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INVITED REVIEW

ALK-rearrangement in non-small-cell lung cancer (NSCLC)

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Abstract

The *ALK* gene encodes a transmembrane tyrosine kinase receptor. ALK is physiologically expressed in the nervous system during embryogenesis, but its expression decreases postnatally. *ALK* first emerged in the field of oncology in 1994 when it was identified to fuse to *NPM1* in anaplastic large-cell lymphoma. Since then, *ALK* has been associated with other types of cancers, including non-small-cell lung cancer (NSCLC). More than 19 different ALK fusion partners have been discovered in NSCLC, including *EML4*, *KIF5B*, *KLC1*, and *TPR*. Most of these *ALK* fusions in NSCLC patients respond well to the ALK inhibitor, crizotinib. In this paper, we reviewed fusion partner genes with *ALK*, detection methods for *ALK*-rearrangement (*ALK*-R), and the ALK-tyrosine kinase inhibitor, crizotinib, used in NSCLC patients.

The *ALK* gene

The *ALK* gene is located on the short arm of chromosome 2 (2p23), belongs to the insulin receptor superfamily, and encodes for the ALK protein (Fig 1a). ALK is a transmembrane tyrosine kinase receptor, and like other receptor tyrosine kinases, it has an extracellular domain, a transmembrane segment, and a cytoplasmic receptor kinase segment (Fig 1a–c).[1,2] *ALK* expression occurs in the nervous system during embryo genesis and decreases in postnatal life. Therefore, in human adults, low levels of ALK protein are produced only in rare, scattered neural and endothelial cells and in pericytes in the brain.[3,4]

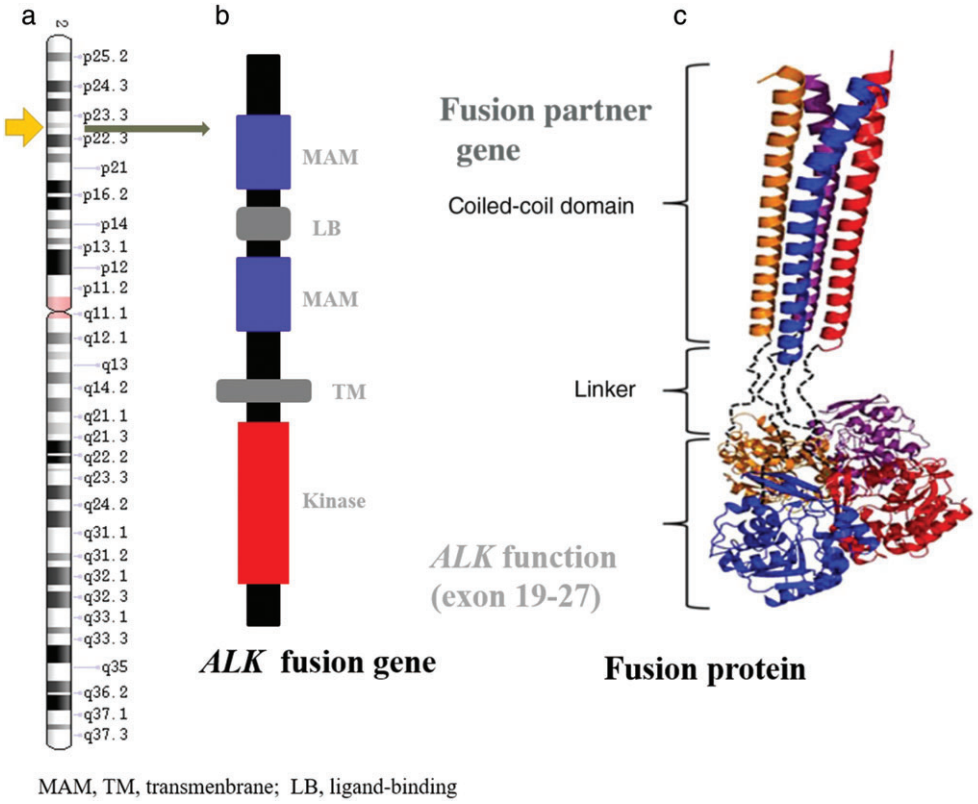

Figure 1 (**a**) The *ALK* gene location in the genome; (**b**) structural organization of ALK protein; and (**c**) the domain of the fusion protein.

Types of oncogenesis in *ALK*

There are three types of *ALK* gene mutations: rearrangement (ALK-R), amplification (ALK-A), and point mutation.

Most mutations of the *ALK* gene are in the form of a translocation with another partner gene leading to a fusion oncogene. This fusion gene then becomes overly expressed in cancers. In 1994, *ALK* was originally identified in anaplastic large-cell lymphoma as a fusion partner of nucleophosmin (NPM-ALK) resulting from a chromosomal translocation.[5] Subsequently, ALK-rearrangement (ALK-R) was identified in many different cancers, including inflammatory myofibroblastic tumors, diffuse large B-cell lymphoma, non-small-cell lung cancer (NSCLC), and esophageal squamous cell, colorectal, and breast carcinomas.[6,7] ALK rearrangements create an oncogenic ALK tyrosine kinase that activates many downstream signaling pathways resulting in increased cell proliferation and survival.[8] Additional gene partners have been discovered in fusion oncogenes with the *ALK* gene, including *TPM3*, *TFG*, *CLTCL1*, and *ATIC* (Table 1).[9]

Another type of *ALK* gene mutation is ALK-A. The oncogenic mechanism of ALK-A was first described in NB cell lines in 2002. The study showed that ALK-A leads to constitutive activation, resulting in the selective activation of SHcC, a docking protein close to the substrate of the ALK receptor.[10] Several studies have reported extra copies of the *ALK* gene in inflammatory breast cancer, NSCLC, anaplastic large-cell lymphoma, and pulmonary sarcomatoid carcinoma.

Table 1 ALK gene mutations and the disease they represent

ALK-R		ALK-A	
Disease	Partner Gene	(disease)	Main point mutation
Anaplastic large cell lymphoma	*NPM1*	Inflammatory breast cancer	L1196M
Inflammatory myofibroblastic tumors	*TPM3/4*	Small cell lung cancer	C1156Y
Diffuse large B-cell lymphoma	*TFG*	Anaplastic large cell lymphoma	G1269A
Non-small cell lung cancer	*EML4*	Pulmonary sarcomatoid carcinoma	F1174L
Esophageal squamous cell carcinoma	*CLTCL1*	Rhabdomyosarcoma	L1152R
Colorectal carcinoma	*ATIC*	Carcinoma of the esophagus	F1245C
Renal medullary carcinoma	*VCL*	Adult renal cell carcinoma	G1201E

The last type of *ALK* gene mutation is point mutation. Secondary resistance is an acquired mechanism after the tumor has been exposed to an ALK inhibitor[2] and most types of resistance are caused by mutations in the target *ALK* gene, resulting in an inability to inhibit the encoded tyrosine kinase.[11] The first drug resistance point mutations identified were C1156Y and L1196M.[12] Subsequently, several other point mutations conferring drug resistance have been identified, including: G1269A, F1174L, 1151Tins, L1152R, S1206Y, I1171T, G1202, D1203N, and V1180L.[11–14]

ALK rearrangement in non-small cell lung cancer (NSCLC)

Non-small-cell lung cancer accounts for approximately 80–85% of lung cancers and is a leading cause of cancer-related mortality in both men and women worldwide.[15–18] *ALK* gene rearrangement is a driving mutation underlying the development of NSCLC, and has been identified in 5–6% of NSCLC cases.[19] Notwithstanding the substantial evidence linking activated ALK to tumor genesis in these rare tumors, it is fair to say that the considerable current enthusiasm for ALK as a target for cancer therapy is largely driven by the relatively recent finding of a recurring *ALK* gene translocation in a significant subset of NSCLC.[20,21] *ALK* rearrangement appears to be more common in younger patients and never or light smokers diagnosed with adenocarcinoma. Data from several patient series has shown that the median age of *ALK* positive NSCLC patients is 55 years and approximately 70% of these patients are never smokers. The incidence of *ALK* positive NSCLC among men and women is similar across the world.[22,23]

ALK mutations were first described in NSCLC in 2007 when a subset (7%) of Japanese patients were found to have EML4 rearrangement with ALK leading to the fusion oncogene EML4-ALK.[24] This rearrangement was an inversion rearrangement from inv.(2) (p21;p23) that results in EML4 replacing the extracellular and intramembranous parts of ALK and fusing with the juxtamembrane domain. The *EML4-ALK* fusion gene represents a new molecular target. It has been reported that the incidence of *ALK* rearrangement ranges from approximately 3% to 13% in unselected or selected patients with NSCLC.[23,25–27] Because of the different breakpoints on *EML4*, several variants of the *EML4-ALK* mutation have been described (Table 2).[27–29] *EML4-ALK* variants with differing frequencies are V1 (54.5%), V2 (10%), V3a/V3b (34%), and V5a (1.5%).[28,29]

EML4-ALK translocation can result in constitutive ALK kinase activity and represents an oncogenic addiction pathway in lung cancer. The *EML4-ALK* gene induced tumor formation in nude mice.[24,30] *EML4-ALK* possesses potent oncogenic activity both in vitro and in vivo, and the tumor can quickly be reduced after the administration of ALK-tyrosine kinase inhibitors (TKIs).[24,31]

Table 2 *EML4-ALK* variant fusions

Variants	EML4-ALK Fusion Types	Number of types	Frequency (%)
E13;A20	E13;A20(variant 1), E13;ins69A20, E13;ins69A20, E13;exoc6bA20	4	33
E6;A20	E6;A20(variant 3a), E6ins33;A20 (variant 3b), E6;ins18A20	3	29
E20;A20	E20;A20(variant 2), E20;ins18A20	2	9
E18;A20	E18;A20(variant 5′)	1	2
E14;A20	E14;ins11del49A20 (variant 4), E14del12A20 (variant 7), E14;del14A20, E14;del36A20,E14;del38A20, E14ins21;del113A20	5	3
E15;A20	E15del19;del20A20 (variant 4′), E15del60;del71A20	2	2
E2;A20	E2;A20(variant 5a), E2;ins117A20(variant 5a/b)	2	2
E17;A20	E17;ins68A20, E17ins65;A20, E17;ins30A20 (variant 8a), E17del58;ins39A20, E17ins61;ins34A20 (variant 8b)	5	1
E3;A20	E3;ins69A20(variant 6), E3;ins53A20	2	1
E6;A19	E6;A19	1	< 1
E21;A20	E21;A20	1	< 1
E10del54E13;A20	E10del54E13;A20	1	< 1
E6;A17	E6;A17	1	< 1
Total		30	

EML4-ALK fusion protein serves as a therapeutic target for an ALK-TKI, and has shown promising results when used to treat NSCLC patients carrying *ALK* rearrangement.[32–35] Over the last few years, ALK inhibitors have shown significant benefits in the management of *ALK*-positive NSCLC compared to conventional chemotherapy.[21,34,36]

Rearrangements of the *ALK* gene with partner genes other than *EML4* have been described, namely, *KIF5B*, *KLC1*, *TFG*, *TPR*, *HIP1*, *STRN*, *DCTN1*, *SQSTM1*, *NPM1*, *BCL11A*, and *BIRC6* (Table 3).[37–50] Targeted therapeutic agents, including the TKI crizotinib, have shown clinical efficacy in treating NSCLC patients harboring *EML4-ALK* gene fusion.[34] Furthermore, a previous study demonstrated that crizotinib is also effective at treating tumors harboring *ALK* fused with other partner genes, including *NPM1* and *BCL11A*.[34] In addition, other not-yet-characterized fusions may also exist in solid tumors, including lung cancer.[51]

ALK rearrangement detection methods in NSCLC patients

ALK rearrangements may involve distinct break points and multiple fusion partners. Therefore, routine *ALK* testing presents a significant technical challenge. There are four primary methods of detecting *ALK* rearrangement: fluorescence in situ hybridization (FISH), immunohistochemical (IHC), reverse transcriptase-PCR (RT-PCR) and next generation sequencing (NGS). Each of these methods has both advantages and limitations.

Fluorescence in situ hybridization break-apart assay is considered the gold standard for the evaluation of *ALK* status and is the first approved diagnostic test for ALK rearrangement to detect break-apart signals, although IHC and RT-PCR have also been evaluated for this purpose, with the former approved by the United States Food and Drug Administration (US FDA) in June 2015 (Table 4).[19,52] FISH relies on a spatial separation of the 5′- and 3′- portions of the *ALK* gene upon rearrangement, and produces characteristic spilt *ALK*-specific signals in case of the translocation. The FISH break-apart assay is currently the most reliable approach to *ALK* testing, but has a number of critical disadvantages. In particular, FISH requires significant time input of extensively trained personnel and cannot be subjected to reasonable automation; furthermore, it demonstrates relatively high failure rates in some sample series and may provide poorly interpretable results in a noticeable fraction of NSCLC cases.[53–55] Despite these challenges, FISH is still regarded as the gold standard assay for the detection of *ALK* rearrangements and a comparator for the other ALK detection methods.

The development of highly sensitive *ALK* diagnostic antibodies has offered an opportunity to detect *ALK*-driven tumors by a standard IHC method. One of the main advantages of IHC in comparison to FISH and RT-PCR is the detection of the ALK protein, which is the target of ALK inhibitors. Other advantages of IHC are its low cost, short turnaround time, and ease of operation for users. The principle of IHC is based on the fact that activating *ALK* rearrangements are accompanied by significant overexpression of the catalytic portion of this tyrosine kinase. IHC is generally capable of producing highly reliable results when performed in reference laboratories; however, it requires the standardization of reagents and protocols across pathology laboratories.[55–58] The Ventana ALK assay used D5F3 antibody is a resultful method of detecting *ALK* rearrangement. The Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ, USA) was approved by the US FDA in 2015 as a companion detection test for the use of crizotinib.[59] Several studies have found that there is high concordance between Ventana IHC and FISH.[60,61] A research analysis of 46 *ALK*-positive patients reported sensitivity and specificity of

Table 3 Fusion details of *ALK* partner genes

Fusion partner gene	Reported year	Oncogenetic driver	TKI PFS	Variants	FISH result	IHC result	First report (ref)
EML4	2007	Yes	—	> 30 types	—	—	24
TFG	2007	—	—	T6;A20	—	—	39
KIF5B	2009	Yes	—	K24;A20/K15:A20	Positive	Positive	40
KLC1	2012	Yes	—	K9:A20	Positive	Positive	41
PTPN3	2012	—	Unknown	P2;A10–11;P3	—	—	45
HIP1	2014	Yes	5M	H21;A20/H28;A20/ H30:A20	—	—	50
TPR	2014	—	Unknown	T15;A20	Positive	Positive	49
BIRC6	2015	Yes	> 9M	B10;A20	Negative	Positive	48
DCTN1	2015	—	Unknown	D26;A20	Negative	—	37
SQSTM1	2015	—	Unknown	S5;A20	Negative	—	37
PRKAR1A	2016	Yes	7M	P5;A20	Positive	Positive	44
PPM1B	2016	Yes	Sensitivity	P1;A20	Negative	—	44
EIF2AK3	2016	Yes	28M	E2;A20	Negative	Negative (D5F3 and 5A4)	44
BCL11A	2017	Yes	> 6M	B4;A20	—	—	43
CEBPZ	2017	Yes	Unknown	C3;A20	Negative/fused signals	Positive	42
PICALM	2017	Yes	Unknown	P19;A20	Negative/fused signals	Positive	42
GCC2	2017	—	—	G12;A20	Positive	Positive	47
LMO7	2017	—	—	L15;A20	Positive	Positive	47
PHACTR1	2017	—	—	PH7;A20	Positive	Positive	47
CMTR1	2017	No	Drug resistant	C2;A20	Negative	Positive	Under review

FISH, fluorescence in situ hybridization IHC, immunohistochemical.

Table 4 Comparison of the four methods used to detect ALK fusion

	FISH	IHC	RT-PCR	NGS
Fusion types detectable	No fusion specification	No fusion specification	Only *EML4-ALK* fusion	All kinds of fusion
Sensitivity	10–15%	5–10%	1–5%	1–5%
Time used for analysis	2–3 days	0.5 days	1 days	5–7 days
Cost	Medium (~$349)	Low (~$31.5)	Medium (~$879)	High (~$945)
Is FFPE material applicable?	Yes	Yes	Yes	Yes
Is fresh tissue material applicable?	No	No	Yes	Yes
Amount of material required	One tissue section (3 μm thick)	One tissue section (3 μm thick)	0.1–0.5 μg of RNA	2–3 μg of DNA
Possibility to see large range of other gene mutations in one analysis	No	No	No	Yes
Requirement for technical skill	Medium	Low	Medium	High
Requirement for diagnostician	High	Medium	Medium	High
Applicability to average pathology laboratory	Most laboratories	All laboratories	Some laboratories	Some laboratories

FFPE, formalin fixed paraffin-embedded; FISH, fluorescence in situ hybridization IHC, immunohistochemical; NGS, next generation sequencing; RT, reverse transcriptase.

the Ventana IHC of 100% and 98.2%, respectively, and the concordance rate between FISH and Ventana IHC was 98.4%.[62] Although the sensitivity of IHC is high for detecting ALK fusion, FISH-positive/IHC-negative cases responding to ALK inhibitors have been reported in the literature.[63]

Reverse transcriptase-PCR based assays have not been as widely used as FISH and IHC for *ALK* testing in NSCLC. However, conventional RT-PCR has significant advantages compared to FISH and IHC. First of all, while FISH and IHC detect relatively indirect signs of the presence of *ALK* translocation, RT-PCR usually reveals the exact variant of the rearrangement and therefore provides definitive evidence of ALK fusion. Furthermore, RT-PCR has high sensitivity and specificity, with a rapid turnaround time and ease of analysis, and can detect a small number (1%) of *ALK*-driven NSCLC cells in the presence of normal tissues.[64–66] In addition, RT-PCR analysis utilizes the same technical platform as other kinds of molecular NSCLC diagnosis, for example *EGFR* testing. Finally, a number of commercial RT-PCR kits for detection of ALK rearrangements have been developed recently, including: the ALK RGQ RT-PCR Kit (Qiagen, Valencia, CA, USA); the EML4-ALK Fusion Gene Detection Kit (Amoy Diagnostics, Xiamen, China); and EML4 ALK Gene Fusion, PCR (Quest Diagnostics, Secaucus, NJ, USA).[37,67] However, there are some disadvantages of this platform. First, this method of analysis of RNA samples yields a poor quality of RNA obtained from formalin fixed paraffin-embedded specimens and can only detect known fusion variants. Second, the high sensitivity may lead to a false-positive result. In a previous study, the sensitivity and specificity of RT-PCR were 95.5% and 87.0%, respectively, and the concordance rate between FISH and RT-PCR was 89.0%.[62]

Next generation sequencing is a promising method for detecting ALK gene rearrangements. The great advantage of the NGS platform is the detection of known *ALK* gene fusions. NGS is also superior to other methods because it allows for simultaneous screening of novel ALK fusion partners as well as other lung cancer related gene mutations, fusions, and amplifications. However, there are still many challenges to overcome before this method can be applied to normal laboratory diagnosis of pathology. For example, expertise is needed to analyze and interpret the results, and the cost and turnaround time are high.[68] As NGS has not yet been approved by the US FDA, it can only be used in conjunction with other methods.

All four methodologies show good sensitivity, specificity, and concordance when artifacts were characterized and excluded. However, the choice of diagnostic methodology for ALK rearrangement detection in clinical practice remains a matter of debate. In ambiguous cases at least two of the four methods should be used to confirm ALK rearrangement.

ALK inhibitors

Crizotinib

ALK rearrangements in NSCLC have introduced new treatment options for advanced NSCLC with the use of *ALK*-TKIs.[24] *ALK* fusion proteins can activate many different interconnected and overlapping pathways, such as Ras/Raf/MEK/ERK1/2, JAK/STAT, PI3K/Akt, and PLC-γ pathways, all of which are involved in cell migration, proliferation, and survival.[8] In addition, several *ALK* fusion partners have been identified. However, regardless of the involved partners, all chimeras retain the *ALK* gene kinase domain responsible for the constitutive activation of *ALK* signaling pathways.[8]

Crizotinib is the first *ALK* inhibitor to enter clinical trials. Crizotinib is a multi-targeted TKI[69] with activity against *MET*, *ALK*, and *ROS1*, and was approved by the US FDA in 2011 for metastatic NSCLC positive for *ALK*

rearrangements.[34,70,71] Crizotinib has a reported response rate of over 60% and a disease control rate of up to 90%.[34] Furthermore, median progression-free survival (PFS) exceeds nine months, and median overall survival is almost 75% after one year in *ALK*-rearranged NSCLC.[72] Comparison of crizotinib treatment with a historical control is instructive.[38] Therefore, from identification to inhibitor approval, the story of *ALK* in NSCLC stands as a testament of the promises of targeted molecular medicine.[22]

Next generation ALK inhibitors

Unfortunately, almost all patients treated with crizotinib develop tumor progression. As such, potent inhibitors of ALK that can overcome resistance to crizotinib are needed. Several agents have been evaluated in patients with crizotinib refractory NSCLC. Ceritinib and alectinib are currently approved in the US, and brigatinib has received breakthrough designation by the US FDA. The response rates with these agents in patients with crizotinib drug resistance are 50–55%, and the median PFS rates are 6.9 for ceritinib, 8.9 for alectinib, and 15.6 months for brigatinib.[73–75] Ensartinib is another next generation ALK inhibitor. Ensartinib activity was not only observed in crizotinib-resistant patients but also yielded results in patients who had previously been administered more than two alternate ALK inhibitors.[76]

The ASCEND-4 trial evaluated the effects of ceritinib or chemotherapy in randomized ALK-positive treatment naïve patients.[77] The median PFS with ceritinib was 16.6 versus 8.1 months in patients treated with chemotherapy. In the ASCEND-5 trial, patients who initially received chemotherapy and crizotinib were randomized for further treatment of ceritinib or chemotherapy. Results demonstrated a significant improvement in PFS, with a median of 5.4 months after the administration of ceritinib compared to 1.6 months with chemotherapy.[78] These data reveal that ceritinib is the preferred treatment for ALK-positive NSCLC patients.

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Disclosure

No authors report any conflict of interest.

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