becoming increasingly important to monitor the molecular changes occurring in 135 NSCLC patients in "real-time" especially when selecting subsequent lines of 136 treatment.⁵ In addition, the degree of benefit from ALKi varies widely between NSCLC patients, despite sharing molecular drivers. Longitudinal biopsies upon 137 138 disease progression have been useful in identifying the molecular mechanisms 139 driving resistance to ALKi. Yet, repeated tissue biopsies are not always feasible and 140 sampling of a single tumor site may not reflect the spatial heterogeneity of resistance 141 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.

147 It is now well admitted that liquid biopsy (LB) is a simple, non-invasive and easily 148 repeatable alternative to tumor biopsies to assess the tumor's molecular status and 149 to monitor genetic changes in "real-time".⁷ Among the LB components, circulating 150 tumor cells (CTCs) contain tumorigenic cell clones with high relevance for metastatic 151 dissemination.⁸ CTCs are likely released from distinct metastatic sites and may 152 inform on the genomic heterogenic picture of the metastatic disease. In contrast to 153 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 156 157 with CTCs collected from patients with ALK-positive NSCLC at baseline by using a 158 filter-based technique for CTC enrichment and ALK testing by FISH and/or 159 immunocytochemistry (ICC).¹⁰⁻¹² To confirm these previous retrospective feasibility 160 studies, we performed the first multi-center prospective clinical validation of the use 161 of CTCs as a surrogate for tumor tissue for ALK testing, thus aiding in 162 prognostication and therapy selection at baseline and during treatment with ALKi of 163 patients with stage IIIB/IV NSCLC.

164

165 MATERIALS AND METHODS

166 **Patients and samples**

This multi-center prospective observational study (NCT02372448) was conducted 167 168 across 9 French clinical institutions from January 23, 2015 through November 21, 169 2019 in accordance with the Declaration of Helsinki and the European Directive and 170 Good Clinical Practices. The study was approved by the Ethics Committee (CPP 171 Sud-Méditerranée V) and authorized by the National Security Agency for Medicines 172 and Health Products (ANSM). Informed-written consent was obtained from all 173 patients. Eligible patients had histologically confirmed stage IIIB/IV NSCLC (**Table 1**). 174 A few number of patients had initially diagnosed stage I-IIIA NSCLC, enrolled in the 175 study at the time of the metastatic relapse (**Table 1**). For these patients, a re-biopsy 176 was performed to evaluate the ALK status. These patients may have had prior 177 chemotherapy delivered as part of their primary treatment but must have completed 178 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; Abott Molecular Inc., Des Plaines, USA),

187 performed according to pre-specified standard operating procedures in ISO 15189

- 188accreditedlaboratories(Accreditationn°8-3034;189https://tools.cofrac.fr/annexes/sect8/8-3034.pdf).
- 190 Thirty ml of peripheral blood was collected in EDTA or Streck tubes at baseline (V0) 191 and at 6 (V1) and 12 (V2) weeks on ALKi initiation and/or at disease progression.¹³ 192 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 193 194 senior thoracic cytopathologist using light microscopy in two steps: (i) screening at × 195 20 magnification to perform cells localization and detection, then, (ii) observation at × 196 100 magnification using an oil immersion for detailed cytomorphological analysis. 197 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 198 199 the nuclear contour; (iii) presence of a well defined and visible cytoplasm; and, (iv) 200 high nuclear-to-cytoplasmic ratio (>0.8).
- 201 Consequently, CT scans and/or MRIs were performed at baseline and during follow-202 up. The response to ALKi (**Supplementary Table 1**) was evaluated according to the 203 "Response Evaluation Criteria in Solid Tumors" (RECIST) version 1.1. The disease 204 control rate was defined as a complete or partial response or stable disease based 205 on RECIST.
- 206

207 ALK immunocytochemistry and FISH on ISET-enriched CTCs

208 All ISET filters were sent to the coordinating team at the CHU Nice for centralized 209 ALK analysis. ICC and FISH were performed on CTCs isolated using the ISET 210 method on unstained spots of the corresponding filters containing CTCs detected on 211 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 212 213 a primary antibody against the ALK protein (pre-diluted, clone D5F3; Ventana) for 32 214 min at room temperature. FISH analysis was carried out using a break-apart probe 215 for the ALK gene (Vysis ALK Break Apart Rearrangement Probe Kit, Abbott 216 Molecular Inc.) in accordance with the manufacturer's instructions. Cells showing at 217 least one split signal or single 3' signals were considered positive for an ALK 218 rearrangement. ISET filters were examined independently and blinded to clinical, 219 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

221 control in every run.¹⁰

222

223 Statistical analysis

224 The performance of ALK assessment on ICC or FISH was compared to the paired 225 tumor biopsy and equivalent method using sensitivity and specificity analysis. The 226 same analysis was carried out first on the population with available CTCs (n=185 227 patients and n=97 analyzed for ICC and FISH respectively) and second on the entire 228 population including patients with no available CTCs (n=203 patients analyzed). In 229 this latter case, ALK assessment was not available by using ICC or FISH methods, 230 we used the worst scenario by considering all ALK positive cases on the biopsy as 231 false negative with ICC and FISH and all ALK negative on the biopsy as false 232 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).

242

243 **RESULTS**

244 **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 = 71 254 vs. 21; P = 0.0098) (**Fig. 1A**). CTCs were not detected in 16/203 (8%) patients, 255 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
- 257 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
- 259 (21%) *ALK*-positive patients (**Fig. 1B**).
- 260
- 261

262 **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.

- 269 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).

275

276 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).

289 In addition, we considered the dynamic change in the CTC counts under treatment 290 as a possible parameter to evaluate. Patients were assigned into two groups: 13 291 patients presented stable or increased numbers of ALK-CTCs (median increase, 36; 292 range, 2–290), whereas 37 patients presented a decrease in ALK-CTCs (median 293 decrease, -7; range, -250 to -280). However, we did not observe a statistically 294 significant association between the dynamic change in the CTC number on ALKi and 295 response to treatment with a univariate analysis (P=0.268; Fig. 4, Supplemental 296 Fig. S2, and Supplemental Fig. S3).

297

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.

304 Currently, the eligibility of NSCLC patients for ALKi relies on an assay performed 305 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 306 307 currently performed on small biopsies or fine-needle aspirates and can be hindered 308 by the limited quantity of tissue and/or the number of tumor cells. Tumor tissue is 309 difficult to obtain from patients with advanced/metastatic lung cancer for whom 310 surgery is rarely a component of the treatment care. Thus, a subset of NSCLC 311 patients can be deprived of potentially more efficient therapies. Finding alternative 312 and more effective means of diagnosing and monitoring ALK rearrangements are 313 critical issues to identify the patients who may benefit from treatment with ALKi. For 314 instance, detection of ALK fusions in circulating tumor DNA (ctDNA) has been 315 previously tested using next-generation sequencing (NGS-, RT-PCR or RNA-Seq 316 with variable sensitivities (70%-80%) for the different assays.^{17, 18} Thus, in contrast to 317 EGFR mutations, detection of ALK rearrangements with ctDNA is rarely implemented 318 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 pancancer 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% 331 332 to 100 % of late stages lung cancer patients represent a non-invasive and easily 333 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 334 335 the range of sensitivity described for the detection of CTCs by ISET approach in 336 NSCLC patients.^{14, 22-24} The lack of CTC detection in these patients could be related 337 to either technical or biological hurdles. It may be that patients truly present negative 338 for CTCs, or CTCs may be lost at different times during the filtration and staining 339 process, (i) through the pores of 8 µm during the procedure of filtration, and/or (ii) 340 during the sequential washes used during the staining procedure performed after 341 filtration.^{23, 25}The high sensitivity and specificity of ISET were two essential starting 342 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 343 344 status between CTCs and the matched tumor tissue sample in a limited series of 87 345 patients with advanced NSCLC, with only 5 patients harboring an ALK 346 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

352 Our study presents some limitations. One ISET spot contains on average 353 5,000 white blood cells, mainly leukocytes, and only a small number of CTCs.²⁶ 354 Consequently, the manual analysis of one ISET spot to identify *ALK*-rearranged 355 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}

369 However, the analysis of ctDNA requires extensive deep sequencing of genomic DNA for reliable detection of the chromosomal break-point.³¹ Moreover, 370 371 digital-droplet PCR, BEAMing and NGS have been reported to have a promising 372 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 373 374 comparisons using CTCs, ctDNA and ctRNA to better characterize the presence of 375 ALK rearrangements and to validate the clinical utility of detecting these genomic 376 variants in plasma, notably in naïve treated patients with no tumor tissue 377 accessibility.

378 In conclusion, this multi-center study is unique as no CTC-based ALK 379 rearrangement assay has yet been independently validated with clinical samples. 380 The development of a non-invasive predictive test through the genomic analysis of 381 CTCs is a clinically relevant goal for non-invasive stratification of cancer patients, 382 avoiding morbidity related to lung biopsy and surgery. Analysis with a blood sample 383 should allow patient eligibility to targeted therapies to be determined. Small-sized 384 samples with a low percentage of tumor cells and poor biopsy quality from NSCLC 385 patients can often jeopardize optimal treatment management; CTC-based ALK 386 testing can be useful to guide the choice of anti-ALK targeted therapy.

388

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394 **Conflicts of Interest:**

M. Ilié reports speaker's bureau honoraria from Roche, Merck & Co, AstraZeneca,
 Bristol-Myers Squibb and Boehringer-Ingelheim, outside the submitted work.

J. Mazières reports commercial research grants from AstraZeneca and Roche,outside the submitted work.

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M. Poudenx reports speaker's bureau honoraria from Roche, Bristol-Myers Squibb,
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Merck Sharp & Dohme, outside the submitted work.

405 D. Moro-Sibilot reports commercial research grants and honoraria from Roche,
406 Bristol-Myers Squibb, Merck Sharp & Dohme and Eli Lilly & Co, outside the
407 submitted work.

F. Barlesi reports speaker's bureau honoraria from AstraZeneca, Bristol-Myers
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work.

415 Simon Heeke has received honoraria from Qiagen, and Boehringer Ingelheim,416 outside the submitted work.

417 Paul Hofman has received honoraria from Roche, AstraZeneca, Bristol-Myers
418 Squibb, Pfizer, Novartis, Bayer, Merck, MSD, Qiagen, Illumina, ThermoFisher
419 Scientific, and Biocartis, outside the submitted work.

420 All other authors have no conflict of interest to declare.

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518 Legend to Tables

519

520 **Table 1.** Main clinicopathological data of the 203 NSCLC patients included in this 521 study.

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

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526 Legend to Figures

527 **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

- 529 follow-up « visit 1 » after six weeks (V1) and follow-up « visit 2 » after 12 weeks (V2).
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531 **Figure 2.** Representative images of *ALK*-positive (**A-C**) and *ALK*-negative (**D-F**) 532 NSCLC cases.

A. Circulating tumor cells showing malignant cyto-morphological criteria isolated by
 the ISET method (original magnification ×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
×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 ×1000; bar: 20 µm).

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

544 **E.** Circulating tumor cells showing lack of staining (score 0) (D5F3 mAb, 545 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 nuclei without the
 rearrangement (arrows; original magnification ×1000; bar: 20 μm).

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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.

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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.

563 The color of the lines is highlighting the respective TKI used at baseline for the 564 treatment of each patient. The dots highlight the response according to RECIST v1.1 565 at the two respective follow up visits (Visit 1 and Visit 2). Only patients with an ALK-566 positive tumor are included in the analysis.

Figure 1



Figure 2







Figure 4



Table ⁻	1
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	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.				

Tab	le	2
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	ALK + CTCs < median	ALK + CTCs > median	<i>P</i> -value
	n (%)	n (%)	
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%)	