

Solicited Review Article

The role of anaplastic lymphoma kinase in pediatric cancers

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In recent years, the discovery of a variety of genetic alterations in different malignancies leading to oncogenesis has provided insight into the complexity of tumorigenesis and the development for target-specific therapies with the objective of improving clinical outcomes.⁽¹⁾ Among them, the breakthrough of the *BCR-ABL* fusion in patients with chronic myeloid leukemia has become a paradigm for personalized or precision medicine.⁽²⁾ In current clinical practice, personalized cancer therapy is well established for a number of gene targets, including various kinase encoded genes.⁽¹⁾ The anaplastic lymphoma kinase (*ALK*) gene encodes a transmembrane receptor tyrosine kinase (RTK), which belongs to the insulin receptor superfamily.⁽³⁾ *ALK* was first identified as part of the nucleophosmin (*NPM*)-*ALK* gene fusion transcript, which is derived from the t(2;5)(p23;q35) translocation that is involved in the pathogenesis of a subset of cases of anaplastic large-cell lymphoma (ALCL).^(4,5) *ALK* has been found to be rearranged, mutated, or amplified in a variety of tumors, including neuroblastoma, inflammatory myofibroblastic tumor (IMT), and non-small-cell lung cancer (NSCLC).^(6–11) This pivotal discovery has designated the ALK protein as a potentially relevant biomarker and therapeutic target in a wide variety of solid tumors and hematological malignancies in which ALK is a critical mediator of carcinogenesis. In fact, dramatic responses to ALK inhibitors have been documented in NSCLC, ALCL, and IMT

The anaplastic lymphoma kinase (*ALK*) gene was initially identified as a fusion partner of the nucleophosmin gene in anaplastic large-cell lymphoma with t(2;5)(p23;q35) translocation, and then described with different genetic abnormalities in a number of tumors. Although *ALK* is known to be involved in the pathogenesis of neuroblastoma through activating mutations or gene amplification, its role in the pathogenesis of other pediatric cancers is still elusive. In addition to neuroblastoma, the high-grade amplification of *ALK* has been described in a subset of rhabdomyosarcoma cases. Normal *ALK* protein expression is restricted to the nervous systems of adult mammals, but the aberrant expression of *ALK* has been observed in a variety of pediatric cancers, including glioma and Ewing sarcoma. The discovery of oncogenic activation of *ALK* in neuroblastoma suggests that this cancer could be potentially treated with an *ALK* inhibitor, as could other cancers, such as non-small-cell lung cancer and anaplastic large-cell lymphoma. However, cellular responses to mutant *ALK* are complex when compared to rearranged *ALK*, and treatment remains a challenge. This review focuses on the biology of *ALK* in pediatric cancers and possible therapeutic strategies for *ALK*-associated tumors.

patients.^(12,13) The identification of recurrent oncogenic alterations of *ALK* in ALCL, IMT, and neuroblastoma has highlighted the importance for *ALK* in histologically diverse pediatric cancers. Therefore, there is a need to better understand the role of *ALK* in cancer biology to optimize treatment strategies for pediatric cancers.

This review summarizes the recent discoveries of the oncogenic roles of *ALK* in pediatric cancers.

Structure, Function, and ALK Signaling

ALK encodes a highly conserved, 1620-amino acid RTK, which is located on chromosome 2p23.2.⁽³⁾ Together with leukocyte receptor tyrosine kinase and reactive oxygen species, *ALK* belongs to the insulin receptor superfamily of cellular transmembrane receptors that display intrinsic tyrosine kinase activity.⁽⁵⁾ The structure of this gene product includes an extracellular domain (ECD), a single transmembrane region, and an intracellular kinase domain.⁽³⁾ The *ALK* ECD is unique among RTK family members, containing a glycine-rich region and a low-density lipoprotein receptor class A domain sandwiched between two meprin, A-5 protein, and receptor protein tyrosine phosphatase mu (MAM) domains (Fig. 1).⁽³⁾ The *ALK* ECD can be divided into several regions with presumed functions of ligand binding, interactions with potential co-receptors and

secreted regulatory proteins, and dimerization, all of which may potentially relay conformational changes to initiate the activation of the intracellular kinase domain.⁽³⁾ The activation of endogenous ALK requires ligand-dependent receptor dimerization and autophosphorylation. The binding site for two putative ALK ligands, pleiotrophin and midkine, has been mapped between residues 391 and 401.^(14,15) More recently, augmentor α and β (FAM150) have been established as ALK ligands.⁽¹⁶⁾ However, the mechanism by which ALK is physiologically activated has not been completely elucidated. The ALK intracellular domains are composed of a tyrosine kinase region with three phosphorylation sites (Y1278, Y1283, and Y1283), followed by a carboxyl-terminal lobe containing interaction sites for phospholipase C- γ and Src homology 2 domain-containing (SHC).⁽¹⁷⁾

Expression of ALK in humans is limited to rare neural cells and scattered pericytes, as well as to endothelial cells after birth, implying a role in neural development and differentiation.⁽³⁾ However, the native function of ALK in humans is still unclear. At present, the distribution and function of the ALK

gene have been investigated in a number of model systems. A single ALK family member has been found in worms (*SCD-2*) and flies (*Alk*).^(18,19) In *Caenorhabditis elegans*, SCD-2 signaling is required for the integration of sensory input but is not essential for development.⁽¹⁸⁾ In *Drosophila melanogaster*, *Alk* is activated by its ligand Jelly belly (*Jeb*) and regulates the development of the gut musculature and neuronal circuitry within the visual system.⁽²⁰⁾ Flies lacking *Alk* die due to a lack of founder cell specification in the embryonic visceral muscle.⁽¹⁹⁾ Mice with a homozygous deletion of the *Alk* tyrosine kinase domain have a normal appearance and no obvious tissue abnormalities, but preliminary observations have revealed an increase in the number of progenitor cells in the hippocampus and modifications of adult brain function.⁽²¹⁾ Additionally, results in ALK gain-of-function, knock-in mice have revealed a role for ALK in neurogenesis and neuroblastoma progression combined with *MYCN* overexpression.⁽²²⁾

The ALK enzyme can activate other signaling pathways, including the PI3K-AKT, JAK-STAT, CRKL-C3G, MEKK2/3-MEK5-ERK, and MAPK pathways, which in turn transmit a

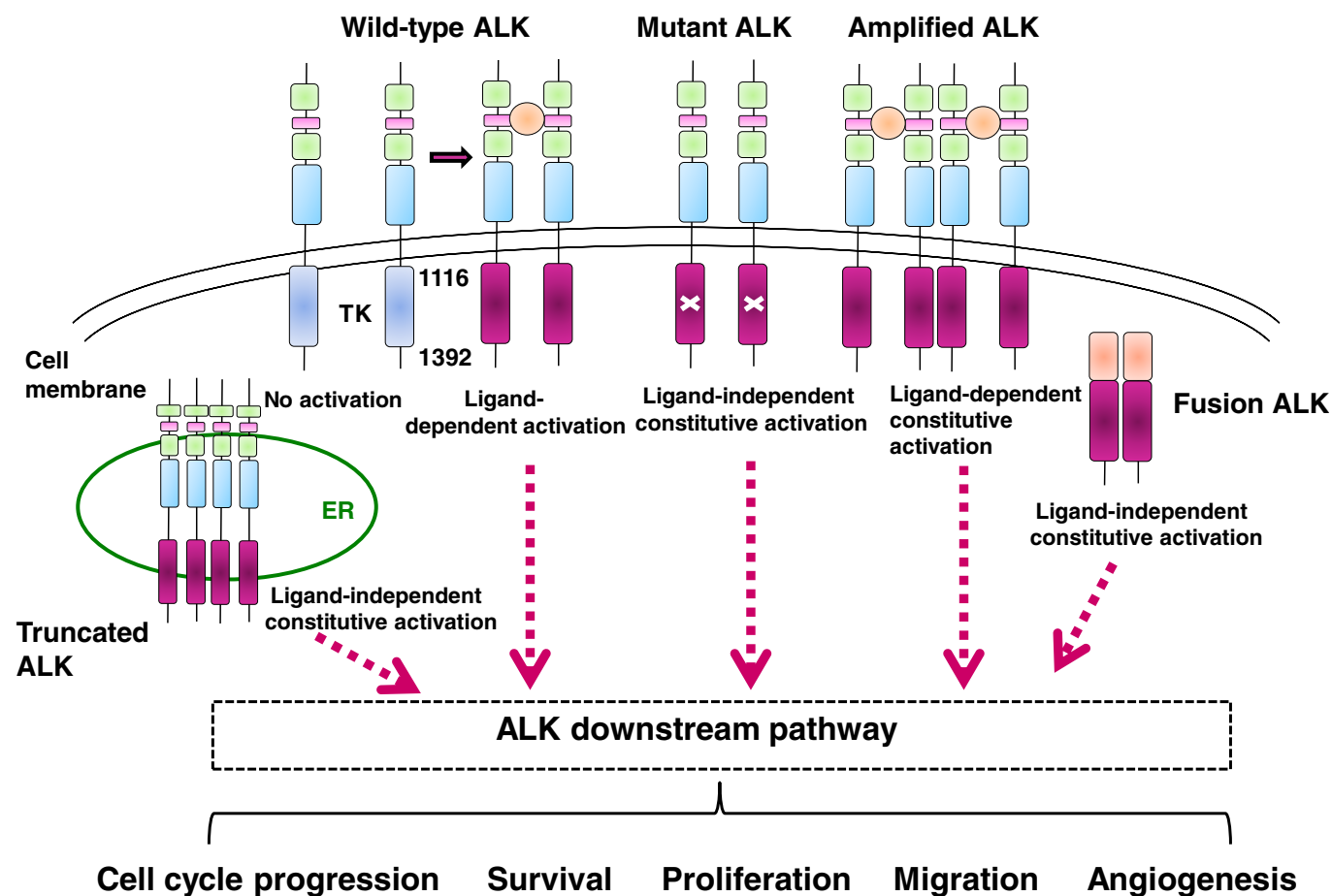


Fig. 1. Anaplastic lymphoma kinase (ALK) signaling in normal and cancer cells. Normal activation of ALK through ligand binding is shown (the ligand is indicated in orange). The full-length ALK receptor is a classical receptor tyrosine kinase, composed of an amino-terminal extracellular domain and intracellular tyrosine kinase domain (inactive in blue and active in red), and connected by a single transmembrane domain. The ALK extracellular domain contains two MAM domains (in green), one LDL domain (in pink), and a glycine-rich region (in light blue). ALK mutations result in ligand-independent constitutive activation of the downstream ALK pathway, whereas ALK amplification results in ligand-dependent constitutive activation of ALK signaling. In expressed ALK fusion proteins, ligand-independent dimerization with the oligomerization domains of partner genes leads to the constitutive activation of the ALK pathway. An aberrant form of ALK that lacks exons 2 and 3 was amplified, leading to the high-level expression of an N-terminal truncated kinase. This short form of ALK is mainly located at endoplasmic reticulum and aberrantly activates the STAT3 pathway from ER. TK, tyrosine kinase.

signal downstream through the signal pathway to the nucleus where the expression of different genes are controlled (Fig. 1).⁽²³⁾ The activation of adaptor proteins and other cellular proteins, such as protein tyrosine phosphatase, non-receptor type 11, SRC, fibroblast growth factor receptor substrate 2, and SHC, has been observed downstream of ALK, implicating roles for these alternative pathways.⁽¹⁷⁾ Other reported ALK downstream targets are Bcl-2-like protein 11, p27, Cyclin D2, nuclear interacting partner of ALK, Ras-related C3 botulinum toxin substrate 1, cell division cycle 42, p130CAS, Src homology region 2 domain-containing phosphatase-1, and PIKFYVE.^(23–26)

ALK Rearrangements in Pediatric Cancers

Various chromosomal rearrangements have been reported at the *ALK* locus on chromosome 2q23.2, leading to the generation of fusion genes that encode the entire intracellular domain of *ALK* at the 3'-end fused to various 5'-end partner genes. Recent genome-wide studies have revealed the presence of nearly 30 different *ALK* fusion partner genes in multiple types of cancer (Table 1). Even though a large number of different N-terminal partners have been identified, they all share common oncogenic features. The subsequent expression of fusion proteins is regulated by the promoter of the N-terminal partner, which is generally expressed in various normal tissues and can lead to the ectopic expression of ALK kinase.⁽⁴⁰⁾ As in other RTK-related, oncogenic fusions, the N-termini of the partner genes are characterized by the presence of oligomerization

domains, which are essential for the oncogenic potential of the fusion protein. The role of ALK fusion proteins as oncogenic drivers has been well established in preclinical models, including transgenic mouse models.⁽⁷⁾

Anaplastic large-cell lymphoma. Anaplastic large-cell lymphoma is a rare type of T-cell non-Hodgkin's lymphoma that is characterized by large cells with variable shapes (anaplastic patterns) expressing the membrane receptor CD30, which is a member of the nerve growth factor/tumor necrosis factor receptor family.⁽⁴¹⁾ Anaplastic large-cell lymphoma accounts for 2–5% of all non-Hodgkin's lymphoma, although its prevalence is higher in children and young adults with a frequency of 10–15%.⁽⁴¹⁾ Up to 90% of all pediatric ALCL cases have *ALK* rearrangements that lead to the ectopic overexpression of ALK kinase, whereas only approximately 30% of adult cases harbor these same genetic abnormalities.⁽⁴²⁾ The most recurrent rearrangements of *ALK* in ALCL is the *NPM-ALK* fusion, accounting for 75–80% of all ALK-positive ALCLs, followed by the tropomyosin 3 (*TPM3-ALK*) fusion, with a frequency of 12–18%.^(4,5,43) *NPM*, located on chromosome 5q35, is an abundant, nucleolar phosphoprotein that shuttles between the nucleus and cytoplasm;⁽⁴⁴⁾ it is involved in numerous cellular processes, including ribonucleoprotein transport, centrosome duplication, and control of genomic stability.⁽⁴⁴⁾ Tropomyosin 3 is a member of the tropomyosin family of actin-binding proteins, located on chromosome 1q25.⁽⁴⁵⁾ Tropomyosins are components of cytoskeletal microfilaments, providing actin filament stability and regulating interactions with other actin-binding proteins.⁽⁴⁵⁾ Other fusions have been found at a much lower frequency (<2%) and include TRK-fused gene (TFG)-*ALK*, clathrin heavy chain (CLTC)-*ALK*, and TPM4-*ALK*.^(23,27–29) These fusions possess subtly different characteristics when transfected into NIH3T3 fibroblasts and implanted as xenografts. The effects of the different ALK N-terminal partners were assessed by expressing five ALK fusion variants in NIH3T3 cells.⁽⁴⁰⁾ *NPM*-, TFG-, CLTC-, and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) -*ALK* were found to increase proliferation and soft agar colony formation, whereas TPM3-*ALK* had a stronger effect on invasion. The expressed TPM3-*ALK* protein was subsequently shown to co-immunoprecipitate endogenous tropomyosin, further supporting an effect on cytoskeleton organization with a concomitant decrease in cell adhesion.^(30,46) Transfection of the different *ALK* fusions in NIH3T3 cells led to the development of tumors in nude mice, but *NPM-ALK*- and *TFG-ALK*-transfected cells gave rise to more rapidly growing tumors.⁽²⁷⁾ Notably, several ALK fusions, such as TPM3/4-*ALK*, CLTC-*ALK*, and Ran-binding protein 2 (RANBP2)-*ALK*, have been detected in IMTs and diffuse B-cell large cell lymphoma, implicating a preferential choice for the *ALK* rearrangement partner, regardless of the affected organs or tissues. Importantly, clinical outcomes for ALK-positive ALCL patients approach the 70–80% cure rate, compared to 15–45% for ALK-negative ALCL patients.^(41,42)

Inflammatory myofibroblastic tumors. Inflammatory myofibroblastic tumors are rare benign or locally aggressive soft-tissue mesenchymal neoplasms.⁽⁴⁷⁾ They occur primarily in children and young adults, but can develop at any age. Histologically, IMTs are characterized by the presence of a dense, inflammatory infiltrate amid spindle cells in a myxoid to collagenous stroma.⁽⁴⁷⁾ Surgical resection is usually the first line of treatment, but many cases develop a more aggressive phenotype with occurrence of metastases. Approximately 50% of IMTs possess chromosomal rearrangements involving the 2q23.2 region, resulting in *TPM3-4-ALK* fusion transcripts.⁽³⁰⁾

Table 1. Chromosomal rearrangements involving *ALK* locus in pediatric cancers

Cancer type	<i>ALK</i> fusion gene	Chromosomal abnormalities	Reference no.
ALCL	<i>TPM3-ALK</i>	t(1;2) (q25;p23)	(23)
	<i>ATIC-ALK</i>	inv(2) (p23;q35)	(23)
	<i>TFG-ALK</i>	t(2;3) (p23;q21)	(27)
	<i>NPM1-ALK</i>	t(2;5) (p23;q35)	(4, 5)
	<i>TRAF1-ALK</i>	t(2;9) (p23;q32.2)	(28)
	<i>CLTC-ALK</i>	t(2;17) (p23;q23.1)	(23)
	<i>RNF213-ALK</i>	t(2;17) (p23;q25.3)	(29)
	<i>TPM4-ALK</i>	t(2;19) (p23;q13.1)	(23)
	<i>MYH9-ALK</i>	t(2;22) (p23;q12.3)	(23)
	<i>MSN-ALK</i>	t(2;X) (p23;q12)	(23)
IMT	<i>TPM3-ALK</i>	t(1;2) (q25;p23)	(30)
	<i>RANBP2-ALK</i>	t(2;2) (p23;q13)	(31)
	<i>ATIC-ALK</i>	inv(2) (p23;q35)	(32)
	<i>SEC31A-ALK</i>	t(2;4) (p23;q21.22)	(33)
	<i>CARS-ALK</i>	t(2;11) (p23;p15.4)	(23)
	<i>PPFIBP1-ALK</i>	t(2;12) (p23;p11)	(34)
	<i>CLTC-ALK</i>	t(2;17) (p23;q23.1)	(23)
	<i>TPM4-ALK</i>	t(2;19) (p23;q13.1)	(30)
RCC	<i>TPM3-ALK</i>	t(1;2) (q25;p23)	(36,37)
	<i>EML4-ALK*</i>	inv(2) (p21;p23)	(35)
	<i>STRN-ALK</i>	t(2;2) (p23;p22)	(37)
	<i>VCL-ALK</i>	t(2;10) (p23;q22)	(36)
PTC	<i>EML4-ALK</i>	inv(2) (p21;p23)	(39)
	<i>STRN-ALK</i>	t(2;2) (p23;p22)	(38,39)
	<i>GTF21RD1-ALK</i>	t(2;7) (p23;q11.23)	(39)

*Reported only in adult cases. ALCL, anaplastic large cell lymphoma; IMT, inflammatory myofibroblastic tumor; PTC, papillary thyroid cancer; RCC, renal cell carcinoma.

Currently, several other ALK fusions, including cysteinyl-TRNA synthetase (CARS)-ALK, CLTC-ALK, ATIC-ALK, RANBP2-ALK, and SEC31L1-ALK, have been identified at frequencies <5%.^(6,31–34) With the exception of RANBP2-ALK, which is localized to the nuclear membrane when expressed, the other expressed fusion proteins display a typical cytoplasmic staining. Anaplastic lymphoma kinase expression is generally seen in younger patients and correlates with local recurrence rather than with distant metastasis formation; thus, ALK fusions are thought to be associated with better prognosis in cases of IMT.⁽⁴⁷⁾ Published studies suggest that the identification of the ALK gene rearrangements is useful to differentiate IMTs from other spindle cell neoplasms of soft tissues and viscera.

Other pediatric and adolescent cancers with ALK fusions. Renal cell carcinoma (RCC) is rare in children; it accounts for approximately 2–4% of all pediatric renal tumors.⁽³⁵⁾ Initially, ALK rearrangements were described in pediatric RCC cases, but further investigations showed that a small proportion of adult RCCs belong to this ALK-rearranged category.⁽³⁶⁾ ALK-rearranged RCC is a distinct type of disease included in the so-called class of emerging/provisional RCCs.^(36,48) Several gene partners, including *VCL*, *TPM3*, striatin (*STRN*), echinoderm microtubule-associated protein like 4 (*EML4*), and *HOOK1*, have been reported in cases of RCC to date.^(37,48–50) Histologically, ALK-rearranged RCCs most commonly feature a solid architecture with occasional trabecular and tubular features, and are composed of sheets of epithelioid cells with abundant pale eosinophilic cytoplasm and frequent intracytoplasmic lumina.⁽⁴⁸⁾ The clinical course for ALK-rearranged RCCs is frequently indolent;

however, in adult patients, progression is frequently more aggressive.⁽⁴⁸⁾

Additionally, in adult cases of thyroid cancer, various ALK fusions, including EML4-ALK, glutamine-fructose-6-phosphate transaminase 1 (GFPT1)-ALK, TFG-ALK, and STRN-ALK, have been detected. These ALK fusions have been found in approximately 2% of patients with papillary thyroid cancer and medullary thyroid carcinoma.^(38,51) Although rare, ALK rearrangements, such as *STRN-ALK* and *EML4-ALK*, have been observed in pediatric papillary thyroid cancer cases as well.⁽³⁹⁾

Furthermore, ALK rearrangements occur in approximately 10–20% of spitzoid tumors (Spitz nevi, atypical Spitz tumors, and spitzoid melanomas), which are melanocytic neoplasms with distinctive histopathological features such as increased cell size and an epithelioid or spindle morphology.⁽⁵²⁾ Spitzoid tumors are more common in children and adolescents, but can occur at all ages. Those with ALK fusions show unique histopathologic features, which promise to improve the classification of these diagnostically challenging tumors.

ALK mutations in pediatric cancers. Gain-of-function mutations in the full-length ALK were first described in sporadic and familial neuroblastomas, common childhood solid tumors.^(8–11) Subsequently, activating ALK mutations were reported in adult cases of anaplastic thyroid cancer.⁽⁵³⁾ In addition, next-generation sequencing revealed the presence of several mutations in the ALK kinase domain in pediatric cases with rhabdomyosarcoma, primitive neuroectodermal tumor, and osteosarcoma (Fig. 2). Most of these mutations are located in the kinase domain and can be classified into three groups: ligand-independent mutations (F1174I, F1174S, F1174L, and R1275Q), ligand-dependent mutations (D1091N, T1151M, and

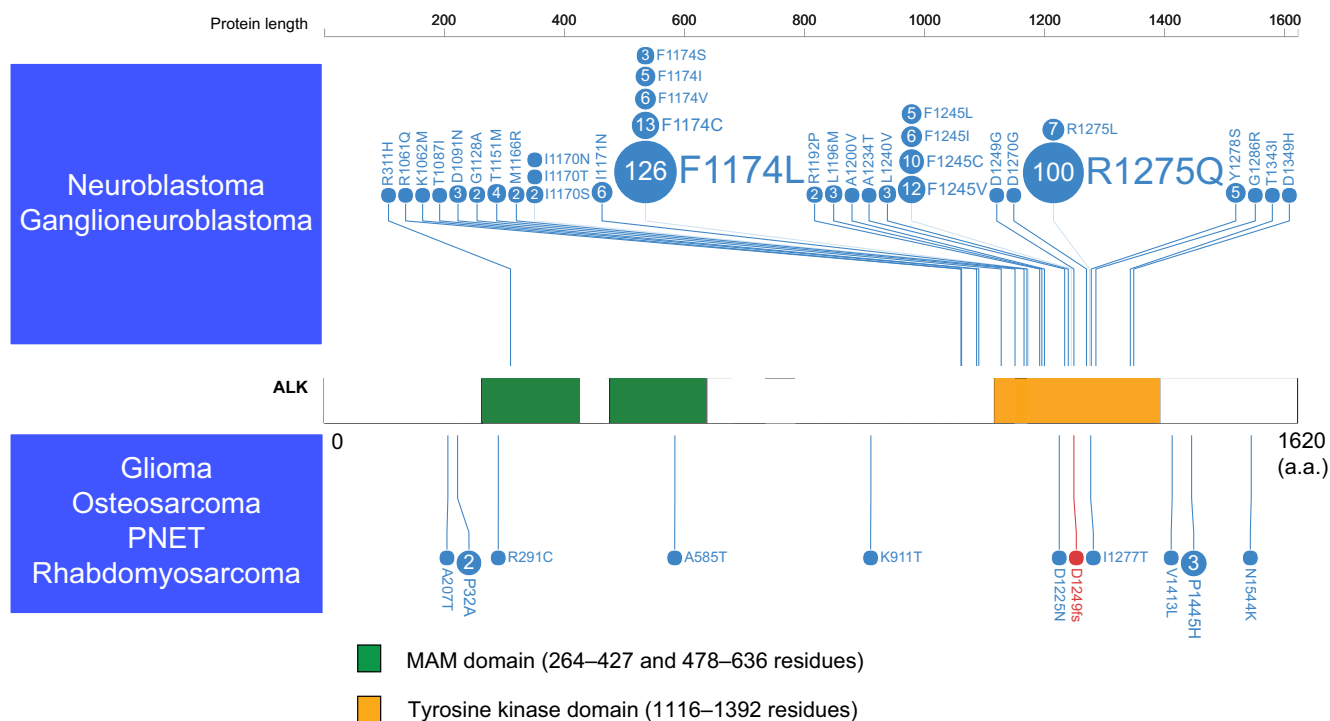


Fig. 2. Anaplastic lymphoma kinase (ALK) mutations detected in pediatric cancers. The nucleotide changes detected in neuroblastoma, ganglioneuroblastoma, glioma, osteosarcoma, primitive neuroectodermal tumor, and rhabdomyosarcoma are shown. COSMIC frequencies of ALK mutations from published reports with functional and/or therapeutic significance are shown. A missense mutation is indicated by a blue circle, and a frameshift mutation is indicated by a red circle. The numbers in the circles are the reported mutation numbers. The green box indicates MAM domains, and the orange box indicates the tyrosine kinase domain. a.a., amino acids; PNET, primitive neuroectodermal tumor.

A1234T), and a kinase-dead mutation (I1250T) (Fig. 2). *ALK* mutations are occasionally acquired in *ALK* fusion genes as a result of resistance to *ALK* inhibitors in cases of NSCLC as well as ALCL.^(54,55)

Neuroblastoma. Neuroblastoma is the most common pediatric extracranial solid tumor, a disease of the sympathoadrenal lineage of the neural crest, accounting for approximately 15% of all pediatric oncology deaths.^(56,57) This disease has a broad spectrum of clinical behaviors, ranging from localized disease with spontaneous regression to aggressive clinical course and death from progressive disease.^(56,57) Although mostly sporadic, neuroblastomas may occur in familial or syndromic contexts.⁽⁵⁷⁾ The best characterized genetic alteration associated with a poor prognosis of neuroblastoma is the amplification of the *MYCN* oncogene.⁽⁵⁶⁾ A loss of heterozygosity on chromosome 11q was also found to correlate with poor prognosis in neuroblastoma.⁽⁵⁸⁾

Previously, we and other groups discovered *ALK* to be a major oncogene target in sporadic and familial neuroblastoma cases.^(8–11) In familial cases, germline *ALK* mutations were observed in 50% of pedigree, whereas approximately 10% of sporadic cases showed *ALK* oncogenic mutations. Greater than 90% of all *ALK* mutations occurred within the kinase domain, which clearly showed two mutation hot spots at positions F1174 and R1275.⁽⁵⁹⁾ Mutations of the F1245 residue were also frequently observed in neuroblastoma, after the more common F1174 and the R1275 mutations. The R1275 mutation is more frequently observed in familial cases compared to the F1174 mutation, which is a common mutation in sporadic cases.⁽⁵⁹⁾ Intriguingly, the R1275 mutation is adjacent to the corresponding L858R mutation in the epidermal growth factor receptor, which is the most common epidermal growth factor receptor mutation in lung cancer.⁽⁶⁰⁾ According to insulin RTK homology models, F1174 is located at the end of the C α 1 helix, whereas the other two common mutations are located in the two β sheets: before the catalytic loop (β 6) (F1245) and within the activation loop (β 9) (R1275).⁽⁶⁰⁾ The R1192P mutation, which has only been reported in familial cases, is found in the β 4 strand of the kinase domain.⁽⁶⁰⁾ Expression of the F1174L and K1062M mutants in NIH3T3 cells induces transformation capacity; cells transduced with mutant proteins display increased colony formation in soft agar and tumor generation in nude mice, and mutant kinases show increased autophosphorylation and *in vitro* kinase activity compared to wild-type.⁽⁸⁾ In accordance with these findings, molecules located downstream of *ALK*, including AKT, STAT3, and

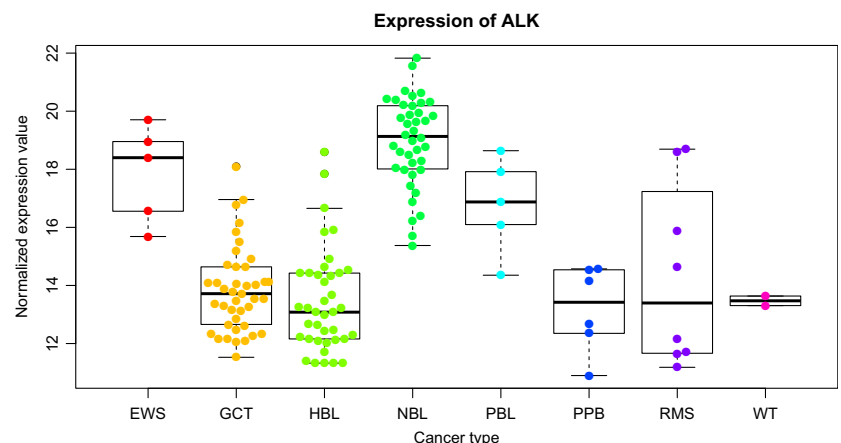
ERK, were found to be activated in cells expressing mutant *ALK* (Fig. 3).^(8–11) Furthermore, both F1174L and R1275Q mutants promote cytokine-dependent growth of BaF3 cells, an immortalized murine bone marrow-derived pro-B-cell line whose growth and proliferation depend on the presence of interleukin-3.⁽¹¹⁾ Importantly, RNAi-mediated *ALK* knockdown resulted in reduced cell proliferation in a cell line harboring the F1174L mutation, but these effects were less clear in wild-type *ALK*-expressing neuroblastoma cells.^(8,9) *ALK* mutations were found to highly correlate with *MYCN* amplification: approximately 50% of *ALK* mutations coexist with *MYCN* amplification.^(8,61) Transgenic and knock-in *ALK* mutation models highlight the synergy between *ALK* and *MYCN* in the pathogenesis of neuroblastoma.^(22,62)

ALK Amplification, Copy Number Gain, and Rare Structural Variants

ALK gene amplification occurs in a variety of cancers and is recognized as a therapeutic target. In neuroblastoma, high-grade amplification of *ALK* has been found in cell lines and primary tissues, and the subsequent overexpression of *ALK* protein correlates with tumorigenesis.⁽²³⁾ Constitutive activation of *ALK* results in the phosphorylation of downstream molecules, including SHC and MAPK pathway-related proteins, in neuroblastoma cell lines.⁽¹⁷⁾ As with *ALK* mutation, amplification of *ALK* also strongly correlates with *MYCN* amplification.⁽⁶²⁾ In addition, recurrent copy number gain of chromosome 2p23.2 involving the *ALK* locus has been reported in neuroblastoma with a frequency of 35–40%.^(8,62) High *ALK* expression has been shown to be tightly associated with *ALK* amplification/mutation, as well as copy number gain of the *ALK* locus, suggesting the upregulation of *ALK* expression by increasing DNA dose.⁽⁶²⁾

Amplification or copy number gain of the *ALK* locus, as reported in a subset of rhabdomyosarcoma cases, is also intriguing from a therapeutic perspective. Rhabdomyosarcoma is the most common soft-tissue sarcoma in children and can be divided into two histological subtypes, embryonal (ERMS) and alveolar (ARMS).⁽⁶³⁾ Alveolar rhabdomyosarcoma shows an aggressive phenotype, whereas embryonal has a more favorable prognosis.⁽⁶³⁾ We previously found that *ALK* inhibitors were effective in select rhabdomyosarcoma cell lines with high *ALK* expression levels, suggesting a possible therapeutic target for *ALK* inhibitors in rhabdomyosarcoma.⁽⁶⁴⁾ Compared with TAE684 and 2,4-pyrimidinediamine derivative (2,4-PDD), the

Fig. 3. Anaplastic lymphoma kinase (*ALK*) expression in pediatric cancers detected by RNA sequencing. Box plots of the *ALK* mRNA expression in pediatric cancers are shown. *ALK* expression, detected by RNA sequencing, was normalized and analyzed using variance-stabilizing transformations (VST). EWS, Ewing sarcoma; GCT, germ cell tumor; HBL, hepatoblastoma; NBL, neuroblastoma; PBL, pancreaticoblastoma; PPB, pleuropulmonary blastoma; RMS, rhabdomyosarcoma; WT, Wilms' tumor.



different pharmacological behavior of crizotinib may be related to MET inhibition, the specific activity of crizotinib, because the rhabdomyosarcoma cell lines examined also express MET to varying degrees.⁽⁶⁴⁾ Given that *ALK* amplification or copy number gain is predominantly detected in ARMS (6–17%),^(64,65) rhabdomyosarcoma cases with a poor prognosis may benefit from ALK inhibitors.

We found another mechanism of ALK activation observed in a neuroblastoma-derived cell line (NB-1): A short form of the ALK protein ($ALK^{\text{del}2-3}$, with a truncated extracellular domain) is overexpressed, because of the amplification of an abnormal *ALK* gene that lacks exons 2 and 3.⁽⁶⁶⁾ Functional analysis revealed that $ALK^{\text{del}2-3}$ underwent autophosphorylation in NB-1 and NIH3T3 cells, demonstrating enhanced kinase activity and promoting downstream signaling pathways, such as the STAT3 pathway. These results confirm the oncogenic activity of $ALK^{\text{del}2-3}$.⁽⁶⁶⁾ Interestingly, two separate studies described aberrant ALK proteins with truncated extracellular domains, Del 1-5 and Del 4-11 variants, leading to constitutive kinase activity in four neuroblastoma specimens, including three primary cases. These truncated proteins resulted from chromosomal rearrangements involving the *ALK* locus.^(67,68) Our data, together with these results, indicate that, although rare, truncated ALK proteins are involved in the pathogenesis of neuroblastoma, and patients with these rare types of *ALK* alterations may benefit from targeted treatments using ALK inhibitors.

Expression of Full-length ALK in Pediatric Cancers

Expression of full-length ALK protein has been detected in a variety of human cancer cell lines and tumor specimens, such as rhabdomyosarcoma, glioblastoma, and Wilms' tumor.^(65,69,70) In our cohort of 146 cases with pediatric cancers, expression of full-length *ALK* was observed across various pediatric solid tumors, such as rhabdomyosarcoma, Ewing sarcoma, and pancreatoblastoma (Fig. 3). Previously, a genome-wide study revealed *ALK* as a target gene of *PAX3-FOXO1*, the recurrent gene fusion in ARMS.⁽⁷¹⁾ Quantitative assessment of *ALK* mRNA expression in rhabdomyosarcoma revealed that high *ALK* expression correlated with a negative prognosis, independent of fusion status.⁽⁷²⁾ The wide distribution of ALK expression in pediatric solid tumors suggests that ALK inhibitor-based therapies may benefit patients with these particular tumors.

Targeted Anticancer Therapies

Recently, several ALK inhibitors were developed and examined in preclinical and clinical trials.⁽⁷³⁾ Initial studies were carried out using less potent ALK inhibitors, such as WHI-P154, pyridines, and heat shock protein 90.^(74,75) Subsequently, more potent and specific ALK inhibitors, such as diaminopyrimidines or aminopyrimidines including NVP-TAE684, 2,4-PDD, and crizotinib (PF-2341066), have been developed.^(7,73) NVP-TAE684 is a highly potent and specific NPM-ALK inhibitor but is not currently being developed clinically. Such

selective inhibitors can lead to the potent suppression of cell growth in tumors expressing ALK fusion proteins. Crizotinib is an inhibitor of both MET and ALK, and is the first ALK inhibitor to be tested in phase I–II clinical trials of pediatric patients including relapsed solid tumors and ALCL.⁽¹³⁾ Crizotinib was generally well tolerated in pediatric patients and showed excellent antitumor activity in patients with ALCL and IMT.⁽⁹⁾ Although neuroblastoma cell lines harboring *ALK* mutations or amplification were highly vulnerable to ALK inhibitors, such as NVP-TAE684, such a robust response to crizotinib has not been observed in neuroblastoma patients with *ALK* mutations.^(8,9) Thus, it will be important to study neuroblastoma cells expressing mutant ALK to determine whether an ALK inhibitor alone, or in combination with other kinase inhibitors, is sufficient for growth inhibition. Further development of ALK inhibitors, alone and in combination with conventional chemotherapies, is likely to be used in the near future. The development of mAbs against ALK should be investigated, as they may be particularly useful for tumors that have acquired resistance to ALK inhibitors.

Conclusion

This review provides an understanding of the role of ALK in pediatric cancers and the preclinical proof-of-principle for the use of ALK as a therapeutic target. There is a strong rationale and significant enthusiasm for the use of ALK inhibitors as targeted therapies against tumors harboring oncogenic fusions or activating mutations. The results of the first clinical trial with crizotinib have shown promise in highly refractory patients with tumors harboring ALK fusion proteins; these results support the ongoing efforts to select patients for subsequent trials based on *ALK* translocation status. Additional clinical trials of other ALK inhibitors, such as alectinib and loratinib, in neuroblastoma patients will be crucial to overcoming neuroblastomas harboring *ALK* mutations.⁷⁶

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References

1 McDermott U, Settleman J. Personalized cancer therapy with selective kinase inhibitors: an emerging paradigm in medical oncology. *J Clin Oncol* 2009; **27**: 5650–9.

2 Gambacorti-Passerini C, Aroldi A, Cordani N, Piazza R. Chronic myeloid leukemia: Second-line drugs of choice. *Am J Hematol* 2016; **91**: 67–75.
3 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.

- 4 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.
- 5 Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S. Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. *Oncogene* 1994; **9**: 1567–74.
- 6 Bridge JA, Kanamori M, Ma Z *et al.* Fusion of the *ALK* gene to the clathrin heavy chain gene, *CLTC*, in inflammatory myofibroblastic tumor. *Am J Pathol* 2001; **159**: 411–5.
- 7 Soda M, Takada S, Takeuchi K *et al.* A mouse model for EML4-*ALK*-positive lung cancer. *Proc Natl Acad Sci U S A* 2008; **105**: 19893–7.
- 8 Chen Y, Takita J, Choi YL *et al.* Oncogenic mutations of *ALK* kinase in neuroblastoma. *Nature* 2008; **455**: 971–4.
- 9 Mossé YP, Laudenslager M, Longo L *et al.* Identification of *ALK* as a major familial neuroblastoma predisposition gene. *Nature* 2008; **455**: 930–5.
- 10 Janoueix-Lerosey I, Lequin D, Brugères L *et al.* Somatic and germline activating mutations of the *ALK* kinase receptor in neuroblastoma. *Nature* 2008; **455**: 967–70.
- 11 George RE, Sanda T, Hanna M *et al.* Activating mutations in *ALK* provide a therapeutic target in neuroblastoma. *Nature* 2008; **455**: 975–8.
- 12 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.
- 13 Mossé YP, Lim MS, Voss SD *et al.* Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncol* 2013; **14**: 472–80.
- 14 Stoica GE, Kuo A, Aigner A *et al.* Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J Biol Chem* 2001; **276**: 16772–9.
- 15 Stoica GE, Kuo A, Powers C *et al.* Midkine binds to anaplastic lymphoma kinase (*ALK*) and acts as a growth factor for different cell types. *J Biol Chem* 2002; **277**: 35990–8.
- 16 Reshetnyak AV, Murray PB, Shi X *et al.* Augmentor α and β (FAM150) are ligands of the receptor tyrosine kinases *ALK* and *LTK*: hierarchy and specificity of ligand-receptor interactions. *Proc Natl Acad Sci U S A* 2015; **112**: 15862–7.
- 17 Miyake I, Hakomori Y, Shinohara A *et al.* Activation of anaplastic lymphoma kinase is responsible for hyperphosphorylation of ShcC in neuroblastoma cell lines. *Oncogene* 2002; **21**: 5823–34.
- 18 Reiner DJ, Ailion M, Thomas JH, Meyer BJC. *elegans* anaplastic lymphoma kinase ortholog SCD-2 controls dauer formation by modulating TGF- β signaling. *Curr Biol* 2008; **18**: 1101–9.
- 19 Lorén CE, Englund C, Grabbe C, Hallberg B, Hunter T, Palmer RH. A crucial role for the Anaplastic lymphoma kinase receptor tyrosine kinase in gut development in *Drosophila melanogaster*. *EMBO Rep* 2003; **4**: 781–6.
- 20 Bazigou E, Apitz H, Johansson J *et al.* Anterograde Jelly belly and *Alk* receptor tyrosine kinase signaling mediates retinal axon targeting in *Drosophila*. *Cell* 2007; **128**: 961–75.
- 21 Englund C, Lorén CE, Grabbe C *et al.* Jeb signals through the *Alk* receptor tyrosine kinase to drive visceral muscle fusion. *Nature* 2003; **425**: 512–6.
- 22 Ueda T, Nakata Y, Yamasaki N *et al.* *ALK(R1275Q)* perturbs extracellular matrix, enhances cell invasion and leads to the development of neuroblastoma in cooperation with *MYCN*. *Oncogene* 2016; **35**: 4447–58.
- 23 Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer* 2008; **8**: 11–23.
- 24 Dupuis-Coronas S, Lagarrigue F, Ramel D *et al.* The nucleophosmin-anaplastic lymphoma kinase oncogene interacts, activates, and uses the kinase PIKfyve to increase invasiveness. *J Biol Chem* 2011; **286**: 32105–14.
- 25 Piazza R, Magistroni V, Mogavero A *et al.* Epigenetic silencing of the proapoptotic gene *BIM* in anaplastic large cell lymphoma through an MeCP2/SIN3a deacetylating complex. *Neoplasia* 2013; **15**: 511–22.
- 26 Kramer M, Ribeiro D, Arsenian-Henriksson M, Deller T, Rohrer H. Proliferation and Survival of Embryonic Sympathetic Neuroblasts by *MYCN* and Activated *ALK* Signaling. *J Neurosci* 2016; **36**: 10425–39.
- 27 Hernández L, Pinyol M, Hernández S *et al.* *TRK*-fused gene (*TFG*) is a new partner of *ALK* in anaplastic large cell lymphoma producing two structurally different *TFG-ALK* translocations. *Blood* 1999; **94**: 3265–8.
- 28 Feldman AL, Vasmataz G, Asmann YW *et al.* Novel *TRAF1-ALK* fusion identified by deep RNA sequencing of anaplastic large cell lymphoma. *Genes Chromosomes Cancer* 2013; **52**: 1097–102.
- 29 van de Krogt JA, Vanden Bempt M, Finalet Ferreiro J *et al.* *ALK*-positive anaplastic large cell lymphoma with the variant *RNF213*-, *ATIC*- and *TPM3-ALK* fusions is characterized by copy number gain of the rearranged *ALK* gene. *Haematologica* 2017; pii: haematol.2016.146571. <https://doi.org/10.3324/haematol.2016.146571>.
- 30 Lawrence B, Perez-Atayde A, Hibbard MK *et al.* *TPM3-ALK* and *TPM4-ALK* oncogenes in inflammatory myofibroblastic tumors. *Am J Pathol* 2000; **157**: 377–84.
- 31 Ma Z, Hill DA, Collins MH *et al.* Fusion of *ALK* to the Ran-binding protein 2 (*RANBP2*) gene in inflammatory myofibroblastic tumor. *Genes Chromosomes Cancer* 2003; **37**: 98–105.
- 32 Debiec-Rychter M, Marynen P, Hagemeyer A, Pauwels P. *ALK-ATIC* fusion in urinary bladder inflammatory myofibroblastic tumor. *Genes Chromosomes Cancer* 2003; **38**: 187–90.
- 33 Panagopoulos I, Nilsson T, Domanski HA *et al.* Fusion of the *SEC31L1* and *ALK* genes in an inflammatory myofibroblastic tumor. *Int J Cancer* 2006; **118**: 1181–6.
- 34 Takeuchi K, Soda M, Togashi Y *et al.* Pulmonary inflammatory myofibroblastic tumor expressing a novel fusion, *PPFIBP1-ALK*: reappraisal of anti-*ALK* immunohistochemistry as a tool for novel *ALK* fusion identification. *Clin Cancer Res* 2011; **17**: 3341–8.
- 35 Chen YB, Xu J, Skanderup AJ *et al.* Molecular analysis of aggressive renal cell carcinoma with unclassified histology reveals distinct subsets. *Nat Commun* 2016; **7**: 13131.
- 36 Cajaiba MM, Jennings LJ, Rohan SM *et al.* *ALK*-rearranged renal cell carcinomas in children. *Genes Chromosomes Cancer* 2016; **55**: 442–51.
- 37 Thorner PS, Shago M, Marrano P, Shaikh F, Somers GR. *TFE3*-positive renal cell carcinomas are not always *Xp11* translocation carcinomas: report of a case with a *TPM3-ALK* translocation. *Pathol Res Pract* 2016; **212**: 937–42.
- 38 Kelly LM, Barila G, Liu P *et al.* Identification of the transforming *STRN-ALK* fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. *Proc Natl Acad Sci U S A* 2014; **111**: 4233–8.
- 39 Vanden Borre P, Schrock AB, Anderson PM *et al.* Pediatric, adolescent, and young adult thyroid carcinoma harbors frequent and diverse targetable genomic alterations, including Kinase fusions. *Oncologist* 2017; **22**: 255–63.
- 40 Armstrong F, Duplantier MM, Tremat P *et al.* Differential effects of *X-ALK* fusion proteins on proliferation, transformation, and invasion properties of NIH3T3 cells. *Oncogene* 2004; **23**: 6071–82.
- 41 Chihara D, Fanale MA. Management of anaplastic large cell lymphoma. *Hematol Oncol Clin North Am* 2017; **31**: 209–22.
- 42 Medeiros LJ, Elenitoba-Johnson KS. Anaplastic large cell lymphoma. *Am J Clin Pathol* 2007; **127**: 707–22.
- 43 Cools J, Wlodarska I, Somers R *et al.* Identification of novel fusion partners of *ALK*, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. *Genes Chromosomes Cancer* 2002; **34**: 354–62.
- 44 Box JK, Paquet N, Adams MN *et al.* Nucleophosmin: from structure and function to disease development. *BMC Mol Biol* 2016; **17**: 19.
- 45 Hitchcock-DeGregori SE, Barua B. Tropomyosin structure, function, and interactions: a dynamic regulator. *Subcell Biochem* 2017; **82**: 253–84.
- 46 Armstrong F, Lamant L, Hieblot C, Delsol G, Touriol C. *TPM3-ALK* expression induces changes in cytoskeleton organisation and confers higher metastatic capacities than other *ALK* fusion proteins. *Eur J Cancer* 2007; **43**: 640–6.
- 47 Palaskar S, Koshti S, Maralingannavar M, Bartake A. Inflammatory myofibroblastic tumor. *Contemp Clin Dent* 2011; **2**: 274–7.
- 48 Jeanneau M, Gregoire V, Desplechain C *et al.* *ALK* rearrangements-associated renal cell carcinoma (*RCC*) with unique pathological features in an adult. *Pathol Res Pract* 2016; **212**: 1064–6.
- 49 Debelenko LV, Raimondi SC, Daw N *et al.* Renal cell carcinoma with novel *VCL-ALK* fusion: new representative of *ALK*-associated tumor spectrum. *Mod Pathol* 2011; **24**: 430–42.
- 50 Kusano H, Togashi Y, Akiba J *et al.* Two cases of renal cell carcinoma harboring a novel *STRN-ALK* fusion gene. *Am J Surg Pathol* 2016; **40**: 761–9.
- 51 Ji JH, Oh YL, Hong M *et al.* Identification of driving *ALK* fusion genes and genomic landscape of medullary thyroid cancer. *PLoS Genet* 2015; **11**: e1005467.
- 52 Amin SM, Haugh AM, Lee CY *et al.* A comparison of morphologic and molecular features of *BRAF*, *ALK*, and *NTRK1* fusion spitzoid neoplasms. *Am J Surg Pathol* 2017; **41**: 491–8.
- 53 Guan J, Wolfstetter G, Siaw J *et al.* Anaplastic lymphoma kinase L1198F and G1201E mutations identified in anaplastic thyroid cancer patients are not ligand-independent. *Oncotarget* 2017; **8**: 11566–78.
- 54 Choi YL, Soda M, Yamashita Y *et al.* EML4-*ALK* mutations in lung cancer that confer resistance to *ALK* inhibitors. *N Engl J Med* 2010; **363**: 1734–9.
- 55 Sakamoto H, Tsukaguchi T, Hiroshima S *et al.* CH5424802, a selective *ALK* inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* 2011; **19**: 679–90.
- 56 Bown N. Neuroblastoma tumour genetics: clinical and biological aspects. *J Clin Pathol* 2001; **54**: 897–910.
- 57 Maris JM. Recent advances in neuroblastoma. *N Engl J Med* 2010; **362**: 2202–11.
- 58 Atiyeh EF, London WB, Mossé YP *et al.* Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med* 2005; **353**: 2243–53.
- 59 Carpenter EL, Mossé YP. Targeting *ALK* in neuroblastoma—preclinical and clinical advancements. *Nat Rev Clin Oncol* 2012; **9**: 391–9.

- 60 Lee CC, Jia Y, Li N *et al.* Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. *Biochem J* 2010; **430**: 425–37.
- 61 De Brouwer S, De Preter K, Kumps C *et al.* Meta-analysis of neuroblastomas reveals a skewed ALK mutation spectrum in tumors with MYCN amplification. *Clin Cancer Res* 2010; **16**: 4353–62.
- 62 Berry T, Luther W, Bhatnagar N *et al.* The ALK(F1174L) mutation potentiates the oncogenic activity of MYCN in neuroblastoma. *Cancer Cell* 2012; **22**: 117–30.
- 63 El Demellawy D, McGowan-Jordan J, de Nanassy J, Chernetsova E, Nasr A. Update on molecular findings in rhabdomyosarcoma. *Pathology* 2017; **49**: 238–46.
- 64 Nishimura R, Takita J, Sato-Otsubo A *et al.* Characterization of genetic lesions in rhabdomyosarcoma using a high-density single nucleotide polymorphism array. *Cancer Sci* 2013; **104**: 856–64.
- 65 Yoshida A, Shibata T, Wakai S *et al.* Anaplastic lymphoma kinase status in rhabdomyosarcomas. *Mod Pathol* 2013; **26**: 772–81.
- 66 Okubo J, Takita J, Chen Y *et al.* Aberrant activation of ALK kinase by a novel truncated form ALK protein in neuroblastoma. *Oncogene* 2012; **31**: 4667–76.
- 67 Cazes A, Louis-Brennetot C, Mazot P *et al.* Characterization of rearrangements involving the ALK gene reveals a novel truncated form associated with tumor aggressiveness in neuroblastoma. *Cancer Res* 2013; **73**: 195–204.
- 68 Fransson S, Hansson M, Ruuth K *et al.* Intragenic anaplastic lymphoma kinase (ALK) rearrangements: translocations as a novel mechanism of ALK activation in neuroblastoma tumors. *Genes Chromosom Cancer* 2015; **54**: 99–109.
- 69 Gustafson S, Medeiros LJ, Kalhor N, Bueso-Ramos CE. Anaplastic large cell lymphoma: another entity in the differential diagnosis of small round blue cell tumors. *Ann Diagn Pathol* 2009; **13**: 413–27.
- 70 Le Rhun E, Chamberlain MC, Zairi F *et al.* Patterns of response to crizotinib in recurrent glioblastoma according to ALK and MET molecular profile in two patients. *CNS Oncol* 2015; **4**: 381–6.
- 71 Cao L, Yu Y, Bilke S *et al.* Genome-wide identification of PAX3-FKHR binding sites in rhabdomyosarcoma reveals candidate target genes important for development and cancer. *Cancer Res* 2010; **70**: 6497–508.
- 72 van Gaal JC, Flucke UE, Roeffen MH *et al.* Anaplastic lymphoma kinase aberrations in rhabdomyosarcoma: clinical and prognostic implications. *J Clin Oncol* 2012; **30**: 308–15.
- 73 Ardini E, Magnaghi P, Orsini P, Galvani A, Menichincheri M. Anaplastic Lymphoma Kinase: role in specific tumours, and development of small molecule inhibitors for cancer therapy. *Cancer Lett* 2010; **299**: 81–94.
- 74 Amin HM, Medeiros LJ, Ma Y *et al.* Inhibition of JAK3 induces apoptosis and decreases anaplastic lymphoma kinase activity in anaplastic large cell lymphoma. *Oncogene* 2003; **22**: 5399–407.
- 75 Sequist LV, Gettinger S, Senzer NN *et al.* Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J Clin Oncol* 2010; **28**: 4953–60.
- 76 Roskoski R. Anaplastic lymphoma kinase (ALK) inhibitors in the treatment of ALK-driven lung cancers. *Pharmacol Res* 2017; **117**: 343–56.