ALK immunohistochemistry positive, FISH negative NSCLC is infrequent, but associated with impaired survival following treatment with crizotinib


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ABSTRACT

Objective: Metastasized non-small cell lung cancer (NSCLC) with an anaplastic lymphoma kinase (ALK) rearrangement is usually sensitive to a range of ALK-tyrosine kinase inhibitors. ALK-positive NSCLC have been identified in pivotal phase III trials with fluorescence in situ hybridization (ALK FISH +). These tumors are also

☆ Key message: This study is the first comparative analysis of metastasized discordant ALK IHC positive, FISH negative with concordant ALK and FISH positive non-small cell lung cancer. The prognosis of the discordant cases is worse than of concordant cases. A suitable predictive testing strategy may be to screen first with IHC and then confirm with FISH instead of considering ALK IHC equivalent to ALK FISH according to the current ESMO and CAP/AMP/IASLC guidelines.

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1. Introduction

In 2007, the first anaplastic lymphoma kinase (ALK) fusion was described in non-small cell lung cancer (NSCLC) [1]. In 2013, a phase 3 study demonstrated a significant improvement in progression free survival (PFS) and overall survival (OS) in patients with metastasized ALK positive lung cancer treated with crizotinib compared to chemotherapy [2]. Subsequently, testing for ALK aberrations in patients with metastasized adenocarcinoma of the lung was recommended by international guidelines [3, 4].

When testing for ALK rearrangements, both ALK fluorescence in situ hybridization (FISH) and ALK immunohistochemistry (IHC) may be used. Although in many studies a high association has been shown between immunohistochemistry positive (IHC+) and ALK FISH positivity (FISH+) [5], occasional discrepant cases may occur [6-9]. Cases with positive ALK FISH and negative ALK IHC do not seem to respond on treatment with ALK tyrosine kinase inhibitor [10, 11]. As testing with IHC is preferred over testing by FISH for ALK fusions, it is likely that discordant cases with ALK IHC positivity and negative ALK FISH (ALK IHC+ FISH-) will occur in practice.

Case of patients reports with discordant ALK IHC+ FISH- tests show response to crizotinib [7, 12-16]. However, a comparative study with treatment outcome is lacking.

The aim of this study was to prospectively collect a cohort of ALK IHC+ NSCLC cases and after validation compare within this group response to crizotinib treatment of ALK FISH+ cases with ALK FISH- cases.

2. Materials and methods

A prospective multicenter investigator initiated study on ALK IHC+ metastasized (M+) NSCLC was started across Europe on April 1, 2014. Monthly, the number of ALK IHC tests on M+ NSCLC and number ALK IHC+ was recorded per center until June 2016, providing prevalence. Entry of individual ALK IHC+ cases in central database with clinical information was possible until November 2017. The ALK antibodies 5A4 or D5F3 were allowed for local testing in NSCLC. The study required local a) ALK IHC+ metastatic NSCLC, b) ALK FISH was optional for local testing; c) central validation for ALK IHC and FISH testing, d) treatment with crizotinib and minimal follow-up at 12 weeks. As the outcome of ALK FISH could be positive or negative, patients were stratified into ALK IHC positive and FISH positive (IHC+ FISH+) and ALK IHC positive and FISH negative (IHC+ FISH-). This study was approved by the VU University Medical Center (VUMc) institutional review board. Patient informed consent was locally arranged. Entry into the study was possible by the treating physician (oncologist/pulmonologist, who was not always aware of availability of tissue sections for validation) or via the pathologist (who was not always aware of the treatment details). Therefore, two data sets were initially compiled and subsequently merged. During final analysis March 2019 from most, but not all patients all required information was available.

2.1. Clinical data

Collection of clinical data and validation data was performed in parallel. The clinical database contained 66 NSCLC cases with local data on testing, of which 5 with unknown IHC status and one without follow-up information. The following parameters were recorded: age, gender, smoking history, WHO performance status, clinical-stage at start of crizotinib treatment, response assessment according to Response Evaluation Criteria in Solid Tumors (RECIST) at 12 weeks after start on crizotinib, site primary lung cancer, date of first NSCLC diagnosis, comorbidities, other malignancy, sample type, sample site, histological diagnosis, local ALK IHC test used, local outcome IHC test, ALK FISH test used, local outcome FISH test, testing for EGFR, KRAS, HER2, PI3KCA, RET, BRAF, ROS1, progression free survival (PFS) and overall survival (OS).

2.2. Validation data

For validation of ALK testing blank histological sections were submitted to VUmc Amsterdam for validation with two ALK IHC assays and an ALK FISH assay. The ALK D5F3 antibody was performed according assay of supplier (Roche Ventana, land) in Groningen, NL (ES). The ALK 5A4 was done according a previously described protocol [17], performed in Amsterdam, NL (ET). The ALK FISH assay was performed in Antwerp (PP) with the Vysis ALK test (Abbott Molecular Inc. Des Plaines, IL, USA). In time 5 batches of sections were distributed to Groningen and Antwerp. Testing evaluation was performed blinded for clinical data. In case of limited number of slides, the order of ALK validation was i) 5A4, ii) D5F3 and iii) FISH. Upon receipt in Amsterdam, slides were sent within 3 months in batches to Groningen, Netherlands, and Antwerp, Belgium.

2.3. Statistics

The prevalence of ALK IHC+ was calculated based on the number of monthly recorded ALK IHC tests per laboratory, and ALK IHC+ outcome. Clinicopathologic parameters were summarized for local test outcome and after central validation. Overall survival (OS) was defined as start of treatment with crizotinib until death, and patients alive at their last follow-up time were censored. PFS was defined as start of treatment with crizotinib until progressive disease or death. OS and PFS were compared with Kaplan-Meier curves and the log-rank test. Statistical analyses (BW [18]) were carried out by SPSS for Windows and Mac version 22 (IBM Corp., Armonk, NY, USA). The significance level was set at 0.05
3. Results

In total 3523 ALK IHC tests were recorded in a period of 25 months, of which 94 were ALK IHC+, resulting in a prevalence of 2.7%.

In total 72 ALK IHC + M + NSCLC cases were signed up in the central database, see supplemental figure for consort diagram in Fig. 1.

3.1. Validation

After initial registration, blank slides were centrally received for validation of 72 cases in which the original (i.e. local test) diagnosis was ALK IHC + M + NSCLC. The outcome of the local ALK FISH analysis resulted in 48 concordant (ALK IHC + FISH+) and 16 discordant (ALK IHC + FISH-) cases. In 8 cases the ALK FISH was unknown/ uninformative.

The results of central validation for all 3 assays is shown in supplemental table S1. Note that due to limited availability of tumor in the remaining of the formalin fixed and paraffin embedded samples, not all cases could be adequately examined for validation purposes. In 54 of the 62 cases (87%) ALK IHC + was confirmed with 5A4 IHC and in 41 of the 55 cases (75%) with the D5F3 IHC.

The distribution of cases with outcome of IHC and FISH validation is shown in supplemental table S2. Of the 55 cases with a test outcome, slightly more cases were positive for 5A4 than D5F3.

3.2. Clinical data and treatment

The clinicopathological data for locally and central validated ALK testing performed ALK tests are shown in Table 1. All patients were stage IV. There are no major differences between the clinicopathological variables (gender, age, performance status, treatment).

Information about crizotinib treatment and ALK test results in the local institution was available for 58 IHC + cases. ALK FISH was positive in 44 cases (76%), negative in 8 cases (14%), ‘uninformative’ in 2 cases (3%) and ‘missing’ in 4 cases (7%). Of the 52 cases with ALK FISH test result, RECIST determined response at 12 weeks was missing in 1 case. Forty-five out of 52 patients were still on treatment after 12 weeks.

After central testing the median follow-up time for concordant cases was 54 weeks [6–188], and for discordant cases 40 weeks [4–125].

The overall survival between patients with ALK IHC + FISH + and ALK IHC + FISH- tumors did not differ significantly according to local testing: 1 year OS were 89% and 71% for ALK concordant and discordant cases, respectively (HR = 1.7; 95% CI = 0.45–6.3; p = 0.42). OS, however, was significantly better for concordant cases than discordant cases, 85% versus 40% at 1 year, after central validation (HR = 4.3; 95% CI = 1.2–15.4; p = 0.012, Fig. 1A).

The PFS at 1 year by local ALK testing for ALK concordant and discordant was 68% and 50%, respectively (HR = 0.75; 95% CI: 0.30–2.6; p = 0.83). For centrally ALK validated cases, the PFS at 1 year for ALK concordant and discordant was 58% and 20%, respectively (HR = 2.4; 95% CI: 0.78–7.3; p = 0.11, Fig. 1B).

4. Discussion

This study showed a better overall survival for ALK IHC and FISH
concordant cases as compared to discordant cases after central validation, but not according to local testing.

The 1-year PFS for ALK IHC and FISH concordant cases treated with crizotinib (68% median) is similar as reported in the literature [19–22]. Although in our study the number of discordant ALK IHC + FISH- cases is low, their 1-year overall survival was significantly lower than in concordant cases. In a post-hoc analysis of the ALEX phase 3 trial, where patients with ALK-IHC positive NSCLC, assessed with D5F3 assay, showed better efficacy for alectinib than for crizotinib, [20] a subset of cases with discordant ALK IHC + FISH- also revealed a lower response rate than in the concordant cases [23]. This was in accordance with our findings. The difference between these two studies (Alex post-hoc analysis and our study) on the one hand and the case reports on ALK IHC + FISH- NSCLC showing a treatment response on the other hand can be explained by publication bias for the latter.

The prevalence of ALK IHC + NSCLC of 2.6% in this study by multiple institutions in Europe is in line with that reported in the literature. In a meta-analysis of 27 studies comparing clinicopathological characteristics of patients with NSCLC having an EML4-ALK fusion gene, the frequency of ALK positive lung cancer was 6.8% (range 2.4%–32.6%) [24]. In consecutively tested pulmonary adenocarcinomas series ranging from 1.9%–5% [21,25,26] and in a series of consecutive resection specimen ranging from 4.4 to 8.6% [17,27–29].

Literature comparison of ALK IHC and FISH testing reveals an impressive high concordance [30,31]. However, the discordant ALK IHC + FISH- are in this context at population level (metastasized adenocarcinomas of the lung) hidden in the specificity, ranging for 5A4 from 96 to 100% and for D5F3 from 95 to 100% with one outlier of 82% [31]. A recent review [5], comprised 18 studies with 5.5% ALK IHC positivity out of 10404 NSCLC cases, of which 0.7% discordant IHC + FISH- of the tested NSCLC. Remarkably, when expressed on test outcome level (as a fraction of ALK IHC + positive cases), the number of discordant ALK IHC + FISH- is 13%. In our study, at population level, the frequency of discordant ALK IHC + FISH- cases in stage IV NSCLC is lower (0.1%).

To understand the nature of the ALK IHC + FISH- discordancy, analysis with an orthogonal method is useful. In most cases not enough tumor material was available for further analysis. Explanations for ALK IHC + FISH- include (1) false-negative interpretation of FISH results, especially for results that are close to the threshold of 15% sections [32]; (2) counting in FISH normal cells as tumor cells; (3) double rearrangement involving ALK, reducing the visible distance of the two FISH probes [33]; (4) amplification of the ALK gene (which has been associated with ALK protein expression in some but not all cases), possibly leading to 1+ or 2+ staining [34,35]; (5) false-positive IHC staining with less specific antibodies (e.g. 1A4 [36]) (6) false positive interpretation of ALK IHC results due to high signal enhancement [37]; (7) Infrequently, ALK IHC may be positive in high grade neuroendocrine carcinomas of e.g. lung [38–40] and Merkel cell carcinomas [IASLC atlas [31] chapter 4] and (8) an indeterminate mechanism.

The central validation of the assays revealed surprising discordances with local testing in a small number of cases with respect to false positive IHC and false negative FISH. In daily practice these discordances may be addressed by participation in external quality assessment schemes [37]. However, these schemes do not always have a sufficient amount of material from the informative cases for distribution to a large number of laboratories.

The fact that the remaining tumor material was often not sufficient for the validation process of ALK IHC and FISH testing is a major limitation of this paper. For a portion of the cases, sufficient blank histological slides were only available for validation of one or two of the three assays. This is explained by the small biopsies, where most of the sample was used for primary diagnostic and predictive testing and very little or no tumor was left in the remaining of the block. This also prevented inclusion of several local ALK IHC + cases into the study. A selection bias by tissue sample size is not excluded, as larger samples are likely to be overrepresented (see table 3). The use of the remaining archival part of the small biopsies may, at least in part, be circumvented by better tissue management, where during the first cutting of the small biopsy sample, blank slides are set aside. These can be used, depending of the histological diagnosis, for future diagnostic, predictive and research purposes [41].

In conclusion, ALK IHC + FISH- NSCLC is infrequent and associated with a worse prognosis on personalized treatment. In combination with a similar trend in ALK FISH+IHC- discordant cases [10], a suitable predictive testing strategy may be to screen first with IHC and then confirm with FISH instead of considering ALK IHC equivalent to ALK FISH according to the current ESMO [42,43] and CAP, AMP, IASLC [44] guidelines.

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Declaration of competing interest

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Appendix A. Supplementary data

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References


