

## REVIEW

# Anaplastic lymphoma kinase in human cancer

Antonella Barreca<sup>1</sup>, Elena Lasorsa<sup>1</sup>, Ludovica Riera<sup>1</sup>, Rodolfo Machiorlatti<sup>1</sup>, Roberto Piva<sup>1,2</sup>, Maurilio Ponzoni<sup>3</sup>, Ivo Kwee<sup>4</sup>, Francesco Bertoni<sup>4</sup>, Pier Paolo Piccaluga<sup>5</sup>, Stefano A Pileri<sup>5</sup>, Giorgio Inghirami<sup>1,2</sup> and The European T-Cell Lymphoma Study Group<sup>†</sup>

<sup>1</sup>Department of Pathology and Center for Experimental Research and Medical Studies (CeRMS), University of Torino, Via Santena 7, Torino 10126, Italy

<sup>2</sup>Department of Pathology, NYU Cancer Center, New York University School of Medicine, New York, New York 10016, USA

<sup>3</sup>Unit of Lymphoid Malignancies, San Raffaele H Scientific Institute, Milan 20132, Italy,

<sup>4</sup>Laboratory of Experimental Oncology and Lymphoma Unit, Oncology Institute of Southern Switzerland (IOSI), Bellinzona 6900, Switzerland

<sup>5</sup>Hematopathology Section, Department of Hematology and Oncological Sciences 'L. and A. Seràgnoli', S. Orsola-Malpighi Hospital, University of Bologna, Bologna 40138, Italy

(Correspondence should be addressed to G Inghirami at Department of Pathology and Center for Experimental Research and Medical Studies (CeRMS), University of Torino; Email: giorgio.inghirami@unito.it)

<sup>†</sup>See Acknowledgements section for details of the European T-Cell Lymphoma Study Group

## Abstract

The receptor tyrosine kinases (RTKs) play a critical role, controlling cell proliferation, survival, and differentiation of normal cells. Their pivotal function has been firmly established in the pathogenesis of many cancers as well. The anaplastic lymphoma kinase (ALK), a transmembrane RTK, originally identified in the nucleophosmin (NPM)–ALK chimera of anaplastic large cell lymphoma, has emerged as a novel tumorigenic player in several human cancers. In this review, we describe the expression of the ALK–RTK, its related fusion proteins, and their molecular mechanisms of activation. Novel tailored strategies are briefly illustrated for the treatment of ALK-positive neoplasms.

*Journal of Molecular Endocrinology* (2011) **47**, R11–R23

## Introduction

Since the seminal description of the nucleophosmin (NPM)–anaplastic lymphoma kinase (ALK) fusion protein in anaplastic large cell lymphoma (ALCL; Morris *et al.* 1994, Shiota *et al.* 1994), many ALK chimeras have been described in inflammatory myofibroblastic tumors (IMTs; Griffin *et al.* 1999), diffuse large B-cell lymphoma (DLBCL; Arber *et al.* 1996), and more recently, in several epithelial neoplasms, including non-small cell lung cancer (NSCLC; Rikova *et al.* 2007, Soda *et al.* 2007), esophageal squamous cell carcinoma (SCC; Jazii *et al.* 2006, Du *et al.* 2007), colon (Lin *et al.* 2009), and breast carcinoma (Lin *et al.* 2009). ALK receptor expression, originally documented in a variety of cancer lines, has been documented in many neuronal tumors (Lamant *et al.* 2000, Miyake *et al.* 2002, 2005, Stoica *et al.* 2002, Osajima-Hakomori *et al.* 2005), glioblastoma (Powers *et al.* 2002, Shao *et al.* 2002, Grzelinski *et al.* 2005, Lu *et al.* 2005), and mesenchymal neoplasms including melanoma (Dirks *et al.* 2002) and rhabdomyosarcoma (Morris *et al.* 1994, 1997, Pulford

*et al.* 1997, Falini *et al.* 1998, Cessna *et al.* 2002, Pillay *et al.* 2002, Li *et al.* 2004). In this context, ALK overexpression or gain of function mutations have been demonstrated to be tumorigenic.

## ALK expression in hematological disorders

ALCL, first described in 1985 (Stein *et al.* 1985), nowadays corresponds to a specific subtype of systemic peripheral T-cell lymphoma (Swerdlow *et al.* 2008). Most ALCL display chromosomal translocations of the *ALK* gene, although a subset, lacking these aberrations, is now recognized as a provisional entity (Swerdlow *et al.* 2008). *ALK* encodes a 210 kDa tyrosine kinase (TK) receptor (CD247) belonging to the insulin growth factor receptor super family. It is expressed at high levels in the nervous system during embryogenesis but only focally in the adult brain (Iwahara *et al.* 1997). Its presence outside of the nervous system is believed to be negligible in normal tissues. Although the physiologic role of ALK receptor in mammals is unknown, it might

be involved in neuronal differentiation, as suggested by its ability to induce neurite outgrowth *in vitro* (Soultou *et al.* 2001) and by its role in synapse formation in *Caenorhabditis elegans* and *Drosophila melanogaster* (Liao *et al.* 2004, Bazigou *et al.* 2007, Reiner *et al.* 2008).

Remarkably, Allouche (2007) has recently demonstrated that ALK (CD246) is a novel dependence receptor. Indeed, the ALK receptor is inactive in the absence of engaging ligand(s) and its expression results in enhanced apoptosis, whereas ALK activation, via a ligand-mediated engagement or as result of ALK fusion proteins, decreases apoptosis (Mourali *et al.* 2006).

Virtually, all ALK chimeras derive from genomic breakpoints, almost invariably located within the intron between the exons 19 and 20 (NM\_004304.3), leading to the fusion of the intracytoplasmic domain of ALK (exons 20–29) with different partners, which provide dimerization domains (Chiarle *et al.* 2008, Fornari *et al.* 2009).

Many ALK-positive (ALK<sup>+</sup>) ALCL express the NPM–ALK fusion protein, derived from the t(2;5)(p23;q25) translocation (Jaffe *et al.* 2001). NPM1 is a multifunctional protein, which acts as a molecular chaperone in the transport of pre-ribosomal particles from the nucleus to the cytoplasm, although it plays a critical role in DNA repair, transcription, and genomic stability as well (Okuwaki 2008). The N-terminus domain of NPM1, within the ALK chimera, provides a dimerization domain, essential for chimera autophosphorylation, allowing the constitutive activation of the kinase and the firing of downstream signaling (Fujimoto *et al.* 1996, Bischof *et al.* 1997, Chiarle *et al.* 2008).

The oncogenic potential of ALK chimeras was first demonstrated *in vivo* in mice undergoing bone marrow transplantation with cells transduced with NPM–ALK construct (Kuefer *et al.* 1997). Similar results were obtained testing the transforming potential of fibroblasts containing NPM–ALK *in vitro* (Bai *et al.* 1998). In 2003, a mouse model was generated in which the expression of NPM–ALK, under the control of the CD4 promoter (Chiarle *et al.* 2003), showed the spontaneous development of T-cell lymphomas and/or plasmacytomas, confirming the lymphomagenic role of NPM–ALK, providing a valuable tool for the study of ALCL. These findings were then confirmed using additional mouse models (Turner & Alexander 2005).

Mutagenesis and functional studies have identified several NPM–ALK interacting molecules such as PLC- $\gamma$ , IRS1, HSP90, GRB2, SHCC, JAK2/JAK3, PI3K, and STAT3/5 (Chiarle *et al.* 2008; Fig. 1).

### Phospholipase C- $\gamma$

NPM–ALK controls cellular proliferation via the phospholipase C- $\gamma$  (PLC- $\gamma$ ) docking in position Y664 of NPM–ALK. PLC- $\gamma$  activation induces the hydrolysis

of phosphatidylinositol (PIP<sub>2</sub>) into inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG), molecules that can modulate the release of Ca<sup>2+</sup> from intracellular compartments and activate the serine/threonine protein kinase C (PKC). Ba/F3 cells (a pro-B line that requires IL3 for survival and growth) can grow in IL3-independent manner following the NPM–ALK transfection, while the use of the NPM–ALK<sup>Y664F</sup> mutant completely disables their growth in the absence of IL3 (Bai *et al.* 1998).

### RAS

ALK<sup>+</sup> ALCL cell growth is largely dependent on the Ras–extracellular signal regulated kinase (ERK) pathway. ALK fusion proteins can engage the effectors IRS1, SHC, and GRB2 lead to the constitutive activation of Ras. Although IRS1 and SHC may not be required for transformation (Fujimoto *et al.* 1996), inhibition of ERK-1 and -2 leads to cell cycle arrest and block of proliferation.

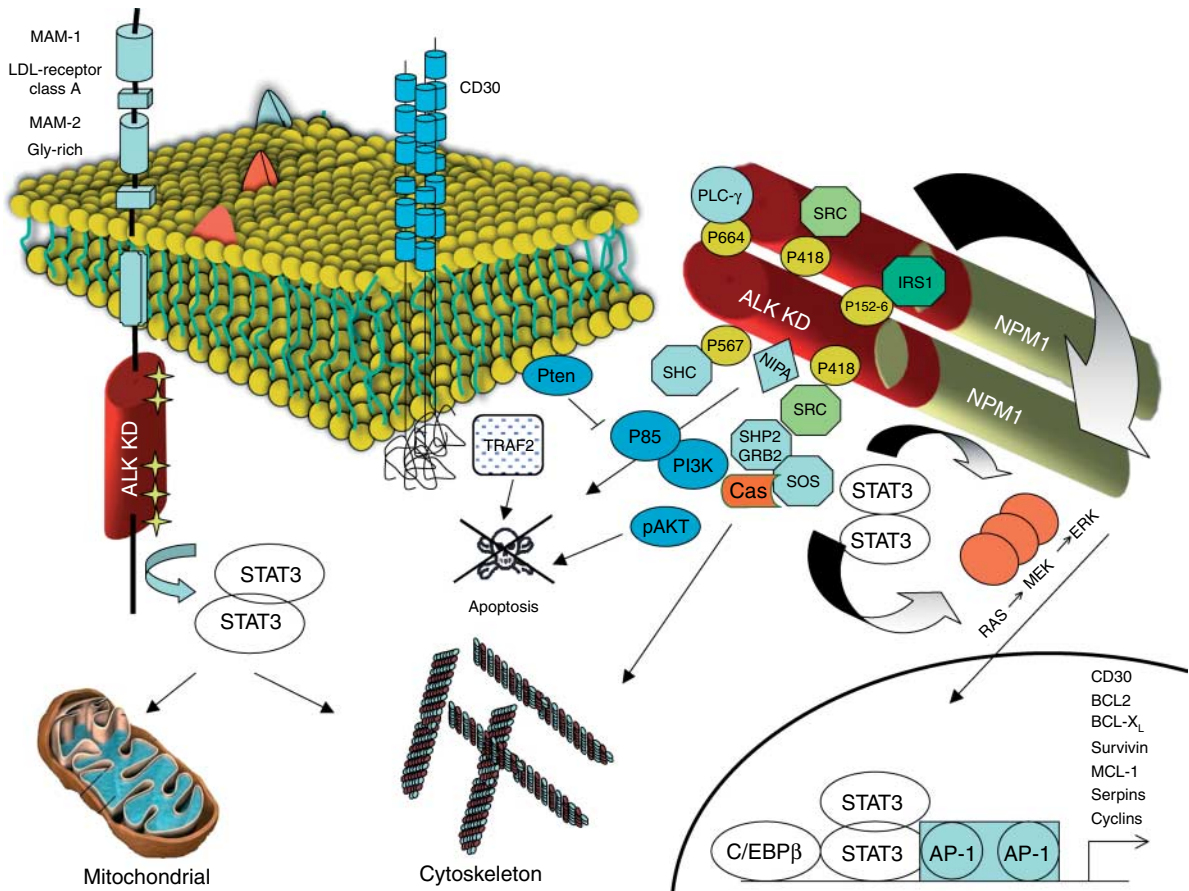
Ras activation via mitogen-activated protein kinases (MAPK), ERK-1, and -2 regulates the phosphorylation of several transcription factors, including the AP-1 complex, which is believed to contribute to the ALCL neoplastic phenotype (i.e. CD30).

### Phosphatidylinositol 3 kinase

NPM–ALK interacts directly and indirectly with PI3K (Bai *et al.* 2000). Following this association, the PI3K catalytic subunit (p110) leads to the activation of the PKB/AKT pathway. AKT, a serine/threonine kinase, is known to provide anti-apoptotic signals regulating several mediators, including caspase 9, BAD, NF- $\kappa$ B, and Fas ligand (Chiarle *et al.* 2008). Moreover, AKT, through the hyperphosphorylation of the transcription factor FOXO3a (Gu *et al.* 2004), increases cyclin D2 and inhibits p27 transcription, forcing G1 phase cell cycle arrest.

### c-Src

c-Src is a TK receptor that plays a relevant role in cell migration, as well as in cell proliferation and growth. Its kinase activity is essential for the integrin-mediated adhesion and for morphological adaptation of cells. c-Src is normally maintained in a catalytically inactive conformation by molecular interactions via its SH2 and SH3 domains. pp60 (c-Src) is activated by NPM–ALK following its association with a tyrosine residue in position 418. Studies taking advantage of Src-specific inhibitors or RNA interference have shown that NPM–ALK-mediated activation of c-Src kinase is important for the growth of NPM–ALK-positive ALCL cells. SRC-family kinases may also contribute to the activation of VAV1, which was directly activated by



**Figure 1** ALK and its signaling transduction pathways. Mutated ALK-R and ALK fusion proteins (NPM-ALK is depicted as representative ALK chimera) can elicit multiple signaling pathways, which are responsible for cell transformation and for the maintenance of the neoplastic phenotype (Chiarle *et al.* 2008, Fornari *et al.* 2009). The ALK-RTK is a tyrosine kinase membrane receptor characterized by an extra-membraneous region, in which distinct domains/regions can be recognized (MAM-1 and -2 LDL-receptor class A, and glycine-rich region), a trans-membrane segment within the lipid bilayer, and an intracytoplasmic segment, which includes the tyrosine catalytic domain and its flanking regions. Several activating mutations of the ALK-RTK have been recently demonstrated within the juxtamembrane, P-loop, kinase domain and end of C helix, and catalytic/activation loops (some of the mutations within the catalytic domains are schematically represented here: stars). These activating mutations lead to the constitutive activation of the receptor in a ligand-independent manner. The kinase activation, in both mut-ALK-RTK and ALK chimeras, is associated with the docking of several adaptors, which in turn fire several signaling pathways. ALK-dependent mitogenic signaling is largely mediated via Ras/MAP kinase pathway through the direct binding of IRS1, SHC, and SRC on specific tyrosine residues within the intracytoplasmic segment of ALK. The SHP2/GRB2 complex interacts with p130Cas, modifying the cytoskeleton organization as well. In the case of ALK-driven phosphatidylinositol 3 kinase (PI3K) activation, a relevant anti-apoptotic signal is generated mainly through pAKT1/2, and its downstream molecules (inhibition of BAD and FOXO3a-mediated transcription). At the same time, the PI3K pathway controls cell cycle progression. An additional oncogenic signal may be provided by PLC- $\gamma$ , which binds directly to activated ALK, and generating diacylglycerol and IP<sub>3</sub> activates PKC and mobilizes calcium stores from the endoplasmic reticulum. A critical oncogenic player is represented by the JAK/STAT3 pathway, which provides essential survival signals and modulates the cellular metabolism regulating the mitochondrial oxidation chain. STAT3 is activated by ALK either directly or through Jak. STAT3 activation is associated with distinct gene expression profile, which can distinguish ALCL from other T-cell neoplasms. The downstream effectors of STAT3 include several members of the BCL2 family (BCL2, BCL-X<sub>L</sub>, and MCL-1) and anti-apoptotic proteins, i.e. survivin and multiple transcription factor (i.e. C/EBP $\beta$ ). Finally, ALK fusion proteins have been shown to upregulate, via Ras and AP-1 transcription factors, the expression of CD30, which provides anti-apoptotic signals through TRAF2.

NPM-ALK, leading to a sustained activation state of Cdc42 in ALCL cells (Ambrogio *et al.* 2008). Cdc42 regulates the shape and migration of ALCL cells and it is necessary for the growth and maintenance of lymphoma cells *in vivo* (Ambrogio *et al.* 2008).

**Signal transducers and activators of transcription**

Signal transducers and activators of transcription (STAT) proteins are a family of transcription factors first characterized for their role in cytokine signaling.

These proteins contain a site for specific tyrosine phosphorylation, which after modification results in a conformational rearrangement and dimerization through phosphotyrosine-SH2 domain interactions (Levy & Darnell 2002). Once STATs are phosphorylated, they dimerize and accumulate in the cell nucleus and bind to enhancer elements of target genes. Zamo *et al.* (2002) have first shown that STAT3 is the key effector molecule of the ALK-mediated signaling in ALCL and its activation is required for the maintenance of the neoplastic phenotype (Chiarle *et al.* 2005). NPM-ALK can directly phosphorylate STAT3 or can activate JAK3, which in turn can contribute to STAT3 activation (Chiarle *et al.* 2008). STAT3 phosphorylation results in an increased expression of BCL2, BCL-X<sub>L</sub>, survivin, and MCL-1 proteins, involved in anti-apoptotic processes. STAT3-mediated signal also leads to an uncontrolled proliferation, acting on cell cycle regulators such as cyclin D3 and c-myc (Amin *et al.* 2003), often overexpressed in ALK<sup>+</sup> lymphoma (Chiarle *et al.* 2003). Cooperation between NPM-ALK and JAK/STAT pathway might also lead in certain context to the STAT5 activation (Nieborowska-Skorska *et al.* 2001), although in T-cell, STAT3 acts as a STAT5 repressor (Zhang *et al.* 2007).

### ALK fusion proteins

In addition to NPM-ALK, many other fusion proteins can be expressed in ALCL, namely ALK lymphoma oligomerization partner on chromosome 17 (ALO17;

Cools *et al.* 2002), TRK-fused gene (TFG; Hernández *et al.* 1999, 2002), moesin (MSN; Tort *et al.* 2001), tropomyosin 3 and 4 (TPM3 and TPM4; Lamant *et al.* 1999, Siebert *et al.* 1999, Meech *et al.* 2001), 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC; Colleoni *et al.* 2000, Ma *et al.* 2000, Trinei *et al.* 2000), non-muscle myosin heavy chain (MYH9; Lamant *et al.* 2003), and clathrin heavy chain (CLTC-ALK; Touriol *et al.* 2000; Table 1).

Virtually, all chimeras have the same ALK intracytoplasmic segment, but they show minor differences in the activation of several intracellular mediators, conceivably as a result of their different/unique subcellular compartmentalization and/or specific substrate interaction(s). Nevertheless, ALK fusion proteins share many common features: i) the transcription of the chimeric protein is driven by an ectopic/partner promoter; ii) the localization of these proteins is largely determined by the N-terminus partner region; iii) the presence of an oligomerization domain by the ALK partner protein, which induces the autophosphorylation and activation of the ALK kinase domain (Bischof *et al.* 1997, Mason *et al.* 1998).

Since the NPM-ALK shows a typical nuclear and cytoplasmic subcellular localization, the absence of nuclear ALK staining suggests the presence of ALK variant proteins (Kinney & Kadin 1999, Morris *et al.* 2001, Pulford *et al.* 2005). Indeed, the systematic application of ALK-specific antibodies has a critical

**Table 1** Chromosomal translocations involving anaplastic lymphoma kinase gene in cancers

Disease	Fusion protein	Chromosomal abnormality	Principal references
ALCL	NPM-ALK	t(2;5)(p23;q35)	Morris <i>et al.</i> (1994) and Shiota <i>et al.</i> (1994)
ALCL	ALO17-ALK	t(2;17)(p23;q25)	Cools <i>et al.</i> (2002)
ALCL	TFG-ALK	t(2;3)(p23;q21)	Hernández <i>et al.</i> (1999, 2002)
ALCL	MSN-ALK	t(2;X)(p32;q11-12)	Tort <i>et al.</i> (2001, 2004)
ALCL	TPM3-ALK	t(1;2)(q25;p23)	Lamant <i>et al.</i> (1999) and Siebert <i>et al.</i> (1999)
ALCL	TPM4-ALK	t(2;19)(p23;p13)	Meech <i>et al.</i> (2001)
ALCL	ATIC-ALK	inv(2)(p23;q35)	Colleoni <i>et al.</i> (2000), Ma <i>et al.</i> (2000), and Trinei <i>et al.</i> (2000)
ALCL	MYH9-ALK	t(2;22)(p23;q11-2)	Lamant <i>et al.</i> (2003)
ALCL	CLTC-ALK	t(2;17)(p23;q23)	Touriol <i>et al.</i> (2000)
IMT	TPM3-ALK	t(1;2)(q25;p23)	Lawrence <i>et al.</i> (2000)
IMT	TPM4-ALK	t(1;19)(p23;p13)	Lawrence <i>et al.</i> (2000)
IMT	CLTC-ALK	t(2;17)(p23;q23)	Bridge <i>et al.</i> (2001) and Patel <i>et al.</i> (2007)
IMT	ATIC-ALK	inv(2)(p23;q35)	Debiec-Rychter <i>et al.</i> (2003)
IMT	SEC31L1-ALK	t(2;4)(p23;q21)	Panagopoulos <i>et al.</i> (2006)
IMT	RANBP2-ALK	t(2;2)(p23;q13) inv(2)(p23;p15;q31)	Ma <i>et al.</i> (2003)
IMT	CARS-ALK	t(2;11;2)(p23;p15;q31)	Cools <i>et al.</i> (2002) and Debelenko <i>et al.</i> (2003)
NSCLC	EML4-ALK	inv(2)(p21;p23)	Rikova <i>et al.</i> (2007) and Soda <i>et al.</i> (2007)
NSCLC	TFG-ALK	t(2;3)(p23;q21)	Rikova <i>et al.</i> (2007)
DLBCL	NPM-ALK	t(2;5)(p23;q35)	Adam <i>et al.</i> (2003) and Onciu <i>et al.</i> (2003)
DLBCL	CLTC-ALK	t(2;17)(p23;q23)	De Paepe <i>et al.</i> (2003)
DLBCL	Unknown	ins(3'ALK)(4q22-24)	Stachurski <i>et al.</i> (2007)
DLBCL	SQSTM1-ALK	t(2;5)(p23-1;q35-3)	Takeuchi <i>et al.</i> (2010)
DLBCL	SEC31A-ALK	ins(4)(2;4)(?;q21) t(2;4)(p24;q21)	Bedwell <i>et al.</i> (2010) and Van Roosbroeck <i>et al.</i> (2010)
SCC	TPM4-ALK	t(2;19)(p23;p13)	Du <i>et al.</i> (2007) and Jazii <i>et al.</i> (2006)
RCC	VCL-ALK	t(2;10)(p23;q22)	Debelenko <i>et al.</i> (2010)



role for the appropriate classification of ALCL, demonstrating that ~60–80% of all ALCL are ALK<sup>+</sup> (Webb *et al.* 2009). It is important to underline that ALK<sup>-</sup> ALCL are indistinguishable from ALK<sup>+</sup> ALCL using morphological criteria alone. Therefore, the expression of ALK has become a key factor, not only for a proper diagnosis, but also for the precise ALCL stratification, providing relevant prognostic and therapeutic information. Since ALCL share a distinct gene expression profile, it has been postulated a putative common origin and/or common transformation pathway(s) for all ALCL (Piva *et al.* 2010). A single ALK lesion, although essential for transformation, requires additional genetic defects, which are however yet to be determined. The actual impact of insect bites in the pathogenesis of ALK<sup>+</sup> ALCL remains to be elucidated (Fornari *et al.* 2009, Lamant *et al.* 2010).

Interestingly, as underlined in the fourth edition of the WHO classification (Swerdlow *et al.* 2008), both ALK<sup>+</sup> and ALK<sup>-</sup> ALCL are characterized by frequent diffusion through sinuses and a cohesive growth pattern that can mimic metastatic carcinoma in the lymph node. They consist of very large lymphomatous elements (up to 60 µm) that in the ALK<sup>+</sup> tumors usually acquire a kidney- or horseshoe-shaped nuclear profile that justifies the term ‘hallmark cells’. Besides the classical type, almost exclusively formed by large cells with a few reactive elements, ALK<sup>+</sup> ALCL display some morphological variants: lympho-histiocytic, small cell, mixed, and Hodgkin-like cells. Under these circumstances, the expression of ALK by the neoplastic cells is of paramount importance for the distinction of the process from a hyperimmune reaction, PTCL-NOS, and nodular sclerosing Hodgkin lymphoma respectively. It is still a matter of debate whether similar variants are also observed in the setting of ALK<sup>-</sup> ALCL: possibly the lympho-histiocytic and Hodgkin-like ones do occur, although their recognition require negativity for PAX5/BSAP and occurrence of T-cell markers and possible clonal TCR rearrangements.

ALK<sup>+</sup> ALCL most frequently occur in the first decades of life with a typical male preponderance, although ALK<sup>+</sup> ALCL can also be seen in older individual at lower frequency; while ALK<sup>-</sup> ALCL arise most commonly in older patients (peak of incidence in the sixth decade) with a lower male preponderance (Shiota *et al.* 1995, Falini *et al.* 1999, Stein *et al.* 2000, Savage *et al.* 2008). ALK<sup>+</sup> ALCL patients have longer disease-free survival and better overall survival (OS) than ALK<sup>-</sup> cases (5 year OS: 70–80 vs 33–49%) following CHOP-based chemotherapy (Brugières *et al.* 1998, 2000, Falini *et al.* 1999, Stein *et al.* 2000, Williams *et al.* 2002, Savage *et al.* 2008), although these differences disappear if ALCL patients are stratified by stage (Savage *et al.* 2008).

Finally, it should be considered that the clinical outcome of ALCL is also influenced by the age of the patients, with a better survival in younger individuals. This may explain the more favorable clinical course of ALK<sup>+</sup> ALCL most frequently occurring in children and young adults.

Notably, an aberrant ALK expression has been detected in a minute subset of B-NHL (Delsol *et al.* 1997, Adam *et al.* 2003, Chikatsu *et al.* 2003, De Paep *et al.* 2003, Gascoyne *et al.* 2003, Onciu *et al.* 2003, Reichard *et al.* 2007). ALK<sup>+</sup> DLBCL often carry the t(2;17) translocation (Clathrin/ALK), while NPM-ALK or SEC31A-ALK proteins are less frequently expressed (Van Roosbroeck *et al.* 2010). Histologically, they display monomorphic, large immunoblastic/plasmablastic cells, which are CD138-, EMA-, CD4-, and cytoplasmic IgA- positive but lack CD30 and B-cell-restricted markers (Delsol *et al.* 1997, Reichard *et al.* 2007). ALK<sup>+</sup> DLBCL are characterized by an aggressive outcome and poor response to treatment (Reichard *et al.* 2007, Stachurski *et al.* 2007, Choung *et al.* 2008, Lee *et al.* 2008, Momose *et al.* 2009).

Finally, Chan *et al.* (2008) have described three cases of systemic histiocytosis, presenting in early infancy, expressing ALK or the TPM3-ALK chimeras. It is unclear whether these disorders are indeed true malignancies or due to an aberrant hyperproliferation of macrophages and dendritic cells, driven by the ectopic ALK expression.

## ALK expression in non-hematological disorders

### ALK in mesenchymal neoplasms

The IMTs are benign lesions of mesenchymal origin, composed of spindle cells, mixed with plasma cells and lymphocytes (Gleason & Hornick 2008), originally thought to represent a reactive post-inflammatory condition rather than a neoplastic process (Umiker & Iverson 1954). In 1999, Griffin *et al.* reported the first ALK gene rearrangements in these disorders. Further studies have subsequently documented the presence of different ALK-fusion proteins, all sharing the ALK kinase domain, fused to different partners, eventually leading to TPM4-ALK (Lawrence *et al.* 2000), ATIC-ALK (Debiec-Rychter *et al.* 2003), CLTC-ALK (Bridge *et al.* 2001, Patel *et al.* 2007), CARS-ALK (Cools *et al.* 2002, Debelenko *et al.* 2003), RANBP2-ALK (Ma *et al.* 2003), and SEC31L1-ALK (Panagopoulos *et al.* 2006) fusion proteins. It is believed that 35–60% of all IMTs display ALK rearrangements, which more often are seen in lesions of young individuals (Lawrence *et al.* 2000, Coffin *et al.* 2001, Cook *et al.* 2001).

Among soft tissue tumors, Cessna *et al.* (2002) first reported two cases of rhabdomyosarcoma (RMS), with embryonal, alveolar features, and the NPM-ALK translocation. Subsequently, using an immunohistochemical approach, ALK expression was confirmed in 53% of alveolar RMS and 23% of embryonal or unclassifiable RMS, which can display *ALK* amplification (Corao *et al.* 2009).

### ALK and neural tumors

Neuroblastoma is the most common extracranial solid tumor of childhood, derived from neural crest cells of the sympatho-adrenal lineage (Park *et al.* 2008). Although the clinical course of these patients is heterogeneous, many neuroblastomas are incurable, with poor long-term survival (Matthay *et al.* 1999), accounting for 15% of all pediatric oncology deaths (Maris *et al.* 2007).

Detectable levels of the ALK-receptor tyrosine kinase (RTK) were first described by Lamant *et al.* (2000) and subsequently high protein levels, due to *ALK* amplification, were documented by several groups (Miyake *et al.* 2002, Osajima-Hakomori *et al.* 2005). In 2008, several groups have showed the presence of ALK mutations in inherited version (Janoueix-Lerosey *et al.* 2008, Mosse *et al.* 2008) as well as in sporadic (Caren *et al.* 2008, Chen *et al.* 2008, George *et al.* 2008, Janoueix-Lerosey *et al.* 2008, Mosse *et al.* 2008) neuroblastoma. The frequency of ALK mutations ranges from 4 to 8% in primary samples, and from 20 to 36% in neuroblastoma cell lines (Caren *et al.* 2008, Chen *et al.* 2008, George *et al.* 2008, Janoueix-Lerosey *et al.* 2008, Mosse *et al.* 2008). Notably, patients carrying mutated ALK-RTK or with over-expressed ALK-RTK have a poor prognosis (Caren *et al.* 2008, Chen *et al.* 2008, George *et al.* 2008, Janoueix-Lerosey *et al.* 2008, Mosse *et al.* 2008, Passoni *et al.* 2009).

Neuroblastoma-associated ALK-RTK mutations induce a constitutive activation of the receptor, which activates several downstream molecules (Osajima-Hakomori *et al.* 2005) imposing a transformed phenotype. Indeed, the genetic (Mosse *et al.* 2008) or pharmacological inhibition of ALK-mutated species (George *et al.* 2008, McDermott *et al.* 2008) results in a decreased tumor growth. On the other hand, the role of wt-ALK-RTK remains elusive, since its expression might be simply linked to lineage constrains and/or unique neuronal differentiation stage(s) (Dirks *et al.* 2002).

Powers *et al.* (2002) first demonstrated that some primary glioblastoma and established cell lines expressed wt-ALK-RTK as well as pleiotrophin (PNT), an ALK-putative ligand. Glioblastoma often displays deregulated RTKs signaling, which plays a key role in their development and tumor outgrowth (Nister *et al.* 1991, Nishikawa *et al.* 1994). Interestingly, the

ribozyme-mediated targeting of ALK was shown to reduce tumor growth of glioblastoma xenografts and increase apoptosis. Finally, the ablation of both PNT and ALK strongly enhances their individual antiproliferative effects (Grzelinski *et al.* 2009).

### ALK in epithelial cancers

In the last decade, it has also become evident that many types of non-lymphoid tumors display a deregulated activation of *ALK*. This was first suggested by the work of Dirks *et al.* (2002), who originally documented the presence of ALK mRNA in many cancer cell lines derived from thyroid, small cell lung, breast carcinoma, and many other tumors.

Among epithelial cancers, lung tumors are the most common cause of cancer death in the world. Approximately, 85% of these neoplasms are represented by NSCLC, while 15% are recognized as small cell lung cancers (Jemal *et al.* 2004, Kamangar *et al.* 2006, Kelleher & Mc Dermott 2010). In 2007, Soda *et al.* first reported a novel echinoderm microtubule-associated protein-like 4 (EML4)-ALK fusion protein in Japanese patients with NSCLC. Shortly thereafter, a second group, using a proteomic approach, described either EML4-ALK or TFG-ALK chimera in ~4% of Chinese lung cancer patients (Rikova *et al.* 2007). Since then, many different EML4-ALK variants have been described (Rikova *et al.* 2007, Soda *et al.* 2007, Fukuyoshi *et al.* 2008, Inamura *et al.* 2008, Koivunen *et al.* 2008, Perner *et al.* 2008, Shinmura *et al.* 2008, Martelli *et al.* 2009, Wong *et al.* 2009). The frequency of EML4-ALK fusion ranges from 0.1 to 7.5% (Fukuyoshi *et al.* 2008, Inamura *et al.* 2008, Koivunen *et al.* 2008, Perner *et al.* 2008, Shinmura *et al.* 2008, Martelli *et al.* 2009, Palmer *et al.* 2009, Wong *et al.* 2009), although Lin *et al.* (2009) and more recently Zhang *et al.* (2010) have detected a higher frequency of ALK<sup>+</sup> NSCLC, using highly sensitive approaches.

Finally, two different variants involving the *KIF5B* and *ALK* genes have been described in a small subset of NSCLC (Takeuchi *et al.* 2009, Wong *et al.* 2011).

Collectively, these studies have pointed out the presence of several shared features among ALK<sup>+</sup> lung cancers: i) ALK fusions are mainly restricted to adenocarcinoma in patients with minimal or absent smoking story and young age of onset; ii) ALK rearrangements are mutually exclusive with other lung-associated genetic abnormalities such as *EGFR* and *KRAS* mutations; and iii) ALK translocations are not influenced by ethnic/racial differences, in contrast with *EGFR* mutations (Paez *et al.* 2004).

Notably, the univocal identification of ALK<sup>+</sup> NSCLC patients remains quite problematic. Indeed, the recognition of ALK translocations by FISH can be technically demanding and sometimes questionable.

Similarly, the detection of ectopic ALK fusion proteins by immunohistochemistry is problematic as well (Inamura *et al.* 2008, Takeuchi *et al.* 2008, Martelli *et al.* 2009), and once FISH, immunohistochemistry, and RT-based approaches are combined, an overall consensus is reached in 80% of the cases (M Volante, personal communication, 24 November 2010). Moreover, normal lung epithelial and lymphoid cells can display ALK genetic lesions (Martelli *et al.* 2009, Sozzi *et al.* 2009).

ALK inhibitors, such as PF-2341066 or NPV-TAE-684 first in mouse models (Christensen *et al.* 2007, Galkin *et al.* 2007, Zou *et al.* 2007, McDermott *et al.* 2008, Soda *et al.* 2008) and more recently in clinical trials, have shown their therapeutic potential. Indeed, the data with crizotinib in a recent Phase II study have demonstrated an objective response rate of 57% and a disease control rate of 87% in NSCLC patients (Kwak *et al.* 2010). These findings are very impressive, although longer follow-up and different clinical trials may be required to conclusively assess the efficacy of a single drug regimen and its efficacy in naïve patients. Finally, the occurrence of ALK overriding resistance has to be precisely appraised and its molecular mechanism(s) dissected (Martinsson *et al.* 2010).

Perez-Pinera *et al.* (2007) first documented the ALK ectopic expression in a very large number of breast neoplasms, demonstrating detectable levels of ALK protein in normal breast epithelium and other non-epithelial elements by immunohistochemistry. Notably, the PNT knockdown in breast cancer cells can result in a decreased tumor growth *in vitro* (Fang *et al.* 1992, Garver *et al.* 1994, Riegel & Wellstein 1994) and *in vivo* (Zhang *et al.* 1997). These findings suggested a pathogenetic role of the wt-ALK-RTK in this disease. Supporting findings have been provided by Lin *et al.* (2009), who have documented the presence of EML4-ALK transcripts in ~2.5% of breast cancers and showed that ALK ablation leads to cell growth impairment. Analogous data have been generated in colon cancers (Lin *et al.* 2009) and very recently in renal cell carcinoma (Debelenko *et al.* 2010). The significance of ALK deregulation in breast and colon tumors remains unclear and its pathogenetic significance needs further confirmation (Fukuyoshi *et al.* 2008).

Finally, among epithelial cancers, squamous cell carcinoma (SCC) of the esophagus (SCCE) represents the sixth most common entity with the highest incidence rates in China, Iran, and developing countries. Deregulated ALK fusion proteins expression has been documented in SCCE, originally in Iranian patients by Jazii *et al.* (2006) and subsequently confirmed in a cohort of Chinese individuals (Du *et al.* 2007).

In conclusion, the list of solid neoplasms positive for ALK is continuously growing (i.e. prostate cancer, etc. E Medico and G Inghirami, personal communication).

These findings will definitively foster the execution of more frequent systematic molecular analyses and the development of reliable clinical diagnostic tests.

## Innovative therapeutic approaches for ALK tumors

The ablation of ALK protein expression was originally obtained by ALK-specific small interfering RNA (siRNA) duplexes or selective ribozyme (Hubinger *et al.* 2003). These original studies showed that the ALK knockdown leads first to a cell cycle arrest, followed by massive apoptosis *in vitro* and/or *in vivo* (Piva *et al.* 2006). These original findings were first confirmed applying ALK-specific small molecules (Wan *et al.* 2006, Galkin *et al.* 2007) and more recently were supported by other novel ATP-competitive inhibitors (Li & Morris 2008, Cheng & Otte 2010). Since then, we have witnessed an increasing interest in this field, strongly encouraged by the discovery of a growing number of ALK<sup>+</sup> cancers (Li & Morris 2008, Webb *et al.* 2009, Cheng & Otte 2010). As a result, the first ALK inhibitor, PF-2341066, an ATP competitor, targeting both c-Met and ALK (Christensen *et al.* 2007), has recently reached the clinical arena in the treatment of ALK<sup>+</sup> NSCLC tumors, and other small molecules have just reached the clinics (LDK378) or are in pre-clinical stages (CEP28122, CEP37440, AP-26113, TAE-684, etc.). Meanwhile, several trials have also been opened for ALCL and neuroblastoma patients (<http://www.ClinicalTrials.gov/>). It is postulated that many compounds could soon reach the clinics (Webb *et al.* 2009, Cheng & Otte 2010).

Since ALK signaling activates multiple downstream molecules, i.e. PI3K/AKT, JAK/STAT3 and 5, mTOR, and SRC, it is reasonable to speculate that several small molecules, targeting key effectors within these pathways, will be investigated in ALK<sup>+</sup> cancer patients. Considering the exquisite oncogenetic addition of ALK<sup>+</sup> ALCL to STAT3 (Piva *et al.* 2006), inhibition of this transcription factor could provide a novel therapeutic avenue. Nevertheless, because there is an enormous redundancy of signal transduction pathways in any given tumor, it is conceivable that we will be obliged to use disease/patient-specific cocktails to successfully knockdown multiple players among different pathways. This might be the case for those neoplasms displaying partial oncogenic addition to ALK and/or capable of executing counteracting resistant mechanisms. In this context, targeting EGFR, c-src, and MEK may also be considered. Finally, immunological strategies, in combination with conventional or small molecule approaches, could be considered to enhance anti-tumor responses or to gain the complete eradication of cancer cells.



## Final remarks

Since the original discovery by Morris *et al.* (1994) of the first ALK translocation, we have witnessed pivotal discoveries that led to a deeper understanding of the mechanisms leading to ALK-mediated transformation and tumor maintenance of ALCL. Now, a similar knowledge is mandatory for all other ALK<sup>+</sup> neoplasms. Dissecting this landscape is essential for the design of tailored therapies, for predicting therapeutic failures, and to overcome them. We hope that a dedicated effort will also be placed to fully understand the physiological role of the ALK receptor and to discover its ligand(s). Understanding the physiological role of ALK will be necessary for the development of clinical-grade diagnostic assays and for the design and implementation of immune-based therapeutic approaches.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## Funding

The study was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC); Fondazione Guido Berlucchi; Ministero dell'Università e Ricerca Scientifica (MIUR); Regione Piemonte; Compagnia di San Paolo, Torino (Progetto Oncologia); and Oncosuisse Grant KLS-02403-02-2009; Fondazione per la Ricerca e la Cura sui Linfomi (Lugano, Switzerland).

## Acknowledgements

The European T-Cell Lymphoma Study Group: Italy: Antonella Barreca, Roberto Chiarle, Giuditta Cuccuru, Giorgio Inghirami, Barbara Martinoglio, Enzo Medico, Elisa Pellegrino, Roberto Piva, Maria Luisa Ruberto, Claudia Voena (University of Torino); Alessandro Fornari and Domenico Novero (ASO Molinette, and San Luigi Gonzaga Torino); Marco Chilosi and Alberto Zamó (University of Verona); Fabio Facchetti and Silvia Lonardi (University of Brescia); Anna De Chiara and Franco Fulciniti (National Cancer Institute, Napoli); Claudio Doglioni and Maurilio Ponzoni (San Raffaele Institute, Milano); Luca Agnelli, Antonino Neri and Katia Todoerti (University of Milan), Pier Paolo Piccaluga and Stefano Pileri (University of Bologna); Brunangelo Falini and Enrico Tiacci (University of Perugia), Belgium: Peter Van Loo, Thomas Tousseyn, and Christiane De Wolf-Peeters (University of Leuven), Germany: Eva Geissinger, Hans Konrad Muller-Hermelink and Andreas Rosenwald (University of Wuerzburg), Spain: Miguel Angel Piris and Maria E Rodriguez (Spanish National Cancer Research Centre, CNIO).

## References

Adam P, Katzenberger T, Seeberger H, Gattenlohner S, Wolf J, Steinlein C, Schmid M, Muller-Hermelink HK & Ott G 2003 A case of a diffuse large B-cell lymphoma of plasmablastic type associated

with the t(2;5)(p23;q35) chromosome translocation. *American Journal of Surgical Pathology* **27** 1473–1476. (doi:10.1097/0000478-200311000-00012)

- Allouche M 2007 ALK is a novel dependence receptor. *Cell Cycle* **6** 1533–1538. (doi:10.4161/cc.6.13.4433)
- Ambrogio C, Voena C, Manazza AD, Martinengo C, Costa C, Kirchhausen T, Hirsh E, Inghirami G & Chiarle R 2008 The anaplastic lymphoma kinase controls cell shape and growth of anaplastic large cell lymphoma through Cdc42 activation. *Cancer Research* **68** 8899–8907. (doi:10.1158/0008-5472.CAN-08-2568)
- Amin HM, Medeiros LJ, Ma Y, Feretzaki M, Das P, Leventaki V, Rassidakis GZ, O'Connor SL, McDonnell TJ & Lai R 2003 Inhibition of JAK3 induces apoptosis and decreases anaplastic lymphoma kinase activity in anaplastic large cell lymphoma. *Oncogene* **22** 5399–5407. (doi:10.1038/sj.onc.1206849)
- Arber DA, Sun LH & Weiss LM 1996 Detection of the t(2;5)(p23;q35) chromosomal translocation in large B-cell lymphomas other than anaplastic large cell lymphoma. *Human Pathology* **27** 590–594. (doi:10.1016/S0046-8177(96)90167-7)
- Bai RY, Dieter P, Peschel C, Morris SW & Duyster J 1998 Nucleophosmin–anaplastic lymphoma kinase of large-cell anaplastic lymphoma is a constitutively active tyrosine kinase that utilizes phospholipase C-gamma to mediate its mitogenicity. *Molecular and Cellular Biology* **18** 6951–6961.
- Bai RY, Ouyang T, Miething C, Morris SW, Peschel C & Duyster J 2000 Nucleophosmin–anaplastic lymphoma kinase associated with anaplastic large-cell anaplastic lymphoma activates the phosphatidylinositol 3-kinase/Akt antiapoptotic signaling pathway. *Blood* **96** 4319–4327.
- Bazigou E, Apitz H, Johansson J, Lorén CE, Hirst EM, Chen PL, Palmer RH & Salecker I 2007 Anterograde Jelly belly and Alk receptor tyrosine kinase signaling mediates retinal axon targeting in *Drosophila*. *Cell* **128** 961–975. (doi:10.1016/j.cell.2007.02.024)
- Bedwell C, Rowe D, Moulton D, Jones G, Bown N & Bacon CM 2010 Cytogenetically complex SEC31A–ALK fusions are recurrent in ALK-positive large B-cell lymphomas. *Haematologica* **96** 343–346.
- Bischof D, Pulford K, Mason DY & Morris SW 1997 Role of the nucleophosmin (NPM) portion of the non-Hodgkin's lymphoma-associated NPM–anaplastic lymphoma kinase fusion protein in oncogenesis. *Molecular and Cellular Biology* **17** 2312–2325.
- Bridge JA, Kanamori M, Ma Z, Pickering D, Hill DA, Lydiatt W, Lui MY, Colleoni GW, Antonescu CR, Ladanyi M *et al.* 2001 Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. *American Journal of Pathology* **159** 411–415. (doi:10.1016/S0002-9440(10)61711-7)
- Brugières L, Deley MC, Pacquement H, Meguerian-Bedoyan Z, Terrier-Lacombe MJ, Robert A, Pondarré C, Leverger G, Devalck C, Rodary C *et al.* 1998 CD30<sup>+</sup> anaplastic large-cell lymphoma in children: analysis of 82 patients enrolled in two consecutive studies of the French Society of Pediatric Oncology. *Blood* **92** 3591–3598.
- Brugières L, Quartier P, Le Deley MC, Pacquement H, Perel Y, Bergeron C, Schmitt C, Landmann J, Patte C, Terrier-Lacombe MJ *et al.* 2000 Relapses of childhood anaplastic large-cell lymphoma: treatment results in a series of 41 children – a report from the French Society of Pediatric Oncology. *Annals of Oncology* **11** 53–58. (doi:10.1023/A:1008352726155)
- Caren H, Abel F, Kogner P & Martinsson T 2008 High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. *Biochemical Journal* **416** 153–159. (doi:10.1042/BJ20081834)
- Cessna MH, Zhou H, Sanger WG, Perkins SL, Tripp S, Pickering D, Daines C & Coffin CM 2002 Expression of ALK1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases. *Modern Pathology* **15** 931–938. (doi:10.1097/01.MP.0000026615.04130.1F)



- Chan JK, Lamant L, Algar E, Delsol G, Tsang WYW & Lee KC 2008 ALK<sup>+</sup> histiocytosis: a novel type of systemic histiocytic proliferative disorder of early infancy. *Blood* **112** 2965–2968. (doi:10.1182/blood-2008-03-147017)
- Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, Wang L, Soda M, Kikuchi A, Igarashi T *et al.* 2008 Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* **455** 971–974. (doi:10.1038/nature07399)
- Cheng M & Otte GR 2010 Anaplastic lymphoma kinase as a therapeutic target in anaplastic large cell lymphoma, non-small cell lung cancer and neuroblastoma. *Anti-Cancer Agents in Medicinal Chemistry* **10** 236–249.
- Chiarle R, Gong JZ, Guasparri I, Pesci A, Cai J, Liu J, Simmons WJ, Dhall G, Howes J, Piva R *et al.* 2003 NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. *Blood* **101** 1919–1927. (doi:10.1182/blood-2002-05-1343)
- Chiarle R, Simmons WJ, Cai H, Dhall G, Zamo A, Raz R, Karras JG, Levy DE & Inghirami G 2005 Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. *Nature Medicine* **11** 623–629. (doi:10.1038/nm1249)
- Chiarle R, Voena C, Ambrogio C, Piva R & Inghirami G 2008 The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nature Reviews. Cancer* **8** 11–23. (doi:10.1038/nrc2291)
- Chikatsu N, Kojima H, Suzukawa K, Shinagawa A, Nagasawa T, Ozawa H, Yamashita Y & Mori N 2003 ALK<sup>+</sup>, CD30<sup>+</sup>, CD20<sup>+</sup> large B-cell lymphoma containing anaplastic lymphoma kinase (ALK) fused to clathrin heavy chain gene (CLTC). *Modern Pathology* **16** 828–832. (doi:10.1097/01.MP.0000081729.40230.1F)
- Choung HS, Kim HJ, Kim WS, Kim K & Kim SH 2008 Cytomorphology and molecular characterization of CLTC-ALK rearrangement in 2 cases of ALK-positive diffuse large B-cell lymphoma with extensive bone marrow involvement. *Korean Journal of Laboratory Medicine* **28** 89–94. (doi:10.3343/kjlm.2008.28.2.89)
- Christensen JG, Zou HY, Arango ME, Li Q, Lee JH, McDonnell SR, Yamazaki S, Alton GR, Mroczkowski B & Los G 2007 Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Molecular Cancer Therapeutics* **6** 3314–3322. (doi:10.1158/1535-7163.MCT-07-0365)
- Coffin CM, Patel A, Perkins S, Elenitoba-Johnson KS, Perlman E & Griffin CA 2001 ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor. *Modern Pathology* **14** 569–576. (doi:10.1038/modpathol.3880352)
- Colleoni GW, Bridge JA, Garicochea B, Liu J, Filippa DA & Ladanyi M 2000 ATIC-ALK: a novel variant ALK gene fusion in anaplastic large cell lymphoma resulting from the recurrent cryptic chromosomal inversion, inv(2)(p23q35). *American Journal of Pathology* **156** 781–789. (doi:10.1016/S0002-9440(10)64945-0)
- Cook JR, Dehner LP, Collins MH, Ma Z, Morris SW, Coffin CM & Hill DA 2001 Anaplastic lymphoma kinase (ALK) expression in the inflammatory myofibroblastic tumor: a comparative immunohistochemical study. *American Journal of Surgical Pathology* **25** 1364–1371. (doi:10.1097/0000478-200111000-00003)
- Cools J, Wlodarska I, Somers R, Mentens N, Pedoutour F, Maes B, De Wolf-Peeters C, Pauwels P, Hagemeijer A & Marynen P 2002 Identification of novel fusion partners of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. *Genes, Chromosomes and Cancer* **34** 354–362. (doi:10.1002/gcc.10033)
- Corao DA, Biegel JA, Coffin CM, Barr FG, Wainwright LM, Ernst LM, Choi JK, Zhang PJ & Pawel BR 2009 ALK expression in rhabdomyosarcomas: correlation with histologic subtype and fusion status. *Pediatric and Developmental Pathology* **12** 275–283. (doi:10.2350/08-03-0434.1)
- Debelenko LV, Arthur DC, Pack SD, Helman LJ, Schrupp DS & Tsokos M 2003 Identification of CARS-ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumor. *Laboratory Investigation* **83** 1255–1265. (doi:10.1097/01.LAB.0000088856.49388.EA)
- Debelenko L, Raimondi SC, Daw N, Shivakumar BR, Huang D, Nelson M & Bridge JA 2010 Renal cell carcinoma with novel VCL-ALK fusion: new representative of ALK-associated tumor spectrum. *Modern Pathology* **24** 430–442. (doi:10.1038/modpathol.2010.213)
- Debiec-Rychter M, Marynen P, Hagemeijer A & Pauwels P 2003 ALK-AT1C fusion in urinary bladder inflammatory myofibroblastic tumor. *Genes, Chromosomes and Cancer* **38** 187–190. (doi:10.1002/gcc.10267)
- Delsol G, Lamant L, Mariamé B, Pulford K, Dastugue N, Brousset P, Rigal-Huguet F, al Saati T, Cerretti DP, Morris SW *et al.* 1997 A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2;5 translocation. *Blood* **89** 1483–1490.
- De Paepe P, Baens M, van Krieken H, Verhasselt B, Stul M, Simons A, Poppe B, Laureys G, Brons P, Vandenberghe P *et al.* 2003 ALK activation by the CLTC-ALK fusion is a recurrent event in B-cell lymphoma. *Blood* **102** 2638–2641. (doi:10.1182/blood-2003-04-1050)
- Dirks WG, Fahrnich S, Lis Y, Becker E, MacLeod RA & Drexler HG 2002 Expression and functional analysis of the anaplastic lymphoma kinase (ALK) gene in tumor cell lines. *International Journal of Cancer* **100** 49–56. (doi:10.1002/ijc.10435)
- Du XL, Hu H, Lin DC, Xia SH, Shen XM, Zhang Y, Luo ML, Feng YB, Cai Y, Xu X *et al.* 2007 Proteomic profiling of proteins dysregulated in Chinese esophageal squamous cell carcinoma. *Journal of Molecular Medicine* **85** 863–875. (doi:10.1007/s00109-007-0159-4)
- Falini B, Bigerna B, Fizzotti M, Pulford K, Pileri SA, Delsol G, Carbone A, Paulli M, Magrini U, Menestrina F *et al.* 1998 ALK expression defines a distinct group of T/null lymphomas with a wide morphological spectrum. *American Journal of Pathology* **153** 875–886. (doi:10.1016/S0002-9440(10)65629-5)
- Falini B, Pulford K, Pucciarini A, Carbone A, De Wolf-Peeters C, Cordell J, Fizzotti M, Santucci A, Pelicci PG, Pileri S *et al.* 1999 Lymphomas expressing ALK fusion protein(s) other than NPM-ALK. *Blood* **94** 3509–3515.
- Fang W, Hartmann N, Chow DT, Riegel AT & Wellstein A 1992 Pleiotrophin stimulates fibroblasts and endothelial and epithelial cells and is expressed in human cancer. *Journal of Biological Chemistry* **267** 25889–25897.
- Fornari A, Piva R, Chiarle R, Novero D & Inghirami G 2009 Anaplastic large cell lymphoma: one or more entities among T-cell lymphoma? *Hematological Oncology* **27** 161–170. (doi:10.1002/hon.897)
- Fujimoto J, Shiota M, Iwahara T, Seki N, Satoh H, Mori S & Yamamoto T 1996 Characterization of the transforming activity of p80, a hyperphosphorylated protein in a Ki-1 lymphoma cell line with chromosomal translocation t(2;5). *PNAS* **93** 4181–4186. (doi:10.1073/pnas.93.9.4181)
- Fukuyoshi Y, Inoue H, Kita Y, Utsumomiya T, Ishida T & Mori M 2008 EML4-ALK fusion transcript is not found in gastrointestinal and breast cancers. *British Journal of Cancer* **98** 1536–1539. (doi:10.1038/sj.bjc.6604341)
- Galkin AV, Melnick JS, Kim S, Hood TL, Li N, Li L, Xia G, Steensma R, Chopiuk G, Jiang J *et al.* 2007 Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK. *PNAS* **104** 270–275. (doi:10.1073/pnas.0609412103)
- Garver RJ Jr, Radford DM, Donis-Keller H, Wick MR & Milner PG 1994 Midkine and pleiotrophin expression in normal and malignant breast tissue. *Cancer* **74** 1584–1590. (doi:10.1002/1097-0142(19940901)74:5 <1584::AID-CNCR2820740514 >3.0.CO;2-V)
- Gascoyne RD, Lamant L, Martin-Subero JI, Lestou VS, Harris NL, Müller-Hermelink HK, Seymour JF, Campbell LJ, Horsman DE, Auvigne I *et al.* 2003 ALK-positive diffuse large B-cell lymphoma is associated with clathrin-ALK rearrangements: report of six cases. *Blood* **102** 2568–2571. (doi:10.1182/blood-2003-03-0786)

- George RE, Sanda T, Hanna M, Fröhling S, Luther W II, Zhang J, Ahn Y, Zhou W, London WB, McGrady P *et al.* 2008 Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* **455** 975–978. (doi:10.1038/nature07397)
- Gleason BC & Hornick JL 2008 Inflammatory myofibroblastic tumours: where are we now? *Journal of Clinical Pathology* **61** 428–437. (doi:10.1136/jcp.2007.049387)
- Griffin CA, Hawkins AL, Dvorak C, Henkle C, Ellingham T & Perlman EJ 1999 Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. *Cancer Research* **59** 2776–2780.
- Grzelinski M, Bader N, Czubyko F & Aigner A 2005 Ribozyme-targeting reveals the rate-limiting role of pleiotrophin in glioblastoma. *International Journal of Cancer* **117** 942–951. (doi:10.1002/ijc.21276)
- Grzelinski M, Steinberg F, Martens T, Czubyko F, Lamszus K & Aigner A 2009 Enhanced antitumorigenic effects in glioblastoma on double targeting of pleiotrophin and its receptor ALK. *Neoplasia* **11** 145–156.
- Gu TL, Tothova Z, Scheijen B, Griffin JD, Gilliland DG & Sternberg DW 2004 NPM–ALK fusion kinase of anaplastic large-cell lymphoma regulates survival and proliferative signaling through modulation of FOXO3a. *Blood* **103** 4622–4629. (doi:10.1182/blood-2003-03-0820)
- Hernández L, Pinyol M, Hernández S, Beà S, Pulford K, Rosenwald A, Lamant L, Falini B, Ott G, Mason DY *et al.* 1999 TRK-fused gene (TFG) is a new partner of ALK in anaplastic large cell lymphoma producing two structurally different TFG–ALK translocations. *Blood* **94** 3265–3268.
- Hernández L, Beà S, Bellosillo B, Pinyol M, Falini B, Carbone A, Ott G, Rosenwald A, Fernández A, Pulford K *et al.* 2002 Diversity of genomic breakpoints in TFG–ALK translocations in anaplastic large cell lymphomas: identification of a new TFG–ALK(XL) chimeric gene with transforming activity. *American Journal of Pathology* **160** 1487–1494. (doi:10.1016/S0002-9440(10)62574-6)
- Hubinger G, Wehnes E, Xue L, Morris SW & Maurer U 2003 Hammerhead ribozyme-mediated cleavage of the fusion transcript NPM–ALK associated with anaplastic large-cell lymphoma. *Experimental Hematology* **31** 226–233. (doi:10.1016/S0301-472X(02)01084-6)
- Inamura K, Takeuchi K, Togashi Y, Nomura K, Ninomiya H, Okui M, Satoh Y, Okumura S, Nakagawa K, Soda M *et al.* 2008 EML4–ALK fusion is linked to histological characteristics in a subset of lung cancers. *Journal of Thoracic Oncology* **3** 13–17. (doi:10.1097/JTO.0b013e31815e8b60)
- Iwahara T, Fujimoto J, Wen D, Cupples R, Bucay N, Arakawa T, Mori S, Ratzkin B & Yamamoto T 1997 Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene* **14** 439–449. (doi:10.1038/sj.onc.1200849)
- Jaffe ES, Harris NL, Stein H & Vardiman JW 2001 *World Health Organization Classifications of Tumors: Pathology and Genetics of Tumors of the Haematopoietic and Lymphoid Tissues*. Lyon: International Agency for Research on Cancer.
- Janoueix-Lerosey I, Lequin D, Brugières L, Ribeiro A, de Pontual L, Combaret V, Raynal V, Puisieux A, Schleiermacher G, Pierron G *et al.* 2008 Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* **455** 967–970. (doi:10.1038/nature07398)
- Jazii FR, Najafi Z, Malekzadeh R, Conrads TP, Ziaee AA, Abnet C, Yazdznbod M, Karkhane AA & Salekdeh GH 2006 Identification of squamous cell carcinoma associated proteins by proteomics and loss of  $\beta$  tropomyosin expression in esophageal cancer. *World Journal of Gastroenterology* **12** 7104–7112.
- Jemal A, Clegg LX, Ward E, Ries LA, Wu X, Jamison PM, Wingo PA, Howe HL, Anderson RN & Edwards BK 2004 Annual report to the nation on the status of cancer, 1975–2001, with a special feature regarding survival. *Cancer* **101** 3–27. (doi:10.1002/cncr.20288)
- Kamangar F, Dores GM & Anderson WF 2006 Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *Journal of Clinical Oncology* **24** 2137–2215. (doi:10.1200/JCO.2005.05.2308)
- Kelleher FC & Mc Dermott R 2010 The emerging pathogenic and therapeutic importance of the anaplastic lymphoma kinase gene. *European Journal of Cancer* **46** 2357–2368. (doi:10.1016/j.ejca.2010.04.006)
- Kinney MC & Kadin ME 1999 The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gene dysregulation. *American Journal of Clinical Pathology* **111** S56–S67.
- Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R *et al.* 2008 EML4–ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clinical Cancer Research* **14** 4275–4283. (doi:10.1158/1078-0432.CCR-08-0168)
- Kuefer MU, Look AT, Pulford K, Behm FG, Pattengale PK, Mason DY & Morris SW 1997 Retrovirus-mediated gene transfer of NPM–ALK causes lymphoid malignancy in mice. *Blood* **90** 2901–2910.
- Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB *et al.* 2010 Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *New England Journal of Medicine* **18** 1693–1703. (doi:10.1056/NEJMoa1006448)
- Lamant L, Dastugue N, Pulford K, Delsol G & Mariame BA 1999 A new fusion gene TPM3–ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. *Blood* **93** 3088–3095.
- Lamant L, Pulford K, Bischof D, Morris SW, Mason DY, Delsol G & Mariame B 2000 Expression of the ALK tyrosine kinase gene in neuroblastoma. *American Journal of Pathology* **156** 1711–1721. (doi:10.1016/S0002-9440(10)65042-0)
- Lamant L, Gascoyne RD, Duplantier MM, Armstrong F, Raghav A, Chhanabhai M, Rajcan-Separovic E, Raghav J, Delsol G & Espinos E 2003 Non-muscle myosin heavy chain (MYH9): a new partner fused to ALK in anaplastic large cell lymphoma. *Genes, Chromosomes and Cancer* **37** 427–432. (doi:10.1002/gcc.10232)
- Lamant L, Pileri S, Sabattini E, Brugières L, Jaffe ES & Delsol G 2010 Cutaneous presentation of ALK-positive anaplastic large cell lymphoma following insect bites: evidence for an association in five cases. *Haematologia* **95** 449–455. (doi:10.3324/haematol.2009.015024)
- Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal Cin P, Pinkus JL, Pinkus GS, Xiao S, Yi ES, Fletcher CD *et al.* 2000 TPM3–ALK and TPM4–ALK oncogenes in inflammatory myofibroblastic tumors. *American Journal of Pathology* **157** 377–384. (doi:10.1016/S0002-9440(10)64550-6)
- Lee HW, Kim K, Kim W & Ko YH 2008 ALK-positive diffuse large B-cell lymphoma: report of three cases. *Hematological Oncology* **26** 108–113. (doi:10.1002/hon.841)
- Levy DE & Darnell JE Jr 2002 Stats: transcriptional control and biological impact. *Nature Reviews. Molecular Cell Biology* **3** 651–662. (doi:10.1038/nrm909)
- Li R & Morris SW 2008 Development of anaplastic lymphoma kinase (ALK) small-molecule inhibitors for cancer therapy. *Medicinal Research Reviews* **28** 372–412. (doi:10.1002/med.20109)
- Li XQ, Hisaoka M, Shi DR, Zhu XZ & Hashimoto H 2004 Expression of anaplastic lymphoma kinase in soft tissue tumors: an immunohistochemical and molecular study of 249 cases. *Human Pathology* **35** 711–721. (doi:10.1016/j.humpath.2003.12.004)
- Liao E, Hung W, Abrams B & Zhen M 2004 An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. *Nature* **430** 345–350. (doi:10.1038/nature02647)
- Lin E, Guan Y, Soriano R, Rivers CS, Mohan S, Pandita A, Tang J & Modrusan Z 2009 Exon array profiling detects EML4–ALK fusion in breast, colorectal, and non-small cell lung cancers. *Molecular Cancer Research* **7** 1466–1476. (doi:10.1158/1541-7786.MCR-08-0522)
- Lu KV, Jong KA, Kim GY, Singh J, Dia EQ, Yoshimoto K, Wang MY, Cloughesy TF, Nelson SF & Mischel PS 2005 Differential induction

- of glioblastoma migration and growth by two forms of pleiotrophin. *Journal of Biological Chemistry* **280** 26953–26964. (doi:10.1074/jbc.M502614200)
- Ma Z, Cools J, Marynen P, Cui X, Siebert R, Gesk S, Schlegelberger B, Peeters B, De Wolf-Peeters C, Wlodarska I *et al.* 2000 Inv(2)(p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase (ALK) tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis. *Blood* **95** 2144–2149.
- Ma Z, Hill DA, Collins MH, Morris SW, Sumegi J, Zhou M, Zuppan C & Bridge JA 2003 Fusion of ALK to the Ran-binding protein 2 (RANBP2) gene in inflammatory myofibroblastic tumor. *Genes, Chromosomes and Cancer* **37** 98–105. (doi:10.1002/gcc.10177)
- Maris JM, Hogarty MD, Bagatell R & Cohn SL 2007 Neuroblastoma. *Lancet* **369** 2106–2120. (doi:10.1016/S0140-6736(07)60983-0)
- Martelli MP, Sozzi G, Hernandez L, Pettirossi V, Navarro A, Conte D, Gasparini P, Perrone F, Modena P, Pastorino U *et al.* 2009 EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. *American Journal of Pathology* **174** 661–670. (doi:10.2353/ajpath.2009.080755)
- Martinsson T, Eriksson T, Abrahamsson J, Caren H, Hansson M, Kogner P, Kamaraj S, Schonherr C, Weinmar J, Ruuth K *et al.* 2010 Appearance of the novel activating F1174S ALK mutation in neuroblastoma correlates with aggressive tumor progression and unresponsiveness to therapy. *Cancer Research* **71** 98–105. (doi:10.1158/0008-5472.CAN-10-2366)
- Mason DY, Pulford KA, Bischof D, Kuefer MU, Butler LH, Lamant L, Delsol G & Morris SW 1998 Nucleolar localization of the nucleophosmin–anaplastic lymphoma kinase is not required for malignant transformation. *Cancer Research* **58** 1057–1062.
- Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM *et al.* 1999 Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-*cis*-retinoic acid. Children's Cancer Group. *New England Journal of Medicine* **341** 1165–1173. (doi:10.1056/NEJM199910143411601)
- McDermott U, Iafraite AJ, Gray NS, Shioda T, Classon M, Maheswaran S, Zhou W, Choi HG, Smith SL, Dowell L *et al.* 2008 Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Research* **68** 3389–3395. (doi:10.1158/0008-5472.CAN-07-6186)
- Meech SJ, McGavran L, Odom LF, Liang X, Meltesen L, Gump J, Wei Q, Carlsen S & Hunger SP 2001 Unusual childhood extramedullary hematologic malignancy with natural killer cell properties that contains tropomyosin 4–anaplastic lymphoma kinase gene fusion. *Blood* **98** 1209–1216. (doi:10.1182/blood.V98.4.1209)
- Miyake I, Hakomori Y, Shinohara A, Gamou T, Saito M, Iwamatsu A & Sakai R 2002 Activation of anaplastic lymphoma kinase is responsible for hyperphosphorylation of ShcC in neuroblastoma cell lines. *Oncogene* **21** 5823–5834. (doi:10.1038/sj.onc.1205735)
- Miyake I, Hakomori Y, Misu Y, Nakadate H, Matsuura N, Sakamoto M & Sakai R 2005 Domain-specific function of ShcC docking protein in neuroblastoma cells. *Oncogene* **24** 3206–3215. (doi:10.1038/sj.onc.1208523)
- Momose S, Tamaru J, Kishi H, Mikata I, Mori M, Toyozumi Y & Itoyama S 2009 Hyperactivated STAT3 in ALK-positive diffuse large B-cell lymphoma with clathrin–ALK fusion. *Human Pathology* **40** 75–82. (doi:10.1016/j.humpath.2008.06.009)
- Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL & Look AT 1994 Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* **263** 1281–1284. (doi:10.1126/science.8122112)
- Morris SW, Naev C, Mathew P, James PL, Kirstein MN, Cui X & Witte DP 1997 ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). *Oncogene* **14** 2175–2188. (doi:10.1038/sj.onc.1201062)
- Morris SW, Xue L, Ma Z & Kinney MC 2001 ALK<sup>+</sup> CD30<sup>+</sup> lymphomas: a distinct molecular genetic subtype of non-Hodgkin's lymphoma. *British Journal of Haematology* **113** 275–295. (doi:10.1046/j.1365-2141.2001.02574.x)
- Mosse YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P *et al.* 2008 Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455** 930–935. (doi:10.1038/nature07261)
- Mourali J, Bénard A, Lourenço FC, Monnet C, Greenland C, Moog-Lutz C, Racaud-Sultan C, Gonzalez-Dunia D, Vigny M, Delsol G *et al.* 2006 Anaplastic lymphoma kinase is a dependence receptor whose proapoptotic functions are activated by caspase cleavage. *Molecular and Cellular Biology* **26** 6209–6222. (doi:10.1128/MCB.01515-05)
- Nieborowska-Skorska M, Slupianek A, Xue L, Zhang Q, Raghunath PN, Hoser G, Wasik MA, Morris SW & Skorski T 2001 Role of signal transducer and activator of transcription 5 in nucleophosmin/anaplastic lymphoma kinase-mediated malignant transformation of lymphoid cells. *Cancer Research* **61** 6517–6523.
- Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK & Huang HJ 1994 A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. *PNAS* **91** 7727–7731. (doi:10.1073/pnas.91.16.7727)
- Nister M, Claesson-Welsh L, Eriksson A, Heldin CH & Westermark B 1991 Differential expression of platelet-derived growth factor receptors in human malignant glioma cell lines. *Journal of Biological Chemistry* **266** 16755–16163.
- Okuwaki M 2008 The structure and functions of NPM1/nucleophosmin/B23, a multifunctional nucleolar acidic protein. *Journal of Biochemistry* **143** 441–448. (doi:10.1093/jb/mvm222)
- Onciu M, Behm FG, Downing JR, Shurtleff SA, Raimondi SC, Ma Z, Morris SW, Kennedy W, Jones SC & Sandlund JT 2003 ALK-positive plasmablastic B-cell lymphoma with expression of the NPM-ALK fusion transcript: report of 2 cases. *Blood* **102** 2642–2644. (doi:10.1182/blood-2003-04-1095)
- Osajima-Hakomori Y, Miyake I, Ohira M, Nakagawara A, Nakagawa A & Sakai R 2005 Biological role of anaplastic lymphoma kinase in neuroblastoma. *American Journal of Pathology* **167** 213–222. (doi:10.1016/S0002-9440(10)62966-5)
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N & Boggon TJ 2004 EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304** 1497–1500. (doi:10.1126/science.1099314)
- Palmer R, Vernersson E, Grabbe C & Hallberg B 2009 Anaplastic lymphoma kinase: signalling in development and disease. *Biochemical Journal* **420** 345–361. (doi:10.1042/BJ20090387)
- Panagopoulos I, Nilsson T, Domanski HA, Isaksson M, Lindblom P, Mertens F & Mandahl N 2006 Fusion of the SEC31L1 and ALK genes in an inflammatory myofibroblastic tumor. *International Journal of Cancer* **118** 1181–1186. (doi:10.1002/ijc.21490)
- Park JR, Eggert A & Caron H 2008 Neuroblastoma: biology, prognosis, and treatment. *Pediatric Clinics of North America* **55** 97–120. (doi:10.1016/j.pcl.2007.10.014)
- Passoni L, Longo L, Collini L, Coluccia AML, Bozzi F, Podda M, Gregorio A, Gambini C, Garaventa A, Pistoia V *et al.* 2009 Mutation-independent anaplastic lymphoma kinase overexpression in poor prognosis neuroblastoma patients. *Cancer Research* **69** 7338–7346. (doi:10.1158/0008-5472.CAN-08-4419)
- Patel AS, Murphy KM, Hawkins AL, Cohen JS, Long PP, Perlman EJ & Griffin CA 2007 RANBP2 and CLTC are involved in ALK rearrangements in inflammatory myofibroblastic tumors. *Cancer Genetics and Cytogenetics* **176** 107–114. (doi:10.1016/j.cancergencyto.2007.04.004)



- Perez-Pinera P, Chang Y, Astudillo A, Mortimer J & Deuel TF 2007 Anaplastic lymphoma kinase is expressed in different subtypes of human breast cancer. *Biochemical and Biophysical Research Communications* **358** 399–403. (doi:10.1016/j.bbrc.2007.04.137)
- Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, Arbogast S, Soltermann A, Weder W, Giordano TJ *et al.* 2008 EML4–ALK fusion lung cancer: a rare acquired event. *Neoplasia* **10** 298–302.
- Pillay K, Govender D & Chetty R 2002 ALK protein expression in rhabdomyosarcomas. *Histopathology* **41** 461–467. (doi:10.1046/j.1365-2559.2002.01534.x)
- Piva R, Chiarle R, Manazza AD, Taulli R, Simmons W, Ambrogio C, D'Escamard V, Pellegrino E, Ponzetto C, Palestro G *et al.* 2006 Ablation of oncogenic ALK is a viable therapeutic approach for anaplastic large-cell lymphomas. *Blood* **107** 689–697. (doi:10.1182/blood-2005-05-2125)
- Piva R, Agnelli L, Pellegrino E, Todoerti K, Grosso V, Tamagno I, Fornari A, Martinoglio B, Medico E, Zamò A *et al.* 2010 Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. *Journal of Clinical Oncology* **28** 1583–1590. (doi:10.1200/JCO.2008.20.9759)
- Powers C, Aigner A, Stoica GE, McDonnell K & Wellstein A 2002 Pleiotrophin signaling through anaplastic lymphoma kinase is rate-limiting for glioblastoma growth. *Journal of Biological Chemistry* **277** 14153–14158. (doi:10.1074/jbc.M112354200)
- Pulford K, Lamant L, Morris SW, Butler LH, Wood KM, Stroud D, Delsol G & Mason DY 1997 Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)–ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* **89** 1394–1404.
- Pulford K, Robertson HM & Jones M 2005 Antibody techniques used in the study of anaplastic lymphoma kinase-positive ALCL. *Methods in Molecular Medicine* **115** 271–294. (doi:10.1385/1-59259-936-2:271)
- Reichard KK, McKenna RW & Kroft SH 2007 ALK-positive diffuse large B-cell lymphoma: report of four cases and review of the literature. *Modern Pathology* **20** 310–319. (doi:10.1038/modpathol.3800742)
- Reiner DJ, Ailion M, Thomas JH & Meyer BJ 2008 *C. elegans* anaplastic lymphoma kinase ortholog SCD-2 controls dauer formation by modulating TGF- $\beta$  signaling. *Current Biology* **18** 1101–1109. (doi:10.1016/j.cub.2008.06.060)
- Riegel AT & Wellstein A 1994 The potential role of the heparin-binding growth factor pleiotrophin in breast cancer. *Breast Cancer Research and Treatment* **31** 309–314. (doi:10.1007/BF00666163)
- Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y *et al.* 2007 Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* **131** 1190–1203. (doi:10.1016/j.cell.2007.11.025)
- Savage KJ, Harris NL, Vose JM, Ullrich F, Jaffe ES, Connors JM, Rimsza L, Pileri SA, Chhanabhai M, Gascoyne RD *et al.* 2008 ALK<sup>-</sup> anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK<sup>+</sup> ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-cell Lymphoma Project. *Blood* **111** 5496–5504. (doi:10.1182/blood-2008-01-134270)
- Shao CK, Su ZL, Feng ZY, Rao HL & Tang LY 2002 Significance of ALK gene expression in neoplasms and normal tissues. *Ai Zheng* **21** 58–62.
- Shinmura K, Kageyama S, Tao H, Bunai T, Suzuki M, Kamo T, Takamochi K, Suzuki K, Tanahashi M, Niwa H *et al.* 2008 EML4–ALK fusion transcripts, but no NPM-, TPM3-, CLTC-, ATIC-, or TFG–ALK fusion transcripts, in non-small cell lung carcinomas. *Lung Cancer* **61** 163–169. (doi:10.1016/j.lungcan.2007.12.013)
- Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T & Mori S 1994 Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. *Oncogene* **9** 1567–1574.
- Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, Mori N, Fujimoto J, Miyauchi J, Mikata A *et al.* 1995 Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity. *Blood* **86** 1954–1960.
- Siebert R, Gesk S, Harder L, Steinemann D, Grote W, Schlegelberger B, Tiemann M, Wlodarska I & Schemmel V 1999 Complex variant translocation t(1;2) with TPM3–ALK fusion due to cryptic ALK gene rearrangement in anaplastic large-cell lymphoma. *Blood* **94** 3614–3617.
- Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K & Hatanaka H 2007 Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. *Nature* **448** 561–566. (doi:10.1038/nature05945)
- Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, Haruta H, Hamada T, Yamashita Y, Ishikawa Y *et al.* 2008 A mouse model for EML4–ALK-positive lung cancer. *PNAS* **105** 19893–19897. (doi:10.1073/pnas.0805381105)
- Souttou B, Carvalho NB, Raulais D & Vigny M 2001 Activation of anaplastic lymphoma kinase receptor tyrosine kinase induces neuronal differentiation through the mitogen-activated protein kinase pathway. *Journal of Biological Chemistry* **276** 9526–9531. (doi:10.1074/jbc.M007333200)
- Sozzi G, Martelli MP, Conte D, Modena P, Pettirossi V, Pileri SA & Falini B 2009 The EML4–ALK but not the fusion protein can be expressed in reactive and neoplastic lymphoid tissues. *Haematologia* **94** 1307–1311. (doi:10.3324/haematol.2009.008045)
- Stachurski D, Miron PM, Al-Homsi S, Hutchinson L, Harris NL, Woda B & Wang SA 2007 Anaplastic lymphoma kinase-positive diffuse large B-cell lymphoma with a complex karyotype and cryptic 3 ALK gene insertion to chromosome 4 q22–24. *Human Pathology* **38** 940–945. (doi:10.1016/j.humpath.2006.12.019)
- Stein H, Mason DY, Gerdes J, O'Connor N, Wainscoat J, Pallesen G, Gatter K, Falini B, Delsol G, Lemke H *et al.* 1985 The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* **66** 848–858.
- Stein H, Foss HD, Dürkop H, Marafioti T, Delsol G, Pulford K, Pileri S & Falini B 2000 CD30<sup>+</sup> anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. *Blood* **96** 3681–3695.
- Stoica GE, Kuo A, Powers C, Bowden ET, Sale EB, Riegel AT & Wellstein A 2002 Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. *Journal of Biological Chemistry* **277** 35990–35999. (doi:10.1074/jbc.M205749200)
- Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J & Vardiman JW 2008 *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: International Agency for Research on Cancer.
- Takeuchi K, Lim Choi Y, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Takada S & Yamashita Y 2008 Multiple reverse transcription-PCR screening for EML4–ALK in fusion transcripts. *Clinical Cancer Research* **14** 6618–6624. (doi:10.1158/1078-0432.CCR-08-1018)
- Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, Takada S, Ueno T, Yamashita Y, Satoh Y *et al.* 2009 KIF5B–ALK, a novel fusion onco-kinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clinical Cancer Research* **15** 3143–3149. (doi:10.1158/1078-0432.CCR-08-3248)
- Takeuchi K, Soda M, Togashi Y, Ota Y, Sekiguchi Y, Hatano S, Asaka R, Noguchi M & Mano H 2010 Identification of a novel fusion, SQSTM1–ALK, in ALK-positive large B-cell lymphoma. *Haematologia* **96** 464–467. (doi:10.3324/haematol.2010.033514)



- Tort F, Pinyol M, Pulford K, Roncador G, Hernandez L, Nayach I, Kluijn-Nelemans HC, Kluijn P, Touriol C, Delsol G *et al.* 2001 Molecular characterization of a new ALK translocation involving moesin (MSN–ALK) in anaplastic large cell lymphoma. *Laboratory Investigation* **81** 419–426.
- Tort F, Campo E & Pohlman B 2004 Heterogeneity of genomic breakpoints in MSN–ALK translocations in anaplastic large cell lymphoma. *Human Pathology* **35** 1038–1041. (doi:10.1016/j.hum-path.2004.05.006)
- Touriol C, Greenland C, Lamant L, Pulford K, Bernard F, Rousset T, Mason DY & Delsol G 2000 Further demonstration of the diversity of chromosomal changes involving 2p23 in ALK-positive lymphoma: 2 cases expressing ALK kinase fused to CLTCL (clathrin chain polypeptide-like). *Blood* **95** 3204–3207.
- Trinei M, Lanfrancone L, Campo E, Pulford K, Mason DY, Pelicci PG & Falini B 2000 A new variant anaplastic lymphoma kinase (ALK)-fusion protein (ATIC–ALK) in a case of ALK-positive anaplastic large cell lymphoma. *Cancer Research* **60** 793–798.
- Turner SD & Alexander DR 2005 What have we learnt from mouse models of NPM–ALK-induced lymphomagenesis? *Leukemia* **19** 1128–1134. (doi:10.1038/sj.leu.2403797)
- Umiker WO & Iverson L 1954 Postinflammatory tumors of the lung; report of four cases simulating xanthoma, fibroma, or plasma cell tumor. *Journal of Thoracic Surgery* **28** 55–63.
- Van Roosbroeck K, Cools J, Dierickx D, Thomas J, Vandenberghe P, Stul M, Delabie J, De Wolf-Peeters C, Marynen P & Wlodarska I 2010 ALK-positive large B-cell lymphomas with cryptic SEC31A–ALK and NPM1–ALK fusions. *Haematologia* **95** 509–513. (doi:10.3324/haematol.2009.014761)
- Wan W, Albom MS, Lu L, Quail MR, Becknell NC, Weinberg LR, Reddy DR, Holskin BP, Angeles TS, Underiner TL *et al.* 2006 Anaplastic lymphoma kinase activity is essential for the proliferation and survival of anaplastic large-cell lymphoma cells. *Blood* **107** 1617–1623. (doi:10.1182/blood-2005-08-3254)
- Webb TR, Slavish J, George RE, Look AT, Xue L, Jiang Q, Cui X, Rentrop WB & Morris SW 2009 Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. *Expert Review of Anticancer Therapy* **9** 331–356. (doi:10.1586/14737140.9.3.331)
- Williams DM, Hobson R, Imeson J, Gerrard M, McCarthy K & Pinkerton CR 2002 Anaplastic large cell lymphoma in childhood: analysis of 72 patients treated on The United Kingdom Children's Cancer Study Group chemotherapy regimens. *British Journal of Haematology* **117** 812–820. (doi:10.1046/j.1365-2141.2002.03482.x)
- Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, Ho KK, Au JS, Chung LP & Pik Wong M 2009 The EML4–ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* **115** 1723–1733. (doi:10.1002/cncr.24181)
- Wong DW, Leung EL, Wong SK, Tin VP, Sihoe AD, Cheng LC, Au JS, Chung LP & Wong MP 2011 A novel KIF5B–ALK variant in non-small cell lung cancer. *Cancer* (doi:10.1002/cncr.25843)
- Zamo A, Chiarle R, Piva R, Howes J, Fan Y, Chilosi M, Levy DE & Inghirami G 2002 Anaplastic lymphoma kinase (ALK) activates Stat3 and protects haematopoietic cells from cell death. *Oncogene* **21** 1038–1047. (doi:10.1038/sj.onc.1205152)
- Zhang N, Zhong R, Wang ZY & Deuel TF 1997 Human breast cancer growth inhibited *in vivo* by a dominant negative pleiotrophin mutant. *Journal of Biological Chemistry* **272** 16733–16736. (doi:10.1074/jbc.272.27.16733)
- Zhang Q, Wang HY, Liu X & Wasik MA 2007 STAT5A is epigenetically silenced by the tyrosine kinase NPM1–ALK and acts as a tumor suppressor by reciprocally inhibiting NPM1–ALK expression. *Nature Medicine* **13** 1341–1348. (doi:10.1038/nm1659)
- Zhang X, Zhang S, Yang X, Yang J, Zhou Q, Yin L, An S, Lin J, Chen S, Xie Z *et al.* 2010 Fusion of EML4 and ALK is associated with development of lung adenocarcinoma lacking EGFR and KRAS mutations and is correlated with ALK expression. *Molecular Cancer* **9** 188–200. (doi:10.1186/1476-4598-9-188)
- Zou HY, Li Q, Lee JH, Arango ME, McDonnell SR, Yamazaki S, Koudriakova TB, Alton G, Cui JJ, Kung PP *et al.* 2007 An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Research* **67** 4408–4417. (doi:10.1158/0008-5472.CAN-06-4443)

Received in final form 28 March 2011

Accepted 18 April 2011

Made available online as an Accepted Preprint 18 April 2011