REVIEW

Anaplastic lymphoma kinase in human cancer

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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.

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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

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be involved in neuronal differentiation, as suggested by its ability to induce neurite outgrowth *in vitro* (Souttou *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^+) 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-γ, IRS1, HSP90, GRB2, SHCC, JAK2/JAK3, PI3K, and STAT3/5 (Chiarle *et al.* 2008; Fig. 1).

Phospholipase C-γ

NPM–ALK controls cellular proliferation via the phospholipase C-γ (PLC-γ) docking in position Y664 of NPM–ALK. PLC-γ activation induces the hydrolysis of phosphatidylinositol (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG), molecules that can modulate the release of Ca^{2+} 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^{Y664F} mutant completely disables their growth in the absence of IL3 (Bai *et al.* 1998).

RAS

ALK^+ 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-kB, 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

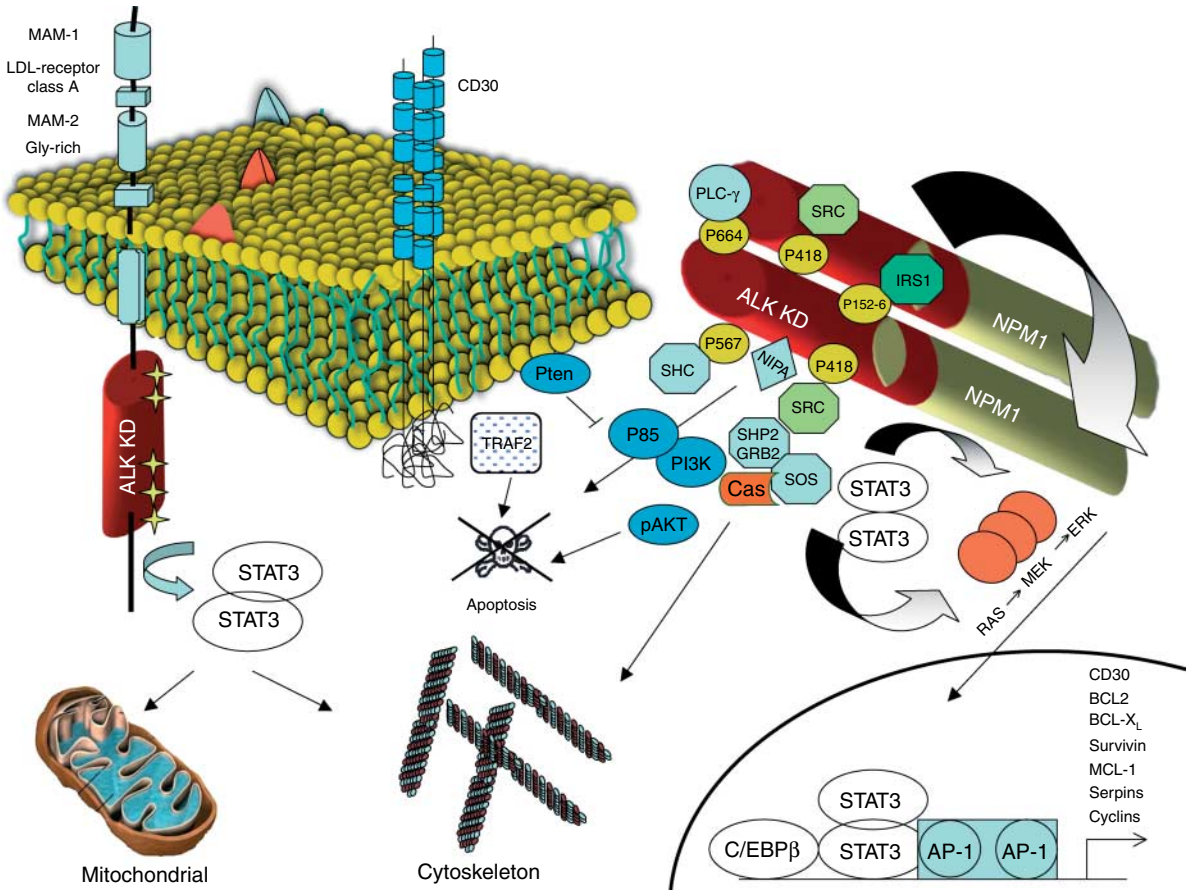

Figure 1 ALK and its signaling transduction pathways. Mutated ALK-R and ALK fusion proteins (NPM–ALK is depicted as representative ALK chimera) can elicit multiple signaling pathways, which are responsible for cell transformation and for the maintenance of the neoplastic phenotype (Chiarle *et al.* 2008, 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-γ, which binds directly to activated ALK, and generating diacylglycerol and IP_3 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, $BCL\text{-}X_L$, and MCL-1) and anti-apoptotic proteins, i.e. survivin and multiple transcription factor (i.e. C/EBPβ). 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_L, 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^+ 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 *et al.* (1994) and Shiota *et al.* (1994)
ALCL	ALO17–ALK	t(2;17)(p23;q25)	Cools *et al.* (2002)
ALCL	TFG–ALK	t(2;3)(p23;q21)	Hernández *et al.* (1999, 2002)
ALCL	MSN–ALK	t(2;X)(p32;q11–12)	Tort *et al.* (2001, 2004)
ALCL	TPM3–ALK	t(1;2)(q25;p23)	Lamant *et al.* (1999) and Siebert *et al.* (1999)
ALCL	TPM4–ALK	t(2;19)(p23;p13)	Meech *et al.* (2001)
ALCL	ATIC–ALK	inv(2)(p23;q35)	Colleoni *et al.* (2000), Ma *et al.* (2000), and Trinei *et al.* (2000)
ALCL	MYH9–ALK	t(2;22)(p23;q11·2)	Lamant *et al.* (2003)
ALCL	CLTC–ALK	t(2;17)(p23;q23)	Touriol *et al.* (2000)
IMT	TPM3–ALK	t(1;2)(q25;p23)	Lawrence *et al.* (2000)
IMT	TPM4–ALK	t(1;19)(p23;p13)	Lawrence *et al.* (2000)
IMT	CLTC–ALK	t(2;17)(p23;q23)	Bridge *et al.* (2001) and Patel *et al.* (2007)
IMT	ATIC–ALK	inv(2)(p23;q35)	Debiec-Rychter *et al.* (2003)
IMT	SEC31L1–ALK	t(2;4)(p23;q21)	Panagopoulos *et al.* (2006)
IMT	RANBP2–ALK	t(2;2)(p23;q13) inv(2)(p23;p15;q31)	Ma *et al.* (2003)
IMT	CARS–ALK	t(2;11;2)(p23;p15;q31)	Cools *et al.* (2002) and Debelenko *et al.* (2003)
NSCLC	EML4–ALK	inv(2)(p21;p23)	Rikova *et al.* (2007) and Soda *et al.* (2007)
NSCLC	TFG–ALK	t(2;3)(p23;q21)	Rikova *et al.* (2007)
DLBCL	NPM–ALK	t(2;5)(p23;q35)	Adam *et al.* (2003) and Onciu *et al.* (2003)
DLBCL	CLTC–ALK	t(2;17)(p23;q23)	De Paepe *et al.* (2003)
DLBCL	Unknown	ins(3'ALK)(4q22–24)	Stachurski *et al.* (2007)
DLBCL	SQSTM1–ALK	t(2;5)(p23·1;q35·3)	Takeuchi *et al.* (2010)
DLBCL	SEC31A–ALK	ins(4)(2;4)(?;q21) t(2;4)(p24;q21)	Bedwell *et al.* (2010) and Van Roosbroeck *et al.* (2010)
SCC	TPM4–ALK	t(2;19)(p23;p13)	Du *et al.* (2007) and Jazii *et al.* (2006)
RCC	VCL–ALK	t(2;10)(p23;q22)	Debelenko *et al.* (2010)

role for the appropriate classification of ALCL, demonstrating that ~60–80% of all ALCL are ALK^+ (Webb *et al.* 2009). It is important to underline that ALK^- ALCL are indistinguishable from ALK^+ 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^+ 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^+ and ALK^- 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^+ 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^+ 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^- 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^+ ALCL most frequently occur in the first decades of life with a typical male preponderance, although ALK^+ ALCL can also be seen in older individual at lower frequency; while ALK^- 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^+ ALCL patients have longer disease-free survival and better overall survival (OS) than ALK^- 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^+ 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 Paepe *et al.* 2003, Gascoyne *et al.* 2003, Onciu *et al.* 2003, Reichard *et al.* 2007). ALK^+ 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^+ 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^+ 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^+ 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^+ 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^+ 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^+ 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^+ cancer patients. Considering the exquisite oncogenetic addition of ALK^+ 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$^+$ 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.

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