CMLS, Cell. Mol. Life Sci. 61 (2004) 2939–2953
1420-682X/04/232939-15
DOI 10.1007/s00018-004-4275-9

CMLS Cellular and Molecular Life Sciences

The emerging normal and disease-related roles of anaplastic lymphoma kinase

K. Pulford [a, *], L. Lamant [b], E. Espinos [b], Q. Jiang [c], L. Xue [c], F. Turturro [d], G. Delsol [b] and S.W. Morris [c]

[a] Leukaemia Research Fund Immunodiagnostics Unit, Nuffield Department, Clinical Laboratory Sciences, Room 5501, Level 5, John Radcliffe Hospital, Oxford OX3 9DU (United Kingdom), Fax: +44 1865 222912, e-mail: karen.pulford@ndcls.ox.ac.uk
[b] INSERM U563, Department of Oncogenesis and signalling in hematopoietic cells, Centre de Physiopathologie de Toulouse-Purpan, Toulouse (France)
[c] Departments of Pathology and Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, Tenessee (USA)
[d] Department of Medicine, Feist-Weiller Cancer Center, LSU Health Sciences Center, Shreveport, Louisiana (USA)

Abstract. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, the normal role of which remains to be completely elucidated. Although work carried out in mammals suggests a function in neural development, results from studies in *Drosophila* indicate an additional role in visceral muscle differentiation. The aberrant expression of full-length ALK receptor proteins has been reported in neuroblastomas and glioblastomas, while the occurrence of ALK fusion proteins in anaplastic large cell lymphoma (ALCL) has resulted in the identification of the new tumor entity, ALK-positive ALCL. ALK represents one of few examples of a receptor tyrosine kinase implicated in oncogenesis in both haematopoietic and non-haematopoietic tumors, given that ALK fusions also occur in the mesenchymal tumor known as inflammatory myofibroblastic tumor (IMT). The study of ALK fusion proteins, besides demonstrating their importance in tumor development, has also raised the possibility of new therapeutic treatments for patients with ALK-positive malignancies.

Key words. ALK; NPM-ALK; ALCL; IMT; lymphoma; receptor tyrosine kinase.

Structure, distribution and function of full-length ALK in normal tissues

ALK is a member of the insulin receptor superfamily of receptor tyrosine kinases (RTKs) and bears significant homology throughout its full extent with leukocyte tyrosine kinase (LTK) [1–3]. The human 6226-bp *ALK* complementary DNA (cDNA) encodes a protein with a predicted mass of 177 kDa, which following post-translational modifications increases to 200 kDa [3–5]. ALK is a single-chain transmembrane protein of 1620 amino acids (aa) in the human (GenBank accession codes: U62540, U66559), 1621 aa in the mouse (Genbank accession code: D83002) and 1701 aa in the fruit fly (GenBank nucleotide sequence accession number AAF36990) [2, 3, 6] and is highly conserved across species. Detailed descriptions of the ALK protein have been provided previously [7–11]. A diagram of the full-length ALK protein is shown in figure 1.

Morris et al. initially described multiple human *ALK* messenger RNA (mRNA) transcripts by Northern blot analysis in a rhabdomyosarcoma cell line, placenta, testis, foetal liver, brain and the enteric innervation [12]. Subsequent in situ hybridisation studies confirmed the presence of *Alk* mRNA in the thalamus, hypothalamus, midbrain, olfactory bulb and selected cranial, as well as dorsal root ganglia of foetal and adult mice [2, 3]. The expression of both *Alk* mRNA and Alk protein decreased until only very low levels were detectable in neonates [2]. Immunocytochemical labelling provided further evidence of the restricted tissue distribution of ALK in normal cells and tissues [5, 13]. Recent studies have described *DAlk* mRNA transcripts and DAlk protein in the

* Corresponding author.

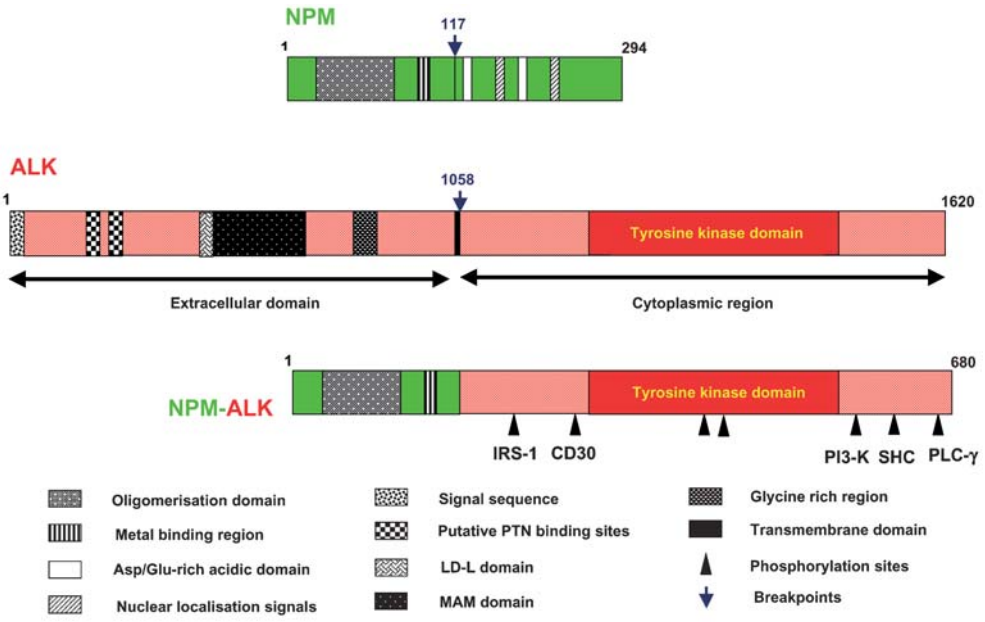

Figure 1. Diagram of the full-length ALK receptor tyrosine kinase and the most common oncogenic ALK fusion protein, NPM-ALK. As a result of the t(2;5)(p23;q35) the entire extracellular region of ALK is lost and is replaced by the amino-terminus of NPM. This region of NPM contains an oligomerisation domain whose presence permits the formation of NPM-ALK homodimers and NPM/NPM-ALK heterodimers. The formation of NPM-ALK homodimers results in dysregulation of the tyrosine kinase domain of ALK with consequent activation of downstream signalling pathways. The binding sites of various important molecules in these signalling pathways are indicated.

brain and ventral nerve in *Drosophila* [6] and in the musculature of the digestive tract throughout embryonic development [14, 15].

The presence of both ALK proteins and transcripts in the brain and spinal cord suggests a role in the normal development and function of the nervous system [2, 3, 5, 6]. Loren et al., studying mutant *Drosophila* strains, reported the absence of normal gut musculature development in flies lacking DAlk, suggesting a vital role for DAlk in the development of the ventral mesoderm in the fly [6, 15] possibly through activation of the *Dumbfounded (Duf)* and *org1* genes [14, 16]. No evidence for an essential role for Alk has been found in knockout mice [L. Xue and S.W. Morris, unpublished].

The pathways by which ALK mediates its effects are still under investigation. Studies using the chimeric ALK proteins IgG2b-ALK and epithelial growth factor receptor (EGFR)-ALK have implicated the mitogen activated protein (MAP) kinase (MAPK) pathway in signalling by the ALK protein [17, 18]. Immunocytochemical techniques demonstrating the co-localisation of DAlk and phosphorylated extracellular signal-related kinase (ERK) proteins in *Drosophila* have provided additional evidence for such an interaction [14, 15].

Iwahara et al. suggested that, since ALK possessed an epithelial growth factor (EGF)-like motif in its extracellular domain, it might itself act as a ligand [2]. Another possibility is that ALK may act as a receptor for a neurotrophic factor(s). This latter hypothesis has been borne out, at least in part, given that recent studies have identified three likely ligands for ALK, two of which, pleiotrophin (PTN) and midkine (MK) in the human, have been shown to have effects upon neural tissues [19, 20]. Questions remain, however, concerning the description of PTN and MK as ALK ligands. For example, the receptor protein tyrosine phosphatase (RPTP) beta/zeta and the heparan sulphate proteoglycan N-syndecan (syndecan 3) have also been described as PTN receptors. More convincing evidence based on in vivo experiments has been obtained for Jeb as a possible DAlk ligand. For example, both Lee et al. [14] and Englund and co-workers [16] observed a similar phenotype in *Jeb* and *DAlk* mutant flies. Immunocytochemical studies demonstrated that, although Jeb and DAlk were expressed in adjacent cells, these proteins were co-localised at areas of contact. Furthermore, the expression of both Jeb and DAlk proteins were essential for the activation of the RAS/MAPK pathway and the normal specification of visceral gut muscle development.

Expression and function of the full-length ALK receptor in tumors

The full-length ALK receptor protein was originally reported in the Rh30 rhabdomyosarcoma-derived cell line [2, 3, 5, 12] and subsequently in a subset of rhabdomyosarcoma tumors [21–23]. ALK receptor protein expression has also been documented in neuroblastomas

and in a number of non-haematopoietic cell lines [4, 20, 24–27].

Lamant et al. were unable to identify any correlation between ALK expression and outcome in a small cohort of neuroblastoma cases [4]. Indeed, the absence of detectable significant levels of endogenously autophosphorylated ALK found in these tumors [4] and in some neuroblastoma-derived cell lines [27] suggests that ALK expression in these malignancies could simply reflect its normal expression in immature neural cells rather than a primary oncogenic role. Furthermore, Dirks et al. [27] found no evidence for the existence of an autocrine or paracrine loop activating ALK in a variety of tumor types. Miyake et al. [24] were unable to detect the formation of stable complexes of ALK with its signalling substrates insulin receptor substrate-1 (IRS-1) and phospholipase C-gamma (PLC-γ) in neuroblastoma. However, Miyake et al. did report amplification of the *ALK* gene and phosphorylation of the encoded ALK protein, as well as its increased binding to SHC in two neuroblastoma-derived cell lines, thus suggesting a pathogenic role for ALK via this mechanism in at least a subset of neuroblastomas. Stoica et al. also described MK-mediated ALK phosphorylation and the activation of phosphoinositide 3-kinase (PI-3) kinase and MAPK in the SH SY-5Y neuroblastoma cell line [20]. Furthermore, Powers et al. reported the degree of ALK expression to be a rate-limiting factor in apoptosis and tumor growth in a glioblastoma cell line [25]. These results support the possibility that endogenous ALK, activated due to amplification of the *ALK* gene and/or by endogenous ligands, might be a contributing factor in the tumorigenesis of some neuroblastomas or other ALK-positive malignancies.

The expression of full-length ALK receptor expression in a subtype of B cell lymphoma [28] will be described in more detail below.

Structure of ALK fusion proteins

In common with other receptor tyrosine kinases, translocations affecting the *ALK* gene at chromosome 2p23 have been described. These abnormalities result in the production of a number of oncogenic ALK fusion proteins, the most common of which is nucleophosmin (NPM)-ALK, which occurs in ~80% of ALK-positive ALCL [7, 8, 29, 30]. The characteristic nuclear and cytoplasmic distribution pattern of the chimaeric NPM-ALK protein means that the presence of the other, so-called variant ALK fusion proteins, can be easily identified through their subcellular distribution in the tumor cells (see table 1). It is also possible that additional ALK fusion proteins remain to be identified. For example, although the t(2;13)(p23;q34) [54] and t(2;22)(p23;q11.2) [55] abnormalities have been reported in non-Hodgkin's lymphome (NHL), the genes and their encoded proteins have not yet been identified. It is of note, however, that the translocation, t(2;22)(p23;q11.2), reported by Park et al., [55] was found to be ALK-negative [Gascoyne R. et al., unpublished communication].

ALK fusion proteins share certain characteristics. With the exception of moesin (MSN)-ALK and the recently identified non-muscle myosin heavy chain (MYH9)-ALK, all ALK fusion protein possess the entire intracytoplasmic region (aa 1058–1620 of the full-length human

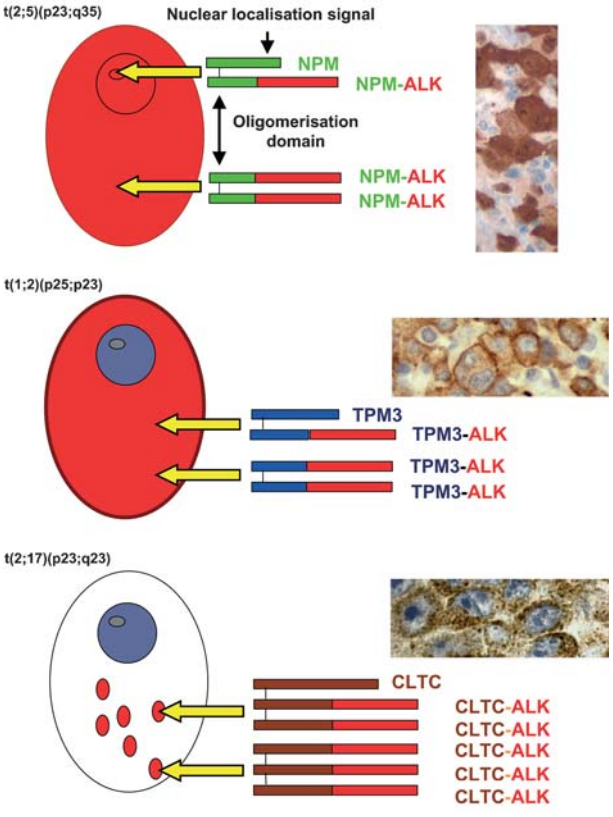

Figure 2. Diagram demonstrating mechanisms for distribution of ALK fusion proteins in neoplastic cells. The right-hand side shows immunoperoxidase labelling of sections of ALK-positive ALCL, while diagrammatic representations of the variant ALK fusion proteins are illustrated on the left. All three partner proteins, NPM, TPM3 and CLTC, shown here contain oligomerisation domains in their N-termini that permit both homodimer and heterodimer formation. NPM-ALK and the t(2;5)(p23;q35): The distribution of the NPM-ALK homodimers are limited to the cytoplasm, but the nuclear localisation motifs present in wild-type NPM permits entry of the NPM/NPM-ALK heterodimers to the nucleus and nucleolus. This characteristic distribution pattern can be seen in the NPM-ALK-positive ALCL case. TPM3-ALK and t(1;2)(q25;p23): The distribution of the TPM3/TPM3-ALK heterodimers and TPM3-ALK/TPM3-ALK homodimers is limited to the cytoplasm of the tumor cells to give a diffuse labelling pattern of the neoplastic cells with stronger membrane labelling. CLTC-ALK and the t(2;17)(p23;q23): Both CLTC/CLTC-ALK heterodimers and CLTC-ALK/CLTC-ALK homodimers are located within the clathrin-coated vesicules of the neoplastic cell to give the characteristic granular distribution of this fusion protein in the cell.

Table 1. Characteristics and subcellular distribution patterns of the ALK fusion proteins

Fusion protein	Chromosomal translocation	Partner protein	Location of fusion protein	Size fusion protein (kDa)	Tumor
NPM-ALK	t(2;5)(p23;q35)	nucleophosmin (NPM)*	nucleus, nucleolus and cytoplasm	80	ALCL and B cell lymphoma [5, 12, 31–33]
TPM3-ALK	t(1;2)(p25;p23) cryptic ALK rearrangement	tropomyosin 3 (TPM)3*	cytoplasm	104	ALCL and IMT [34–36]
TFG-ALK_S, TFG-ALK_L TFG-ALK_{XL}	t(2;3)(p23;q21)	TRK-fused gene (TFG)*	cytoplasm cytoplasm cytoplasm	85 97 113	ALCL [37, 38]
ATIC-ALK	inv(2)(p23q35)	5-aminoimidazole–4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC); also known as PurH	cytoplasm	96	ALCL [39–41]
CLTC-ALK	t(2;17)(p23;q23)	clathrin heavy chain (CLTC)	granular cytoplasmic	250	ALCL, IMT and B cell lymphoma [33, 42–48]
MSN-ALK	t(2;X)(p23;q11–12)	moesin (MSN)	cell membrane-associated	125	ALCL [49]
TPM4-ALK	t(2;19)(p23;p13.1)	tropomyosin 4 (TPM4)	cytoplasm	95–105	ALCL and IMT [35, 50]
ALO17-ALK	t(2;17)(p23;q25)	unknown gene, ALK lymphoma oligomerization partner on chromosome 17 (ALO17)	cytoplasm	not determined	ALCL [44]
RANBP2-ALK	t(2;2)(p23;q13) or inv(2)(p23q11–13)	RAN binding protein 2 (RANBP2), also known as NUP358	periphery of the nucleus	160	IMT [51]
MYH9-ALK	t(2;22)(p23;q11.2)	non-muscle myosin heavy chain gene (MYH9)	cytoplasm	220	ALCL [52]
CARS-ALK	t(2;11;2)(p23;p15;q31)	cysteinyl-tRNA synthetase enzyme (CARS)	cytoplasm	130	IMT [44, 53]

With the exception of MSN-ALK and MYH9-ALK, all of the fusion proteins contain the final 563 amino acids of ALK. MSN-ALK and MYH9-ALK contain the final 567 and 566 amino acids, respectively.
* Partners in other oncogenic fusion proteins.
NPM-ALK represents ~75% of all ALK fusion proteins found in ALK-positive lymphomas, TPM3-ALK is present in ~15% of cases, while ATIC-ALK, TFG-ALK and CLTC-ALK account for ~8% of cases. The other ALK fusion proteins are present in the remaining 2% of cases of ALK-positive lymphomas.

receptor protein) of ALK. The partner protein determines the subcellular localisation of the ALK fusion protein (see fig. 2). The RAN binding protein 2 (RanBP2)-ALK fusion protein, which has thus far been identified only in inflammatory myofibroblastic tumour (IMT) cases (vide infra), possesses a unique staining pattern, with the majority of the ALK immunostaining signal being associated not with the cytoplasm but rather with the nuclear membrane of tumor cells due to hetero-association of RanBP2-ALK with RanBP2, a nuclear pore protein [51]. All fusion proteins, with the apparent exceptions of MSN-ALK and MYH9-ALK, contain an oligomerisation domain in their amino-terminal portion. Oligomerisation of the homodimeric fusion proteins mimics ligand-mediated aggregation of the full-length wild-type receptor, resulting in the constitutive activation of each specific ALK fusion protein [40–42, 49, 56–59]. Variations of this mechanism may occur with the MSN-ALK and MYH9-ALK fusion proteins. For example, the portion of the MYH9 α-helical coiled-coil tail, critical for the self-assembly of two myosin heavy chains, is lacking in the MYH9-ALK protein [52, 60]. In this context, it remains

difficult to explain how this protein is activated and phosphorylated, unless a novel functional oligomerisation domain is ultimately identified in the amino-terminus of MYH9. The ensuing dysregulation of the ALK tyrosine kinase catalytic domain in the ALK fusion proteins then results in the aberrant activation of multiple downstream signalling cascades that are responsible for the neoplastic transformation of cells.

Expression of *ALK* fusion genes in normal tissues

Although the presence of *NPM-ALK* mRNA has been reported in samples of normal peripheral blood [61, 62] and in reactive lymphoid tissue [63] by sensitive reverse transcription-polymerase chain reaction (RT-PCR) methods, the presence of NPM-ALK protein has not, as yet, been identified in normal tissues. This raises questions as to the biologic significance of the expression of the fusion gene in this situation. Nonetheless, these observations are intriguing and suggest that the rearrangements that generate oncogenic fusion genes such as *NPM-ALK* occur on a regular, perhaps ongoing, basis in cells but require other events to become apparent clinically.

Distribution of ALK fusion proteins in haematopoietic tumors (see table 1)

The expression of ALK fusion proteins is one of the rare occurrences in which an aberrant tyrosine kinase has been shown to play a role in lymphoma development. Subsequent immunocytochemical studies have verified the expression of ALK fusion proteins in 60–80% of ALCLs. These CD30-positive ALK-positive lymphoma cells exhibit either a cytotoxic T-cell (85%) or null (lacking expression of both T- and B-cell markers) cell phenotype [7, 64, 65]. There is heterogeneity in the expression of the EMA glycoprotein in these lymphomas [29]. These tumors also commonly express the CD134/OX40 and CXCR3 proteins [66]. Although primary systemic ALCL contains a number of different morphological subtypes, the presence of ALK-positive tumor cells occurs in all of these subtypes [8, 10, 67], suggesting that they are simply morphological variants of the same disease. In keeping with this belief, a highly characteristic 'hallmark' cell with an eccentrically located horseshoe or kidney-shaped nucleus and a prominent eosinophilic region near the Golgi apparatus has been described in the full spectrum of the reported subtypes of ALCL [29]. These 'ALK-positive lymphomas' or 'ALKomas' occur mainly in children and young adults, with a significant proportion between 10 and 19 years of age and a slight male predominance. These lymphomas, although highly aggressive, are associated with a good prognosis, with an overall 5-year survival of 71–80% compared to only 15–46% for ALK-negative ALCL patients [7, 13, 68]. ALK-positive lymphoma has recently been recognised as a distinct entity known as ALK-positive ALCL in the WHO Classification of Non-Hodgkin's Lymphoma [64].

Although the presence of the *NPM-ALK* mRNA was reported by RNA-PCR in Hodgkin's lymphoma (HL) [69], multiple subsequent studies have failed to demonstrate any involvement of the *ALK* gene in these tumors [7, 10]. Another curiosity was the presence of *NPM-ALK* transcripts, but no detectable ALK protein, in a minority of cases of primary cutaneous ALCL [70, 71]. While the inability to detect ALK protein expression in HL and primary cutaneous ALCL suggests that the t(2;5) has no major role in either of these tumors, it is also possible that the expression of *NPM-ALK* mRNA in normal cells, as described above, may have been responsible for the detection of *NPM-ALK* mRNA described in HL [69] and primary cutaneous ALCL [70, 71]. These results suggest that the detection of ALK fusion proteins in tumor cells can serve as a reliable means to distinguish ALK-positive ALCL from other CD30-positive tumors such as lymphomatoid papulosis, primary cutaneous lymphoma, Hodgkin's lymphoma and Hodgkin's disease-like ALCL [7, 8, 64].

Six groups of workers have recently described the presence of the ALK fusion proteins CLTC-ALK [45–48] and NPM-ALK [32, 33] in a rare form of B-cell NHL. These tumors exhibit a similar immunoblastic/plasmablastic morphology and immunophenotype to the ALK-positive B-cell tumors previously described by Delsol et al. in 1997 [28]. Indeed, one of the cases included by Gascoyne et al. [47] in their recent study was described in the original 1997 paper by Delsol and colleagues.

The removal of ALK-negative B-cell tumors with anaplastic morphology to the category of diffuse large B-cell lymphomas has simplified the classification of ALK-negative ALCL. The ALK-negative ALCLs, possessing either a null- or T-cell phenotype are now subdivided into primary cutaneous ALCL and systemic ALK-negative ALCL [28]. The distinction of primary cutaneous ALCL from systemic ALK-negative ALCL is extremely important since the former tumors are responsive to treatment and have a markedly better prognosis compared to systemic ALK-negative ALCLs [70–73].

Distribution of ALK fusion proteins in non-haematopoietic tumors

Griffin et al. [74] identified cytogenetic evidence for rearrangement of the *ALK* gene and ALK protein expression in three cases of inflammatory myofibroblastic tumor (IMT) in 1999. As shown in table 1, subsequent studies have also demonstrated the presence of the lym-

phoma-associated TPM3-ALK, TPM4-ALK and CLTC-ALK fusion proteins in IMTs [35, 43, 75]. Two additional ALK fusions, RanBP2-ALK and CARS-ALK, have been recently described in IMT but not, as yet, in NHL [44, 51, 53]. Thirty-six to 62% of IMT cases express ALK proteins [8, 22, 76–78]. Although preliminary evidence suggests that patients with ALK-positive forms of IMT appear to have an improved prognostic outlook compared to those with ALK-negative tumors [79], more work is required to ascertain whether ALK expression will, in fact prove to identify clinically meaningful subtypes of IMT.

ALK expression has also been reported in a number of mesenchymal tumors such as malignant peripheral nerve sheath tumor (40%) and leiomyosarcoma (10%) [22]. Additional studies of these tumor types are required to assess the role, if any, of either full-length or fusion forms of ALK in the pathogenesis and/or progression of these tumors.

Oncogenic role of the NPM-ALK fusion protein

Although full-length ALK has been implicated in oncogenesis [17, 19, 20, 25, 80], it is the studies on the NPM-ALK fusion protein that have provided the majority of the information concerning the oncogenic properties of ALK and led to the conclusion that NPM-ALK expression is a major causative event in cases of ALK-positive ALCL exhibiting the t(2;5). It is the cytoplasmic localisation of NPM-ALK that appears to be absolutely necessary for its oncogenic effects [81, 82].

The development of in vivo murine models has been instrumental in providing information concerning the oncogenic role of NPM-ALK. The initial study by Kuefer et al. [83] reported that BALB/cByJ mice transplanted with bone marrow from donor mice infected with retroviral constructs containing the human *NPM-ALK* cDNA develop B-cell lymphomas within 4–6 months. Subsequent studies, using either a murine CD4 promoter [84] or a Moloney murine leukemia virus-based vector [85] to drive NPM-ALK expression, reported the development in transgenic mice of short-latency (13–30 weeks mean survival time) NPM-ALK-positive T-cell tumors which were more typical of human ALK-positive ALCL. However, a subset of these mice also manifested B-cell tumors with a phenotype reminiscent of human ALK-positive B-cell lymphomas described above. Miething et al. [86], using a retroviral vector to program high levels of NPM-ALK expression, also reported the induction of B-cell tumors in some animals transplanted with marrow transduced at a low multiplicity of infection. These authors were also the first to report ALK-positive tumors expressing myeloid/macrophage-associated antigens such as CD11b (Mac-1) and Gr-1. In an effort to obtain more representative murine models of the human lymphomas, Turner et al. used a *Vav* gene promoter with the aim of switching on NPM-ALK production in a wide range of haematopoietic cells at an early stage of their differentiation [87]. This study also resulted in the occurrence of two tumor types, the first being a more aggressive tumor of B-cell phenotype, while tumor cells in the second type, which affected mainly the liver and spleen, expressed the Mac-1 myeloid antigen. Since Mac-1 expression is not limited to myeloid cells, both Turner et al. [87] and Miething et al. [86] proposed that these tumors might have arisen from cells expressing a less differentiated B-cell phenotype. Of note from all of these in vivo experiments is the suggestion of a relationship between the degree of the induced expression of NPM-ALK and the phenotype of the resulting tumor.

Details of the more extensively studied pathways involving the complex interactions of the NPM-ALK fusion protein with its various downstream signalling molecules such as GRB2, PLC-γ, PI3-kinase, IRS-1 and SHC have been the subject of extensive reviews [8–11, 75], and only recent developments concerning the oncogenic role of NPM-ALK will be described below.

Additional evidence linking ALK proteins with the RAS/MAPK pathway was found in *Drosophila*, where DAlk was necessary for ERK activation in the developing mesoderm [6, 14, 16] and in the developing eye disc [6]. Activation of SHC and the MAPK pathways, involving C-Jun N-terminal kinase (JNK) and ERK, was also detected in NPM-ALK expressing tumors obtained from in vivo studies in transgenic mice [87]. It should be noted, however, that although SHC and IRS-1 have been implicated in oncogenesis, their exact role is unknown, and it is not yet clear whether their activation by NPM-ALK or the variant ALK fusions is essential for cellular transformation.

Turturro et al. [88], using adenovirally induced expression of the cyclin-dependent kinase inhibitor $p27^{Kip1}$, were able to demonstrate a downregulation of the NPM-ALK/PLC-γ pathway in the NPM-ALK-positive SU-DHL-1 cell line. It is of note that this effect, and the induction of apoptosis, were observed only in the SU-DHL-1 cells and not in the Karpas 299 cell line, despite both of these lines having been derived from t(2;5)-positive ALCL. Rassidakis et al. have subsequently reported differences in the levels of expression of $p27^{Kip1}$, as well as the c-Jun activation domain binding protein-1 (JAB1) involved in the regulation of $p27^{Kip1}$ protein degradation by proteasomal pathways, in five different cell lines derived from ALK-positive ALCL [89], thus further emphasising the biochemical heterogeneity of these cells.

A recent study by Cussac et al. described the involvement of the pp60 SRC kinase in the mitogenic potential of NPM-ALK [90]. Site-directed mutagenesis studies and the use of SRC kinase inhibitors or pp60c-SRC interfer-

ence RNA strongly inhibited the proliferation of NPM-ALK-positive cells. Although these authors were able to identify NPM-ALK as a substrate for pp60c-SRC, further work is necessary to elucidate the intermediary proteins implicated in this pathway.

Although NPM-ALK Tyr 418 residue was proposed as a potential PI3-kinase binding site because it is found within a sequence context typical of previously described PI3-kinase-binding motifs, its substitution with phenylalanine did not reduce the binding and activation of PI3-kinase or the activation of AKT [91]. One possible reason for this observation could be that an adaptor protein(s) could function as intermediary linking molecule(s) between NPM-ALK and the p85 regulatory subunit of PI3-kinase. In support of this hypothesis, a recent study has highlighted the possibility that pp60c-SRC is activated in these cells [90]. *In vitro* studies have also demonstrated that the anti-apoptotic (and proliferation-enhancing) effects of NPM-ALK that occur via AKT can also be mediated, in part, through AKT-induced inhibition of the activity of the forkhead family transcription factor FOXO3a (or FKHRL1) [92]. It should be noted that PI3-kinase involvement in NPM-ALK-mediated apoptosis is not always predictable. For example, PI3-kinase is not implicated in the inhibition of doxorubicin- or etoposide-induced apoptosis despite activation of AKT/PKB [91]. Furthermore, NPM-ALK appears not to be the only tyrosine kinase in ALCL interacting with the PI3-kinase/AKT pathway since comparable levels of phosphorylation of the p85 subunit of PI3-kinase have been reported in both ALK-positive and -negative ALCL cell lines [27].

CD30 expression is, by definition, present on all ALK-positive ALCK, and a possible link between the CD30 cell surface receptor and NPM-ALK has been reported [93]. Binding of CD30 ligand to CD30 elicits a wide range of cellular responses depending upon the cell type under study [94–96]. One possible explanation to account for this difference in responses to CD30 ligand is the presence of high levels of BCL-3 protein in ALK-positive ALCL cells compared to those in HL-derived-cell lines [97, 98]. The degree of CD30 activation could also be another contributing factor for the variation in cellular responses to CD30 [99, 100]. The functional significance of the association for NPM-ALK and CD30 remains to be clearly established.

The constitutive activation of STAT3 has now been described in NPM-ALK-transfected cell lines as well as in ALCL cell lines and tumor biopsies [101, 102], and Zamo et al. reported the nuclear expression of STAT3 to be indicative of its activated status [101]. The upregulation of STAT3 activation in NPM-ALK transfectants was not dependent upon the presence of JAK3 [101], a finding confirmed in a later study by Amin et al. [103]. However, it is possible that NPM-ALK may bind directly to STAT3. The activation of STAT3 due to NPM-ALK enhanced the expression of the anti-apoptotic protein BCL-X(L) and is in keeping with the recent report by Khoury et al., [104], who described a correlation between the lack of STAT3 activation and improved prognosis in ALK-positive ALCL. The reports of the constitutive expression and association with STAT3 of protein phosphatase 2A (the positive regulator of STAT3 activation) and lack of the protein inhibitor of activated STAT3 (the STAT3 antagonist) in NPM-ALK-positive cells provides additional evidence for a central role for STAT3 in ALK-positive ALCL [102].

STAT5 is the only other STAT protein that exhibits a significant and consistent level of activation being implicated in both mitogenic and anti-apoptotic pathways in NPM-ALK-positive cells [105]. One possible mechanism by which STAT5 mediates these effects is via the tyrosine kinase JAK2 [106]. Another possible pathway involves the activation of anti-apoptotic genes such as *BCL-X(L)* [107]. Coluccia et al. [107] also identified BCL-X(L) as a possible therapeutic target for ALK-positive tumors. Mice injected with NPM-ALK transfected Ba/F3 cells containing a doxycycline-inducible BCL-X(L) antisense transgene were protected from tumor development following doxycycline administration. Rassidakis et al. [108], however, reported the anti-apoptotic BCL-2 and BCL-X(L) proteins to be less commonly expressed at high levels in ALK-positive lymphomas than the pro-apoptotic BAX and BCL-X(S) proteins. Villalva and colleagues [109] also found the expression of BCL-2 to be rare in ALK-positive ALCLs. Thus, our current understanding of the consequences of, and requirement for, the STAT5 activation pathway in the genesis and progression of ALK-positive ALCL requires further elucidation.

A relationship between NPM-ALK, STAT5 and RAD51 (involved in homology-dependent recombinational repair of DNA double-strand breaks) has recently been described [110]. In this study, the use of STAT5 dominant-negative constructs inhibited the upregulation of RAD51 in NPM-ALK transfectants. These results suggest that enhanced activity of RAD51 due to transcriptional regulation by STAT5 in ALK-positive cells may have effects upon DNA repair, cell cycle checkpoint control and apoptosis regulation, and may thus contribute to resistance to therapy noted in some tumors [111].

The adenoviral-induced expression of $p27^{Kip1}$ resulted in apoptosis in the NPM-ALK-positive SU-DHL-1 cell line [112]. In contrast, adenovirally mediated p53 expression in these cells produced a delayed but greater degree of cell death. The in vitro use of adenoviral constructs in these studies also demonstrated the selective apoptotic effects of different cyclin-dependent kinase inhibitors, since expression of the p21 and p27 proteins induced greater degrees of apoptosis compared to that produced by p16 [113].

A role for full-length NPM in the activation of p53 has recently been described. The direct interaction of NPM with p53 increased the stability and transcriptional activity of p53 following different types of stress [114]. The absence of the p53 binding site in the NPM-ALK protein may result in the loss of normal p53 function with consequent increased cell survival and growth.

Ouyang et al. recently identified a possible anti-apoptotic role for nuclear interacting protein of anaplastic lymphoma kinase (NIPA) in ALK signalling [115]. The binding of activated ALK protein to the nuclear NIPA protein occurred via a novel phosphotyrosine binding domain and resulted in a delay, but not the complete inhibition of apoptosis, in the IL3-dependent Ba/F3 cell line when deprived of the cytokine. The anti-apoptotic effect of NIPA was shown to be reliant upon its nuclear location.

Both stress-induced apoptosis (primarily involved in drug-induced death) and the CD95/FAS ligand pathway eventually lead to the activation of caspase 3. Ergin et al. [116], studying the effects of the tyrosine kinase inhibitor Herbimycin A on NPM-ALK signalling, reported that its use resulted in increased expression of caspase 3. This observation led these authors to hypothesize that the inhibition of caspase 3 activity may be a critical event in the anti-apoptotic role of NPM-ALK. However, the existence of such a mechanism remains to be identified since evidence obtained from later immunocytochemical studies demonstrated significantly higher levels of active caspase 3 in ALK-positive lymphomas compared to ALK-negative ALCL [117, 118]. It should be noted that whereas NPM-ALK inhibited drug-induced apoptosis in Jurkat T-leukemia cells, it appeared to have no effect in preventing CD95-mediated apoptosis, a result suggesting inhibition of apoptotic effects upstream of caspase 3 rather than of caspase 3 itself [91].

An inverse correlation of the expression of members of the BCL-2 anti-apoptotic family with ALK in cases of ALCL has also been reported in several studies [108, 109, 117]. While the pattern of expression of anti-apoptotic BCL-2 family members may account, in part, for the improved prognostic outlook for ALK-positive ALCL, it should also be remembered that NPM-ALK is also implicated in the anti-apoptotic AKT/PKB and STAT protein pathways. Furthermore, it is possible that other molecules, such as the anti-apoptotic protein MCL-1, may represent an alternative mechanism of overcoming the absence of BCL-2 anti-apoptotic proteins in ALK-positive lymphomas [119, 120].

A recently published proteomics-based analysis of NPM-ALK-associated proteins helps point the way to future signal transduction experiments concerning ALK and exemplifies the complexity of signalling mediated by the chimaeric kinase. A total of 57 proteins was identified as interacting with either the NPM or ALK portions of the fusion protein in a multi-protein signalling complex [121]. Thus, additional studies are clearly needed to elucidate fully the mechanisms employed by ALK proteins to regulate both mitogenicity and apoptosis.

Current treatment for ALK-positive ALCL

Despite the relatively good prognosis of ALK-positive ALCL, ~20–30% of patients with this type of non-Hodgkin's lymphoma fail to respond well to treatment. Thus, the optimal therapeutic approach still remains to be established. Current treatment methods include the use of various combination chemotherapy protocols originally developed for T-cell lymphoblastic tumors and high-grade B-cell NHL [122–125]. Some clinicians also advocate a combination of high-dose chemotherapy followed by autologous stem cell transplantation for patients with ALK-positive ALCL who are considered to be high risk [7, 10]. A recent study by Liso et al. has also highlighted the possibility of using a haploidentical peripheral blood stem cell transplantation as a last form of therapy [126].

The ability to identify those patients who will not respond to treatment at an early stage of their disease is of paramount importance. A high age-related International Prognostic Index (IPI) has been suggested as a reliable indicator of a poor outcome for ALK-positive ALCL patients [13, 68]. An additional marker of poor prognosis includes the expression of CD56 on ALK-positive lymphomas [127], while the low or absent expression of $p27^{Kip1}$ and MUC-1/EMA [128] may constitute markers of good prognosis. Investigations into the levels of apoptosis-regulatory proteins, including caspase 3, granzyme B-specific protease inhibitor 9 (PI9) and BCL-2, in individual cases of ALK-positive ALCL may provide further means of identifying those ALK-positive patients with a poor prognosis [117, 129].

Immunotherapeutic studies in animal models

Although the exact functional relevance of CD30 in ALK-positive ALCL requires further study, therapeutic approaches targeting this molecule hold promise [130–132]. Anti-CD30 antibodies are now being examined in clinical trials for CD30-positive tumors [133, 134]. The 110-kDa CD26 antigen may also represent an additional therapeutic target [135]. Zhang et al. have shown that IL-2Rα (CD25) is a target for radioimmunotherapy in ALK positive ALCL-derived cell line SU-DHL-1-xenografted mice [136].

Gene therapy approaches

Adenoviruses hold potential promise for the treatment of tumors, and a study by Meeker and colleagues suggested that adenoviral vectors could be of value for gene therapy of lymphoproliferative disorders [137]. In 2000, Turturro and co-workers proposed the use of adenovirus as a treatment option for ALK-positive ALCL and identified the presence of the high-affinity receptor for the coxsackie-adenovirus receptor (CAR) 2 and 5 as well as other adenovirus subtypes on the NPM-ALK-positive SU-DHL-1 cell line [138]. They subsequently went on to demonstrate in vivo anti-tumor activity of the same adenoviral vector constructs containing p53 in xenografts derived from SU-DHL-1 cells established in the subcutaneous tissue of nude mice [139]. Similar approaches employing adenovirus-mediated delivery of cyclin-dependent kinase inhibitors such as $p27^{Kip1}$, $p21^{Waf1}$ and $p16^{INK4A}$ have been suggested by these investigators for possible therapeutic purposes in ALK-positive ALCL as well [88, 113]. Although the adenoviral-mediated gene therapy approach for ALK-positive ALCL holds potential, the limitations, pitfalls and suggestions to improve this method as a therapeutic tool have been recently analysed [140]. The ideal viral vector for gene therapy of hematological malignancies must have distinctive features, including the potential for systemic administration, the ability to specifically target transformed cells for high efficiency of infection and, ultimately, an intrinsic ability to cause cytopathic effects or to program the expression of a cytotoxic transgene.

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors have been found to play a valuable role in the treatment of other tumors in which oncogenic tyrosine kinases play a role. Although STI571 (Gleevec or Glivec, Novartis Pharmaceuticals, Basel, Switzerland), an ATP-competitive tyrosine kinase inhibitor [141], has been used with great success in the treatment of chronic myeloid leukaemia expressing the fusion tyrosine kinase BCR-ABL [141], no such role has been found for this compound in ALK-positive ALCL [116]. There is, however, an anecdotal clinical report of a single ALK-positive lymphoma patient from a phase 1 study of the compound UCN-01 (7-hydroxystaurosporine) [142], suggesting the possible effectiveness of other ATP-competitive small molecule inhibitors for the treatment of ALK-positive malignancies. It should, however, be noted that since UCN-01 has the ability to inhibit numerous kinases, the remission of this lymphoma could have been due to the inhibition of kinases other than ALK (indeed the effect of UCN-01 on ALK was not investigated in this report). Since it is probable that continual treatment with one ATP-competitive inhibitor of ALK will select for inhibitor-resistant ALK mutant proteins, similar to the BCR-ABL mutants described following therapy with STI571 [141], therapy with two alternating ALK inhibitors may prove to be optimal once such small molecules are available.

Turturro et al., utilising the ansamycin derivative 17-allylamino-17-demethoxygeldanamycin (17AAG), were also able to demonstrate reduced AKT/PKB activity and increased apoptosis in 17AAG-treated ALK-positive ALCL cell lines [143]. Bonvini et al. [144] demonstrated that the effect of 17AAG on the chimaeric ALK protein was dependent upon the ALK portion of the fusion and not the partner protein.

Ribozyme-mediated therapeutic approaches

Hübinger and co-workers recently described the use of a hammerhead ribozyme-mediated approach for the cleavage of the *NPM-ALK* fusion transcript in NPM-ALK-positive ALCL-derived cell lines [145]. Although in vitro assays showed essentially complete degradation of *NPM-ALK*, repeated transfections of the ribozyme for 96 h did not produce a significant reduction in the levels of endogenous NPM-ALK protein in the Karpas 299 cells. It is possible that the long half-life of NPM-ALK (approximately 48 h), when combined with the high steady-state levels of the fusion protein expressed in ALK-positive ALCLs, may complicate any future treatment approaches using ribozymes. These observations may also have implications for the use of techniques such as RNA interference in this disease. However, ribozymes may represent a therapeutic option in those diseases, such as certain brain tumors, in which expression of the full-length ALK protein occurs at lower levels. Indeed, Powers et al. [25] were able to reduce the levels of endogenous *ALK* expression in glioblastoma cell lines using *ALK*-targeted ribozymes, while in vivo experiments demonstrated the increased survival of nude mice bearing ribozyme-treated glioblastoma cell line tumor xenografts. Such experiments have also helped provide validation for the full-length ALK receptor as a therapeutic target for other types of intervention. For example, an anti-ALK monoclonal antibody specific for the extracellular region of ALK may disrupt PTN and MK-induced ALK receptor-signalling pathways, in a situation analogous to that found with the Herceptin antibody (Genentech, San Francisco, CA), specific for the HER2/NEU receptor tyrosine kinase [146].

Immunotherapy

In contrast to the situation in ALK-positive tumors, ALK expression in normal cells in the adult is limited mainly to rare scattered cells in the brain. The presence of ALK in an immunoprivileged site raises the possibility that ALK proteins could be seen as non-self antigens and could, therefore, be the target of an immune response. The detection of a circulating immunoglobulin G (IgG) antibody response to ALK in patients with ALK-positive ALCL provided support for this hypothesis [147, 148]. Although the exact function of these antibodies to ALK proteins is unknown, these antibodies would seem, at the minimum, to signify the presence of an intact immune system in the patient. Given preliminary evidence indicating that those patients with higher levels of circulating antibodies to ALK tend to fare better clinically [147], a study of the presence of circulating anti-ALK autoantibodies may provide an additional means of monitoring to detect those patients at high risk. There is also increasing evidence to suggest a correlation of antibodies with the presence of cytotoxic CD8-positive T-cells believed to be the major effectors in tumor immunity [149]. Indeed, work by Passoni et al. has identified the ability of ALK peptide sequences to stimulate a cytotoxic T-cell response in normal subjects [150], while Ait-Tahar and colleagues have recently confirmed the presence of a T-cell response in the blood of patients with ALK-positive ALCL [151]. Since the expression of ALK appears to be a primary causative agent in ALCL, ALK would appear to constitute a promising immunotherapeutic target.

Perspectives

Although ascribed a role in neural development in both mammals and *Drosophila*, in addition to a potential role in gut muscle differentiation in *Drosophila*, the normal functions of the full-length ALK receptor remain to be completely elucidated. The aberrant expression of ALK fusion proteins has not only resulted in the diagnosis of a subtype of ALCL, but also provided major insights into the oncogenic mechanisms underlying these tumors. Data from these studies have provided increasing support for the expression of NPM-ALK, and the other ALK fusion proteins, as being a primary causative event in cases of ALK-positive ALCL. The expression of full-length ALK proteins in some neuroblastomas, glioblastomas, and rhabdomyosarcomas and ALK fusions proteins in IMTs means that ALK is one of the few examples of an RTK to be implicated in both haematopoietic and non-haematopoietic tumors. Further analysis of the ALK signalling pathways should be instrumental in the rational development of new therapeutic strategies for ALK-positive tumors. The recognition of ALK proteins by the immune response of patients also suggests ALK to represent an ideal potential target for immunotherapeutic interventions.

Acknowledgements. Financial support for this work was provided by the Leukaemia Research Fund (9047), the National Cancer Institute (R01-CA69129), a National Cancer Institute CORE Grant (CA21765), the American Lebanese Syrian Associated Charities (ALSAC), the Louisiana Gene Therapy Research Consortium, Inc., and Assoociation pour la recherche sur le Cancer (ARECA).

1 Shiota M., Fujimoto J., Semba T., Satoh H., Yamamoto T. and 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
2 Iwahara T., Fujimoto J., Wen D., Cupples R., Bucay N., Arakawa T. et al. (1997) Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene **14:** 439–449
3 Morris S. W., Naeve C., Mathew P., James P. L., Kirstein M. N., Cui X. et al. (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
4 Lamant L., Pulford K., Bischof D., Morris S. W., Mason D. Y., Delsol G. et al. (2000) Expression of the ALK tyrosine kinase gene in neuroblastoma. Am. J. Pathol. **156:** 1711–1721
5 Pulford K., Lamant L., Morris S. W., Butler L. H., Wood K. M., Stroud D. et al. (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
6 Loren C. E., Scully A., Grabbe C., Edeen P. T., Thomas J., McKeown M. et al. (2001) Identification and characterization of DAlk: a novel *Drosophila* melanogaster RTK which drives ERK activation in vivo. Genes Cells **6:** 531–544
7 Stein H., Foss H. D., Durkop H., Marafioti T., Delsol G., Pulford K. et al. (2000) CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood **96:** 3681–3695
8 Morris S. W., Xue L., Ma Z. and Kinney M. C. (2001) Alk+ CD30+ lymphomas: a distinct molecular genetic subtype of non-Hodgkin's lymphoma. Br. J. Haematol. **113:** 275–295
9 Duyster J., Bai R. Y. and Morris S. W. (2001) Translocations involving anaplastic lymphoma kinase (ALK). Oncogene **20:** 5623–5637
10 Falini B. (2001) Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br. J. Haematol. **114:** 741–760
11 Pulford K., Morris S. W. and Turturro F. (2004) Anaplastic lymphoma kinase (ALK) proteins in cell growth and malignancy. J. Cell. Physiol., in press
12 Morris S. W., Kirstein M. N., Valentine M. B., Dittmer K. G., Shapiro D. N., Saltman D. L. et al. (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science **263:** 1281–1284
13 Falini B., Pileri S., Zinzani P. L., Carbone A., Zagonel V., Wolf-Peeters C. et al. (1999) ALK+ lymphoma: clinico-pathological findings and outcome. Blood **93:** 2697–2706
14 Lee H. -H., Norris A., Weiss J. B. and Frasch M. (2003) *Drosophila* jelly belly signals through the receptor tyrosine kinase Alk to specify visceral muscle pioneers. Nature **425:** 507–512
15 Loren C. E., Englund C., Grabbe C., Hallberg B., Hunter T. and Palmer R. H. (2003) A crucial role for the Anaplastic lym-

phoma kinase receptor tyrosine kinase in gut development in *Drosophila melanogaster*. EMBO J. **4:** 1–6
16 Englund C., Loren C. E., Grabbe C., Deleuil F., Varshney G. K. and Palmer R. H. (2003) Jeb signals via the DAlk receptor tyrosine kinase to drive visceral muscle fusion. Nature **425:** 512–516
17 Souttou B., Carvalho N. B., Raulais D. and Vigny M. (2001) Activation of anaplastic lymphoma kinase receptor tyrosine kinase induces neuronal differentiation through the mitogen-activated protein kinase pathway. J. Biol. Chem. **276:** 9526–9531
18 Piccinini G., Bacchiocchi R., Serresi M., Vivani C., Rossetti S., Gennaretti C. et al. (2002) A ligand-inducible epidermal growth factor receptor/anaplastic lymphoma kinase chimera promotes mitogenesis and transforming properties in 3T3 cells. J. Biol. Chem. **277:** 22231–22239
19 Stoica G. E., Kuo A., Aigner A., Sunitha I., Souttou B., Malerczyk C. et al. (2001) Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. J. Biol. Chem. **276:** 16772–16779
20 Stoica G. E., Kuo A., Powers C., Bowden E. T., Buchert-Sale E., Riegel A. T. et al. (2002) Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. J. Biol. Chem. **277:** 35990–35999
21 Falini B., Bigerna B., Fizzotti M., Pulford K., Pileri S. A., Delsol G. et al. (1998) ALK expression defines a distinct group of T/null lymphomas with a wide morphological spectrum. Am. J. Pathol. **153:** 875–886
22 Cessna M. H., Zhou H., Sanger W. G., Perkins S. L., Tripp S., Pickering D. et al. (2002) Expression of ALK1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases. Mod. Pathol. 1**5:** 931–938
23 Pillay K., Govender D. and Chetty R. (2002) ALK protein expresion in rhabdomyosarcomas. Histopathology **41:** 461–467
24 Miyake I., Hakomori Y., Shinohara A., Gamou T., Saito M., Iwamatsu A. et al. (2002) Activation of anaplastic lymphoma kinase is responsible for hyperphosphorylation of ShcC in neuroblastoma cell lines. Oncogene **21:** 5823–5834
25 Powers C., Aigner A., Stoica G. E., McDonnell K. and Wellstein A. (2002) Pleiotrophin signaling through anaplastic lymphoma kinase is rate-limiting for glioblastoma growth. J. Biol. Chem. **277:** 14153–14158
26 Shao C. K., Su Z. L., Feng Z. Y., Rao H. L. and Tang L. Y. (2002) Significance of ALK gene expression in neoplasms and normal tissues. Ai Zheng **21:** 58–62
27 Dirks W. G., Fahrnich S., Lis Y., Becker E., MacLeod R. A. and Drexler H. G. (2002) Expression and functional analysis of the anaplastic lymphoma kinase (ALK) gene in tumor cell lines. Int. J. Cancer **100:** 49–56
28 Delsol G., Lamant L., Mariame B., Pulford K., Dastugue N., Brousset P. 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
29 Benharroch D., Meguerian-Bedoyan Z., Lamant L., Amin C., Brugieres L., Terrier-Lacombe M. J. et al. (1998) ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood **91:** 2076–2084
30 Drexler H. G., Gignac S. M., von Wasielewski R., Werner M. and Dirks W. G. (2000) Pathobiology of NPM-ALK and variant fusion genes in anaplastic large cell lymphoma and other lymphomas. Leukemia **14:** 1533–1559
31 Shiota M., Fujimoto J., Taneka M., Satoh H., Ichinohasama R., Abe M. et al. (1994) Diagnosis of t(2;5)(p23;q35)-associated Ki-1 lymphoma with immunohistochemistry. Blood **84:** 3684–3652
32 Adam P., Katzenberger T., Seeberger H., Gattenlohner S., Wolf J., Steinlein C. et al. (2003) A case of a diffuse large B-cell lymphoma of plasmablastic type associated with the t(2;5)(p23;q35) chromosome translocation. Am. J. Surg. Pathol. **27:** 1173–1176
33 Onciu M., Behm F. G., Downing J. R., Shurtleff S. A., Raimondi S. C., Ma Z. et al. (2003) ALK-positive plasmablastic B-cell lymphoma with expression of the *NPM-ALK* fusion transcript: report of two cases. Blood **102:** 2642–2646
34 Lamant L., Dastugue N., Pulford K., Delsol G. and Mariame B. (1999) A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood **93:** 3088–3095
35 Lawrence B., Perez-Atayde A., Hibbard M. K., Rubin B. P., Dal Cin P., Pinkus J. L., Pinkus G. S. et al. (2000) TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am. J. Pathol. **157:** 377–384
36 Siebert R., Gesk S., Harder L., Steinemann D., Grote W., Schlegelberger B. et al. (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
37 Hernandez L., Pinyol M., Hernandez S., Bea S., Pulford K., Rosenwald A. 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
38 Hernandez L., Bea S., Bellosillo B., Pinyol M., Falini B., Carbone A. 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. Am. J. Pathol. **160:** 1487–1494
39 Colleoni G. W., Bridge J. A., Garicochea B., Liu J., Filippa D. A. and 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). Am. J. Pathol. **156:** 781–789
40 Ma Z., Cools J., Marynen P., Cui X., Siebert R., Gesk S. 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
41 Trinei M., Lanfrancone L., Campo E., Pulford K., Mason D. Y., Pelicci P. G. et al. (2000) A new variant anaplastic lymphoma kinase (ALK)-fusion protein (ATIC-ALK) in a case of ALK-positive anaplastic large cell lymphoma. Cancer Res. **60:** 793–798
42 Touriol C., Greenland C., Lamant L., Pulford K., Bernard F., Rousset T. et al. (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
43 Bridge J. A., Kanamori M., Ma Z., Pickering D., Hill D. A., Lydiatt W. et al. (2001) Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. Am. J. Pathol. **159:** 411–415
44 Cools J., Wlodarska I., Somers R., Mentens N., Pedeutour F., Maes B. et al. (2002) Identification of novel fusion partners of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. Genes Chromosomes Cancer **34:** 354–362
45 Chikatsu N., Kojima H., Suzukawa K., Shinagawa A., Nagasawa T., Ozav H. et al. (2003) ALK+, CD30-, CD20- large B-cell lymphoma containing anaplastic lymphoma kinase (ALK) fused to clathrin heavy chain gene (CLTC). Mod. Pathol. **16:** 828–832
46 De Paepe P., Baens M., van krieken H., Verhasselt B., Stul M., Simons A. et al. (2003) ALK activation by the CTLC-ALK fusion is a recurrent event in B-cell lymphoma. Blood **102:** 2638–2641

47 Gascoyne R. D., Lamant L., Martin-Subero J. I., Lestou V. S., Harris N. L., Müller-Hermelink H. K. et al. (2003) ALK-positive difuse large B-cell lymphoma is associated with *clathrin-ALK* rearrangements: report of six cases. Blood **102:** 2568–2571

48 Reichard K. K., McKenna R. W. and Kroft S. H. (2003) ALK-positive B-cell lymphoma: a report of three cases. Mod. Pathol. **16:** 250A

49 Tort F., Pinyol M., Pulford K., Roncador G., Hernandez L., Nayach I. et al. (2001) Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. Lab. Invest. **81:** 419–426

50 Meech S. J., McGavran L., Odom L. F., Liang X., Meltesen L., Gump J. et al. (2001) Unusual childhood extramedullary hematologic malignancy with natural killer cell properties that contains tropomyosin 4 – anaplastic lymphoma kinase gene fusion. Blood **98:** 1209–1216

51 Ma Z., Hill D. A., Collins M. H., Morris S. W., Sumagi J., Zhon M. et al. (2003) Fusion of ALK to the Ran-binding protein 2 (RANBP2) in inflammatory myofibroblastic tumor. Genes Chromosomes Cancer **37:** 98–105

52 Lamant L., Gascoyne R. D., Duplantier M. M., Armstrong F., Raghab A., Chhanabhai M. et al. (2003) Non-muscle myosin heavy chain (MYH9): a new partner fused to ALK in anaplastic large cell lymphoma. Genes Chromosomes Cancer **37:** 427–432

53 Debelenko L. V., Arthur D. C., Pack S. D., Helman L. J., Schrump D. S. and Tsokos M. (2003) Identification of CARS-ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumour. Lab. Invest. **83:** 1255–1265

54 Sainati L., Montaldi A., Stella M., Putti M. C., Zanesco L. and Basso G. (1990) A novel variant translocation t(2;13)-(p23;q34) in Ki-1 large cell anaplastic lymphoma. Br. J. Haematol. **75:** 621–622

55 Park J. P., Curran M. J., Levy N. B., Davis T. H., Elliott J. H. and Mohandas T. K. (1997) Diffuse large cell, B-cell type lymphoma with a novel translocation (2;22)(p23;q11. 2). Cancer Genet. Cytogenet. **96:** 118–122

56 Fujimoto J., Shiota M., Iwahara T., Seki N., Satoh H., Mori S. et al. (1996) Characterization of the transforming activity of p80, a hyperphosphorylated protein in a Ki-1 lymphoma cell line with chromosomal translocation t(2;5). Proc. Natl. Acad. Sci. USA **93:** 4181–4186

57 Bischof D., Pulford K., Mason D. Y. and Morris S. W. (1997) Role of the nucleophosmin (NPM) portion of the non-Hodgkin's lymphoma-associated NPM-anaplastic lymphoma kinase fusion protein in oncogenesis. Mol. Cell. Biol. 1**7:** 2312–2325

58 Pulford K., Falini B., Cordell J., Rosenwald A., Ott G., Muller-Hermelink H. K. et al. (1999) Biochemical detection of novel anaplastic lymphoma kinase proteins in tissue sections of anaplastic large cell lymphoma. Am. J. Pathol. **154:** 1657–1663

59 Rosenwald A., Ott G., Pulford K., Katzenberger T., Kuhl J., Kalla J. et al. (1999) t(1;2)(q21;p23) and t(2;3)(p23;q21): two novel variant translocations of the t(2;5)(p23;q35) in anaplastic large cell lymphoma. Blood **94:** 362–364

60 Ikebe M., Komatsu S., Woodhead J. L., Mabuchi K., Ikebe R., Saito J. et al. (2001) The tip of the coiled-coil rod determines the filament formation of smooth muscle and non-muscle myosin. J. Biol. Chem. **276:** 30293–30300

61 Biernaux C., Loos M., Sels A., Huez G. and Stryckmans P. (1995) Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood **86:** 3118–3122

62 Trumper L., Pfreundschuh M., Bonin F. V. and Daus H. (1998) Detection of the t(2;5)-associated NPM/ALK fusion cDNA in peripheral blood cells of healthy individuals. Br. J. Haematol. **103:** 1138–1144

63 Maes B., Vanhentenrijk V., Wlodarska I., Cools J., Peeters B., Marynen P. et al. (2001) The NPM-ALK and the ATIC-ALK fusion genes can be detected in non-neoplastic cells. Am. J. Pathol. **158:** 2185–2193

64 Delsol G., Ralfkier E., Stein H., Wright D. and Jaffe E. S. (2001) Anaplastic large cell lymphoma. In: Pathology and Genetics: Tumours of Haematopoietic and Lymphoid tissues, pp. 230–235, Jaffe E. S., Harris N. L., Syein H., Vardiman J. W., (eds), World Health Organization Classification of Tumours, IARC Press, Lyon

65 Falini B. and Mason D. Y. (2002) Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. Blood **99:** 409–426

66 Jones D., O'Hara C., Kraus M. D., Perez-Atayde A. R., Shahsafaei A., Wu L. et al. (2000) Expression pattern of T-cell-associated chemokine receptors and their chemokines correlates with specific subtypes of T-cell non-Hodgkin lymphoma. Blood **96:** 685–690

67 Kinney M. C. and Kadin M. E. (1999) The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gene dysregulation. Am. J. Clin. Pathol. **111:** S56–67

68 Gascoyne R. D., Aoun P., Wu D., Chhanabhai M., Skinnider B. F., Greiner T. C. et al. (1999) Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. Blood **93:** 3913–3921

69 Orscheschek K., Merz H., Hell J., Binder T., Bartels H. and Feller A. C. (1995) Large-cell anaplastic lymphoma-specific translocation (t[2;5] [p23;q35]) in Hodgkin's disease: indication of a common pathogenesis? Lancet **345:** 87–90

70 Beylot-Barry M., Groppi A., Vergier B., Pulford K. and Merlio J. P. (1998) Characterization of t(2;5) reciprocal transcripts and genomic breakpoints in CD30+ cutaneous lymphoproliferations. Blood **91:** 4668–4676

71 Beylot-Barry M., Lamant L., Vergier B., de Muret A., Fraitag S., Delord B. et al. (1996) Detection of t(2;5)(p23;q35) translocation by reverse transcriptase polymerase chain reaction and in situ hybridization in CD30-positive primary cutaneous lymphoma and lymphomatoid papulosis. Am. J. Pathol. **149:** 483–492

72 Beljaards R. C., Meijer C. J., Van der Putte S. C., Hollema H., Geerts M. L., Bezemer P. D. et al. (1993) Primary cutaneous T-cell lymphoma: clinicopathological features and prognostic parameters of 35 cases other than mycosis fungoides and CD30-positive large cell lymphoma. J Pathol **172:** 53–60

73 Paulli M., Berti E., Rosso R., Boveri E., Kindl S., Klersy C. et al. (1993) CD30/Ki-1-positive lymphoproliferative disorders of the skin – clinicopathologic correlation and statistical analysis of 86 cases: a multicentric study from the European Organization for Research and Treatment of Cancer Cutaneous Lymphoma Project Group. J. Clin. Oncol. **13:** 1343–1354

74 Griffin C. A., Hawkins A. L., Dvorak C., Henkle C., Ellingham T. and Perlman E. J. (1999) Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. Cancer Res. **59:** 2776–2780

75 Ladanyi M. (2000) Aberrant ALK tyrosine kinase signaling. Different cellular lineages, common oncogenic mechanisms. Am. J. Pathol. **157:** 341–345

76 Coffin C. M., Patel A., Perkins S., Elenitoba-Johnson K. S., Perlman E. and Griffin C. A. (2001) ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor. Mod. Pathol. **14:** 569–576

77 Cook J. R., Dehner L. P., Collins M. H., Ma Z., Morris S. W., Coffin C. M. et al. (2001) Anaplastic lymphoma kinase (ALK) expression in the inflammatory myofibroblastic tumor: a

comparative immunohistochemical study. Am. J. Surg. Pathol. **25:** 1364–1371
78 Yousem S. A., Shaw H. and Cieply K. (2001) Involvement of 2p23 in pulmonary inflammatory pseudotumors. Hum. Pathol. **32:** 428–433
79 Chan J. K., Cheuk W. and Shimizu M. (2001) Anaplastic lymphoma kinase expression in inflammatory pseudotumors. Am. J. Surg. Pathol. **25:** 761–768
80 Bowden E. T., Stoica G. E. and Wellstein A. (2002) Anti-apoptotic signaling of pleiotrophin through its receptor, anaplastic lymphoma kinase. J. Biol. Chem. **277:** 35862–35868
81 Mason D. Y., Pulford K. A., Bischof D., Kuefer M. U., Butler L. H., Lamant L. et al.. (1998) Nucleolar localization of the nucleophosmin-anaplastic lymphoma kinase is not required for malignant transformation. Cancer Res. **58:** 1057–1062
82 Simonitsch I., Polgar D., Hajek M., Duchek P., Skrzypek B., Fassl S. et al. (2001) The cytoplasmic truncated receptor tyrosine kinase ALK homodimer immortalizes and cooperates with ras in cellular transformation. FASEB J. **15:** 1416–1418
83 Kuefer M. U., Look A. T., Pulford K., Behm F. G., Pattengale P. K., Mason D. Y. et al. (1997) Retrovirus-mediated gene transfer of NPM-ALK causes lymphoid malignancy in mice. Blood **90:** 2901–2910
84 Chiarle R., Gong J. Z. J., Guasparri I., Pesci A., Cai J., Liu J. et al. (2003) NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. Blood **101:** 1919–1927
85 Lange K., Uckert W., Blankenstein T., Nadrowitz B., C., Renauld J.-C., van Snick J. et al. (2003) Overexpression of NPM-ALK induces different types of malignant lymphomas in IL–9 transgenic mice. Oncogene **22:** 517–527
86 Meithing C., Grundler R., Fend F., Hoepfl J., Mugler C., von Schilling C. et al. (2003) The oncogenic fusion protein nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) induces two distinct malignant phenotypes in a murine retroviral transplantation model. Oncogene **22:** 4642–4647
87 Turner S. D., Tooze R., Maclennan K. and Alexander D. R. (2003) *Vav*-promoter regulated oncogenic fusion protein NPM-ALK in transgenic mice causes B-cell lymphmas with hyperactive Jun kinase. Oncogene **22:** 7750–7761
88 Turturro F., Frist A. Y., Arnold M. D., Pal A., Cook G. A. and Seth P. (2001) Comparison of the effects of recombinant adenovirus-mediated expression of wild-type p53 and p27Kip1 on cell cycle and apoptosis in SUDHL-1 cells derived from anaplastic large cell lymphoma. Leukemia **15:** 1225–1231
89 Rassidakis G. Z., Claret F. X., Lai R., Zhang Q., Sarris A. H., McDonnell T. J. et al. (2003) Expression of p27(Kip1) and c-Jun Activation Binding Protein 1 are inversely corrleated in systemic anaplastic large cell lymphoma. Clin. Cancer Res. **9:** 1121–1128
90 Cussac D., Greenland C., Roche S., Bai R. -Y., Duyster J., Morris S. W. et al. (2003) Nucleophosmin-anaplastic lymphoma kinase of anaplastic large cell lymphoma recruits, activates and uses pp60c-src to mediate its mitogenicity. Blood **103:** 1464–1471
91 Greenland C., Touriol C., Chevillard G., Morris S. W., Bai R., Duyster J. et al. (2001) Expression of the oncogenic NPM-ALK chimeric protein in human lymphoid T-cells inhibits drug-induced, but not Fas-induced apoptosis. Oncogene **20:** 7386–7397
92 Gu T. L., Tothova Z., Scheijen B., Griffin J. D., Gilliland D. G. and Sternberg D. W. (2004) The NPM-ALK fusion kinase of anaplastic large cell lymphoma regulates survival and proliferative signals through modulation of FOX03a. Blood **103:** 4622–4629
93 Hübinger G., Scheffrahn I., Muller E., Bai R., Duyster J., Morris S. W. et al. (1999) The tyrosine kinase NPM-ALK, associated with anaplastic large cell lymphoma, binds the intracellular domain of the surface receptor CD30 but is not activated by CD30 stimulation. Exp. Hematol. **27:** 1796–1805
94 Gruss H.-J., Boiani N., Williams D. F., Armitage R. J., Smith E. A. and Goodwin R. G. (1994) Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood **83:** 2045–2091
95 Lee S. Y., Kandala G., Liou M. L., Liou H. C. and Choi Y. (1996) CD30/TNF receptor-associated factor interaction: NF-kappaB activation and binding specificity. Proc. Natl. Acad. Sci. USA 9**3:** 9699–9703
96 Mir S. S., Richter B. W. and Duckett C. S. (2000) Differential effects of CD30 activation in anaplastic large cell lymphoma and Hodgkin disease cells. Blood **96:** 4307–4312
97 Nishikori M., Maesako Y., Ueda C., Kurata M., Uchiyama T. and Ohno H. (2002) High-level expression of BCL3 differentiates t(2;5)(p23;q35)-positive anaplastic large cell lymphoma from Hodgkin's disease. Blood **101:** 2789–2796
98 Rassidakis G. Z., Oyarzo M. P. and Medeiros L. J. (2003) BCL–3 overexpression in anaplastic lymphoma kinase-positive anaplastic large cell lymphoma. Blood 10**2:** 1146–1147
99 Mir S. S., Richter B. W. M. and Duckett C. S. (2001) Strength of CD30 signal determines sensitivity to apoptosis. Blood **99:** 1631–1632
100 Levi E., Wang Z., Petrogiannis-Haliotis T., Pfeifer W. M., Kempf W., Drews R. et al. (2000) Distinct effects of CD30 and Fas signaling in cutaneous anaplastic lymphomas: a possible mechanism for disease progression. J Invest Dermatol **115:** 1034–1040
101 Zamo A., Chiarle R., Piva R., Howes J., Fan Y., Chilosi M. et al. (2002) Anaplastic lymphoma kinase (ALK) activates Stat3 and protects hematopoietic cells from cell death. Oncogene **21:** 1038–1047
102 Zhang Q., Raghunath P. N., Xue L., Majewski M., Carpentieri D. F., Odum N. et al. (2002) Multilevel dysregulation of STAT3 activation in anaplastic lymphoma kinase-positive T/null-cell lymphoma. J. Immunol. **168:** 466–474
103 Amin H. A., Medeiros L. J., Ma Y., Feretski M., Das P., Leventaki V. et al. (2003) Inhibition of JAK3 induces apoptosis and decreases anaplastic lymphoma kinase activity in anaplastic large cell lymphoma. Oncogene **22:** 5399–5407
104 Khoury J. D., Medeiros L. J., Rassidakis G. Z., Yared M. A., Tsioli P., Leventaki V. et al. (2003) Differential expression and clinical significance of tyrosine-phosphorylated STAT3 in ALK+ and ALK-anaplastic large cell lymphoma. Clin. Cancer Res. **9:** 3692–3699
105 Nieborowska-Skorska M., Slupianek A., Xue L., Zhang Q., Raghunath P. N., Hoser G. et al. (2001) Role of signal transducer and activator of transcription 5 in nucleophosmin/anaplastic lymphoma kinase-mediated malignant transformation of lymphoid cells. Cancer Res. **61:** 6517–6523
106 Ruchatz H., Coluccia A. M., Stano P., Marchesi E. and Gambacorti-Passerini C. (2003) Constitutive activation of JAK2 contributes to proliferation and resistance to apoptosis in NPM/ALK-transformed cells. Exp. Hematol. **31:** 309–315
107 Coluccia A. M., Perego S., Cleris L., Gunby R. H., Passoni L., Marchesi E. et al. (2003) Bcl-XL downregulation suppresses the tumorigenic potential of NPM-ALK in vitro and in vivo. Blood **103:** 2787–2794
108 Rassidakis G. Z., Sarris A. H., Herling M., Ford R. J., Cabanillas F., McDonnell T. J. et al. (2001) Differential expression of BCL–2 family proteins in ALK-positive and ALK-negative anaplastic large cell lymphoma of T/null-cell lineage. Am. J. Pathol. **159:** 527–535
109 Villalva C., Bougrine F., Delsol G. and Brousset P. (2001) BCL-2 expression in anaplastic large cell lymphoma. Am. J. Pathol. **158:** 1889–1890
110 Slupianek A., Hoser G., Majsterek I., Bronisz A., Malecki M., Blasiak J. et al. (2002) Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombina-

tion repair, prolongation of G(2)/M phase, and protection from apoptosis. Mol. Cell. Biol. 2**2:** 4189–4201
111 Hoser G. I., Majsterek I., Romana D. L., Slupianek A., Blasiek J. and Skorski T. (2003) Fusion oncogenic tyrosine kinases alter DNA damage and repair after genoxic treatment: role in drug resistance? Leuk. Res. **27:** 267–273
112 Turturro F., Frist A. Y., Arnold M. D., Seth P. and Pulford K. (2001) Biochemical differences between SUDHL-1 and KARPAS 299 cells derived from t(2;5)-positive anaplastic large cell lymphoma are responsible for the different sensitivity to the antiproliferative effect of p27(Kip1). Oncogene **20:** 4466–4475
113 Turturro F., Arnold M. D., Frist A. Y. and Seth P. (2002) Effects of adenovirus-mediated expression of p27Kip1, p21Waf1 and p16INK4A in cell lines derived from t(2;5) anaplastic large cell lymphoma and Hodgkin's disease. Leuk. Lymphoma **43:** 1323–1328
114 Colombo E., Marine J. C., Danovi D., Falini B. and Pelicci P. G. (2002) Nucleophosmin regulates the stability and transcriptional activity of p53. Nat. Cell Biol. **4:** 529–533
115 Ouyang T., Bai R.-Y., Bassermann F., von Klitzing C., Klumpen S., Miething C. et al. (2003) Identification and characterization of a nuclear interacting partner of anaplastic lymphoma kinase (NIPA). J. Biol. Chem. **278:** 30028–30036
116 Ergin M., Denning M. F., Izban K. F., Amin H. M., Martinez R. L., Saeed S. et al. (2001) Inhibition of tyrosine kinase activity induces caspase-dependent apoptosis in anaplastic large cell lymphoma with NPM-ALK (p80) fusion protein. Exp Hematol **29:** 1082–1090
117 ten Berge R. L., Meijer C. J., Dukers D. F., Kummer J. A., Bladergroen B. A., Vos W. et al. (2002) Expression levels of apoptosis-related proteins predict clinical outcome in anaplastic large cell lymphoma. Blood **99:** 4540–4546
118 Drakos E., Rassidakis G., Lai R., Herling M., O'Connor S. L., Schmitt-Graeff A. et al. (2004) Caspase–3 activation in systemic anaplastic large cell lymphoma. Mod. Pathol. **17:** 109–116
119 Rassidakis G. Z., Lai R., McDonnell T. J., Cabanillas F., Sarris A. H. and Medeiros C. J. (2002) Overexpression of Mcl-1 in anaplastic large cell lymphoma cell lines and tumors. Am. J. Pathol. **160:** 2309–2310
120 Villalva C., Trempat P., Greenland C., Thomas C., Girard J. P., Moebius F. et al. (2002) Isolation of differentially expressed genes in NPM-ALK-positive anaplastic large cell lymphoma. Br. J. Haematol. **118:** 791–798
121 Crockett D. K., Lin Z., Elenitoba-Johnson S. J. and Lim M. S. I. (2004) Identification of NPM-ALK interacting proteins by tandem mass spectrometry. Oncogene **23:** 2617–2629
122 Brugieres L., Deley M. C., Pacquement H., Meguerian-Bedoyan Z., Terrier-Lacombe M. J., Robert A. et al. (1998) CD30(+) 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
123 Brugieres L., Quartier P., Le Deley M. C., Pacquement H., Perel Y., Bergeron C. 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. Ann. Oncol. **11:** 53–58
124 Fiorani C., Vinci G., Sacchi S., Bonaccorsi G. and Artusi T. (2001) Primary systemic anaplastic large-cell lymphoma (CD30+): advances in biology and current therapeutic approaches. Clin. Lymphoma **2:** 29–37; discussion 38–29
125 Williams D. M., Hobson R., Imeson J., Gerrard M., McCarthy K. and Pinkerton C. R. (2002) Anaplastic large cell lymphoma in childhood: analysis of 72 patients treated on The United Kingdom Children's Cancer Study Group chemotherapy regimens. Br. J. Haematol. **117:** 812–820
126 Liso A., Tiacci E., Binazzi R., Pulford K., Benedetti R., Carotti A. et al. (2004) Haploidentical peripheral-blood-stem transplantation for ALK-positive large-cell lymphoma. The Lancet Oncology **5:** 127–128
127 Suzuki R., Kagami Y., Takeuchi K., Kami M., Okamoto M., Ichinohasama R. et al. (2000) Prognostic significance of CD56 expression for ALK-positive and ALK-negative anaplastic large-cell lymphoma of T/null cell phenotype. Blood **96:** 2993–3000
128 Rassidakis G. Z., Goy A., Medeiros L. J., Jiang Y., Thomaides A., Remache Y. et al.. (2003) Prognostic significance of MUC-1 expression in systemic anaplastic large cell lymphoma. Clin. Cancer Res. **9:** 2213–2220
129 ten Berge R. L., Oudejans J. J., Ossenkoppele G. J. and Meijer C. J. (2003) ALK-negative systemic anaplastic large cell lymphoma: differential diagnostic and prognostic aspects – a review. J. Pathol. **200:** 4–15
130 Pasqualucci L., Wasik M., Teicher B. A., Flenghi L., Bolognesi A., Stirpe F. et al. (1995) Antitumor activity of anti-CD30 immunotoxin (Ber-H2/saponin) in vitro and in severe combined immunodeficiency disease mice xenografted with human CD30+ anaplastic large-cell lymphoma. Blood **85:** 2139–2146
131 Tian Z. G., Longo D. L., Funakoshi S., Asai O., Ferris D. K., Widmer M. et al. (1995) In vivo antitumor effects of unconjugated CD30 monoclonal antibodies on human anaplastic large-cell lymphoma xenografts. Cancer Res. **55:** 5335–5341
132 Pfeifer W., Levi E., Petrogiannis-Haliotis T., Lehmann L., Wang Z. and Kadin M. E. (1999) A murine xenograft model for human CD30+ anaplastic large cell lymphoma. Successful growth inhibition with an anti-CD30 antibody (HeFi-1). Am. J. Pathol. **155:** 1353–1359
133 Koon H. B. and Junghans R. P. (2000) Anti-CD30 antibody-based therapy. Curr. Opin. Oncol. **12:** 588–593
134 Wahl A. F., Klussman K., Thompson J. D., Chen J. H., Francisco L. V., Risdon G. et al. (2002) The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease. Cancer Res. **62:** 3736–3742
135 Ho L., Aytac U., Stephens L. C., Ohnuma K., Mills G. B., McKee K. S. et al. (2001) In vitro and in vivo antitumor effect of the anti-CD26 monoclonal antibody 1F7 on human CD30+ anaplastic large cell T-cell lymphoma Karpas 299. Clin. Cancer Res. **7:** 2031–2040
136 Zhang M., Zhang Z., Garmestani K., Schultz J., Axworthy D. B., Goldman C. G. et al. (2003) Pretarget radiotherapy with an anti-CD25 antibody-streptavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. Proc. Natl. Acad. Sci. **100:** 1891–1895
137 Meeker T. C., Travis Lay L., Wroblewski J. M., Turturro F., Li Z. and Seth P. (1997) Adenoviral vectors efficiently target cell lines derived from selected lymphocytic malignancies, including anaplastic large cell lymphoma and Hodgkin's disease. Clin. Cancer Res. **3:** 357–364
138 Turturro F., Seth P. and Link C. J. Jr (2000) In vitro adenoviral vector p53-mediated transduction and killing correlates with expression of coxsackie-adenovirus receptor and alpha(nu)-beta5 integrin in SUDHL-1 cells derived from anaplastic large-cell lymphoma. Clin. Cancer Res. **6:** 185–192
139 Turturro F., Heineke H. L., Drevyanko T. F., Link C. J. Jr and Seth P. (2000) Adenovirus-p53-mediated gene therapy of anaplastic large cell lymphoma with t(2;5) in a nude mouse model. Gene Ther. **7:** 930–933
140 Turturro F. (2003) Recombinant adenovirus-mediated cytotoxic gene therapy of lymphoproliferative disorders: is CAR important for the vector to ride? Gene Therapy **10:** 100–104
141 Druker B. J. (2002) Perspectives on the development of a molecularly targeted agent. Cancer Cell **1:** 31–36
142 Sausville E. A., Arbuck S. G., Messmann R., Headlee D., Bauer K. S., Lush R. M. et al. (2001) Phase I trial of 72-hour

continuous infusion UCN-01 in patients with refractory neoplasms. J. Clin. Oncol. **19:** 2319–2333
143 Turturro F., Frist A. Y. and Arnold M. D. (2001) Effects of the inhibition of NPM-ALK kinase activity by 17-Allylamino-17-demethoxygeldanamycin (17-AAG). Blood 98 (11):p 456a, abstract 1944
144 Bonvini P., Gastaldi T., Falini B. and Rosolen A. (2002) Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), a novel Hsp90-client tyrosine kinase: down-regulation of NPM-ALK expression and tyrosine phosphorylation in ALK(+) CD30(+) lymphoma cells by the Hsp90 antagonist 17-allylamino,17-demethoxygeldanamycin. Cancer Res. **62:** 1559–1566
145 Hübinger G., Wehnes E., Xue L., Morris S. W. and Maurer U. (2003) Hammerhead ribozyme-mediated cleavage of the fusion transcript NPM-ALK associated with anaplastic large cell lymphoma. Exp. Hematol. **31:** 226–233
146 Cho H. -S., Mason K., Ramifan K. X., Stanley A. M., Gabelli S. B., Denney D. W. J. et al. (2003) Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature **421:** 756–760
147 Pulford K., Falini B., Banham A. H., Codrington D., Roberton H., Hatton C. et al. (2000) Immune response to the ALK oncogenic tyrosine kinase in patients with anaplastic large-cell lymphoma. Blood **96:** 1605–1607
148 Pulford K., Roberton H., Banham A. H., Hatton C. S. and Mason D. Y. (2002) Immunochemical studies of antigenic lymphoma-associated proteins. Br. J. Haematol. **116:** 135–141
149 Jäger E., Nagata Y., Gnjatic S., Wada H., Stockert E., Karbach J. et al. (2000) Monitoring CD8 T cell responses to NY-ESO-1: correlation of humoral and cellular immune responses. Proc. Natl. Acad. Sci. USA **97:** 4760–4765
150 Passoni L., Scardino A., Bertazzoli C., Gallo B., Coluccia A. M., Lemonnier F. A. et al. (2002) ALK as a novel lymphoma-associated tumor antigen: identification of 2 HLA-A2. 1-restricted CD8+ T-cell epitopes. Blood **99:** 2100–2106
151 Ait-Tahar K., Banham A. H., Roberton H., Hatton C. S. R. and Pulford K. (2003) T and B cell response to the ALK protein in patients with ALK-positive ALCL. Blood **102:** 859a