Oncogenic Anaplastic Lymphoma Kinase Rearrangements in Lymphoma

Katrien Van Roosbroeck[1] and Iwona Wlodarska[2]

1. PhD Student; 2. Staff Member, Centre for Human Genetics, University Hospital of Gasthuisberg, Catholic University of Leuven

DOI: 10.17925/EOH.2009.03.1.50

Abstract

Lymphomas expressing anaplastic lymphoma kinase (ALK) represent two distinct lymphoma entities: ALK-positive T-/null-cell anaplastic large cell lymphoma (ALK+ ALCL) and ALK-positive large B-cell lymphoma (ALK+ LBCL). In both subtypes, the inappropriate expression of ALK is driven by 2p23/*ALK*-involving chromosomal translocations found to target several partner genes. These translocations lead to constitutively activated and oncogenic ALK fusions, of which nucleophosmin (NPM1)-ALK associated with t(2;5)(p23;q35) is the most common. Recently, various ALK fusions, including those previously described in lymphomas, have been identified in several types of non-haematological malignancy. Identification of further types of ALK+ tumours is clinically important because in future these patients may benefit from targeted therapy, already applied in neoplasms driven by, for example, the mutated ABL1, KIT and PDGFRA/B tyrosine kinases. In this article, we will focus mainly on oncogenic ALK rearrangements in lymphomas and their molecular consequences.

Keywords

Oncogene, anaplastic lymphoma kinase, anaplastic large cell lymphoma, large B-cell lymphoma

Disclosure: The authors have no conflicts of interest to declare.

Received: 21 May 2009 **Accepted:** 16 July 2009

Correspondence: Iwona Wlodarska, Centre for Human Genetics, KU Leuven, Gasthuisberg, Herestraat 49, Box 602, B-3000 Leuven, Belgium.

E: Iwona.Wlodarska@uz.kuleuven.ac.be

Anaplastic Lymphoma Kinase

Located at 2p23, the anaplastic lymphoma kinase (*ALK*) gene codes for a receptor tyrosine kinase (RTK). This gene was initially identified in the chimeric nucleophosmin (*NPM*)-*ALK* fusion driven by the t(2;5)(p23;q35), a recurrent translocation in anaplastic large cell lymphoma (ALCL).[1] The *ALK* gene contains 6,226bp and encodes a 177kDa protein that is transformed to a mature ALK RTK of approximately 200kDa by post-translational modifications such as N-glycosylation.[2] In common with other RTKs, ALK consists of a large extracellular ligand-binding domain, a lipophilic transmembrane-spanning segment and a cytoplasmic tyrosine kinase catalytic region (see *Figure 1*). Due to the significant homology of the extracellular domain to leukocyte tyrosine kinase (LTK), ALK is classified in the insulin-receptor superfamily of RTKs; the function of the ALK receptor is still poorly characterised. Expression of ALK in normal tissues is restricted to rare scattered neural cells, pericytes and endothelial cells in the brain.[1–3] Recent data suggest that ALK plays a role in embryonic neural development and differentiation.[4,5]

Anaplastic Lymphoma Kinase Rearrangements in Lymphoma

Anaplastic Lymphoma Kinase-positive Anaplastic Large Cell Lymphoma

ALK+ ALCL (informally ALKoma) accounts for approximately 60–80% of all ALCL cases.[4,6,7] In the World Health Organization (WHO) classification of haematolymphoid neoplasms, ALK+ ALCL is recognised as a separate entity among mature T-cell lymphomas.[8] These tumours form a homogenous group in terms of the immunophenotype and clinical behaviour, but demonstrate a heterogeneous morphology. The common type, composed of atypical large, pleomorphic cells with an abundant cytoplasm, is seen in approximately 80% of cases, and morphological variants, including lymphohistiocytic, small- and giant-cell types, are observed in the remaining cases.[9,10] ALK+ ALCL cases exhibit a cytotoxic T-cell or null (i.e. lacking expression of both T- and B-cell markers) phenotype and frequently express epithelial membrane antigen (EMA) and CD30. The disease tends to occur in the first three decades of life, with mean age at onset <30 years, and shows a slight male predominance (male to female [M:F] ratio of 1.5–1).[4,6–18] Although ALK+ ALCLs are highly aggressive, patients with this lymphoma have a more favourable prognosis compared with ALK-negative ALCL (ALK- ALCL) patients; the overall five-year survival rate approaches 80% in ALK+ ALCL and 48% in ALK- ALCL.[4,8,15,17] Multivariate analysis has shown that good prognosis of patients with ALK+ ALCL strongly correlates with expression of ALK, but not with younger age and/or presence in low international prognostic index risk groups.[4,15] The inappropriate expression of ALK in ALK+ ALCL derives from chromosomal translocations targeting the 2p23/*ALK* locus. Occurring in 84% of ALK+ ALCL, the most frequent, t(2;5)(p23;q35), results in the fusion of the amino-terminal end of NPM (currently designated as NPM1) with the intracytoplasmic portion of ALK.[1,8,19,20] Variant translocations, seen in the remaining ALK+ ALCL cases, target several partner genes including *TPM3*/1q21, *ATIC*/2q35, *TFG*/3q12, *CLTC*/17q23, *ALO17*/17q25, *MYH9*/22q12, *MSN*/Xq11 and *SEC31A*/4q21 (see *Table 1*). Four fusions, TFG-ALK, MSN-ALK, ALO17-ALK and MYH9-ALK, have been exclusively observed in ALK+ ALCL[21] (see *Table 1*). Given that normal lymphoid cells do not express the ALK protein, immunostaining with ALK-specific antibodies is routinely used for the

© TOUCH BRIEFINGS 2009

diagnosis of ALK+ tumours (see *Figure 2*). Additional diagnostic techniques include conventional cytogenetics, fluorescence *in situ* hybridisation (FISH) (see *Figure 3*) and molecular analysis.

Anaplastic Lymphoma Kinase-positive Large B-cell Lymphoma

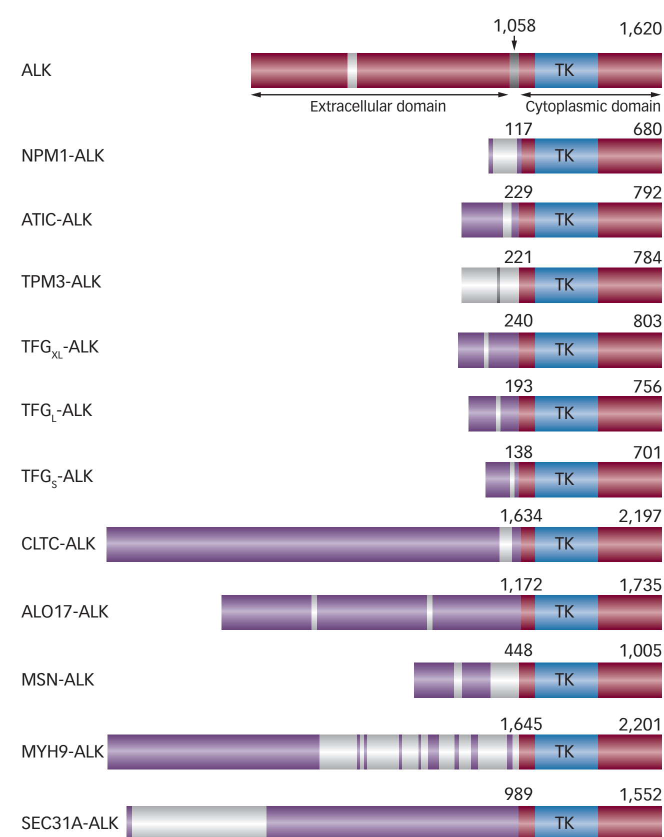
Subsequent studies showed that ALK expression is not restricted to ALCL but also hallmarks other tumours, including large B-cell lymphoma (LBCL). The first series of ALK-expressing LBCL was reported by Delsol et al.[22] in 1997. To date, 54 ALK+ LBCL cases have been described.[23–25] The recent WHO classification of haematolymphoid neoplasms considers ALK+ LBCL a distinct lymphoma entity.[8] Cytologically, the lymphoma cells display either an immunoblastic or a plasmablastic morphology; occasional binucleated or multinucleated cells mimicking Hodgkin and Reed-Sternberg (H/RS) cells may also be seen.[26] ALK+ LBCL shows a distinct immunophenotype, characterised by the strong expression of ALK and EMA and expression of plasma cell markers such as CD138/VS38 and MUM1. Notably, the neoplastic cells have lost expression of B-cell-lineage-associated leukocyte antigens such as CD19, CD20 and CD79a, suggesting terminal plasma cell differentiation.[22,23] The disease spans all age groups (nine to 72 years of age, with a median age of 38 years), but approximately 22% of the cases occurred in the paediatric population (<18 years of age). The lymphoma is more common in men than women (M:F ratio of 3:1).[23] Most patients with ALK+ LBCL presented with stage III/IV disease and their clinical outcome was worse than ALK+ ALCL, particularly in the paediatric population. Despite aggressive treatment, approximately half of the patients died of disease three to 33 months after therapy. No difference in survival between paediatric and adult cases was found, despite more intensive therapies in the paediatric population.[23]

Similar to ALK+ ALCL, the aberrant ALK expression in ALK+ LBCL is also driven by 2p23/*ALK* translocations, of which t(2;17)(p23;q23) is the most frequent (65%). This aberration leads to a chimaeric CLTC-ALK protein and manifests as a distinctive granular cytoplasmic expression of ALK[27–35] (see *Table 1* and *Figure 3*). Interestingly, only a few ALK+ LBCL cases (13%) showed the t(2;5)(p23;q35)/*NPM1-ALK* rearrangement, commonly occurring in ALK+ ALCL[25,36–38] (see *Table 1*). Recently, we have identified a new cryptic SEC31A-ALK fusion in an ALK+ LBCL case generated by complex rearrangements targeting the respective loci at 4q21 and 2p23.[25] A functional analysis of the SEC31A-ALK protein and its downstream effectors revealed that this fusion is a constitutively activated tyrosine kinase responding *in vitro* to the ALK inhibitor NVP-TAE-684.

Anaplastic Lymphoma Kinase Rearrangements in Non-haematological Malignancies

Apart from ALK+ ALCL and LBCL, various tumours of mesenchymal and epithelial origin, including inflammatory myofibroblastic tumour (IMT),[39–41] lung cancer[42–53] and oesophageal squamous cell carcinoma (SCC),[54,55] were found to implicate the *ALK* gene and inappropriately express the ALK protein (see *Table 1*). Particularly interesting is the finding of the same translocations and/or ALK fusions in ALK+ malignancies of different origin (see *Table 1*). TPM3-ALK,[56–59] ATIC-ALK,[60] CLTC-ALK[59,61,62] and SEC31A-ALK[63] were reported in lymphomas and IMT, and TPM4-ALK has been found in IMT and SCC.[57,59,64] So far, CARS-ALK[65,66] and RANBP2-ALK[62,67,68] have only been found in IMT, and EML4-ALK and KIF5B-ALK in non-small-cell lung carcinoma (see *Table 1*).[42–53] Recently, oncogenic mutations of the ALK kinase and amplification of *ALK* have been discovered in neuroblastoma tumours.[69–75] In addition, deregulated expression of full-length ALK messenger RNA (mRNA) and/or protein has been described in a variety of primary tumours and cancer cell lines including glioblastoma,[76,77] rhabdomyosarcoma,[78] Ewing sarcoma and retinoblastoma[76] and breast cancer.[79] Additional studies are required to assess the possible role of full-length ALK and ALK fusion forms in the pathogenesis and progression of these non-haematopoietic tumours.

Figure 1: Schematic Diagrams of the Known Anaplastic Lymphoma Kinase Fusion Proteins in Lymphoma



With the exception of MSN-anaplastic lymphoma kinase (ALK) and MYH9-ALK, the ALK portion present in the fusions is identical, comprising the final 563 cytoplasmic amino acid residues of ALK. MSN-ALK and MYH9-ALK contain the terminal 557 and 556 ALK amino acid residues, respectively. The numbers correspond to the number of amino acid residues of the partner protein present in the fusion, and the total number of amino acids included in each fusion protein. The normal ALK protein is shown at the top and consists of a large extracellular domain, a 27-amino-acid transmembrane domain (dark grey box) and a cytoplasmic domain containing a tyrosine kinase (TK) catalytic domain. The breakpoint mostly occurs at amino acid 1,058, designated by the arrow, immediately after the transmembrane domain, leaving the cytoplasmic domain and the tyrosine kinase catalytic domain intact. The light-grey boxes are putative oligomerisation domains, i.e. nucleoplasmin domain (NPM1), dimerisation domain (ATIC), coiled coils (TPM3, TFG, MSN, ALO17 and MYH9), multimerisation domain (CLTC) and WD40-like repeats (SEC31A).

Mechanism of Anaplastic Lymphoma Kinase Fusion Kinase Activation

Under normal conditions, activation of RTKs such as ALK depends on ligand binding. Two putative ALK ligands have been proposed, pleiotrophin (PTN) and midkine (MK),[80,81] which show similar expression patterns to ALK (high levels during neural development and decreased expression following birth),[82–85] indicating that PTN, MK and ALK could be important for normal neural function. Stoica and co-workers showed that PTN-induced mitogenesis resulted in increased phosphorylation of ALK substrates in ALK-transfected cells,[80] and that MK binds to ALK-transfected 32Dcl3 cells.[81] Furthermore, ALK is likely to be involved in PTN-stimulated phosphorylation and activation of PI3K, MAPK and PKB/AKT anti-apoptotic proteins.[77,86]

Table 1: Recurrent Chromosomal Rearrangements Involving Anaplastic Lymphoma Kinase in Lymphoma and Non-haematopoietic Tumours

Chromosomal Translocation	Partner Protein	Fusion Protein	Molecular Weight of Hybrid Protein (kDa)	Cellular Localisation	Associated Disease	References
t(2;5)(p23;q35)	NPM	NPM-ALK	80	Nuclear, nucleolar and diffuse cytoplasmic	ALK$^+$ ALCL ALK$^+$ LBCL	1, 25, 36–38, 157
inv(2)(p23;q35)	ATIC	ATIC-ALK	96	Diffuse cytoplasmic IMT	ALK$^+$ ALCL	60, 158–161
t(1;2)(q25;p23)* t(1;2)(q21;p23)	TPM3	TPM3-ALK	104	Diffuse cytoplasmic with peripheral intensification	ALK$^+$ ALCL IMT	41, 56, 58, 59, 162–165
t(2;3)(p23;q21)†	TFG TFG Xlong TFG long TFG short	TFG-ALK 113 97 85		Diffuse cytoplasmic	ALK$^+$ ALCL	166, 167
t(2;17)(p23;q23) t(2;17;7)(p23;q23;q?22)	CLTC	CLTC-ALK	250	Granular cytoplasmic	ALK$^+$ ALCL ALK$^+$ LBCL IMT	27–35, 59, 61, 62, 65, 168
t(2;19)(p23;p13.1)	TPM4	TPM4-ALK	95	Diffuse cytoplasmic SCC Unusual extramedullary haematological malignancy	IMT 57, 59, 64, 169, 170	41, 54, 55,
t(2;X)(p23;q11)	MSN	MSN-ALK	125	Cell-membrane-associated	ALK$^+$ ALCL	88, 89
t(2;17)(p23;q25)	ALO17	ALO17-ALK	ND	Cytoplasmic	ALK$^+$ ALCL	65
t(2;11;2)(p23;p15;q31)	CARS	CARS-ALK	130	Cytoplasmic	IMT	65, 66
t(2;2)(p23;q13) der(2)inv(2)(p23;q12) del(2)(p11.1p11.2)	RANBP2	RANBP2-ALK	160	Nuclear-membrane-associated	IMT	62, 67, 68
t(2;22)(p23;q11.2)§	MYH9	MYH9-ALK	220	Diffuse cytoplasmic	ALK$^+$ ALCL	87
t(2;4)(p23;q21)	SEC31A	SEC31A-ALK	138	Diffuse cytoplasmic ALK$^+$ LBCL	IMT	25, 63
inv(2)(p21;p23)	EML4 Variant 1 Variant 2 Variant 3 (a/b) Variant 4 Variant 5 (a/b) Variant 6 Variant 7	EML4-ALK 120 147 90/91 123 71/75 119 122		Cytoplasmic	NSCLC	42–51
t(2;10)(p23;p11)	KIF5B	KIF5B-ALK	168	Diffuse cytoplasmic, sometimes with intracytoplasmic macroglobular spots or perinuclear halo	Lung adenocarcinoma	52

*According to Ensembl release 54, May 2009: TPM3 located on chromosome 1q21.3; †According to Ensembl release 54, May 2009: TFG located on chromosome 3q12.2; §According to Ensembl release 54, May 2009: MYH9 located on chromosome 22q12.3.
ALK$^+$ = anaplastic lymphoma kinase-positive; ALCL = anaplastic large cell lymphoma; LBCL = large B-cell lymphoma; IMT = inflammatory myofibroblastic tumour; SCC = squamous cell carcinoma; NSCLC = non-small-cell lung cancer; NPM = nucleophosmin.

Molecular studies show that tyrosine-kinase-associated chromosomal translocations in cancer lead to ligand independence of these kinases that then function as constitutively activated tyrosine kinases. With the exception of MSN-ALK and MYH9-ALK, all of the ALK fusion proteins contain the same ALK region, i.e. amino acid residues 1,058–1,620 of the full-length wild-type receptor.[87–89] This region contains the complete intracellular part of the receptor, including the tyrosine kinase catalytic domain responsible for the kinase activity (see *Figure 1*). This C-terminal ALK fragment is fused to the N-terminal end of the partner protein that mediates constitutive self-association of the ALK fusion, mimicking ligand binding. This can be achieved by oligomerisation via a motif such as a coiled-coil domain that is present in the N-terminal portion of the TFG, TPM3, TPM4, MSN, ALO17, RANBP2 and EML4 partner proteins, a nucleoplasmin domain seen in NPM1, WD40-like repeats observed in SEC31A and a di- or multimerisation domain found in ATIC and CLTC, respectively (see *Figure 1*).[90]

The subcellular localisation of the ALK fusion protein is dependent on the partner protein. The distribution of NPM1-ALK homodimers is limited to the cytoplasm, but the nuclear localisation motifs present in the wild-type NPM1 permit entry of the NPM1/NPM1-ALK heterodimers to the nucleus and nucleolus that is manifested by the unique nuclear and cytoplasmic ALK immunostaining (see *Table 1* and *Figure 2*).[13] The vast majority of variant ALK fusions are localised in the cytoplasm (see *Table 1* and *Figure 2*); notably, CLTC-ALK shows a characteristic granular cytoplasmic distribution (see *Figure 2*) due to its localisation within the clathrin-coated vesicles of the neoplastic cell.[91] In contrast, the MSN-ALK fusion is expressed at the cell membrane, probably because of the association of moesin with other cell membrane proteins.[92]

Mouse Models of Nucleophosmin–Anaplastic Lymphoma Kinase-induced Lymphoma

In this article, we focus on the mouse models of NPM1-ALK-induced lymphoma that were the main focus of investigations. Of note, however, a few variant fusions have also been used to generate transgenic mice, i.e. TPM-ALK[93] and EML4-ALK.[49,94]

Chimaeric Mouse Models

The initial study investigating the tumorigenic capacity of NPM1-ALK *in vivo* was performed by Kuefer et al.[95] The established NPM1-ALK bone marrow transplant mouse model resulted in the development of B-lymphoid immunoblastic tumours with a relatively long latency (four to six months) and moderate penetrance of tumour development. This phenotype was unexpected, as at that time the only known human ALK+ lymphomas were T-/null-cell ALCL. In subsequent retroviral transduction experiments, Miething et al.[96] used retroviral constructs with a promoter that drives higher expression of the chimeric kinase. When bone marrow was transfected with high retroviral titres, the mice developed fatal haematopoietic malignancies possessing a myeloid/ macrophage cell phenotype with a short latency (three to four weeks). The use of low titres of retrovirus gave rise to the development of B-lymphoid tumours with a longer latency (12–16 weeks). Overall, there seems to be a relationship between the degree of induced NPM1-ALK expression and the phenotype of the resulting tumour, with higher levels of NPM1-ALK expression leading to a myeloid/macrophage malignancy and lower levels in B-cell lymphoid tumours.

To generate experimental T-lymphoid malignancies, Miething and co-workers used Cre-conditional NPM1-ALK retrovirus to transduce bone marrow from two lines of Cre-transgenic mice.[97] Lysozyme M-promoter Cre mice expressed Cre recombinase from the myeloid-specific lysozyme M promoter and developed a fatal histiocytic malignancy after four to six weeks. Granzyme B-promoter mice expressed Cre recombinase from the T-cell active granzyme B gene promoter and showed a mixed T-NHL/histiocytic phenotype with the same latency (four to six weeks).

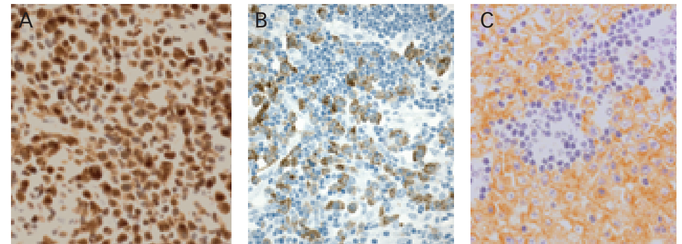
Transgenic Mouse Models

Several groups have reported transgenic NPM1-ALK mouse models in which expression of the fusion is driven from different promoters. In one transgenic CD4-NPM1-ALK mouse line in which NPM1-ALK expression is driven by the T-cell-specific CD4 promoter, CD30+ T-cell lymphomas arose, whereas a second line showed tumours with a plasma cell phenotype, suggesting leakiness of the CD4 promoter.[98] Use of the haematopoietic-cell-specific Vav-promoter and the T-cell-specific CD2-promoter to express NPM1-ALK in transgenic mice resulted in both the development of B-cell tumours, more specifically plasmacytomas (high-copy-number mice) and putative peritoneal B1-cell-derived tumours (low-copy-number mice) in Vav-NPM1-ALK mice,[99] and diffuse large B-cell lymphoma and histiocyte/T-cell-rich B-cell lymphoma in CD2-NPM1-ALK mice.[100] All three models showed a long latency ranging from four to five months (CD4-NPM1-ALK) to three to nine months (Vav-NPM1-ALK) and more (CD2-NPM1-ALK). In contrast, Lck-NPM1-ALK mice, with NPM1-ALK expression from theT-cell specific Lck gene promoter, developed large T-cell lymphoblastic lymphomas after only eight weeks.[101]

Xenograft Mouse Models

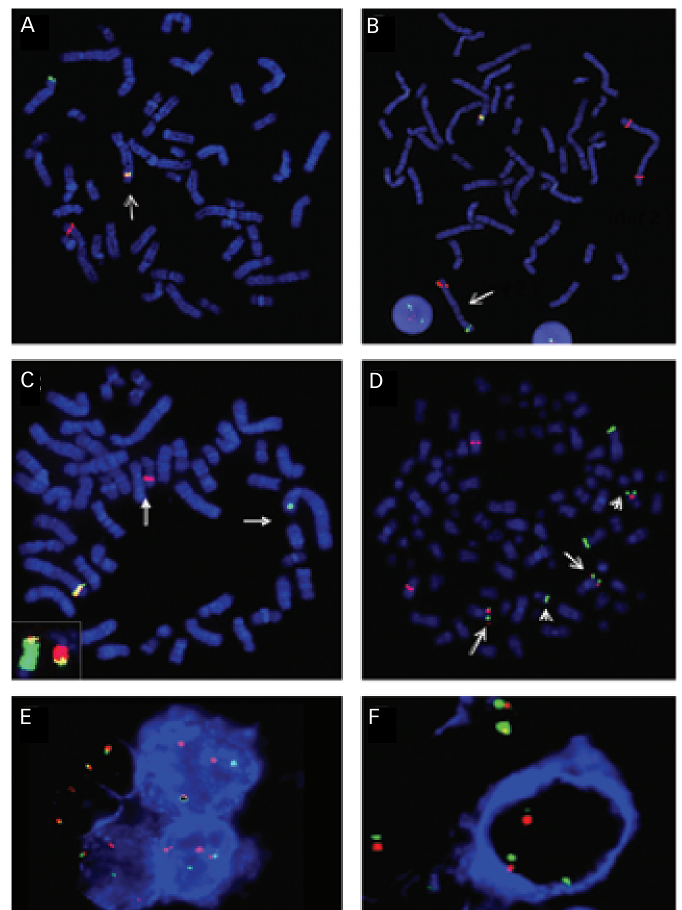
Several studies have been published in which xenograft mouse models of ALK-mediated tumorigenesis are described. JB6, COST, Karpas-299

Figure 2: Examples of Different Patterns of Anaplastic Lymphoma Kinase Immunostaining in Anaplastic Lymphoma Kinase+ Lymphomas



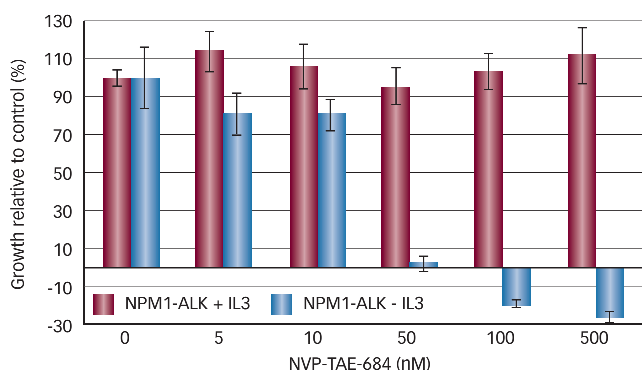
Note the cytoplasmic and nuclear localisation of anaplastic lymphoma kinase (ALK) hallmarking the t(2;5)/nucleophosmin (NMP1)-ALK (A), the cytoplasmic ALK staining in a case with inv(2)/ATIC-ALK (B) and the granular cytoplasmic ALK staining characteristic for the t(2;17)/CLTC-ALK (C). Figure courtesy of C De Wolf-Peeters and P De Paepe.

Figure 3: Fluorescent *In Situ* Hybridisation Analysis of Anaplastic Lymphoma Kinase+ Lymphomas with 2p23/*ALK* Aberrations



*A: Anaplastic lymphoma kinase-positive anaplastic large cell lymphoma (ALK+ ALCL) with a typical t(2;5)(p23;q35)/*NPM1-ALK *fusion analysed with probes for the 3'end of* ALK *(red) and the 5'end region of* NPM1 *(green). Note a co-localised signal on the der(5)t(2;5). B: ALK+ ALCL with inv(2)(p23q35)/ATIC-ALK (B) and additional i(2)(q10) analysed with a commercial LSI ALK dual colour, break apart probe. Note the split ALK signals on the short (p23) and long (q35) arms of inv(2) and two extra copies of the 3' end of ALK (red) on both arms of i(2)(q10). C: ALK+ LBCL with a t(2;17)(p23;q23)/*CLTC-ALK *fusion analysed with LSI ALK showing a normal fused signal on chromosome 2, the green signal (5'ALK) on der(2) and the red signal (3'ALK) on der(17). Whole chromosome painting probes for chromosome 2 (green) and 17 (red) were used to show the t(2;17) involving terminal regions of both chromosomes (inset). D: ALK+ LBCL with a cryptic SEC31A-ALK rearrangement generated by the insertion of the 5'end of* SEC31A *to the 3'end of* ALK*, which is translocated at 3q27 and duplicated on der(20).[25] Note the normal 5'*SEC31A *(red) and 3'*ALK *(green) signal at 4q21 and 2p23, respectively, and the co-localised signals on the der(3) (long arrow) and the der(20) (short arrow) in a tetraploid tumour cell. E: ALK+ LBCL interphase cells immunostained with anti-ALK serum (blue) and hybridised with LSI ALK. Note one fused (normal ALK), one green (5'ALK) and duplicated red (3'ALK) signals in the atypical cells expressing ALK. Normal cells with two fused ALK signals are ALK-negative. F: ALK+ LBCL interphase cells immunostained with CD138 specific antibody (blue) and hybridised with LSI ALK. Only CD138+ neoplastic cells displayed an aberrant fluorescent* in situ *hybridisation pattern.*

Figure 4: Sensitivity of Ba/F3 Cells Expressing *NPM1-ALK* to NVP-TAE-684



Under normal conditions, mouse haematopoietic Ba/F3 cells are dependent on interleukin 3 (IL3) for their growth. The introduction of an oncogene transforms them to growth factor independence. IL3-independent Ba/F3 cells expressing nucleophosmin-anaplastic lymphoma kinase (NPM1-ALK) are sensitive to the ALK inhibitor NVP-TAE-684 with an inhibitory concentration (IC_{50}) between 10 and 50nM of inhibitor (blue bars). When IL3 is added, the NPM1-ALK-expressing Ba/F3 cells do not depend anymore on the oncogenic fusion for their growth and are not affected by the presence of NVP-TAE-684 (black bars). For the dose-response curves, Ba/F3 cells were incubated in the presence of NVP-TAE-684 for 24 hours and viable cell numbers were determined using AQueousOne Solution (Promega). Cell survival in the absence of inhibitor was set at 100%, the mean of four independent measurements is displayed ± standard error of the mean (SEM).

and Ba/F3 cells expressing NPM1-ALK were used to test whether tumours formed in immunocompromised severe combined immunodeficiency (SCID) mice. Localised subcutaneous xenografted tumours and systemic lymphomatous disease could be established in all SCID mouse models.[102–105] In addition, the mice benefited from treatment with ALK inhibitors, suggesting ALK is a good therapeutic target.

Anaplastic Lymphoma Kinase Signalling in Cancer

Research on ALK signalling in cancer has mainly been focused on the effect of NPM1-ALK on signalling pathways. The observation that NPM1 and almost all variant partners of ALK contain some form of an oligomerisation motif suggests that the mechanism underlying ALK tyrosine activation and the activated signal transduction pathways are probably similar for all ALK chimaeric proteins.[106]

The oncogenic potential of ALK fusions translates into enhanced cell proliferation and survival, cytoskeletal rearrangements and changes in cell shape.[95,98,107,108] The aberrant tyrosine kinase activity triggers several different pathways that are interconnected and overlapping. The rat sarcoma viral oncogene homologue (RAS)/mitogen-activated protein kinase (MAPK) and phospholipase C-γ (PLC-γ) pathways are important in mitogenesis, whereas phosphatidylinositol 3-kinase (PI3K/AKT) and STAT pathways are of primordial importance in cell survival and phenotypic changes. Tandem mass spectrometry has identified 46 putative NPM1-ALK-interacting proteins, suggesting the NPM1-ALK signalosome may be considerably more complex than previously assumed.[109]

Enhancement of Proliferation

Activation of the RAS/extracellular signal-regulated kinase (ERK) pathway is the main cause of the increased growth of ALCL cells. In these cells, NPM1-ALK acts as a docking molecule for several downstream adaptors or scaffolding molecules, such as insulin receptor substrate 1 (IRS1), SRC homology 2 domain-containing transforming protein (SHC) and growth factor receptor-bound protein 2 (GRB2), which bind to specific autophosphorylated tyrosine residues of ALK and induce the RAS/ERK pathway.[92,110] Recently, it has been shown that NPM1-ALK-activated tyrosine phosphatase SHP2 stimulates ALCL growth through ERK1/2 phosphorylation.[111] Also, NPM1-ALK-mediated mammalian target of rapamycin (mTOR) activation has been demonstrated to be transduced by the RAS/ERK signal transduction pathway.[112] Activation of the PLC-γ pathway is also thought to play an important role in mitogenesis induced by ALK signalling. PLC-γ binds directly to NPM1-ALK via interaction between the SH2 domain of PLC-γ and tyrosine residue Tyr664 of NPM1-ALK. Mouse Ba/F3 and Rat 1a cells expressing a mutant NPM1-ALK that cannot phosphorylate Tyr664 were unable to bind and activate PLC-γ, emphasising the importance of NPM1-ALK Tyr664.[113]

Studies indicate that NPM1-ALK interacts with a nuclear-interacting partner of ALK (NIPA), initially described as an anti-apoptotic protein.[114] More recent studies, however, consider NIPA as an F-box-containing protein controlling mitotic entry[115] that is involved in cyclin B1 proteasome degradation.[116] Approximately 50% of ALCL cases show loss of the tyrosine phosphatase SHP1 that acts as a tumour suppressor gene via inactivation of the JAK3-STAT3 pathway, the abrogation of which usually correlates with uncontrolled cell growth.[117,118]

Levantaki et al. showed that expression of NPM1-ALK induced phosphorylation of JUN N-terminal kinase (JNK) and cJun was linked to a dramatic increase of activator protein 1 (AP-1)-transcription-factor activity. This may contribute to uncontrolled cell cycle progression and oncogenesis.[119]

Regulation of Apoptosis

Apart from an increase in cell proliferation, cancer cells need to suppress apoptosis in order to become tumorigenic. NPM1-ALK has been shown to activate PI3K in ALCL through binding to its regulatory p85 subunit, resulting in phosphorylation of the serine/threonine kinase AKT.[120,121] AKT then phosphorylates the pro-apoptotic protein BAD, leading to its dissociation from the anti-apoptotic protein $BC\text{-}_{XL}$, which can then block cell death.[122,123] The anti-apoptotic (and proliferation-enhancing) effect of NPM1-ALK via AKT can also, in part, be mediated through AKT-induced inhibition of forkhead family transcription factor (FOXO3a) activity, promoting cell-cycle progression.[124,125] In addition, PI3K/AKT activation induces phosphorylation of the cyclin-dependent kinase inhibitor 27, increasing its proteasomal degradation and promoting cell-cycle progression.[126] Recent evidence points to a role of the PI3K/AKT pathway to cause constitutive activation of the dual specificity phosphatase CDC25A, leading to enhanced proliferation.[127] Also, a positive synergistic effect between the sonic hedgehog (SHH)/GLI1 and PI3K/AKT pathways has been described that contributes to the lymphomagenic effect of NPM1-ALK.[128]

The activated phosphorylated form of the STAT3 transcription factor that has been detected in 84% of ALK^+ ALCL tumour samples, as well as in cell lines and in thymocytes and tumour tissues of NPM1-ALK transgenic mice[98,129–133] leads to the enhanced expression of anti-apoptotic proteins, cell-cycle regulators and immunosuppressive proteins such as $BCL\text{-}_{XL}$, survivin, cyclin D3, C/EBPb, myeloid leukaemia sequence 1 (MCL1) and PD-L1 (CD274).[132,134–136] A pro-proliferative and anti-apoptotic role for STAT5B in NPM1-ALK-mediated signalling has been described by Nieborowska-Skorska and colleagues.[137] However, later studies showed that this mechanism of activation requires JAK21.[38] In contrast, Zhang et al.[139] suggested that STAT5 acts as a tumour suppressor in ALCL as it is epigenetically silenced by the NPM1-ALK tyrosine kinase, but when re-expressed it reciprocally inhibits NPM1-ALK signalling.

Cell Migration and Cytoskeletal Rearrangements

In vitro studies showed that various ALK fusions increase the migration rate of lymphoid cells and change their shape,[107,111] and that lymphoid cells become anaplastic in the presence of transforming ALK proteins.[140] Notably, inhibition of ALK-kinase activity by small-molecule inhibitors dramatically reduces ALCL-cell motility and reverts morphology from anaplastic to spherical.[141] Several lines of evidence indicate that NPM1-ALK activates proteins involved in the regulation of actin filament assembly and cytoskeletal rearrangements, such as p130Cas,[107] SHP2,[111] pp60Src,[142] Rac1[143] and Cdc42,[144] influencing the morphology, migration and transformation of ALK-expressing tumour cells.

Modulation of the Anaplastic Large Cell Lymphoma Immunophenotype

ALCL is characterised by high expression of CD30, which is also strongly expressed by the neoplastic (H/RS) cells of Hodgkin's lymphoma (HL).[18] Transduction of NPM1-ALK into HL-derived cell lines induces an ALCL-like phenotype and morphology. These observations suggest a role for CD30 in the development of the ALCL phenotype.[140] In addition, NPM1-ALK impedes CD30 signalling and nuclear factor kappa-B (NF-κB) activation dependent on both ALK kinase activity and the N-terminal NPM1 domain.[140]

Treatment

Both ALK-expressing ALCL and LBCL are conventionally treated by combination chemotherapy. In a minor group of ALK+ LBCL patients, chemotherapy in combination with radiotherapy (15%) or radiotherapy alone (2%) was given.[23] ALK is a strong candidate for targeted treatments. As numerous malignancies showing aberrant ALK expression have been reported, the search for new ALK inhibitors is rising.[145–147] Recently, several small-molecule inhibitors displaying selective and potent ALK inhibition in both *in vitro* tumour models and *in vivo* xenograft ALCL-tumour models have been described, among them, NVP-TAE-684, fused pyrrolocarbazole (FP)-derived small molecules (CEP-14083 and CEP-14513) and PF-2341066.[25,102,148,149] Inhibition of NPM1-ALK as well as SEC31A-ALK-expressing Ba/F3 cells by NVP-TAE-684 suggests that it can be used in the therapy of patients with both classic and variant ALK fusions[25] (see *Figure 4*). Given that virtually all ALCLs express CD30, this molecule is a promising target for therapy with anti-CD30 monoclonal antibodies. A recent phase I multidose study of SGN-30, a chimaeric monoclonal antibody for the treatment of CD30(+) malignancies, holds promising results.[150]

Recent studies demonstrated that ALK is spontaneously immunogenic in ALCL cells resulting in B-cell, cytotoxic T-cell (CTL) and CD4 T-helper response.[151–154] Chiarle and colleagues[155] showed that DNA vaccination with plasmids encoding portions of the cytoplasmic ALK domain protects mice from local and systemic lymphoma growth; hence, ALK vaccination following chemotherapy and/or pharmacological inhibition of ALK can be an interesting approach to treat ALK-expressing malignancies, such as ALK+ ALCL and ALK+ LBCL. Genetic targeting of ALK through small interfering RNA (si)RNA and single-stranded antisense oligodeoxynucleotides (ASO) may become important in the future; however, *in vivo* delivery of these molecules remains an unsolved problem.[156] Finally, molecules acting downstream of ALK signalling, such as PI3K/AKT, JAK/STAT3, PLC-γ and RAS/MAPK, can also be potential targets of selective inhibition. However, because several signalling cascades are interconnected and are not dependent only on ALK signalling, it remains to be elucidated whether targeting these proteins will result in tumour eradication. ■

Katrien Van Roosbroeck is a PhD student at the Centre for Human Genetics at the University Hospital Gasthuisberg of the Catholic University of Leuven. She works in the Laboratory for Clinical and Molecular Cytogenetics of Malignancies and focuses on the identification and characterisation of aberrant tyrosine kinases in leukaemia and lymphoma. Ms Van Roosbroeck obtained her MBE in cell and gene biotechnology at the Catholic University of Leuven.

Iwona Wlodarska is a staff member at the Centre for Human Genetics at the University Hospital Gasthuisberg of the Catholic University of Leuven. Her research is focused on cytogenetic and molecular mechanisms underlying lymphomagenesis. She has published more than 130 peer-reviewed papers and contributed to the identification of novel anaplastic lymphoma kinase (ALK) rearrangements in lymphoma. She is a member of the European Hematology Association (EHA) and the American Society of Hematology (ASH). Previously, Dr Wlodarska worked at the Institute of Oncology in Warsaw and moved to Leuven in 1989. She obtained her PhD from the Institute of Experimental Immunology at the Polish Academy of Science in Wroclaw.

1. Morris SW, Kirstein MN, Valentine MB, et al., *Science*, 1994;263:1281–4.
2. Morris SW, Naeve C, Mathew P, et al., *Oncogene*, 1997;14:2175–88.
3. Iwahara T, Fujimoto J, Wen D, et al., *Oncogene*, 1997;14:439–49.
4. Falini B, Pileri S, Zinzani PL, et al., *Blood*, 1999;93:2697–2706.
5. Pulford K, Lamant L, Morris SW, et al., *Blood*, 1997;89:1394–1404.
6. Falini B, Bigerna B, Fizzotti M, et al., *Am J Pathol*, 1998;153:875–86.
7. Jaffe ES, Harris NL, Stein H, Vardiman JW, *World Health Organization Classification of Tumors: Tumors of the Haematopoietic and Lymphoid Tissues*, Lyon: International Agency for Research on Cancer (IARC), 2001.
8. Swerdlow SH, Campo E, Harris NL, et al., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, Lyon: International Agency for Research on Cancer (IARC), 2008.
9. Benharroch D, Meguerian-Bedoyan Z, Lamant L, et al., *Blood*, 1998;91:2076–84.
10. Stein H, Foss HD, Durkop H, et al., *Blood*, 2000;96:3681–95.
11. Falini B, Pileri S, Stein H, et al., *Hum Pathol*, 1990;21:624–9.
12. Falini B, Pileri S, Pizzolo G, et al., *Blood*, 1995;85:1–14.
13. Falini B, *Br J Haematol*, 2001;114:741–60.
14. Falini B, Mason DY, *Blood*, 2002;99:409–26.
15. Gascoyne RD, Aoun P, Wu D, et al., *Blood*, 1999;93:3913–21.
16. Morris SW, Xue L, Ma Z, Kinney MC, *Br J Haematol*, 2001;113:275–95.
17. Shiota M, Nakamura S, Ichinohasama R, et al., *Blood*, 1995;86:1954–60.
18. Stein H, Mason DY, et al., *Blood*, 1985;66:848–58.
19. Lamant L, Meggetto F, al Saati T, et al., *Blood*, 1996;87:284–91.
20. Mason DY, Bastard C, Rimokh R, et al., *Br J Haematol*, 1990;74:161–8.
21. Huret JL, Senon S, Bernheim A, Dessen P, *Cell Mol Biol (Noisy-le-grand)*, 2004;50:805–7.
22. Delsol G, Lamant L, Mariame B, et al., *Blood*, 1997;89:1483–90.
23. Beltran B, Castillo J, et al., *J Hematol Oncol*, 2009;2:11.
24. Li S, *Int J Clin Exp Pathol*, 2009;2:508–18.
25. Van Roosbroeck K, Cools J, Dierickx D, et al., *Haematologica*, 2009; in press.
26. Reichard KK, McKenna RW, Kroft SH, *Mod Pathol*, 2007;20:310–19.
27. Bubala H, Maldyk J, Wlodarska I, et al., *Pediatr Blood Cancer*, 2006;46:649–53.
28. Chikatsu N, Kojima H, Suzukawa K, et al., *Mod Pathol*, 2003;16:828–32.
29. Choung HS, Kim HJ, Kim WS, et al., *Korean J Lab Med*, 2008;28:89–94.
30. De Paepe P, Baens M, van Krieken H, et al., *Blood*, 2003;102:2638–41.
31. Gascoyne RD, Lamant L, Martin-Subero JI, et al., *Blood*, 2003;102:2568–73.
32. Gesk S, Gascoyne RD, Schnitzer B, et al., *Leukemia*, 2005;19:1839–40.
33. Isimbaldi G, Bandiera L, d'Amore ES, et al., *Pediatr Blood Cancer*, 2006;46:390–91.
34. McManus DT, Catherwood MA, Carey PD, et al., *Hum Pathol*, 2004;35:1285–8.
35. Momose S, Tamaru J, Kishi H, et al., *Hum Pathol*, 2009;40:75–82.
36. Adam P, Katzenberger T, Seeberger H, et al., *Am J Surg Pathol*, 2003;27:1473–6.
37. Onciu M, Behm FG, Downing JR, et al., *Blood*, 2003;102:2642–4.
38. Rudzki Z, Rucinska M, Jurczak W, et al., *Pol J Pathol*, 2005;56:37–45.
39. Cessna MH, Zhou H, Sanger WG, et al., *Mod Pathol*, 2002;15:931–8.
40. Griffin CA, Hawkins AL, Dvorak C, et al., *Cancer Res*, 1999;59:2776–80.
41. Li XQ, Hisaoka M, Shi DR, et al., *Hum Pathol*, 2004;35:711–21.
42. Choi YL, Takeuchi K, Soda M, et al., *Cancer Res*, 2008;68:4971–6.

43. Fukuyoshi Y, Inoue H, Kita Y, et al., *Br J Cancer*, 2008;98:1536–9.
44. Inamura K, Takeuchi K, Togashi Y, et al., *J Thorac Oncol*, 2008;3:13–17.
45. Koivunen JP, Mermel C, Zejnullahu K, et al., *Cancer Res*, 2008;14:4275–83.
46. Martelli MP, Sozzi G, Hernandez L, et al., *Am J Pathol*, 2009;174:661–70.
47. Perner S, Wagner PL, Demichelis F, et al., *Neoplasia*, 2008;10:298–302.
48. Shinmura K, Kageyama S, Tao H, et al., *Lung Cancer*, 2008;61:163–9.
49. Soda M, Choi YL, Enomoto M, et al., *Nature*, 2007;448:561–6.
50. Takeuchi K, Choi YL, Soda M, et al., *Clin Cancer Res*, 2008;14:6618–24.
51. Wong DW, Leung EL, So KK, et al., *Cancer*, 2009;115:1723–33.
52. Takeuchi K, Choi YL, Togashi Y, et al., *Clin Cancer Res*, 2009;15:3143–9.
53. Boland JM, Erdogan S, Vasmatzis G, et al., *Hum Pathol*, 2009 (Epub ahead of print).
54. Du XL, Hu H, Lin DC, et al., *J Mol Med*, 2007;85:863–75.
55. Jazii FR, Najafi Z, Malekzadeh R, et al., *World J Gastroenterol*, 2006;12:7104–12.
56. Kinoshita Y, Tajiri T, et al., *Pediatr Surg Int*, 2007;23:595–9.
57. Lawrence B, Perez-Atayde A, *Am J Pathol*, 2000;157: 377–84.
58. Milne AN, Sweeney KJ, O'Riordain DS, et al., *Hum Pathol*, 2006;37:112–16.
59. Yamamoto H, Kohashi K, Oda Y, et al., *Pathol Int*, 2006;56:584–90.
60. Debiec-Rychter M, Marynen P, Hagemeijer A, Pauwels P, *Genes Chromosomes Cancer*, 2003;38:187–90.
61. Bridge JA, Kanamori M, Ma Z, et al., *Am J Pathol*, 2001;159:411–15.
62. Patel AS, Murphy KM, Hawkins AL, et al., *Cancer Genet Cytogenet*, 2007;176:107–14.
63. Panagopoulos I, Nilsson T, Domanski HA, et al., *Int J Cancer*, 2006;118:1181–6.
64. Hisaoka M, Shimajiri S, et al., *Pathol Int*, 2003;53:376–81.
65. Cools J, Wlodarska I, Somers R, et al., *Genes Chromosomes Cancer*, 2002;34:354–62.
66. Debelenko LV, Arthur DC, Pack SD, et al., *Lab Invest*, 2003;83:1255–65.
67. Chen ST, Lee JC, *Hum Pathol*, 2008;39:1854–8.
68. Ma Z, Hill DA, Collins MH, et al., *Genes Chromosomes Cancer*, 2003; 37:98–105.
69. Caren H, Abel F, Kogner P, *Biochem J*, 2008;416:153–9.
70. Chen Y, Takita J, Choi YL, et al., *Nature*, 2008;455:971–4.
71. George RE, Sanda T, Hanna M, et al., *Nature*, 2008;455:975–8.
72. Janoueix-Lerosey I, Lequin D, Brugieres L, et al., *Nature*, 2008;455:967–70.
73. Lamant L, Pulford K, Bischof D, et al., *Am J Pathol*, 2000;156:1711–21.
74. Mosse YP, Laudenslager M, Longo L, et al., *Nature*, 2008;455:930–35.
75. Osajima-Hakomori Y, Miyake I, Ohira M, *Am J Pathol*, 2005;167:213–22.
76. Dirks WG, Fahrnich S, et al., *Int J Cancer*, 2002;100:49–56.
77. Powers C, Aigner A, Stoica GE, et al., *J Biol Chem*, 2002;277:14153–8.
78. Pillay K, Govender D, Chetty R, *Histopathology*, 2002;41:461–7.
79. Perez-Pinera P, Chang Y, Astudillo A, et al., *Biochem Biophys Res Commun*, 2007;358:399–403.
80. Stoica GE, Kuo A, Aigner A, et al., *J Biol Chem*, 2001;276: 16772–9.
81. Stoica GE, Kuo A, Powers C, et al., *J Biol Chem*, 2002;277: 35990–98.
82. Kadomatsu K, Huang RP, Suganuma T, et al., *J Cell Biol*, 1990;110:607–16.
83. Kadomatsu K, Muramatsu T, *Cancer Lett*, 2004;204: 127–43.
84. Schulte AM, Wellstein A, In: Bicknell R, Lewis CM, Ferrara N (eds), *Tumour Angiogenesis*, Oxford, New York, Tokyo: Oxford University Press, 1997.
85. Zou P, Muramatsu H, Miyata T, Muramatsu T, Midkine, *J Neurochem*, 2006;99:1470–79.
86. Bowden ET, Stoica GE, Wellstein A, *J Biol Chem*, 2002;277:35862–8.
87. Lamant L, Gascoyne RD, Duplantier MM, et al., *Genes Chromosomes Cancer*, 2003;37:427–32.
88. Tort F, Pinyol M, Pulford K, et al., *Lab Invest*, 2001;81:419–26.
89. Tort F, Campo E, Pohlman B, Hsi E, *Hum Pathol*, 2004;35: 1038–41.
90. Hubbard TJ, Aken BL, Ayling S, et al., *Nucleic Acids Res*, 2009;37:D690–D697.
91. Pulford K, Lamant L, Espinos E, et al., *Cell Mol Life Sci*, 2004;61:2939–53.
92. Pulford K, Morris SW, Turturro F, *J Cell Physiol*, 2004;199:330–58.
93. Giuriato S, Faumont N, Bousquet E, et al., *Cancer Biol Ther*, 2007;6:1318–23.
94. Soda M, Takada S, Takeuchi K, et al., *Proc Natl Acad Sci U S A*, 2008;105:19893–7.
95. Kuefer MU, Look AT, Pulford K, et al., *Blood*, 1997;90:2901–10.
96. Miething C, Grundler R, Fend F, et al., *Oncogene*, 2003;22:4642–7.
97. Miething C, Grundler R, Mugler C, et al., *ASH Annual Meeting Abstracts*, 2004;104:348.
98. Chiarle R, Gong JZ, Guasparri I, et al., *Blood*, 2003;101:1919–27.
99. Turner SD, Tooze R, Maclennan K, Alexander DR, *Oncogene*, 2003;22:7750–61.
100. Turner SD, Merz H, Yeung D, Alexander DR, *Anticancer Res*, 2006;26:3275–9.
101. Jager R, Hahne J, Jacob A, et al., *Anticancer Res*, 2005;25:3191–6.
102. Galkin AV, Melnick JS, Kim S, et al., *Proc Natl Acad Sci U S A*, 2007;104:270–75.
103. Lamant L, Espinos E, Duplantier M, et al., *Leukemia*, 2004;18:1693–8.
104. Pasqualucci L, Wasik M, Teicher BA, et al., *Blood*, 1995;85:2139–46.
105. Piva R, Chiarle R, Manazza AD, et al., *Blood*, 2006;107: 689–97.
106. Vega F, Orduz R, Medeiros LJ, *Pathology*, 2002;34:397–409.
107. Ambrogio C, Voena C, Manazza AD, et al., *Blood*, 2005;106:3907–16.
108. Wellmann A, Doseeva V, Butscher W, et al., *FASEB J*, 1997;11:965–72.
109. Crockett DK, Lin Z, Elenitoba-Johnson KS, Lim MS, *Oncogene*, 2004;23:2617–29.
110. Fujimoto J, Shiota M, Iwahara T, et al., *Proc Natl Acad Sci U S A*, 1996;93:4181–6.
111. Voena C, Conte C, Ambrogio C, et al., *Cancer Res*, 2007;67:4278–86.
112. Marzec M, Kasprzycka M, Liu X, et al., *Oncogene*, 2007;26:5606–14.
113. Bai RY, Dieter P, Peschel C, et al., *Mol Cell Biol*, 1998;18:6951–61.
114. Ouyang T, Bai RY, Bassermann F, et al., *J Biol Chem*, 2003;278:30028–36.
115. Bassermann F, von Klitzing C., Munch S, et al., *Cell*, 2005;122:45–57.
116. Bassermann F, von Klitzing C, Illert AL, et al., *J Biol Chem*, 2007;282:15965–72.
117. Honorat JF, Ragab A, Lamant L, et al., *Blood*, 2006;107:4130–38.
118. Han Y, Amin HM, et al., *Blood*, 2006;108:2796–2803.
119. Leventaki V, Drakos E, Medeiros LJ, et al., *Blood*, 2007;110:1621–30.
120. Bai RY, Ouyang T, Miething C, et al., *Blood*, 2000;96:4319–27.
121. Slupianek A, Nieborowska-Skorska M, Hoser G, et al., *Cancer Res*, 2001;61:2194–9.
122. Datta SR, Brunet A, Greenberg ME, *Genes Dev*, 1999;13:2905–27.
123. Grad JM, Zeng XR, Boise LH, *Curr Opin Oncol*, 2000;12:543–9.
124. Brunet A, Bonni A, Zigmond MJ, et al., *Cell*, 1999;96:857–68.
125. Gu TL, Tothova Z, Scheijen B, et al., *Blood*, 2004;103:4622–9.
126. Rassidakis GZ, Feretzaki M, et al., *Blood*, 2005;105:827–9.
127. Fernandez-Vidal A, Mazars A, Gautier EF, et al., *Cell Cycle*, 2009;8:1373–9.
128. Singh RR, Cho-Vega JH, Davuluri Y, et al., *Cancer Res*, 2009;69: 2550–58.
129. Chiarle R, Simmons WJ, Cai H, et al., *Nat Med*, 2005;11:623–9.
130. Khoury JD, Medeiros LJ, Rassidakis GZ, et al., *Clin Cancer Res*, 2003;9:3692–9.
131. Lai R, Rassidakis GZ, Medeiros LJ, et al., *Am J Pathol*, 2004;164:2251–8.
132. Zamo A, Chiarle R, Piva R, et al., *Oncogene*, 2002;21:1038–47.
133. Zhang Q, Raghunath PN, Xue L, et al., *J Immunol*, 2002;168:466–74.
134. Coluccia AM, Perego S, Cleris L, et al., *Blood*, 2004;103:2787–94.
135. Marzec M, Zhang Q, Goradia A, et al., *Proc Natl Acad Sci U S A*, 2008;105:20852–7.
136. Piva R, Pellegrino E, Mattioli M, et al., *J Clin Invest*, 2006;116:3171–82.
137. Nieborowska-Skorska M, Slupianek A, Xue L, et al., *Cancer Res*, 2001;61:6517–23.
138. Ruchatz H, Coluccia AM, Stano P, et al., *Exp Hematol*, 2003;31:309–15.
139. Zhang Q, Wang HY, Liu X, *Nat Med*, 2007;13:1341–8.
140. Horie R, Watanabe M, Ishida T, et al., *Cancer Cell*, 2004;5:353–64.
141. Chiarle R, Voena C, Ambrogio C, et al., *Nat Rev Cancer*, 2008;8:11–23.
142. Cussac D, Greenland C, Roche S, et al., *Blood*, 2004;103:1464–71.
143. Colomba A, Courilleau D, Ramel D, et al., *Oncogene*, 2008;27:2728–36.
144. Ambrogio C, Voena C, Manazza AD, et al., *Cancer Res*, 2008;68:8899–8907.
145. Li R, Xue L, Zhu T, et al., *J Med Chem*, 2006;49:1006–15.
146. Zhu T, Yan Z, Chucholowski A, et al., *J Comb Chem*, 2006;8:401–9.
147. Li R, Morris SW, *Med Res Rev*, 2008;28:372–412.
148. Wan W, Albom MS, Lu L, et al., *Blood*, 2006;107:1617–23.
149. Zou HY, Li Q, Lee JH, et al., *Cancer Res*, 2007;67:4408–17.
150. Bartlett NL, Younes A, Carabasi MH, et al., *Blood*, 2008;111:1848–54.
151. Ait-Tahar K, Cerundolo V, Banham AH, et al., *Int J Cancer*, 2006;118:688–95.
152. Ait-Tahar K, Barnardo MC, Pulford K, *Cancer Res*, 2007;67:1898–1901.
153. Passoni L, Gallo B, Biganzoli E, et al., *Haematologica*, 2006;91:48–55.
154. Pulford K, Falini B, Banham AH, et al., *Blood*, 2000;96:1605–7.
155. Chiarle R, Martinengo C, Mastini C, et al., *Nat Med*, 2008;14:676–80.
156. Kim DH, Rossi JJ, *Nat Rev Genet*, 2007;8:173–84.
157. Drexler HG, Gignac SM, et al., *Leukemia*, 2000;14:1533–59.
158. Colleoni GW, Bridge JA, Garicochea B, et al., *Am J Pathol*, 2000;156:781–9.
159. Ma Z, Cools J, Marynen P, et al., *Blood*, 2000;95:2144–9.
160. Matsubara K, Tanaka T, Taki T, et al., *Rinsho Ketsueki*, 2008;49:325–30.
161. Trinei M, Lanfrancone L, Campo E, et al., *Cancer Res*, 2000;60:793–8.
162. Elenitoba-Johnson KS, Crockett DK, Schumacher JA, et al., *Proc Natl Acad Sci U S A*, 2006;103:7402–7.
163. Lamant L, Dastugue N, Pulford K, et al., *Blood*, 1999;93:3088–95.
164. Reading NS, Jenson SD, Smith JK, Lim MS, Elenitoba-Johnson KS. *J Mol Diagn*, 2003;5:136–40.
165. Siebert R, Gesk S, Harder L, et al., *Blood*, 1999;94:3614–17.
166. Hernandez L, Pinyol M, Hernandez S, et al., *Blood*, 1999;94:3265–8.
167. Hernandez L, Bea S, Bellosillo B, et al., *Am J Pathol*, 2002;160:1487–94.
168. Touriol C, Greenland C, Lamant L, et al., *Blood*, 2000;95:3204–7.
169. Liang X, Meech SJ, Odom LF, et al., *Am J Clin Pathol*, 2004;121:496–506.
170. Meech SJ, McGavran L, Odom LF, et al., *Blood*, 2001;98:1209–16.