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Lung Cancer (HA Wakelee, Section Editor)

Diagnosis and Treatment of ALK Aberrations in Metastatic NSCLC

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Opinion statement

There has been rapid progress in the use of targeted therapies for ALK-positive which has led to improve dramatically PFS and OS in the metastatic ALK-rearranged NSCLC patients. There are several molecules now available (crizotinib, ceritinib, brigatinib, alectinib, and lorlatinib) and others in development. Such an improvement in treatment efficacy has even more highlighted the importance of an adequate identification of ALK alterations. Efficient and easily accessible testing tools are required to identify eligible patients in a timely fashion. Different methods for detecting ALK+ NSCLC patients are now available, with fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) currently representing validated diagnostic techniques for the initial assessment of ALK status. Furthermore the widespread use of next-generation sequencing to detect other possible different activating mutations has allowed to identify individual ALK fusion variants. Several more expensive and time-consuming methods are also available nowadays which have the advantage to detect even rarer uncommon ALK fusion variants and mutations in tumour or blood samples. A review of the evolving testing-treatment landscape is needed to highlight the importance of properly diagnosing and treating this group of patients.

Introduction

Over the course of the past decade, there have been two major breakthroughs in the treatment of non-small-cell lung cancer (NSCLC). The first is the use of immune-checkpoint inhibitors (ICPIs), leading to a 5-year survival of 16–29.6% [1, 2]. The second, more specific to adenocarcinoma (ADC), is the identification and treatment of alterations driving tumourigenesis. These oncogenic drivers are more frequent among non-smokers and younger patients and are predictive of greater response and survival when treated with targeted tyrosine-kinase inhibitors (TKIs) [3–6]. Genetic alterations and their products can be identified through several techniques including immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS). For ADCs, the most common therapeutic targets are epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) rearrangements, BRAF mutations, and ROS proto-oncogene 1 (ROS1) rearrangements, with other alterations of MET, RET, NTRK, and HER2 showing various degrees of response to TKIs [7, 8].

ALK rearrangements are found in approximately 5% of unselected advanced NSCLCs [9]. Never or light smokers have a 22% probability of ALK positivity and, if EGFR mutated patients are excluded from this group, the frequency reaches 33% [9, 10]. ADCs represent approximately 97% of NSCLC patients with ALK alterations (ALK+), while squamous cell carcinomas (SCC) comprise 3%. Additionally, ALK+ NSCLCs often have signet ring or acinar histopathological features [11].

In the present review, we will discuss the currently available methods for detecting ALK+ NSCLC patients and the clinical implications in today's treatment landscape.

Biology of ALK

ALK is a transmembrane receptor tyrosine kinase involved in development, subsequently silenced in adult tissues. The ALK receptor tyrosine kinase consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain [12]. ALK can activate multiple signalling cascades, such as the PI3K-AKT, CRKL-C3G, MEKK2/3-MEK5-ERK5, JAK-STAT, and MAPK pathways [13]. Since the initial nucleophosmin (NPM)-ALK fusion protein described in anaplastic large cell lymphoma (ALCL) in 1994 [14], several ALK gene alterations have been identified across different tumour types, including point mutations, deletions, and rearrangements leading to ALK reactivation. Gene rearrangements occur between a 5′ fusion partner and its promoter encoding the 3′ ALK kinase domain. A broad spectrum of ALK fusions, entailing the fusion of the kinase domain of ALK and the amino-terminal portion of various protein partners (e.g., KIF5B-ALK, TFG-ALK, and KLC1-ALK) can occur in cancer. Nearly 30 different ALK fusion protein partners have been described, suggesting the *ALK* locus may be prone to translocation. The echinoderm microtubule-associated protein-like 4 (EML4)-ALK is the most prevalent in NSCLC [15]. At least 15 EML4-ALK variants have been described in lung cancer with variants 1, 2, and 3a/b accounting for approximately 90% of them. ALK fusion proteins could interfere with different signalling outputs, depending upon several properties such as protein stability and subcellular localisation. EMLs are believed to represent a class of microtubule destabilizers. The consequent ALK expression can activate multiple downstream known cancer signalling pathways, such as PI3K/AKT, JAK/STAT, and RAS/RAF/MEK/ERK [16]. ALK overexpression has also been reported,

Table 1. Advantages and disadvantages of different ALK testing methods

Method	Advantages	Disadvantages	FDA approval
FISH	Small tissue sample needed	False positives (5′ deletions, mixed patterns) Quantity of tumour cell nuclei needed for analysis (≥ 50) Technical issues (results confirmation by a second reader when 10 to 50% positive nuclei, repeat testing, borderline cut-off)	Yes
CISH	Reading with a light microscope Lower cost than FISH Analysis of morphologic features Signals are persistent over time	5% false negatives as compared to FISH	No
IHC	Identification of some false negatives by FISH Cost Small tissue sample needed Detection of ALK rearrangements regardless of the variant or the fusion partner	Low sensitivity with antibodies other than the mouse 5A4 assay and the rabbit D5F3 Confirmation with another validated method needed in the case of weak/moderate staining due to technical issues (inadequate fixation, artefacts)	Yes
RT-PCR	High specificity	Low sensitivity for standard translocations and uncommon variants related to the choice of primers in the assay Tissue input, particularly if other genes need to be tested	No
Tissue NGS	High sensitivity with multiplexed PCR amplicon-based or hybrid capture-based techniques: allows the detection of ALK variants, mutations, and other actionable alterations	More expensive than each singleplex assays (as FISH or IHC) Adequate tumour sample quantity needed Longer turnaround time The amplicon-based focused panel technique is insufficient for fusion-detection	Yes
Liquid biopsy	Non-invasive, easily repeatable Early detection of *ALK* resistance	Not routinely available Quality of the results strongly related to some pre-analytical parameters, tumour stage, and tumour shedding of cfDNA or CTCs.	No

Abbreviations: *CISH* chromogenic in situ hybridization, *FISH* fluorescence in situ hybridization, *IHC* immunohistochemistry, *NGS* next-generation sequencing, *RT-PCR* reverse transcriptase-polymerase chain reaction

although its role in tumourigenesis and progression is still being investigated [17]. When tumour growth is driven by constitutive activation of the *ALK* fusion oncogene, they are susceptible to an ATP analogue inhibitor of ALK.

ALK testing

Given the progress in ALK inhibition and its impact on survival and quality of life, it is imperative not to miss a diagnosis of ALK+ in

advanced NSCLC. Routine testing for this oncogenic alteration in advanced ADC is recommended by the National Comprehensive Cancer Network (NCCN) and the European Society of Medical Oncology (ESMO) guidelines [18]. Both recommend systematically testing for ALK by FISH, but state that IHC with high-performance ALK antibodies is acceptable as an alternative to FISH, or as part of a multiplex strategy with NGS [17, 18]. Given the major clinical implications of an ALK rearrangement, testing correctly is essential, as outlined 2 years ago in this journal by Niu et al [19]. Here, we will examine currently available ALK-testing methods (Table 1).

FISH

ALK break-apart FISH was the only approved method for the identification of ALK rearrangements at the time of approval of the first-generation ALK TKI, crizotinib. It is important to note that different fusion partners can lead to alternative ALK rearrangements [20]. An ALK break-apart DNA probe labels the telomeric 3′ part of the fusion breakpoint in orange and the centromeric 5′ part in green. ALK+ is diagnosed if there is a split pattern (an orange signal without the green signal) in at least 15% of tumour cells [21]. This is the technique and cut-off used in all crizotinib trials.

There is a small risk of both false positives and negatives, particularly when there is only a small number of tumour cells [22]. A mixed pattern with both split and orange signals can occur. Patients with 5′ deletion are more frequently ALK-negative by NGS and IHC than split signal FISH, with a discordance of approximately 20%, and a higher risk of false positives [21, 23]. Similarly, in the mixed pattern, ALK is negative by NGS and IHC [21]. Thus, among the heterogeneous group of ALK+ by FISH, these conditions could represent false positive results at least partially explaining non-responders to ALK TKIs.

Other procedural issues regarding ALK FISH pertain to the quantity of tissue needed for adequate testing and the cut-off for a positive result. The FDA-approved assay is validated for a minimum of 50 tumour cell nuclei [20]. Generally, the accepted cut-off to confirm ALK is that at least 15% of tumour cells show a rearrangement. FISH is technically difficult, can require repeat testing, and can yield borderline cut-off values. In spite of this, only small quantities of tissue are usually required for FISH assays.

Chromogenic in situ hybridization (CISH)

Like FISH, CISH detects copy number alterations using labelled DNA probes. It combines in situ hybridization with antibodies or avidin conjugated with enzymes, allowing for a chromogenic reaction similar to IHC staining that can be read with a light microscope. CISH costs less than FISH and allows for the analysis of morphological features in parallel to gene amplification. Furthermore, contrary to FISH, CISH signals do not diminish over time, thus can be archived and reanalysed [24, 25]. Unfortunately, in spite of these theoretical

advantages, CISH failed to detect 5% of FISH-positive ALK cases and is not, therefore, considered a standard assay [26].

IHC

The ALK fusion protein in NSCLC is less expressed than in ALCL, for which ALK IHC was first designed [27]. Initial results using the same antibody as in ALCL, the mouse anti-human CD246, showed very low sensitivity in NSCLC. Various antibodies were unsuccessful until two clones demonstrated sensitivity and specificity ranging from 95 to 100% compared to FISH. The first was the mouse 5A4 assay, the second the rabbit D5F3, and the latter currently representing the main antibody clone used clinically for the detection of ALK by IHC. Their validity was confirmed by studies showing tumour response to ALK inhibitors in IHC-positive, FISH-negative NSCLC patients [28].

ALK+ by IHC is represented by strong granular cytoplasmic staining with or without membrane accentuation. In case of weak staining, or poor quality staining due to inadequate fixation, preservation, or significant artefacts, a second validated method should be used [29].

Given the favourable cost-benefit profile, many centres adopted ALK IHC testing. This trend is echoed in French and American guidelines [30]. The French guidelines suggest IHC testing to be completed by FISH only in case of weak to moderate staining, but not in negative or strongly positive staining. Since ALK proteins are not expressed on normal tissues, even a minimal expression can be detected by IHC [31]. IHC can also be performed on small tissue samples and could theoretically detect ALK rearrangements regardless of the variant or fusion partner, though, in practice, these factors influence protein expression and location [20].

In the updated 2018 guidelines from the College of American Pathologists, the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology, IHC is considered an equivalent alternative to FISH. Based on data from 19 studies, the pooled sensitivity for IHC was over 96%, and specificity, over 99% [30].

Moreover, a recently identified ALK transcript, the ALK de novo alternative transcription initiation (ATI), arises independently of ALK genomic aberrations through alternative transcription and can be detected by IHC, but not by FISH. These patients benefit from ALK TKI and would hitherto have been overlooked, and represent an advantage to protein-based detection [32].

Polymerase chain reaction (PCR)

Reverse transcriptase (RT)-PCR techniques can be used to diagnose ALK rearrangements, usually through the detection of specific messenger RNA transcripts as a singleplex assay [33].

While RT-PCR is not currently indicated as the recommended method for detecting ALK+, it has been shown to be comparable to IHC [34]. The specificity is nearly 100%, but sensitivity is variable and a negative result must be confirmed [35].

Fusion partner-specific PCR techniques depend on the choice of primers in the assay and can lead to low sensitivity. When EML4-ALK transcripts were

retrospectively analysed in FISH-positive tumours, a rate of 31–33% false negatives was reported [36]. Thus, while the specificity of RT-PCR is high, the sensitivity, even for standard translocations, is low. Furthermore, due to the many EML4-ALK variants and fusions of ALK to other partners such as TGF and KIF5B, tailoring a multiplexed RT-PCR to be extensive enough for a screening purpose is quite challenging [37, 38].

Tumour NGS

A more recent development is the use of NGS testing for ALK fusions, simultaneously screening for other driver mutations in NSCLC. There are two main NGS techniques currently in use, amplicon-based and hybrid capture-based. They both create a library of amplified DNA, sequenced as single molecules. The amplicon sequencing generates results using multiple PCR reactions. It is fast, is easy to perform, and can detect mutations that occur less frequently, making it ideal for analysing hotspots, but it is often insufficient for fusion detection. The hybrid capture-based NGS uses hybridization to generate a DNA library. It is slower to perform, requiring more complex steps, but is more sensitive in detecting rearrangements such as *ALK* [30].

The advantage of NGS is that it can detect a fusion between any partners. However, like with PCR, if a focused panel is used, fusion partners must be specified beforehand. Multiplexed PCR amplicon-based targeted NGS is an effective approach based on the interrogation of fusion transcripts involving many known driver genes and partners, as well as 3′/5′ expression ratios of oncogenic kinases [39]. In the absence of known fusions, NGS can still detect strong differential expression between the 3′-end and 5′-end domains of ALK. This indicates ALK fusion with a novel partner or an unknown breakpoint [21]. This approach allows for the detection of ALK variants, associated with different resistance mechanisms and sensitivity to ALK TKIs, potentially tailoring the selection of the most appropriate upfront ALK TKI [40••]. For instance, patients whose NSCLCs harbour the *EML4-ALK* variant 3 are more likely to develop ALK resistance mutations (e.g., the ALK G1202R) compared to those with the EML4-ALK variant 1. Furthermore, they tend to have more favourable outcomes when treated with a third-generation ALK TKI, which has been demonstrated to overcome the *ALK* G1202R-mediated resistance mutation [41••].

It is noteworthy that the use of NGS can elucidate critical diagnostic information that would otherwise be missed. By performing a broad hybrid capture-based NGS analysis in lung adenocarcinoma samples from patients with a less than 15 pack-year smoking history and negative results by non-NGS methods for alterations in 11 genes (mutations in EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, and AKT1 and fusions involving ALK, ROS1, and RET), Drilon et al. reported that 26% of patients had actionable alterations, while an additional 39% had genomic alterations warranting possible inclusion in clinical trials [42].

Although NGS is more expensive, requires a more significant sample than FISH or IHC and has a longer turnaround time, it could replace many time, samples, and money-consuming singleplex assays needed for metastatic

nonsquamous NSCLC (e.g., testing for EGFR, ALK, ROS1, and others, including NTRK) or the expensive FISH that is often used as a confirmatory test after IHC.

Circulating-free (cf) and circulating-tumour (ct) DNA or RNA testing by liquid biopsy (LB)

Recent studies have evaluated the use of a LB approach to detect an *ALK* rearrangement and/or the emergence of ALK resistance mutations. LB can either involve the study of circulating tumour cells (CTCs) by FISH and IHC, or the isolation of free DNA or RNA, or RNA in a complex with proteins in an exosome or in association with platelets from plasma samples [43]. Whether LB should be considered a complementary or alternative method for the assessment of *ALK* remains to be established. cfDNA was shown to be able to track the evolution of resistance to TKIs during therapy. ALK fusions were found in 86% or patients and resistance mutations in 50%, with 100% concordance between plasma and tissue-detected alterations [44].

LB represents a non-invasive, easily repeatable approach for early detection of *ALK* resistance mutations and a possible alternative to a tissue biopsy in the presence of inaccessible tumour sites, a fragile patient or poor quality of extracted RNA. Up to 25% of biopsies are inadequate for molecular or genomic testing [45]. The role of LB could become increasingly relevant for monitoring acquired mutations during ALK TKIs, as these could reorient the treatment choice [46]. In addition to detecting resistance mutations, monitoring cfDNA by NGS or digital droplet PCR may be a promising marker for monitoring response to TKIs [47]. As a tissue biopsy evaluates a single lesion, it may not be representative of the entire tumoural landscape due to heterogeneity. Plasma sampling could overcome this limitation. In a retrospective analysis, 96 NSCLC were found to harbour ALK fusions in cfDNA. Interestingly, 26% of patients were ALK+ in cfDNA while the tissue was inadequate for analysis and a further five were positive while tissue-negative. Of these, three received TKIs and responded to therapy [48]. This could be an argument for a complementary role of cfDNA analysis.

However, LB in CTCs is not routinely available and the quality of the results is strongly related to pre-analytical parameters including the sample volume, the collection tubes, the time to plasma RNA extraction due to rapid blood RNA degradation, the centrifugation procedure, and storage conditions. In addition, the detection of *EML4-ALK* rearrangements by RT-PCR in CTCs is low (around 20–25%) [49]. Finally, there are inconsistencies in the number of CTCs, amount of free tumour RNA, or platelet-associated tumour RNA, which can vary widely on an individual patient basis and on the disease tumour stage. All of these parameters must be taken into account when using LB [50].

Discordant results

As previously mentioned, there can be discordant results between ALK FISH, IHC, and NGS assays. Both borderline FISH-positive assays and false-positive 5′ deletion patterns can result in true negative IHC [21, 23, 30]. Nonetheless,

Camidge et al. found no correlation between FISH patterns and response to crizotinib, suggesting further assessment is required [51].

Using NGS as a reference, Pekar-Zlotin et al. reported a 42.9% sensitivity and 97.7% specificity of FISH for ALK, and high concordance between NGS and IHC by the D5F3 antibody [34]. In discordant results between NGS and FISH, Dacic et al. found that IHC had a very high concordance with NGS, with all cases with a split signal yielding negative IHC tests. Furthermore, mutational analysis of these cases revealed other oncogenic mutations such as KRAS and ATM mutations [21].

The question is how patients respond to treatment when there are diagnostic discrepancies. In a multicentre study of 3244 consecutive NSCLC patients, 4.6% (150 patients) were ALK+ by FISH and/or IHC. There was significant discordance with 36 FISH+/IHC–, 19 FISH–/IHC+, and 15 FISH non-contributive/IHC+ cases. Thus, each technique would have detected 80/150 ALK+ cases. No correlation between the percentage of rearranged tumour cells by FISH and the intensity of IHC staining was observed. While different antibodies were used, retesting IHC–/FISH+ cases with the D5F3 antibody led to the detection of ALK in additional 5/17 cases. Different fusion partners are associated with distinct ALK protein expression and stability, potentially leading to IHC false-negatives. In addition to pre-analytical issues, for IHC+/FISH– samples, the discordance could lie in different mechanisms that lead to an ALK protein expression, such as amplifications or point mutations, rather than rearrangements. Nevertheless, a high response rate to crizotinib was observed in all subgroups of discordant patients. The authors concluded that FISH and IHC are complementary in order to avoid overlooking patients amenable to ALK TKIs [52]. Currently, ALK IHC with the D5F3 clone or NGS (tumour or cfDNA) are the most robust platforms for testing of ALK, with the former approach favoured for singleplex testing or in resource-restricted environments, and the latter favoured as part of a multiplex testing strategy in nonsquamous metastatic NSCLC.

ALK inhibition

Retrospective studies found ALK+ NSCLC to be more sensitive to pemetrexed-based chemotherapy [53]. Preclinical data followed by early phase clinical trials showed the efficacy of crizotinib, a TKI targeting ALK, in ALK+ NSCLC patients. This led to its accelerated approval by the Food and Drug Administration (FDA) in 2011 and the European Medicine Agency (EMA) in 2012. In 2013, the phase III PROFILE1007 trial of crizotinib versus chemotherapy as a second-line in advanced ALK+ NSCLC favoured crizotinib with an objective response rate (ORR) of 65% versus 20% and a clear progression-free survival (PFS) benefit [54]. In the final results, there was no overall survival (OS) advantage, perhaps due to crossover [55]. It was in 2014 that the landmark PROFILE 1014 trial proved the superiority of crizotinib over chemotherapy in the first-line, forever changing the treatment algorithm and prognosis of these patients. The ORR was 74 versus 45%, PFS was 10.9 versus 7 months, and 1-year survival was 84 versus 79% [56]. Final OS results showed a median OS of 47.5 months in the chemotherapy cohort, with 84% of patients receiving crizotinib in subsequent lines, while the median OS was not reached in the crizotinib group. Four-year survival was 56.6% in the crizotinib group and 49.1% in the chemotherapy

group [57]. The improved OS and tolerable safety profile made ALK TKI standard of care.

Although OS data with second- and third-generation TKIs are not yet available, a French retrospective study reported a median OS of 16.6 months in 318 patients with ALK+ NSCLC treated with crizotinib. Of 265 patients with progressive disease (PD), 105 received supportive care, 74 chemotherapy, and 84 next-generation ALK TKIs. The latter group had the best prognosis, with a median post-PD survival of 25 months and median OS from the diagnosis of metastatic disease of 89.6 months, or 7.5 years [58]. Despite all the possible limitations of such a retrospective study, its results are unprecedented in the field of metastatic lung cancer, further highlighting the importance of correctly diagnosing ALK+ NSCLC.

Second-generation ALK TKI

Thirty-one percent of ALK+ NSCLC patients (95% CI, 23 to 39) treated with crizotinib have PD at 18 months [56]. Progression on first-line crizotinib can be attributed to various escape mechanisms. Several acquired ALK mutations (e.g., L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, and G1269A) can confer resistance to crizotinib. Resistance can also arise from off-target bypass mechanisms, such as EGFR, KRAS, or KIT mutations [59, 60]. Furthermore, under first-generation TKIs, up to 50% of patients will experience a central nervous system (CNS) progression leading to high morbidity and mortality, due to their poor penetration across the blood-brain barrier (BBB) [61].

Second-generation ALK TKIs were developed to overcome acquired resistance to crizotinib and penetrate the BBB, demonstrating longer-term chemotherapy-free treatment of ALK+ patients [62, 63]. Ceritinib, the first second-generation ALK TKI, is effective both after crizotinib and chemotherapy, conferring a survival benefit [62].

Second-generation ALK inhibitors are also effective in patients without identified resistance mutations, perhaps confirming that they suppress ALK more effectively than crizotinib [30]. The next step was to test these second-generation TKIs as first-line treatment. Ceritinib is superior to chemotherapy [64]. However, as there is no direct comparison to crizotinib, and as ceritinib appears to have a less favourable toxicity profile than crizotinib, it is rarely used in the first-line setting.

Front-line alectinib compared to crizotinib in Japanese patients showed lower grade 3–4 adverse events (26% versus 52%), a 93.5% ORR and improved PFS [65]. These results were mirrored in the Asian ALESIA trial and the global phase III ALEX trial, comparing alectinib to crizotinib, confirming the magnitude of the benefit of this new ALK TKI [43, 66]. The updated results showed a median PFS of 34.8 months versus 10.9 months in the alectinib versus crizotinib groups, respectively, with an 83% ORR to alectinib and excellent safety profile [67]. OS data are still immature, but based on a substantial PFS advantage and with robust CNS activity, alectinib has become the standard of care for first-line ALK+ NSCLC.

However, resistance to second-line ALK TKIs occurs and is associated with specific mutations (G1202R, G1202del, I1171N, V1180L, S1206Y, E1201K), for which not all TKIs are equally effective. The latest second-

generation TKI, brigatinib, showed in vitro and in vivo activity against the G1202R, a frequent escape mutation associated in nearly 50% of alectinib-refractory tumours [68]. It is worth noting that in spite of these promising results, G1202R has been detected in brigatinib-resistant tumour biopsies, raising the question of how clinically active this drug is against this solvent front mutation [46]. The ALTA-1L trial of brigatinib versus crizotinib was clearly positive for PFS (12-month PFS rate of 67% for brigatinib and 43% for crizotinib) with an intracranial response rate of 78% for brigatinib compared to 29% for crizotinib [69].

Third-generation ALK TKI

Lorlatinib is a third-generation TKI with broad activity including against ALK resistance mutations including G1202R. Lorlatinib demonstrated improved ORR in ALK+ treatment-naive patients (90%), in those who had progressed on crizotinib (69.5%), second-generation ALK TKIs (32.1%), or after up to three previous ALK TKIs (38.7%). Substantial CNS activity was seen with an intracranial response rate of 87% in patients treated with prior crizotinib alone, 55.6% in patients who received second-generation ALK TKI, and 53.1% in patients who received multiple ALK TKIs [35, 70••, 71]. After second-generation ALK TKIs, the ORR was twice as high among patients with detectable mutations in plasma or tissue genotyping than those without, namely 62% versus 32% in the plasma and 69% versus 27% in tissue-detected mutation carriers [72]. The presence of ALK mutations at progression is therefore predictive of response and, depending on available therapeutic options, may play a role in the treatment algorithm. This approach is currently being investigated in the ALK Master Protocol in which several ALK inhibitors (alectinib, brigatinib, ceritinib, crizotinib, ensartinib, lorlatinib) have been assessed in specific mutations. Lorlatinib was recently FDA-approved after the failure of second-generation TKIs, in the second- or third-line setting.

Finally, lorlatinib is currently being investigated as a front-line therapy against crizotinib. As with second-generation ALK TKIs that yielded superior results to crizotinib, this may enlarge the therapeutic options, but confirmatory front-line trials against the current standard of care, alectinib, would be required for change of practice.

Immune checkpoint blockade in ALK+ NSCLC

In a retrospective study among 22 *EGFR*+ and 6 *ALK*+ NSCLC patients treated with PD-1 blockade after TKI treatment, ORR was 3.6%, reinforcing the hypothesis that driver mutation induced-tumourigenesis is less sensitive to immune checkpoint inhibition and acquired resistance needs to be sought and addressed with new targeted therapies. Unsurprisingly, few of these patients had tumours with high levels of tumour infiltrating lymphocytes, suggesting that the microenvironment is not conducive to immune checkpoint inhibition [73–75]. Furthermore, subgroup analysis of the CheckMate 057 trial showed that in the 4% of patients harbouring

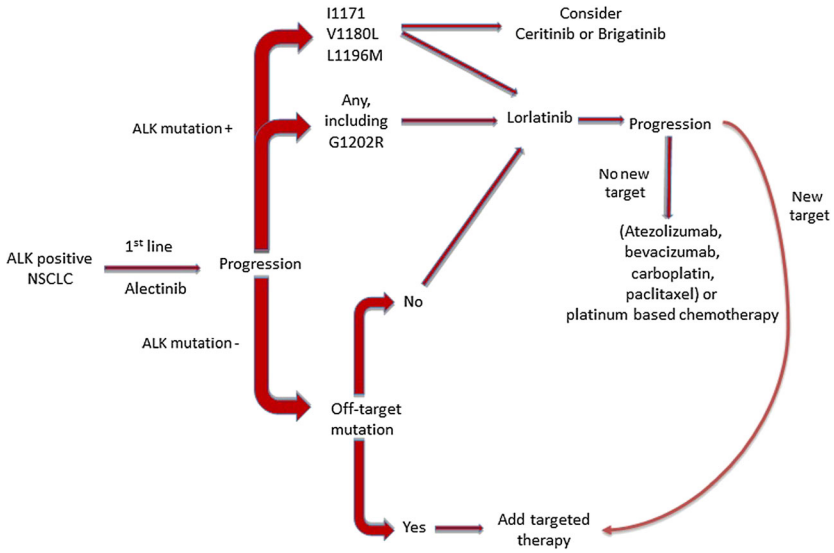

Fig. 1. Treatment algorithm for ALK-rearranged NSCLC patients.

ALK translocations, regardless of PD-L1 expression, the anti-PD-1 checkpoint inhibitor, nivolumab, did not confer a benefit compared to docetaxel [76].

Similarly, the EGFR-addicted NSCLC showed a lack of sensitivity to PD-1 blockade in the Checkmate 057 and KEYNOTE-010 trials, and in a recent meta-analysis comparing immune-checkpoint inhibitors (ICPIs) (nivolumab [$n = 292$], pembrolizumab [$n = 691$], and atezolizumab [$n = 144$]) against docetaxel ($n = 776$), ICPIs significantly prolonged OS over that with docetaxel in the *EGFR* wild-type subgroup ($n = 1362$, HR = 0.66, 95% CI 0.58–0.76, $p < 0.0001$) but not in the *EGFR*-mutant subgroup ($n = 186$, HR = 1.05, 95% CI 0.70–1.55, $p < 0.81$; treatment-mutation interaction $p = 0.03$) [77]. In the phase 2 ATLANTIC trial, the efficacy of durvalumab was evaluated in NSCLC patients treated with at least two lines of prior systemic therapy. One hundred eleven patients harboured either EGFR or ALK alterations, of which 15 were ALK+ and 1 was EGFR+ and ALK+. The ORR among EGFR+ patients with PD-L1 >25% was 14.1%, yet there were no responses in ALK+ patients [78].

This confirms that mechanisms of acquired resistance to the first-line TKI should be elucidated to guide the selection of second-line treatment for these patients and that targeted therapy, rather than immune checkpoint blockade, remains the preferred option in patients with oncogene-addicted tumours.

Finally, an arm of the phase 3 IMpower150 trial evaluated the safety and efficacy of chemotherapy with atezolizumab and bevacizumab in non-squamous metastatic NSCLC. Thirteen percent of patients harboured ALK or EGFR alterations, pretreated with TKIs. Comparing this arm to the chemotherapy and bevacizumab arm, there was a 46% reduction in the risk of mortality in the interim analysis. It is unclear whether the benefit is derived from the addition of antiangiogenic or chemotherapy to ICPIs in these oncogene-

addicted tumours [79]. Though these data are preliminary, this may become an enticing alternative to chemotherapy alone once patients exhaust all available TKI options.

Treating advanced ALK+ NSCLC today

Given the above-listed currently available data, there are multiple therapeutic options. Thanks to a greater understanding of the efficacy of different agents in ALK+ patients, the treatment sequencing is constantly evolving, as we can note when comparing recommendations from 2 years ago to our current algorithm [80] .

Alectinib is now considered the standard of care front-line TKI, when available. In an unrestricted setting, we would recommend front-line alectinib, followed by tissue or LB at progression. In case of an on-target mutation, which is one affecting the ALK pathway, or the absence of an off-target escape mechanism, we would then proceed with lorlatinib, the efficacy of which was demonstrated by Shaw et al [81]. In case of specific on-target mutations with known greater sensitivity to ceritinib, such as ALK I1171, V1180L, or L1196M mutations, ceritinib or brigatinib could be an alternative to lorlatinib [46] and should be the preferred option if the latter is unavailable. The premise of resistance mutation-guided ALK TKI therapy is currently being investigated in the ongoing ALK Master Protocol.

On the other hand, if an escape mechanism such as an EGFR mutation or MET amplification was detected, we would recommend adding targeted therapy or, for MET amplification, switching to crizotinib to target both ALK and MET pathways.

Upon secondary progression, in the absence of a new target, we would recommend a platinum-based doublet. Despite limited data, an alternative is the IMpower150 regimen of atezolizumab, bevacizumab, carboplatin, and paclitaxel [82], though it should be noted that it is not FDA approved in this setting (Fig. 1).

Finally, should progression be oligo-metastatic, local ablative treatment should be considered, in order to prolong the benefit targeted therapy [82].

Conclusions and future directions

Unfortunately, the majority of patients with ALK-rearranged NSCLC eventually develop a resistance to ALK TKIs. Resistance may be intrinsic, or primary, with no evidence of anti-tumour activity of the ALK TKI, or may develop after an initial tumour response. The development of more powerful treatments and proper use of ALK inhibitors based on better knowledge and adequate identification of ALK alterations and resistance mechanisms are key factors to improve patient outcomes.

The molecular link between ALK variants, higher incidence of resistance mutations and the differential response to TKIs [41••] supports IHC or NGS-based detection of ALK status to guide the appropriate treatment strategy for ALK+ mNSCLC patients. Thus, the widespread use of NGS for the molecular characterization of NSCLCs before the start of systemic therapy could allow for the selection of the most appropriate upfront ALK TKI, in addition to detecting

other oncogenic drivers [3].

The identification and monitoring of new resistance mechanisms during second and third-generation ALK TKIs represent another relevant issue. Whether this evidence could also apply to ALK+ NSCLC, suggesting a possible efficacy of ICPI in selected patients, needs to be further investigated. In this setting, the validation of the LB approach for the detection of resistance mutations occurring with upfront use of new-generation ALK TKIs and possibly of blood TMB could play a key role.

In conclusion, the use of IHC and NGS are overtaking FISH as the standard diagnostic techniques for the initial assessment of ALK status in advanced NSCLC, thanks to the ease and cost-efficiency of the former and versatility of the latter. We believe that multiplexed NGS will become increasingly prevalent as part of a broader strategy for identifying drivers mutations at diagnosis, as well as for detecting ALK resistance mechanisms in tissue or blood at later stages, influencing the choice of TKIs and improving cancer care in advanced NSCLC.

Compliance with Ethical Standards

Conflict of Interest

Alex Friedlaender has received compensation from Roche, Pfizer, Astellas and BMS for advisory roles.
Giuseppe Banna declares that he has no conflict of interest.
Sandip Patel has received compensation from AstraZeneca, Bristol-Myers Squibb, Illumina, Nektar Therapeutics, and Tempus for service on advisory boards, and his university receives research funding from Bristol-Myers Squibb, Eli Lilly, Fate Therapeutics, Incyte, AstraZeneca/MedImmune, Merck, Pfizer, Roche/Genentech, Xcovery, Fate Therapeutics, Genocea, and Iovance Biotherapeutics.
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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Banna GL, Passiglia F, Colonese F, et al. Immune-checkpoint inhibitors in non-small cell lung cancer: a tool to improve patients' selection. Crit Rev Oncol Hematol. 2018;129:27–39.
2. Brahmer J HL, Jackman DM, et al. Five-year follow-up from the CA209–003 study of nivolumab in previously treated advanced non-small cell lung cancer: Clinical characteristics of long-term survivors. American Association for Cancer Research Annual Meeting, Washington, DC 2017 [abstract CT077].
3. Mok TS, Wu Y-L, Thongprasert S, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361:947–57.
4. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012;13:239–46.

5. Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-lung 3 and LUX-lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol. 2015;16:141–51.
6. Landi L, Tiseo M, Chiari R, et al. Activity of the EGFR-HER2 dual inhibitor afatinib in EGFR-mutant lung cancer patients with acquired resistance to reversible EGFR tyrosine kinase inhibitors. Clin Lung Cancer. 2014;15:411–7e414.
7. Tsao AS, Scagliotti GV, Bunn PA Jr, et al. Scientific advances in lung Cancer 2015. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2016;11:613–38.
8. Pikor LA, Ramnarine VR, Lam S, Lam WL. Genetic alterations defining NSCLC subtypes and their therapeutic implications. Lung Cancer. 2013;82:179–89.
9. Addeo A, Tabbo F, Robinson T, et al. Precision medicine in ALK rearranged NSCLC: a rapidly evolving scenario. Crit Rev Oncol Hematol. 2018;122:150–6.
10. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique Clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the Western population. Clin Cancer Res. 2009;15:5216–23.
11. Iwahara T, Fujimoto J, Wen D, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene. 1997;14:439–49.
12. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. Nat Rev Cancer. 2013;13:685–700.
13. Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 1994;263:1281–4.
14. Hallberg B, Palmer RH. The role of the ALK receptor in cancer biology. Ann Oncol. 2016;27:iii4–iii15.
15. von Laffert M, Warth A, Penzel R, et al. Multicenter immunohistochemical ALK-testing of non-small-cell lung cancer shows high concordance after harmonization of techniques and interpretation criteria. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2014;9:1685–92.
16. Guan J, Umapathy G, Yamazaki Y, et al. FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase. eLife. 2015;4:e09811.
17. Wu YL, Planchard D, Lu S, et al. Pan-Asian adapted clinical practice guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. Annals of Oncology : official journal of the European Society for Medical Oncology. 2019;30:171–210.
18. von Laffert M, Warth A, Penzel R, et al. Multicenter immunohistochemical ALK-testing of non-small-cell lung cancer shows high concordance after harmonization of techniques and interpretation criteria. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2014;9:1685–92.
19. Niu X, Chuang JC, Berry GJ, Wakelee HA. Anaplastic lymphoma kinase testing: IHC vs. FISH vs. NGS. Curr Treat Options in Oncol. 2017;18:71.
20. Weickhardt AJ, Aisner DL, Franklin WA, et al. Diagnostic assays for identification of anaplastic lymphoma kinase-positive non-small cell lung cancer. Cancer. 2013;119:1467–77.
21. Dacic S, Villaruz LC, Abberbock S, et al. ALK FISH patterns and the detection of ALK fusions by next generation sequencing in lung adenocarcinoma. Oncotarget. 2016;7:82943–52.
22. Camidge DR, Kono SA, Flacco A, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2010;16:5581–90.
23. Gao X, Sholl LM, Nishino M, et al. Clinical implications of variant ALK FISH rearrangement patterns. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2015;10:1648–52.
24. Lambros MB, Natrajan R, Reis-Filho JS. Chromogenic and fluorescent in situ hybridization in breast cancer. Hum Pathol. 2007;38:1105–22.
25. Tanner M, Gancberg D, Di Leo A, et al. Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples. Am J Pathol. 2000;157:1467–72.
26. Kim H, Yoo SB, Choe JY, et al. Detection of ALK gene rearrangement in non-small cell lung cancer: a comparison of fluorescence in situ hybridization and chromogenic in situ hybridization with correlation of ALK protein expression. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2011;6:1359–66.
27. Hutarew G, Hauser-Kronberger C, Strasser F, et al. Immunohistochemistry as a screening tool for ALK rearrangement in NSCLC: evaluation of five different ALK antibody clones and ALK FISH. Histopathology. 2014;65:398–407.
28. Marchetti A, Di Lorito A, Pace MV, et al. ALK protein analysis by IHC staining after recent regulatory changes: a comparison of two widely used approaches, revision of the literature, and a new testing algorithm. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2016;11:487–95.
29. Cutz JC, Craddock KJ, Torlakovic E, et al. Canadian anaplastic lymphoma kinase study: a model for multicenter standardization and optimization of ALK testing in lung cancer. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2014;9:1255–63.

30. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung Cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med. 2018;142:321–46.
31. Palmer RH, Vernersson E, Grabbe C, Hallberg B. Anaplastic lymphoma kinase: signalling in development and disease. Biochem J. 2009;420:345–61.
32. Wiesner T, Lee W, Obenauf AC, et al. Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. Nature. 2015;526:453–7.
33. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer. 2009;115:1723–33.
34. Pekar-Zlotin M, Hirsch FR, Soussan-Gutman L, et al. Fluorescence in situ hybridization, immunohistochemistry, and next-generation sequencing for detection of EML4-ALK rearrangement in lung cancer. Oncologist. 2015;20:316–22.
35. Soda M, Isobe K, Inoue A, et al. A prospective PCR-based screening for the EML4-ALK oncogene in non-small cell lung cancer. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2012;18:5682–9.
36. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med. 2010;363:1693–703.
37. Peled N, Palmer G, Hirsch FR, et al. Next-generation sequencing identifies and immunohistochemistry confirms a novel crizotinib-sensitive ALK rearrangement in a patient with metastatic non-small-cell lung cancer. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2012;7:e14–6.
38. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res. 2008;68:4971–6.
39. Beadling C, Wald AI, Warrick A, et al. A multiplexed amplicon approach for detecting gene fusions by next-generation sequencing. The Journal of Molecular Diagnostics : JMD. 2016;18:165–75.
40.•• Früh M, Peters S. EML4-ALK variant affects ALK resistance mutations. J Clin Oncol. 2018;36:1257–9.
The manuscript has highlighted clearly the importance of detecting and understand the role of the ALK variants and their clinical implications.
41.•• Lin JJ, Zhu VW, Yoda S, et al. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung Cancer. Journal of Clinical Oncology : official journal of the American Society of Clinical Oncology. 2018;36:1199–206.
Important manuscript that gave an insight on the resistance mechanism in ALK NSCLC patients.
42. Drilon A, Wang L, Arcila ME, et al. Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in lung adenocarcinomas otherwise negative for such alterations by other genomic testing approaches. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2015;21:3631–9.
43. Dagogo-Jack I, Brannon AR, Ferris LA, et al. Tracking the evolution of resistance to ALK tyrosine kinase inhibitors through longitudinal analysis of circulating tumor DNA. JCO Precis Oncol. 2018;2018.
44. Meric-Bernstam F, Brusco L, Shaw K, et al. Feasibility of large-scale genomic testing to facilitate enrollment onto Genomically matched clinical trials. Journal of Clinical Oncology : official journal of the American Society of Clinical Oncology. 2015;33:2753–62.
45. Gainor JF, Dardaei L, Yoda S, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung Cancer. Cancer Discov. 2016;6:1118–33.
46. Bordi P, Tiseo M, Rofi E, et al. Detection of ALK and KRAS mutations in circulating tumor DNA of patients with advanced ALK-positive NSCLC with disease progression during Crizotinib treatment. Clin Lung Cancer. 2017;18:692–7.
47. McCoach CE, Blakely CM, Banks KC, et al. Clinical utility of cell-free DNA for the detection of ALK fusions and genomic mechanisms of ALK inhibitor resistance in non-small cell lung Cancer. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2018;24:2758–70.
48. Nilsson RJ, Karachaliou N, Berenguer J, et al. Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer. Oncotarget. 2016;7:1066–75.
49. Hofman P. ALK status assessment with liquid biopsies of lung Cancer patients. Cancers. 2017;9.
50. Camidge DR, Theodoro M, Maxson DA, et al. Correlations between the percentage of tumor cells showing an anaplastic lymphoma kinase (ALK) gene rearrangement, ALK signal copy number, and response to crizotinib therapy in ALK fluorescence in situ hybridization-positive nonsmall cell lung cancer. Cancer. 2012;118:4486–94.
51. Cabillic F, Gros A, Dugay F, et al. Parallel FISH and immunohistochemical studies of ALK status in 3244 non-small-cell lung cancers reveal major discordances. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2014;9:295–306.
52. Camidge DR, Kono SA, Lu X, et al. Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2011;6:774–80.

53. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368:2385–94.
54. Shaw AT, Janne PA, Besse B, et al. Crizotinib vs chemotherapy in ALK+ advanced non-small cell lung cancer (NSCLC): final survival results from PROFILE 1007. J Clin Oncol. 2016;34:9066–6.
55. Solomon BJ, Mok T, Kim D-W, et al. First-line Crizotinib versus chemotherapy in ALK-positive lung Cancer. N Engl J Med. 2014;371:2167–77.
56. Solomon BJ, Kim DW, Wu YL, et al. Final overall survival analysis from a study comparing first-line Crizotinib versus chemotherapy in ALK-mutation-positive non-small-cell lung Cancer. Journal of Clinical Oncology : official journal of the American Society of Clinical Oncology. 2018;36:2251–8.
57. Duruisseaux M, Besse B, Cadranel J, et al. Overall survival with crizotinib and next-generation ALK inhibitors in ALK-positive non-small-cell lung cancer (IFCT-1302 CLINALK): a French nationwide cohort retrospective study. Oncotarget. 2017;8:21903–17.
58. Huang D, Kim DW, Kotsakis A, et al. Multiplexed deep sequencing analysis of ALK kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment. Genomics. 2013;102:157–62.
59. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2012;18:1472–82.
60. Yoshida T, Oya Y, Tanaka K, et al. Clinical impact of crizotinib on central nervous system progression in ALK-positive non-small lung cancer. Lung Cancer. 2016;97:43–7.
61. Shaw AT, Kim TM, Crino L, et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2017;18:874–86.
62. Novello S, Mazieres J, Oh IJ, et al. Alectinib versus chemotherapy in crizotinib-pretreated anaplastic lymphoma kinase (ALK)-positive non-small-cell lung cancer: results from the phase III ALUR study. Annals of Oncology : official journal of the European Society for Medical Oncology. 2018;29:1409–16.
63. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. Lancet. 2017;389:917–29.
64. Hida T, Nokihara H, Kondo M, et al. Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. Lancet. 2017;390:29–39.
65. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus Crizotinib in untreated ALK-positive non-small-cell lung Cancer. N Engl J Med. 2017;377:829–38.
66. Zhou C, Kim SW, Reungwetwattana T, et al. Alectinib versus crizotinib in untreated Asian patients with anaplastic lymphoma kinase-positive non-small-cell lung cancer (ALESIA): a randomised phase 3 study. The lancet. Respir Med. 2019;7:437–46.
67. Camidge DR, Peters S, Mok T, et al. Updated efficacy and safety data from the global phase III ALEX study of alectinib (ALC) vs crizotinib (CZ) in untreated advanced ALK+ NSCLC. J Clin Oncol. 2018;36:9043–3.
68. Lin JJ, Zhu VW, Schoenfeld AJ, et al. Brigatinib in patients with Alectinib-refractory ALK-positive NSCLC. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2018;13:1530–8.
69. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus Crizotinib in ALK-positive non-small-cell lung Cancer. N Engl J Med. 2018;379:2027–39.
70.•• Zou HY, Friboulet L, Kodack DP, et al. PF-06463922, an ALK/ROS1 inhibitor, overcomes resistance to first and second generation ALK inhibitors in preclinical models. Cancer Cell. 2015;28:70–81.

Manuscript that showed the importance to better understand the resistant mechanism to be able to overcome them.

71. Shaw AT, Felip E, Bauer TM, et al. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. Lancet Oncol. 2017;18:1590–9.
72. Shaw AT, Solomon BJ, Besse B, et al. ALK Resistance Mutations and efficacy of Lorlatinib in advanced anaplastic lymphoma kinase-positive non-small-cell lung Cancer. Journal of Clinical Oncology : official journal of the American Society of Clinical Oncology. 2019:JCO1802236.
73. Gainor JF, Shaw AT, Sequist LV, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung Cancer: a retrospective analysis. Clinical Cancer Research : an official journal of the American Association for Cancer Research. 2016;22:4585–93.
74. Addeo A, Banna GL. PROS: should immunotherapy be incorporated in the treatment of oncogene-driven lung cancer? Transl Lung Cancer Res. 2018;7:S287–9.
75. Addeo A, Banna GL. PROS: should immunotherapy be incorporated in the treatment of oncogene-driven lung cancer? Transl Lung cancer Res. 2018;7:S283–6.
76. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung Cancer. N Engl J Med. 2015;373:1627–39.
77. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung Cancer-a meta-analysis. Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer. 2017;12:403–7.
78. Garassino MC, Cho BC, Kim JH, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. Lancet Oncol. 2018;19:521–36.

79. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 2018;378:2288–301.
80. Gadgeel SM. Sequencing of ALK inhibitors in ALK+ non-small cell lung Cancer. Curr Treat Options in Oncol. 2017;18:36.
81. Shaw AT, Solomon BJ, Besse B, et al. ALK resistance mutations and efficacy of Lorlatinib in advanced anaplastic lymphoma kinase-positive non–small-cell lung Cancer. J Clin Oncol. 2019;37:1370–9.
82. Basler L, Kroeze SG, Guckenberger M. SBRT for oligoprogressive oncogene addicted NSCLC. Lung Cancer. 2017;106:50–7.

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