Review Article

ALK fusions turn sixteen in lung cancer: a review on their biology, detection and therapy

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Abstract: During the last two decades, the field of targeted therapy against oncogene-driven tumors has witnessed meaningful and accelerated advancements. Ever since the discovery of *ALK* (anaplastic lymphoma kinase) rearrangements as driver events for non-small cell lung cancer (NSCLC) in 2007, targeting ALK has become a model of precision oncology. Especially, the recognition of crizotinib as an active and effective tyrosine kinase inhibitor (TKI) for ALK-positive NSCLC defined the proof-of-concept that ALK inhibition had important clinical value. Furthermore, a better understanding of crizotinib liabilities and resistance mechanisms prompted the development of the next generations of ALK inhibitors, leading to a shift in treatment strategy, from a sequential approach to a "new generation agents upfront" one. The continuous interrogation of resistance mechanisms to the novel inhibitors is the key feature to improving patients' outcomes through the development of novel treatment strategies. From this perspective, the evolution of ALK inhibition in NSCLC could be defined as a cornerstone for the clinical improvements achievable in patients suffering from oncogene-driven tumors. In this review, we approach the entity of *ALK* fusions in lung cancer reporting the mechanisms conferring oncogenic competence, and describing the molecular diagnostic tools to detect them in the clinical practice. We then report how new generations of ALK TKIs are developed in a continuous effort to overcome the resistance to previous agents, and how these novel drugs have shaped the current therapeutic landscape for patients with ALK-positive lung cancer.

Keywords: Anaplastic lymphoma kinase (ALK); tyrosine kinase inhibitors (TKIs); alectinib; brigatinib; lorlatinib

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Introduction

The identification of molecular events driving malignant cell fate, from initiation to aggressiveness and metastasis, fostered the development of targeted anticancer agents improving patients' outcomes. Moving from the seminal model of chronic myeloid leukemia driven by *BCR*::*ABL*

* The senior author of this review sadly passed away before its publication. His enthusiasm, dedication and competence as a doctor and a researcher will not be forgotten. The authors and the guest editors remember Dr. Giulio Rossi as an extraordinary colleague, mentor, and friend.

fusion, susceptible to the inhibition by imatinib and following generations of tyrosine kinase inhibitors (TKIs) (1), several targetable drivers were identified in non-small cell lung cancer (NSCLC). It is estimated that approximately 50% of non-squamous NSCLC harbor an oncogenic driver (2), translating in the possibility that half of the patients suffering from NSCLC can benefit from targeted treatments. Chronologically, after the recognition of mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) as the molecular marker of sensitivity to EGFR-TKI (3,4) In NSCLC, *ALK* (anaplastic lymphoma kinase) rearrangements emerged as the second targetable driver and especially, as the first one as a gene fusion (5). ALK inhibition in NSCLC represents a model for precision oncology, given the impressive clinical benefit achievable in patients suffering from ALK-positive NSCLC after the introduction of specific TKI. The understanding of the biological underpinnings of ALK as an oncogenic driver and the corresponding molecular elements responsible for resistance to targeted agents are the core elements for improving outcomes in this molecularly-defined subset of patients. The evolution in the field of ALK inhibition in NSCLC can be considered a model for: (I) additional fusion-driven lung cancer (namely ROS1-positive NSCLC); (II) other ALK-dependent malignancies, where *ALK* alterations are pathognomonic [i.e., anaplastic large cell lymphoma (ALCL), neuroblastoma, inflammatory myofibroblastic tumor] (6); (III) diseases harboring *ALK* fusions in a relatively small fraction of cases, detectable with the wide utilization of molecular diagnostic techniques across histologies, suggesting a tumor "agnostic" treatment (7); (IV) generally, oncogene-driven malignancies in which the development of targeted agents and treatment strategies can retrace the one adopted for *ALK*-rearranged NSCLC. In this review, we aim to provide the context for which *ALK* fusions in lung tumors are such a model for precision oncology, approaching their biological features and addressing pragmatic aspects, namely in terms of molecular diagnostic and treatment.

Biology of *ALK* fusion in NSCLC

Defining ALK as a driver oncogene: historical evidence

ALK gene was firstly identified in the genetic context of a gene fusion in the pathognomonic ALCL. The recognition of a chromosomal translocation between the short arm of chromosome 2 and the long arm of chromosome 5, t(2;5)(p23;q35), led to the identification of the fusion gene involving *NPM* (nucleophosmin, mapped on 5q35) and *ALK* (2p23) (8).

The discovery and initial characterization of *ALK* fusions as oncogenic events in NSCLC date back to 2007. Starting from a surgical patient sample, Soda *et al.* identified an *ALK* fusion with *EML4* (echinoderm microtubule-associated protein-like 4) gene (*EML4*::*ALK*) after RNA extraction, cloning procedures and retroviral infection in 3T3 fibroblasts (5). Using a phosphoproteomic approach, Rikova *et al.* recognized high-level of ALK phosphorylation across a wide number of lung cancer tumor specimens and cell lines, in which the presence of *ALK* fusions (with either *EML4* or *TGF* as partner genes) was confirmed (9). Of interest, this latter study was the first to identify *ROS1* fusions across malignancies, paving the way for the development of precision medicine in this molecular subtype as well. Soda *et al.* confirmed the transforming activity of ALK fusions (with both *EML4* and *NPM* as partner genes) in 3T3 mouse fibroblasts, by *in vitro* and *in vivo* growth assays. Moreover, Ba/F3 models engineered to express EML4::ALK were able to proliferate in the absence of IL-3, suggesting the oncogenic competence of the fusion protein (5).

ALK fusions in NSCLC: from structure to function

As anticipated, the genomic events leading to *ALK* fusion belong to chromosomal translocations. The variety of *ALK* fusion partners in NSCLC is extremely rich and constantly updated (10). With regard to the most frequent fusion partner of *ALK* in NSCLC, *EML4* maps on the short arm of chromosome 2 (2p), implying that an intrachromosomal rearrangement occur in this case (5).

While *EML4* gene has different breakpoints (giving rise to different fusion variants, from v1 to v5), *ALK* breakpoint is conserved, located after the juxta-membrane domain and, most importantly, before the tyrosine kinase domain (*Figure 1*). Therefore, the fusion transcript contains the N-terminal, 5' extremity of *EML4* and the C-terminal, 3' extremity of *ALK*. This general structure of the fusion transcript is maintained whichever the *ALK* fusion partner and the disease, in case of inter- or intra-chromosomal rearrangements. The key event is indeed represented by the conservation of a fully intact tyrosine kinase domain of ALK (*Figure 1*).

ALK fusions harbor oncogenic potential for two main reasons. The first explanation can be found in the physiological role and the expression of ALK protein in mammalian tissues. Whereas ALK seems to have a role

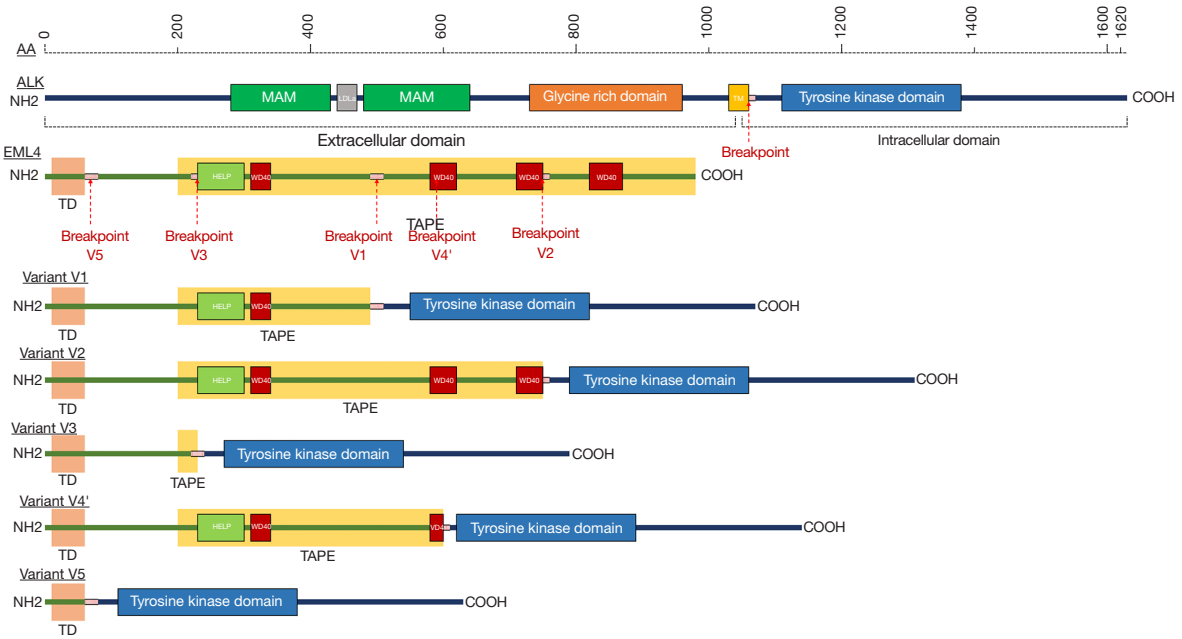


Figure 1 Schematic representation of *ALK*, *EML4* and *EML4::ALK* fusions. ALK domain: meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu (MAM), low density lipoprotein domain (LDLa), transmembrane domain (TM). EML4 domain: trimerisation domain (TD), hydrophobic EML protein (HELP), tandem atypical propeller in EMLs (TAPE). Genomic breakpoints are hatched in red.

in central and peripheral nervous system development, mRNA expression in adult human tissues is limited to the enteric innervation, brain, testis and placenta. ALK protein is detected in brain neurons, pericytes and endothelial cells (11,12). ALK expression across the body is indeed regulated by a negative promoter. The chromosomic rearrangements generating gene fusions release the tyrosine kinase domain of ALK from this transcriptional control. The second reason relies on the activation of ALK signaling in the presence of receptor dimerization. Through its coiled-coil domain, EML4 sustains dimerization of the EML4::ALK fusion protein, leading to constitutive intracellular signaling (5). This latter is mediated by molecular pathways such as MAPK, PI3K/mTOR, JAK/STAT, PLCγ, relying on the cascade phosphorylation of effectors and mediators towards the cellular nucleus, where transcription factors implied in transformation are recruited, starting and maintaining a malignant phenotype (13).

Molecular diagnostics for the detection of *ALK* fusions in NSCLC

Since the original report by Soda *et al.* in 2007 (5), several studies have investigated the presence of *ALK* rearrangements in NSCLC with different techniques (14-16). *ALK* fusions mainly occur in adenocarcinomas showing a solid, acinar or cribriform pattern, often accompanied by the finding of a proportion of "signet ring" cells with intracytoplasmic mucus production (17-19). *ALK*-rearranged adenocarcinomas generally express TTF1 and napsin A and are negative for p63/p40 (17,19). Nevertheless, none of these morphologic or immunohistochemical features are *per se* indicative of the presence of *ALK* rearrangement, and even different histologies of NSCLC, including squamous cell carcinoma, should be tested for *ALK* fusions when dealing with light/never smokers or young patients, particularly in small biopsies when an adenocarcinoma component could be missed (20). At the molecular level, although *ALK* rearrangements are regarded as essentially mutually exclusive with genetic alterations in other oncogenic drivers (21), reports demonstrated the occurrence of concomitant *ALK* rearrangements with *EGFR*, *KRAS* or other druggable or non-druggable oncogenic drivers (22-24).

Approval for the use of ALK inhibitors in routine management of patients with ALK-positive NSCLC by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) is regulated

Table 1 Characteristics of different technologies used in routine practice to detect *ALK* gene fusion in NSCLC

Methods	Sensibility	Specificity	Advantages	Limitations	Tumor sampling	Principal setting
IHC	>95%	>95%	The only IHC test with direct access to TKI in case of positivity; rapid TAT; limited amount of tissue; each batch in automated immunostainer; standardized protocol; technique available worldwide in routine practice; easy and objective interpretation by pathologists	Single test; surrogate detection method (heterogeneous partner gene-dependent performance); be aware on primary Ab clone used (ALK D5F3 companion diagnostic is the most performant); does not recognize gene fusion partner or variant	FFPE tissue (including cell block) and cytology	ALK rearrangement screening tool; orthogonal technique flanking NGS or other methods in indeterminate/ doubtful results
FISH	85–90%	95%	*In situ* assay; correspondence with morphology; utilization of smeared cytological samples, not suitable for IHC; better understanding of the biology behind the rearrangement (deletion/insertion/inversion); quantitative evaluation of the percentage of rearranged cells	Expensive method; false positives/false negatives; long TAT (workflow: 2 days); require expertise (subjective interpretation); single gene assay; do not recognize gene fusion partner or variant	FFPE tissue (including cell block) and cytology	Orthogonal technique flanking NGS or other methods in indeterminate/ doubtful results
RT-PCR and multiplex RT-PCR	92.4% (FFPE)	97.8% (FFPE)	Simple and relatively inexpensive method Rapid TAT (workflow: 2–3 h + extraction time)	Variable yield in RNA extraction from FFPE samples; detect only known and common fusion partner, miss rare variants (only imbalance expression detected)	FFPE tissue (including cell block) and cytology; plasma	
NGS	100%	97.7%	Require small RNA amount; qualitative and quantitative assay; detect known and novel fusion partner, complex fusions and rare variants, multiplex assay; dual detection strategy (imbalance expression and specific fusion partner)	Variable yield in RNA extraction from FFPE samples; laborious method; long TAT (workflow: 3–4 days + extraction time) require expertise	FFPE tissue (including cell block) and cytology; plasma DNA	
IdyllaTM*	97%	100%	User friendly; direct FFPE tissue input (does not require RNA extraction); minimal tissue requirement; rapid TAT (workflow: 3 h); multiplex assay	Detect only known and common fusion partner, miss rare variants	FFPE tissue (including cell block) and cytology	
NanoString*	97%	99%	Multiplex assay testing; dual detection strategy (imbalance expression and specific fusion partner)	Technologies not fully implemented in the clinical setting; require expertise; high cost	FFPE tissue	

*, clinical utility and performance of an ultrarapid multiplex RNA-based assay for detection of ALK, ROS1, RET, and NTRK1/2/3 rearrangements and MET Exon 14 skipping alterations. NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; TKI, tyrosine kinase inhibitor; TAT, tourn-around-time; FFPE, formalin-fixed paraffin-embedded; NGS, next-generation sequencing; FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase polymerase chain reaction.

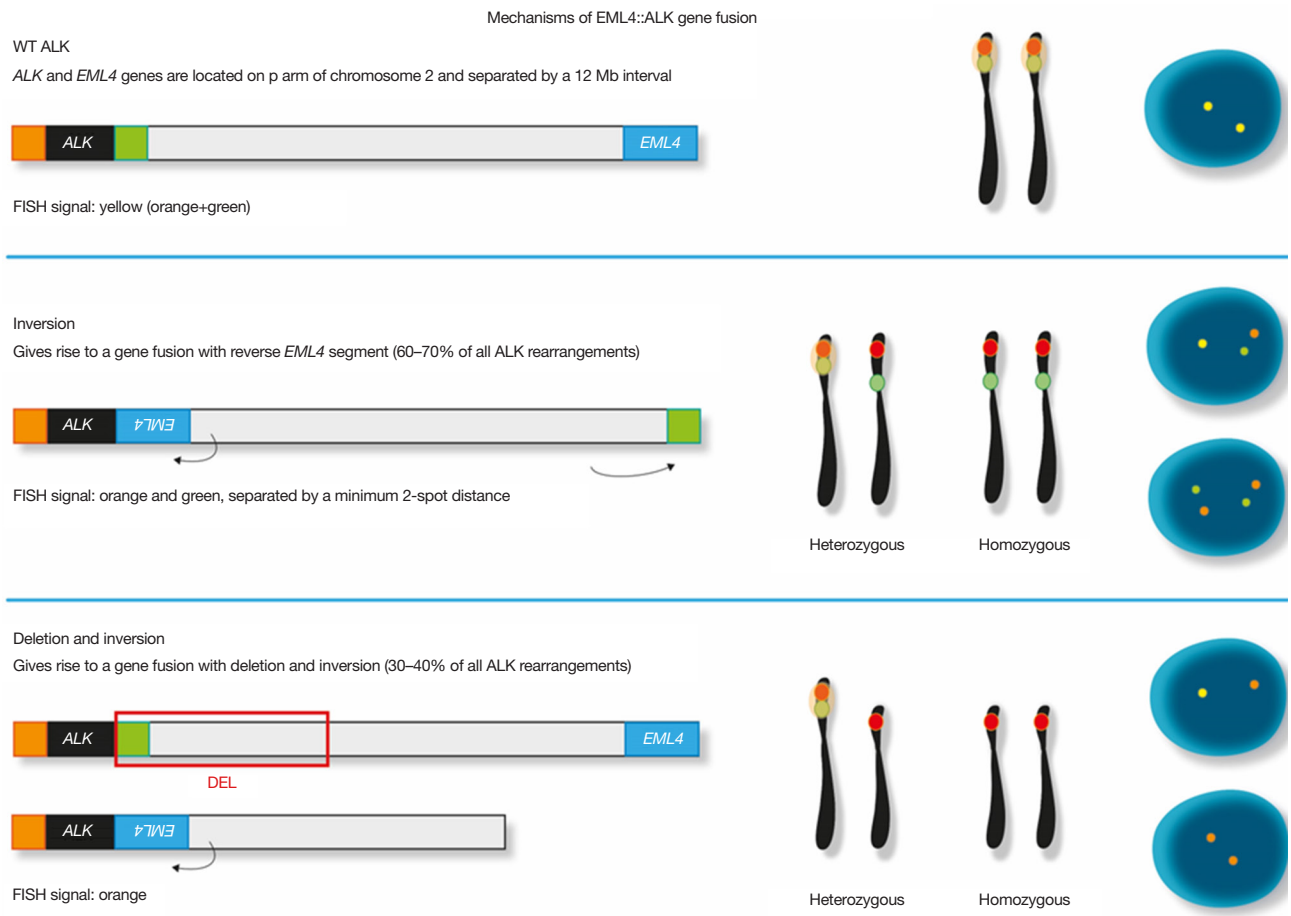


Figure 2 Schematic representation of the FISH read-outs of *EML4::ALK* gene fusions. FISH, fluorescence in situ hybridization; ALK, anaplastic lymphoma kinase.

by the preliminary detection of *ALK* gene fusion by using fluorescence in situ hybridization (FISH) assay, immunohistochemistry (IHC), extractive technologies or integrated approaches (*Table 1*) (14,16).

FISH

In brief, FISH has been initially considered the gold standard technique in the first clinical trials with crizotinib (14-16,25), but this method is technically challenging, somehow difficult to interpret, then requiring specific expertise, and relatively expensive. ALK Break Apart FISH Probe Kit was used to detect ALK breakage and a minimum of 50 non-overlapping cancer cell nuclei should be examined for each case. The interpretation of FISH signals is performed according to the criteria suggested by the international guidelines (14-16,25), and the rearrangement-positive cells were defined as those with split signals or isolated red (3') signals with a frequency ≥15% of tumor cells (*Figures 2,3*).

IHC

Evidence of the role of IHC in detection of ALK protein expression and its advantages over FISH in terms of availability and costs subsequently emerged (14,26-29). Among various primary antibodies clones, D5F3 showed the best performance, particularly when used inside the Ventana ALK (D5F3) companion diagnostic (CDx) (*Figure 4*) (27-31). Of note, several experiences have underlined the superiority of IHC over FISH in predicting ALK positivity and efficacy of ALK inhibitors (32-34).

In the recent past, several algorithms incorporating the coordinated use of FISH and IHC have been developed aiming at the correct identification of *ALK* gene fusion in

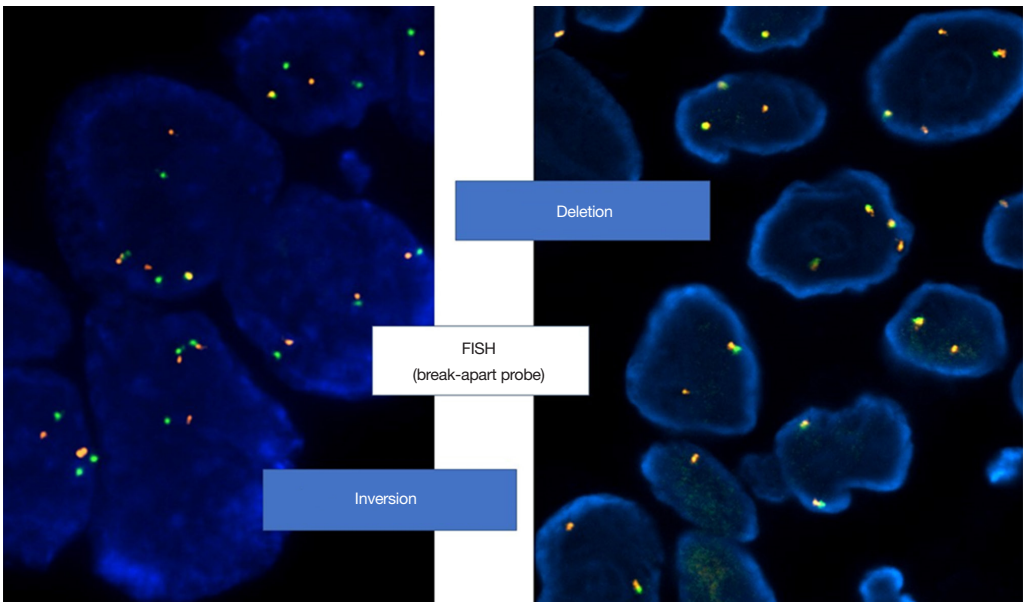


Figure 3 ALK positivity by FISH may be quoted evidencing split signals by inversion with separate green and red spots (rearranged cells have a distance of at least two spots between green and red signals, on left) or a single red spot due to deletion (on right). ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization.

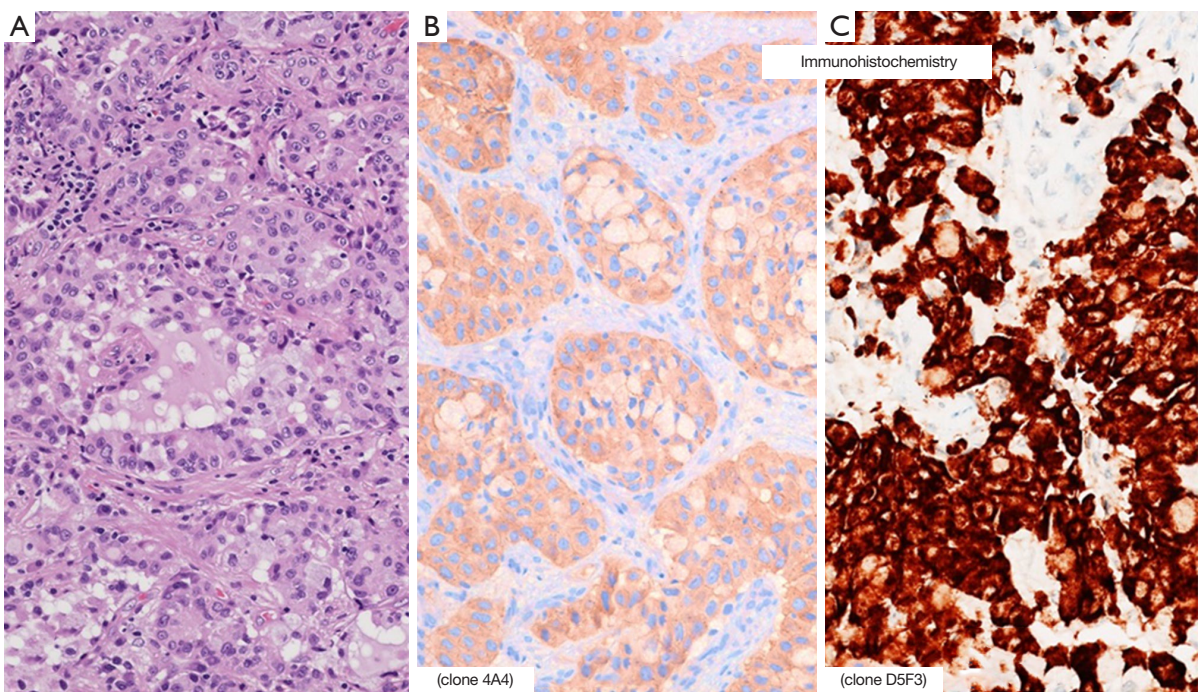


Figure 4 An invasive adenocarcinoma with signet-ring cells (A, haematoxylin-eosin stain ×200) expressing ALK protein with a diffuse but moderate intensity using clone 4A4 (B, immunohistochemistry, ×200) and diffuse, strong intensity using clone D5F3 (C, immunohistochemistry, ×250). ALK, anaplastic lymphoma kinase.

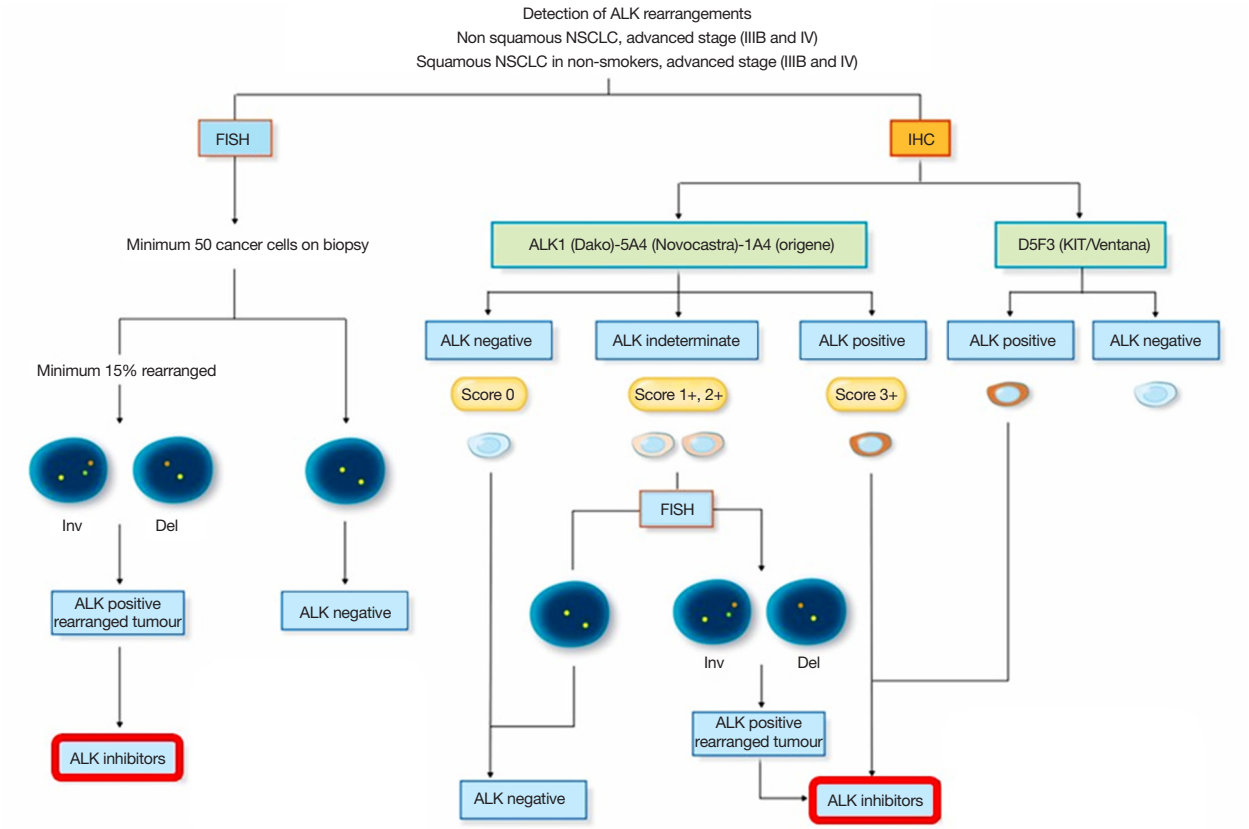


Figure 5 Proposal of an algorithm for the molecular diagnosis of *ALK* fusions in NSCLC. NSCLC, non-small cell lung cancer; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ALK, anaplastic lymphoma kinase.

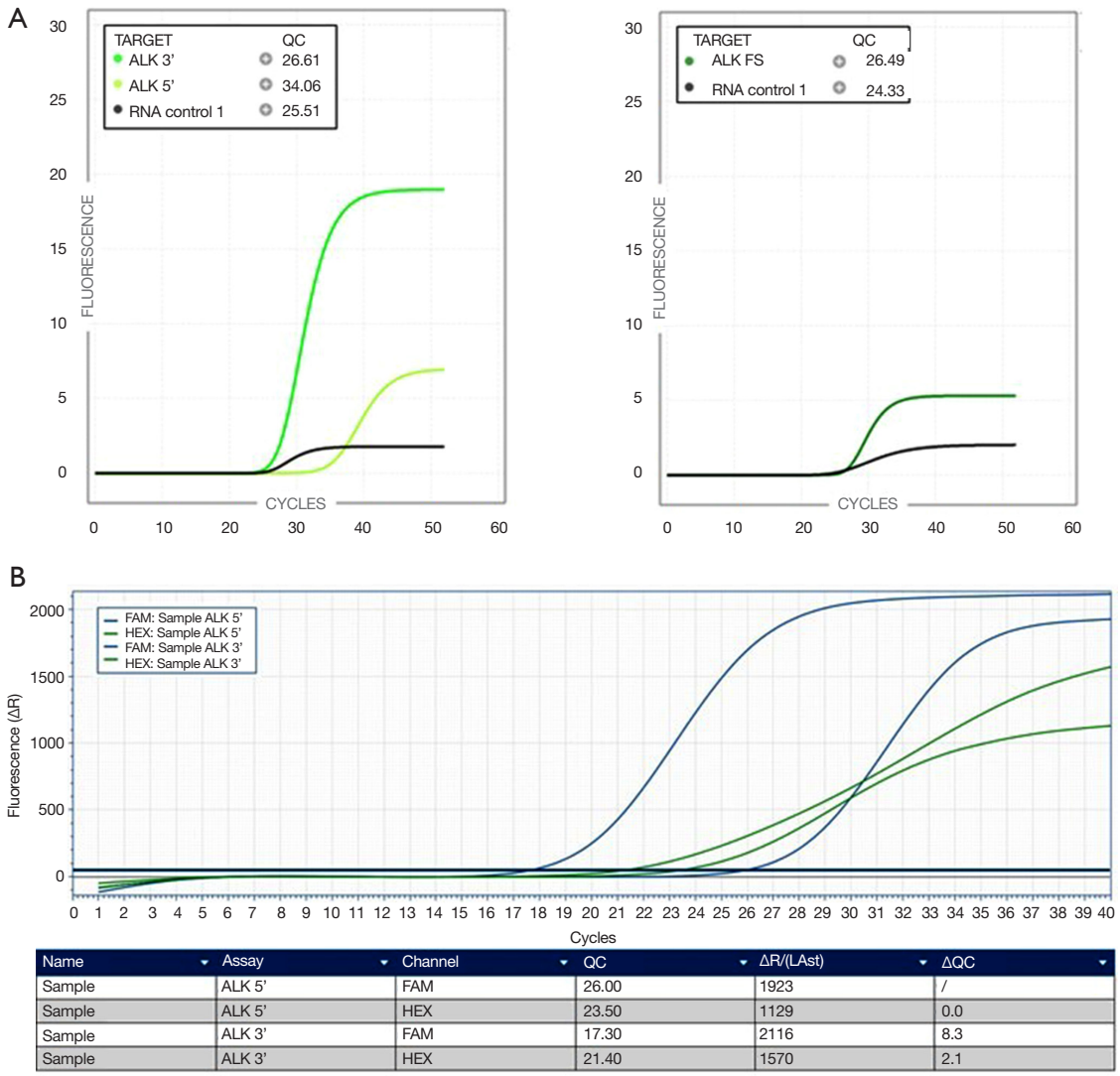
every single patient potentially candidate to ALK inhibitors (*Figure 5*).

Reverse transcriptase polymerase chain reaction (RT-PCR)

Among extractive molecular technologies, RT-PCR is a reliable, sensitive and specific method to detect *ALK* gene rearrangements. On the other hand, this technique requires some skills and availability of good quality RNA, since tumor tissue generally derive from formalin-fixed, paraffin-embedded (FFPE) samples (30). In addition, uncommon variants of *ALK* gene fusion may be uncovered. A meta-analysis on 18 articles including 21 studies and involving 2,800 samples from NSCLC patients showed an overall pooled sensitivity of 92.4% and specificity of 97.8% (35).

Nano String assay is a technology that detects known fusion gene transcripts on FFPE tissue by combination of 3' overexpression and fusion-specific detection probes (36). This technology acts as a multiplexed mRNA-based assay and shows a great concordance rate when compared with IHC (98%) and FISH (87.5–100%) for ALK gene fusion, with a specificity of 98.8%. Nevertheless, the assay requires a high content of RNA (100–200 nanograms) and data on cytology samples have not been reported so far (36,37).

More recently, Idylla™ GeneFusion assay (a rapid and fully-automated platform simultaneously detecting *ALK*, *ROS1*, *RET* and *NTRK1/2/3* and *MET* exon 14 skipping mutations) showed a 100% sensitivity in detecting fusions of *ALK*, *ROS1*, *RET*, *NTRK1*, and *MET* exon 14 skipping and 83% sensitivity for *NTRK2/3* fusions, when compared with next generation sequencing (NGS) (*Figure 6*) (38-40). Specificity was 100% in detecting fusions of *ROS1*, *RET*, *NTRK2/3*, and *MET* exon 14 skipping, while 98% was reported for *ALK* (38-40). The assay is very versatile, successfully performing with biopsy and cell blocks even when the sample has 5% of tumor content or cytology smears with at least 300 cells and extracted RNA of 20 ng (39).



Name	Assay	Channel	QC	ΔR/(LAst)	ΔQC
Sample	ALK 5'	FAM	26.00	1923	/
Sample	ALK 5'	HEX	23.50	1129	0.0
Sample	ALK 3'	FAM	17.30	2116	8.3
Sample	ALK 3'	HEX	21.40	1570	2.1

Figure 6 RT-PCR: Idylla *GeneFusion ASSAY* (Idylla™ GeneFusion) with plots showing (A, on right) *ALK* fusion-specific amplicon (ALK FS) and (A, on left) 3'/5'Expression Imbalance (ALK 3' and ALK 5'). *EasyPGX ready ALK/ROS1/RET/MET* showing ALK 3'-5' Expression Imbalance (FAM, blue curves) and corresponding endogenous control gene (HEX, green curves) (B). ALK, anaplastic lymphoma kinase; RT-PCR, reverse transcriptase polymerase chain reaction; FAM, fluorescein amidites; HEX, hexachlorofluorescein; QC, quality control.

Next generation sequencing

In agreement with international recommendations, ALK testing is just one among several other druggable targets that should be tested in the current clinical practice, including mutations (*EGFR*, *BRAF*, *MET*, *KRAS*, *HER2)* and rearrangements *(ROS1*, *RET*, *NTRK1/2/3)* (41-43). Then, it is essential to have a throughout technology detecting all predictive molecular biomarkers from the same tumor sample. The development of fusion gene panels available into NGS platforms is becoming relatively cost-effective and this technology is becoming the gold standard permitting to cover all the predictive molecular biomarkers at once (44-46).

Although preanalytical factors (including the expertise of molecular biologist, the sample type, the timing and type of fixation) and postanalytical factors may significantly impact on the quality of the final results, NGS has been suggested as the new "gold-standard" in profiling NSCLC for predictive molecular determinations, including *ALK* gene fusions (46).

Several studies using amplicon-based or hybrid capture-based NGS demonstrated a very high sensitivity (100%) and

A

Reads On Target	% Reads On Target	Control CNIH1_ex1-3	Control SRSF4_ex1-3	Control VPS29_ex2-3
120516	0.4242	21498	16176	39830

Result	Alk 3'-5' Expression Imbalance analysis	Warning
Presenza di fusioni del gene Alk	Positive	PASS

5' Gene	5' Exon	3' Gene	3' Exon	HGVS Name	Breakpoint	Reads	R	Warning	Fusion Type
EML4	ex13	ALK	ex20	NM_019063.5:r.-259_1489::NM_004304.5:r.3174_*450	chr2:42522656;chr2:29446394	4250	1\6	PASS	Annotated

B

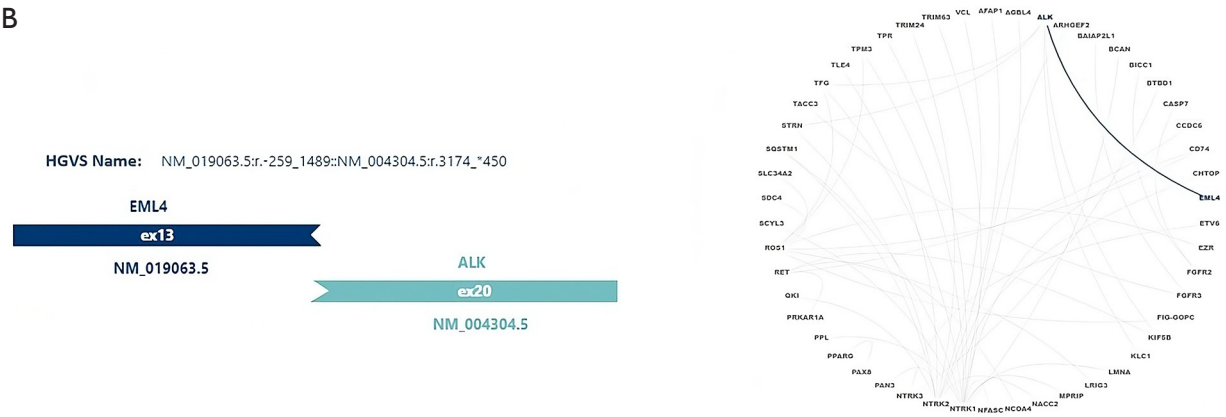


Figure 7 NGS: (myriapod NGS cancer panel RNA) myriapod NGS Data Analysis Software showing quality control metrics (A), ALK 3'-5' expression imbalance analysis and a report of *ALK* fusion-specific partner (B). HGVS, Human Genome Variation Society; NGS, next-generation sequencing.

specificity (>98%) when compared with IHC and/or FISH in ALK-positive NSCLC. Most importantly, NGS permits to discover novel and complex *ALK* gene fusion partners not detected by FISH and/or RT-PCR (*Figure 7*) (46).

Interestingly, in a recent real-world ALK biomarker testing of patients with NSCLC in the United States, among 60,025 eligible patients, tumors from 36,691 (61.1%) patients were tested for *ALK* rearrangements, with 1,042 (2.8%) positive analyses (47). Of note, the rate of ALK testing increased from 33.1% in 2011 to 73.0% in 2019. ALK testing rates increased over time for patients either with non-squamous (41.6–81.6%) and squamous histology (13.6–50.4%) (47). The proportion of tests performed using FISH declined from 83.8% in 2012 to 32.1% in 2019. Conversely, the proportion of tests performed using NGS increased from 0.2% in 2011 to 52.2% in 2019. Again, the proportion of ALK tests performed using liquid biopsy increased from 0.1% in 2011 to 28.2% in 2019, whereas the use of tissue samples decreased from 98.0% in 2011 to 71.2% in 2019 (47). Indeed, the identification of actionable oncogenic drivers, including *ALK* gene fusions, on liquid biopsy could represent another method to investigate an alternative source of tumor tissue, particularly when patients present with a high tumor burden. Although *ALK* rearrangements on liquid biopsy does not represent a real-life practice and may lead to false negative results due to insufficient sensitivity, hybrid-capture NGS on plasma circulating free DNA has demonstrated the feasibility of this approach in determining *EML4*::*ALK* variants in clinical trials (48).

Since the discovery of *EML4*::*ALK* rearrangement, several gene fusions (e.g., *ROS1*, *NTRK1-3*, *RET*, *NRG1*, *NUT*, *FGFR1*, *FGFR2*, *MET*, *BRAF*, *EGFR*, *SMARCA4*) have been identified in lung cancer, also increasing the number of fusion partner genes. These findings, leading to the development of new and promising therapies, mandated the development and implementation of dedicated, comprehensive gene fusions detection methods (38,49). Sequential approaches have been developed using anchored, multiplexed, PCR-based targeted RNA sequencing (RNA NGS) when no oncogenic drivers alterations were detected by DNA-based targeted cancer hotspot NGS, and this strategy was proven particularly efficient in smoking-associated NSCLC (50).

Therapy

Defining ALK as a targetable oncogene: historical evidence

As mentioned in the section dedicated to the biology of *ALK* fusions in NSCLC, their first evidence dates back to 2007. It is also in the same year that we witnessed the first proofs of the possibility of targeted ALK inhibition. The apparently immediate availability of crizotinib (initially known as PF-2341066) was due to its initial development as a MET inhibitor (51). Crizotinib turned out to be active against ALK in models of ALCL (52), neuroblastoma and NSCLC (53). This initial preclinical evidence was rapidly followed by the pivotal phase I clinical trial PROFILE 1001. After defining the maximum tolerated dose of 250 mg twice daily in the dose escalating phase (54), dose expansion cohorts were dedicated to ALK-positive, ROS1-positive, and *MET*-amplified NSCLC (55-57). Positive evidence of the role of crizotinib in ALK-positive ALCL and other ALK-driven diseases was also produced (58-60). Besides providing the proof-of-concept of ALK inhibition in the field of precision medicine, crizotinib represented a crucial agent for the treatment of patients suffering from ALK-positive NSCLC, and still figures among the standards of care for ALCL, neuroblastoma, and inflammatory myofibroblastic tumor.

Crizotinib

Three phase 3 randomized trials defined the role of crizotinib in patients suffering from advanced, ALK-positive NSCLC either after platinum-based regimens [PROFILE 1007 (61)], or as the first-line therapy [PROFILE 1014 (62), PROFILE 1029 (63)]. These trials clearly reported the superiority of crizotinib over chemotherapy in terms of activity [objective response rate (ORR) and progression-free survival (PFS)], while overall survival (OS) did not differ significatively between the two arms. The final OS results of PROFILE 1014 confirmed that the apparent lack of efficacy benefit from crizotinib was due to the fact that the majority of patients initially randomized to chemotherapy received crizotinib at progression (64). The latter study confirmed that the longest survival benefit was obtained by patients receiving crizotinib followed by a new generation ALK TKI.

Resistance mechanisms to crizotinib and development of second-/third-generation inhibitors

The molecular analyses performed on patient samples progressing on crizotinib was frequently associated with functional studies to confirm the putative role of potential mechanism of resistance, and to suggest treatment strategies to restore sensitivity. The evidence of molecular mechanisms of resistance to crizotinib (and lately to new generation inhibitors) relied on case reports, case series and landscape studies, these latter providing a view on the resistance pattern most frequently observed across patients (65-71). Of note, the molecular analyses were mainly performed on tissue biopsies, as circulating tumor DNA (ctDNA) technologies were not fully developed at the time of the major studies of resistance to ALK inhibitors. Nevertheless, liquid biopsy has recently become a valid tool to assess resistance in this setting as well (72).

Schematically, the mechanism of resistance to crizotinib and ALK inhibitors can converge into four main types. Importantly, these categories can be seen applied to other targets and their corresponding inhibitors.

(I) On-target mechanisms of resistance: mutations occurring in the tyrosine kinase domain of ALK, or amplification of the rearranged copy of *ALK* (66,67). In line with other resistance mutations arising in the kinase domain of receptor tyrosine kinases, *ALK* mutations confer resistance by generating a steric hindrance, precluding the TKI from entering into its binding site, and/or by increasing the basal activity of the receptor itself, by increasing ATP-affinity. In contrast with EGFR, where the gatekeeper mutation T790M represent the almost unique mutation observed at resistance to first-/second-generation EGFR-TKI (73,74), ALK-TKI resistance mutations are more variegated. For the scope of this review we mention ALK G1202R as the most recalcitrant (i.e., conferring the highest levels of resistance in functional assays) and the most frequently observed at progression to second-generation ALK inhibitors (69,70).

(II) Off-target mechanisms of resistance: activation of signaling pathways by molecular events (mutations, fusions, amplifications, non-genetic functional hyper-activation) occurring as bypass (e.g., EGFR, MET activation) or downstream alterations (e.g., *RAS*, *SRC* mutations) (66,68,75-77).

(III) Mechanism globally related to cellular plasticity, likely implying epigenetic modifications acting through phenotypic changes such as epithelial-to-mesenchymal transition (69), acquisition of neuroendocrine phenotypes and small-cell lung cancer features (78,79), modulation of expression

and the activity of the effectors and regulators of apoptotic processes (80-82).

(IV) Of greatest importance in the field of ALK-positive NSCLC, central nervous system (CNS) progression. ALK-driven lung cancers have a peculiar brain tropism as shown by the high rate of CNS metastases at diagnosis, and CNS is a frequent site of progression to crizotinib, due to the limited capability of the first-generation TKI to cross the blood-brain barrier (83).

Second- (ceritinib, alectinib, brigatinib, ensartinib), and third- (lorlatinib) generation ALK inhibitors have been developed to satisfy particular characteristics, inspired by the resistance mechanisms just reported:

(I) Activity against mutations occurring in ALK tyrosine kinase domain. While second-generation inhibitors can overcome specific ALK mutations, lorlatinib is active against all the spectrum of resistance mutations, including the mentioned ALK G1202R (against which brigatinib as well could retain activity) (70,84). This statement is true for single ALK mutations, as "compound" mutations (i.e., multiple mutations occurring on the same ALK allele) confer resistance to lorlatinib (see below) (70,71).

(II) Higher potency against the wild-type ALK tyrosine kinase. Crizotinib likely exerts a sub-optimal inhibition of ALK fusions, thus in the case of a bypass such as EGFR, this latter can still confer resistance even in case of a mild activation, sustained by the ALK signaling partially maintained under crizotinib inhibition (66,68). A complete abrogation of ALK phosphorylation with new generation inhibitors limits the occurrence of bypass/downstream resistance to the occurrence of strong mechanisms, capable *per se* of maintaining signaling pathways.

(III) Increased ability to cross the blood-brain barrier, in order to achieve clinically meaningful concentration in the CNS.

(IV) Higher ALK selectivity, sparing other kinases and limiting thus far off-target toxicities.

Across the three generations of ALK inhibitors, a progressive increase of the four characteristics have been achieved, with lorlatinib figuring as the most compelling agent due to its macrocyclic structure. Fourth-generation ALK inhibitors, designed to overcome lorlatinib resistance, have a more tolerable toxicity profile and have recently entered the initial phases of clinical development (see section "Moving beyond second- and third-generation ALK inhibitors").

As mentioned above, the prolonged OS benefits observed in patients suffering from ALK-positive NSCLC were obtained with the sequential administration of crizotinib and new generation ALK TKI. This strategy, sustained by several clinical studies, has been challenged by the introduction of second- or third-generation ALK inhibitors given as upfront therapy (85). Considering that this latter strategy is supported by the main treatment guidelines (86-88), we report here the main results from the clinical trials evaluating alectinib, brigatinib or lorlatinib compared to crizotinib for the first-line treatment of advanced ALK-positive NSCLC. Of note, ceritinib has been evaluated as the upfront ALK inhibitor, providing a median PFS of 16.6 months (89), and is still included in current guidelines for the management of ALK-positive disease. Nevertheless, the sub-optimal comparator arm of ASCEND-4 study (platinum-based chemotherapy), the inferior PFS outcomes compared to the mentioned agents (*Table 2*) and the toxicity profile [then mitigated by the alternative doses-schedules proposed in ASCEND-8 (101)] limit the use of ceritinib in this setting. In addition, the second-generation inhibitor ensartinib has been proven superior to crizotinib in the phase 3 eXalt3 trial (102). Considering it has not been incorporated into clinical guidelines, we address readers to the corresponding publication in extenso of eXalt3 study for ensartinib data.

Activity, efficacy and safety of next-generation inhibitors compared to crizotinib in head-to-head comparisons

Evidence and considerations upon ORR, PFS and OS

Alectinib, brigatinib and lorlatinib have been evaluated as the first ALK inhibitor (a minority of patients having received chemotherapy before) administered in patients suffering from ALK-positive NSCLC in five phase 3 studies, sharing crizotinib as the comparator arm (*Table 2*). Alectinib was tested in ALEX, J-ALEX and ALESIA, brigatinib in ALTA-1L, lorlatinib in CROWN (90,92,95,97,99). The ultimate goal of the strategy of having a new-generation ALK inhibitors upfront, compared to a sequential approach, is to provide longer survival outcomes with a good tolerability profile. Dealing with the first readout of activity, it is worth considering that ORR is still very high with crizotinib (up to 79% in J-ALEX). Still considering the variability between radiological assessments provided

Table 2 Activity and efficacy of next-generation ALK inhibitors compared to crizotinib in phase 3 studies

Study characteristics and outcomes	Alectinib			Brigatinib	Lorlatinib
	ALEX (90,91)	J-ALEX (92-94)	ALESIA (95,96)	ALTA-1L (97,98)	CROWN (99,100)
Enrollment	Global	Japan	Asia	Global	Global
Patients	303	207	187	275	296
Primary endpoint	Investigator-assessed PFS	IRF-assessed PFS	Investigator-assessed PFS	Blinded IRC-assessed PFS	Blinded IRC-assessed PFS
Investigator-assessed median PFS (95% CI)	Alectinib 34.8 (17.7–NE) months	*	Alectinib 41.6 (33.1–58.9) months	Brigatinib 30.8 (21.3–40.6) months	Median FU: 37 months
	Crizotinib 10.9 (9.1–12.9) months		Crizotinib 11.1 (9.1–18.4) months	Crizotinib 9.2 (7.4–12.7) months	Lorlatinib NR (NR-NR)
					Crizotinib 9.1 (7.4–10.9) months
Investigator-assessed PFS (95% CI)	HR 0.43 (0.32–0.58); P<0.0001	*	HR 0.33 (0.23–0.49)	HR 0.43 (0.31–0.58); P<0.0001	HR 0.19 (0.13–0.27)
IRC/IRF-assessed median PFS (95% CI)	*	Alectinib 34.1 (22.1–NE) months	*	Brigatinib 24.0 (18.5–43.2) months	Median FU: 37 months
		Crizotinib 10.2 (8.3–12.0) months		Crizotinib 11.1 (9.1–13.0) months	Lorlatinib NR (NR–NR)
					Crizotinib 9.3 (7.6–11.1)
IRC/IRF-assessed PFS (95% CI)	*	HR 0.37 (0.26–0.52)	*	HR 0.48 (0.35–0.66); P<0.0001	HR 0.48 (0.35–0.66); P<0.0001
Median OS follow-up	48 months	68 months	61 months	40 months	NA (immature data)
Median OS (95% CI)	Alectinib NR	Alectinib NE (70.6 months–NE)	Alectinib NE (NE–NE)	Brigatinib NR	NA (immature data)
	Crizotinib 57.4 (34.6–NR) months	Crizotinib NE (68.5 months–NE)	Crizotinib NE (45.5 months–NE)	Crizotinib NR	
OS (95% CI)	HR 0.67 (0.46–0.98); P=0.0376	HR 1.03 (0.67–1.58); P=0.9105	HR 0.60 (0.37–0.99)	HR 0.81 (0.53–1.22); P=0.305	NA (immature data)
Landmark OS analyses (95% CI)	3-year OS:	5-year OS:	5-year OS:	3-year OS:	NA (immature data)
	Alectinib 67.0% (59.1–74.8%)	Alectinib 60.9% (51.4–70.3%)	Alectinib 66.4% (57.9–74.9%)	Brigatinib 71% (62–78%)	
	Crizotinib 57.0% (42.1–65.9%)	Crizotinib 64.1% (54.9–73.4%)	Crizotinib 56.0% (43.0–69.1%)	Crizotinib 68% (59–75%)	
	4-year OS:			4-year OS:	
	Alectinib 65.3% (55.3–73.3%)			Brigatinib 66% (56–74%)	
	Crizotinib 51.2% (42.1–60.3%)			Crizotinib 60% (51–68%)	
	5-year OS:				
	Alectinib 62.5% (54.3–70.8%)				
	Crizotinib 45.5% (33.6–57.4%)				

In all studies with the exception of J-ALEX, patients were stratified at randomization for the presence of baseline brain metastases. *, data published in the first reports of the corresponding trials, with short follow-up. All the studies had a 1:1 randomization with the exception of ALESIA (2:1). ALK, anaplastic lymphoma kinase; PFS, progression-free survival; IRF, independent review facility; IRC, independent review committee; HR, hazard ratio; 95% CI, 95% confidence interval; NE, not evaluable; FU, follow-up; NR, not reached; OS, overall survival; NA, not available.

by investigators or the independent review committees, ORR are numerically higher with new generation inhibitors (up to 92% with alectinib in J-ALEX). Despite a potential impact on an increased depth of response obtained with new inhibitors, their intracranial activity is likely the major player in the higher ORR. The activity of ALK inhibitors in the ALEX trial is representative of these observations, as the investigator-assessed ORR in the groups with brain metastases is respectively 76.6% and 65.5% with alectinib (n=64) and crizotinib (n=58). In patients without baseline CNS involvement, the two inhibitors obtained overlapping activity: 79.5% in the alectinib arm (n=88) and 78.5% in the crizotinib one (n=93) (48). Besides the increased activity on brain lesions, it is difficult to envisage that novel inhibitors could rescue patients with primary resistance to crizotinib, occurring nevertheless in a minority of cases.

The crucial role of new generation inhibitors is represented by their prolonged PFS compared to crizotinib. Narrowing the range of resistance mechanisms, next-generation ALK TKI eventually turn out in a delayed occurrence of disease progression. Longer PFS (the endpoint of all the studies) was observed across all the trials, both in terms of statistical significance and of clinical relevance.

The benefit in OS, the final clinical readout of interest, is challenging to evaluate across these studies for two main reasons. First, the frequent exposure to next-generation inhibitors at progression to crizotinib for patients initially randomized in the control arms, and the fact that studies were not powered for OS, having been designed with PFS as endpoint. The OS data confirm how the prognosis of patients have evolved since the introduction of ALK inhibitor, as the only median OS reported thus far is for the crizotinib arm in ALEX trial, close to 5 years, whereas median OS is still unreached/not evaluable in the remaining treatment arms across trials (still considering the different follow-up periods) (91).

The best treatment strategy (sequential use of crizotinib and novel inhibitors *versus* novel inhibitors upfront) to be adopted in patients suffering from ALK-positive NSCLC has been a matter of discussion in the last years (85). Despite the methodological challenges in interpreting the OS data in ALEX, J-ALEX, ALESIA, ALTA-1L and CROWN trials (*Table 2*), the clinically meaningful increase in PFS, the activity against CNS disease and the better tolerability profiles favor the upfront use of the novel inhibitors.

Molecular features potentially impacting the activity of ALK inhibitors in this setting have been investigated. The type of *EML4* variant (see section "ALK fusions in NSCLC: from structure to function" and *Figure 1*), as well as the co-occurrence of mutations in *TP53* and *CDKN2A/B* tumor suppressor genes, have been shown to affect the prognosis of patients treated with ALK inhibitors in this setting (48,98,103,104). Nevertheless, these aspects do not harbor a predictive role, that could be of major clinical usefulness in suggesting which patient could benefit from a given treatment strategy/precise inhibitor on the basis of a wide molecular portrait.

Intracranial activity and efficacy

As anticipated above, the intracranial activity of new generation ALK inhibitors plays a crucial role in improving patient outcomes. There is a major difference in the intracranial response obtained with novel inhibitors or crizotinib in case of baseline brain involvement (*Table 3*). With the exception of ALEX trial and the pending results of CROWN, the presence of baseline brain metastases does not even represent a prognostic factor in patients treated with alectinib and brigatinib. All the publications of the trials contain clinically relevant information concerning parameters such as time to intracranial progression in patients with or without baseline CNS involvement, and inhibitors efficacy according to previous brain radiotherapy (93,96,98,100,105,107). As a summary, it can be stated that novel generation inhibitors prevent the onset of intracranial disease in patients without baseline CNS metastases. Further insights on these aspects, of clear clinical interest, go beyond the scope of this review, and readers are invited to refer to the specific publications.

Safety considerations

Overall, new generation inhibitors are considered safer compared to crizotinib. Their global safety profiles are reported in *Table 4*. According to the mentioned clinical trials, grade 3–4 adverse events occur in 37% to 76% of the patients during treatment with new inhibitors; dose reductions (20–44%), interruption (26–72%) and treatment discontinuations (7–14%) are not anecdotical. Despite being frequently represented by laboratory abnormalities, treating physicians should be aware of the expected toxicities of each novel ALK inhibitor, in order to provide an early recognition and management. Grade 3–4 increase in blood levels of creatine phospho-kinase occur in up to 26% of the cases in patients receiving brigatinib, that can cause increase in blood lipase and amylase as well. Of note, no case of pancreatitis and no grade ≥3 myalgia or musculoskeletal

Table 3 Intracranial activity of next-generation ALK inhibitors compared to crizotinib in phase 3 studies, activity and efficacy of next-generation agents in patients with baseline brain metastases

Study characteristics and outcomes	Alectinib						Brigatinib		Lorlatinib	
	ALEX (90,91,105)		J-ALEX (92,94)		ALESIA (95,96)		ALTA-1L (98,106)		CROWN (100)	
IRC/IRF-assessed intracranial ORR	Alectinib 59% (38/64) Crizotinib 26% (15/58)		NA		Alectinib 73% (32/44) Crizotinib 22% (5/23)		Brigatinib 66% (31/47) Crizotinib 14% (7/49)		Lorlatinib 65% (24/37) Crizotinib 18% (7/39)	
Baseline brain metastases in the new-generation ALK inhibitor arm	Yes (N=64)	No (N=88)	Yes (N=14)	No (N=89)	Yes (N=44)	No (N=81)	Yes (N=40)	No (N=97)	Yes (N=37)	No (N=112)
Median PFS (95% CI)	25.4 months (9.2–NE)	38.6 months (22.4–NE)	NA		42.3 months (27.8–60.7)	41.6 months (29.5–64.9)	24.0 months (18.4–NR)	24.0 months (15.7–NR)	NR (18.2 months–NR)	NR (NR–NR)
PFS crizotinib comparator arm (95% CI)	HR 0.37 (0.23–0.58)	HR 0.46 (0.31–0.68)	HR 0.08 (0.01–0.61)	HR 0.39 (0.23–0.64)	HR 0.17 (0.09–0.33)	HR 0.45 (0.29–0.71)	HR 0.25 (0.14–0.46); P<0.0001	HR 0.65 (0.44–0.97); P=0.03	HR 0.21 (0.10–0.44)	HR 0.29 (0.19–0.44)
Median OS	NA		53.9	NE	NE	NE	NR	NR	NA (immature data)	
OS crizotinib comparator arm (95% CI)	HR 0.58 (0.34–1.00); P=0.0477	HR 0.76 (0.45–1.26); P=0.2851	1.56 (0.64-3.80)	0.96 (0.58–1.57)	HR 0.40 (0.19–0.85)	HR 0.81 (0.42–1.55)	HR 0.43 (0.21–0.89); P=0.020	HR 1.16 (0.69–1.93); P=0.603	NA (immature data)	
	Interaction test P=0.4677									
Landmark OS analyses	NA		5-year OS 42.9%	5-year OS 63.7%	5-year OS 63.6%	5-year OS 67.8%	3-year OS 74% (95% CI: 57–85%) 4-year OS 71% (95% CI: 53–83%)	3-year OS 70% (95% CI: 59–78%) 4-year OS 64% (95% CI: 52–74%)	NA (immature data)	

The PFS data reported for studies refer either to investigator assessment (ALEX and ALESIA), IRF (independent review facility, J-ALEX) or BIRC (blinded independent review committee, ALTA-1L and CROWN). The discrepancy between patients evaluated for brigatinib intracranial ORR (n=47) and survival analyses (n=40) in ALTA-1L is explained considering that the 47 patients were considered positive for brain metastases at baseline by BIRC, and the 40 in the PFS/OS analyses correspond to the ones having baseline brain metastases according to investigator assessment. In all studies with the exception of J-ALEX, patients were stratified at randomization for the presence of baseline brain metastases. All the studies had a 1:1 randomization with the exception of ALESIA (2:1). ALK, anaplastic lymphoma kinase; IRC, independent review committee; IRF, independent review facility; ORR, objective response rate; PFS, progression-free survival; 95% CI, 95% confidence interval; HR, hazard ratio; NE, not evaluable; NA, not available; NR, not reached; OS, overall survival.

Table 4 Safety profile of next-generation ALK inhibitors in upfront phase 3 studies

Toxicities	Alectinib			Brigatinib	Lorlatinib
	ALEX (91)	J-ALEX (94)	ALESIA (96)	ALTA-1L (98)	CROWN (100)
Patients	152	103	125	136	149
Serious AE	38.8%	27.2%	28%	NA	38%
Grade 3–5 AE	52%	36.9%	48%	70% (G3–4)	76% (G3–4)
Fatal AE	4.6%	0	4%	8%	7%
Treatment-related, fatal AE	NA	0	NA	0	1%
AE leading to treatment discontinuation	14.5%	11.7%	11.2%	13%	7%
AE leading to dose reduction	20.4%	NA	26.4%	44%	21%
AE leading to dose interruption	26.3%	34%	26.4%	72%	56%
Top G3–4 AEs	Anemia 5.9%	Blood CPK increased 4.9%	Weight increased 8.8%	Blood CPK increased 26%	Hypertriglyceridaemia 23%
	AST increased 5.3%	Interstitial lung disease 4.9%	Blood CPK increased 6.4%	Lipase increased 15%	Hypercholesterolaemia 19%
	ALT increased 4.6%	Rash maculopapular 2.9%	ALT increased 2.4%	Hypertension 14%	Weight gain 20%
	Urinary tract infection 3.9%	Neutrophil count decreased 1.9%	Nausea 0.8%	Amylase increased 6%	Hypertension 11%
	Blood CPK increase 3.3%	QT prolonged 1.9%		Pneumonia 5%	Cardiovascular adverse events 7% (including blood CPK increase)
					CNS adverse events 5%

ALK, anaplastic lymphoma kinase; AE, adverse event; AST, aspartate transaminase; ALT, alanine transaminase; CPK, creatine phosphokinase; CNS, central nervous system.

pain were recorded (98). Lorlatinib is known to increase the blood levels of lipids and weight gain, again without a clear correlation with an increased risk of cardiovascular adverse events. The CNS toxic effects of lorlatinib, mainly in the areas of cognitive and mood effects, were rarely reported as grade 3–4 (107). Nevertheless, their frequent occurrence (39% across all grades) points out the relevance of mild but symptomatic toxicities, even more in patients who hopefully receive targeted therapies for years. Recommendations for the daily management of adverse events from novel ALK inhibitors have been recently published (108-111).

Current clinical scenario of ALK inhibition in NSCLC

The improvement in the clinical management of ALK-positive NSCLC with sequential administration of different generations of ALK inhibitors observed in the "crizotinib era" vouches for the application of the strategy with novel agents. The concepts of treatment "beyond progression" in case of mild, asymptomatic radiological disease progression and of local therapy in case of isolated progression are still valid (112), as the goal is to maintain ALK inhibition as long as possible.

Dealing with the sequential administration of novel

ALK-TKI, brigatinib has been specifically evaluated in patients progressing after ceritinib and alectinib (113,114), but translating the results in the current treatment scenario is challenging, as the patient populations included patients initially treated with crizotinib. For the same reason, evaluating lorlatinib outcomes in the precise patient population pre-treated with alectinib or brigatinib only is not straightforward, due to the relative heterogeneity of patients included in the phase 2 study studying lorlatinib in this setting (115).

It can be estimated nevertheless that alectinib-pretreated patients obtained an ORR and median PFS of approximately 40% and 5–6 months, respectively, with more optimistic results from a recent retrospective study (116). The eminent activity of the third-generation agent in the case of CNS disease (including meningeal metastases) sustain its utilization at progression to second-generation inhibitors (117,118). Even if not supported by formal proofs, the concomitant association of chemotherapy and new generation ALK inhibitors has been reported in some experience, likely with the goal of maintaining an increased control or prevention of CNS disease (119,120).

Moving beyond second- and third-generation ALK inhibitors

Whether in the setting of a sequential treatment encompassing crizotinib, or after an upfront treatment with a second-generation ALK inhibitor, or in the case of the adoption of CROWN results in the clinical setting, lorlatinib represents nowadays the last bulwark of targeted agents in patients with ALK-positive NSCLC.

From a clinical standpoint, after lorlatinib failure, treatment options turn to chemotherapy. Similarly to the *EGFR*-mutated NSCLC field, single-agent anti-PD-1/PD-L1 agents have been proven as globally inactive in ALK-positive NSCLC (121), excluded therefore from the large majority of trials evaluating chemo-immunotherapy regimens. Nevertheless, IMpower150 allowed the inclusion of oncogene-driven diseases. In its *EGFR*-mutant population, the potential synergy of the double blockade of PD-1/PD-L1 and angiogenesis with atezolizumab and bevacizumab, respectively, combined with carboplatin and paclitaxel chemotherapy, is still under debate (122). ALK-positive disease tends to be approached in line with the EGFR-driven one and the evoked combination therapy is mentioned in current guidelines (87). Nevertheless, it should be noted that in IMpower150 only 13 patients with *ALK*-rearranged lung cancer were treated with the four-drug regimen (123). Still with the limitation of a low number of patients, in the GFPC 06-2018 trial, nine patients received the four drugs, obtaining median PFS and OS of 7.3 and 16.8 months, respectively (124). Treatment decision in the post-lorlatinib setting (chemotherapy +/– atezolizumab and bevacizumab) should therefore take into account the limited strength of the supporting evidence, and the tolerability of a four-drug regimen in a setting of pre-treated patients.

The mentioned substantial improvement in the survival outcomes of patients with ALK-positive NSCLC through the last decade serve as an incitement to find additional targeted agents beyond lorlatinib. Fourth-generation agents such as TPX-0131 and NVL-655 have been developed to maintain the characteristics of an optimal ALK inhibitor, and to be active against compound mutations (i.e., double mutations responsible for resistance to lorlatinib, when administered after previous targeted agents) (125). Early clinical studies are ongoing and initial data are eagerly awaited.

Treatment considerations for locally advanced and early-stage ALK-positive NSCLC

The activity and efficacy of ALK inhibitors in the advanced-metastatic disease setting support their putative role in locally-advanced and early disease stages, in line with what has been recently shown in EGFR-driven NSCLC (126,127). Experiences of neo-adjuvant ALK inhibitors have been reported, and this approach is currently been evaluated in clinical trials, where pathological response serves as a surrogate marker of clinical activity (128). Compared to the advanced disease setting, assessing the benefit of an adjuvant approach could be more challenging, having disease-free survival and OS as ultimate goals. Nevertheless, the marked activity of new generation of ALK inhibitors would likely guarantee their competence in preventing/delaying disease onset after surgical treatment.

Of note, just before the final acceptance of this manuscript, the study ALINA was presented at European Society for Medical Oncology (ESMO) 2023 congress. ALINA is a phase 3 study, randomizing patients with resected, ALK-positive, stage IB-IIIA lung cancer (per UICC/AJCC 7th edition), to receive adjuvant chemotherapy or alectinib for two years. The trial met its primary endpoint of disease-free survival, with 94% and 88% of the patients in the alectinib arm not experiencing tumor recurrence at two at three years from randomization, respectively (129).

The limited activity of immunotherapy in advanced disease, and the preliminary proofs of the limited impact of durvalumab in patients with *EGFR*-mutant disease undergoing chemo-radiotherapy (130,131) suggest this could be the case for ALK-rearranged cases. Moreover, the risk of toxicities exacerbated by the sequential use of anti-PD-1/PD-L1 agents and ALK inhibitors should be taken into account when balancing the risk/befit ratio in treatment decision making (121).

These reflections on the potential incorporation of ALK inhibitors in the early/locally-advanced disease stages, and the potential exclusion of immunotherapy approaches, would require ALK testing by IHC in these settings, a procedure that could be easily implemented in the workflow of pathology diagnostic (see section "Molecular diagnostics for the detection of *ALK* fusions in NSCLC").

Conclusions

This review aimed to retrace the history of *ALK* fusions in NSCLC, from their discovery to the latest therapeutic developments. The improvements in the clinical outcomes obtained in the last two decades for patients suffering from ALK-positive lung tumors are the results of the fruitful enlacement between the understanding of biological processes leading to resistance to available ALK inhibitors, and clinical research efforts. We hope that this bond, serving as a model across oncogene-driven tumors, will be maintained in the future, to guarantee the constant improvement of patient outcomes.

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Footnote

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