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Review

The emerging pathogenic and therapeutic importance of the anaplastic lymphoma kinase gene

Fergal C. Kelleher *, *Ray McDermott*

Department of Medical Oncology, Adelaide and Meath Hospital, Dublin 24, Ireland

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ABSTRACT

The anaplastic lymphoma kinase gene (ALK) is a gene on chromosome 2p23 that has expression restricted to the brain, testis and small intestine but is not expressed in normal lymphoid tissue. It has similarity to the insulin receptor subfamily of kinases and is emerging as having increased pathologic and potential therapeutic importance in malignant disease. This gene was originally established as being implicated in the pathogenesis of rare diseases including inflammatory myofibroblastic tumour (IMT) and ALK-positive anaplastic large cell lymphoma, which is a subtype of non-Hodgkin's lymphoma. Recently the number of diseases in which ALK is implicated in their pathogenesis has increased. In 2007, an inversion of chromosome 2 involving ALK and a fusion partner gene in a subset of non-small cell lung cancer was discovered.[1] In 2008, publications emerged implicating ALK in familial and sporadic cases of neuroblastoma, a childhood cancer of the sympatho-adrenal system.[2–5]

Chromosomal abnormalities involving ALK are translocations, amplifications or mutations. Chromosomal translocations are the longest recognised ALK genetic abnormality. When translocations occur a fusion gene is created between ALK and a gene partner. This has been described in ALK-positive anaplastic large cell lymphoma in which ALK is fused to NPM (nucleolar protein gene) and in non-small cell lung cancer where ALK is fused to EML4 (Echinoderm microtubule-associated protein 4). The most frequently described partner genes in inflammatory myofibroblastic tumour are tropomyosin 3/4 (TMP3/4), however in IMTs a diversity of ALK fusion partners have been found, with the ability to homodimerise a common characteristic.[6] Point mutations and amplification of the ALK gene occur in the childhood cancer neuroblastoma. Therapeutic targeting of ALK fusion genes using tyrosine kinase inhibition, vaccination using an ALK specific antigen and treatment using viral vectors for RNAi are emerging potential therapeutic possibilities.

1. The ALK gene in physiology and development

The anaplastic lymphoma kinase (ALK) is a 200 kDa receptor tyrosine kinase that is a member of the insulin receptor superfamily encoded by the ALK gene located on chromosome 2p23[9] (Fig. 2 is illustrative of the ALK gene structure and potential mechanisms of drug resistance).

ALK is normally expressed in the developing nervous system and at lower concentration in the nervous system of adult. Evidence from studies of Drosophila indicates an additional role in visceral muscle differentiation.

* *Corresponding author:* Tel.: +353 86 8332711.
E-mail address: fergalkelleher@hotmail.com (F.C. Kelleher).
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ALK is also a dependence receptor such that in the absence of ligand ALK expression enhances apoptosis via its own cleavage by caspases. Conversely, increased kinase activity in ALK either due to constitutive activation as part of a chimeric fusion gene (e.g. NPM–ALK) or greater ligand availability may lead to increased cell survival and decreased apoptosis.[13] Mourali and co-investigators at INSERM Toulouse, Lyon and Paris, France have identified the following properties of ALK in preclinical experiments or documented the following previously established points regarding ALK (Ref: Table 1).

Deregulated expression of many oncogenes can trigger apoptosis. ALK is a new novel dependence receptor that belongs to the category of oncogenes that induce apoptosis if expressed in an inappropriate cellular context or in the absence of a ligand (Ref: Fig. 1, Table 1). It has been proposed that this arose as a mechanism to abrogate frequent tumour formation if oncogenes became deregulated. An established comparator gene in this respect is *myc*.

2. Anaplastic large cell lymphoma

Anaplastic large cell lymphoma (ALCL) is a rare lymphoma in adults accounting for 2% of cases and is more frequent in the paediatric population comprising 25% of lymphomas in children. It is a form of Non Hodgkin's lymphoma. The immunophenotype of anaplastic large cell/null cell lymphoma is usually CD3+, CD30+, EMA+ and ALK+. Tumour cells will be CD20- and CD15-.[15] The expression of CD30 or Ki-1 is a classic diagnostic immunophenotypic marker and was first described by Stein in 1982. Ki-1 is a 120kd-transmembrane receptor of the tumour necrosis receptor family. It is recognised that 85% of ALK+ ALCL cases have the t (2; 5)(p23; q35) translocation that creates the NPM–ALK chimeric oncogene. Morphologically ALCL has paracortical lymph node involvement with sinusoidal dissemination. The morphological appearance of anaplastic large cell lymphoma is similar to that of carcinomas. The failure to immunostain carcinomas for Ki-1 and not diagnose ALCL is a potential major diagnostic

Table 1 – Anaplastic lymphoma kinase (A dependence receptor).[14]

In the visual system of Drosophila (a subdivision of the nervous system) ALK in the presence of a ligand appears essential for axonal guidance. Conversely in the absence of a ligand ALK can lead to neuronal apoptosis
ALK expression is associated with neural crest derived neuronal tumours including neuroblastomas and glioblastomas
Using 2 cell lines as models expressing wild type ALK it was found that ALK enhances apoptosis. In a lymphoma model/neural model apoptosis was induced by doxorubicin in Jurkat T-lymphoblastic cells and triggered by serum deprivation in 13.S. 1.24 murine immortalised olfactory neuronal cells. Apoptosis was found to be caused by intracellular cleavage of ALK thereby exposing a potentially proapoptotic region within the juxtamemrane intracytoplamic segment of ALK. Doxorubicin induced apoptosis is enhanced in ALK- expressing Jurkat cells
The caspase cleavage site of ALK appears to be the aspartic acid residue at the juxtamemrane site 1160. D1160 mutation abrogates ALK-mediated enhancement of apoptosis
ALK induces cell death in primary cortical neurons in rats
ALK has a proapoptotic activity in the absence of ligand, whereas it is antiapoptotic in the presence of its ligand and when the kinase is intrinsically activated

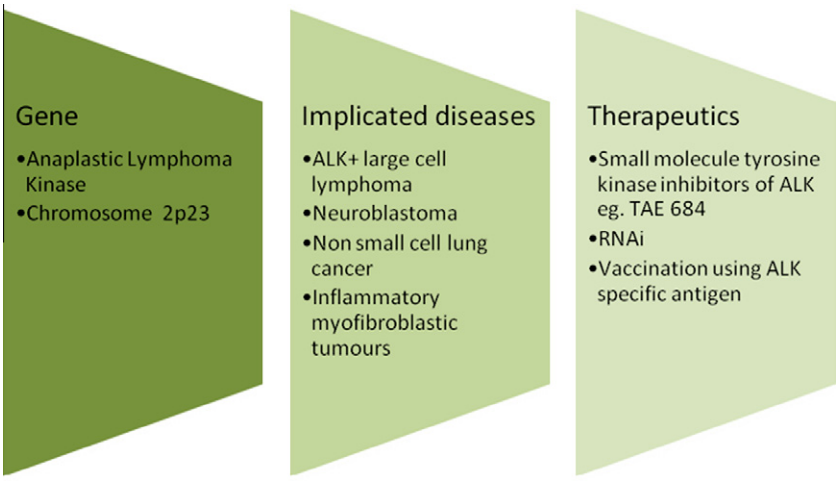

Fig. 1 – ALK and pathobiology. Note: aberrant ALK expression has also been reported in other tumours, such as glioblastoma's and squamous cell carcinomas.[7,8] Furthermore ALK mRNA transcripts were identified by Northern blotting of rhabdomyosarcoma cell lines, enteric nervous tissue, foetal liver and placenta. Separate in situ hybridisation in foetal and adult murine studies descried ALK in the hypothalamus, thalamus, midbrain, olfactory bulbs, dorsal root ganglia and some cranial ganglia. ALK (mRNA and translated protein) diminished to low levels in the neonatal period.[10–12]

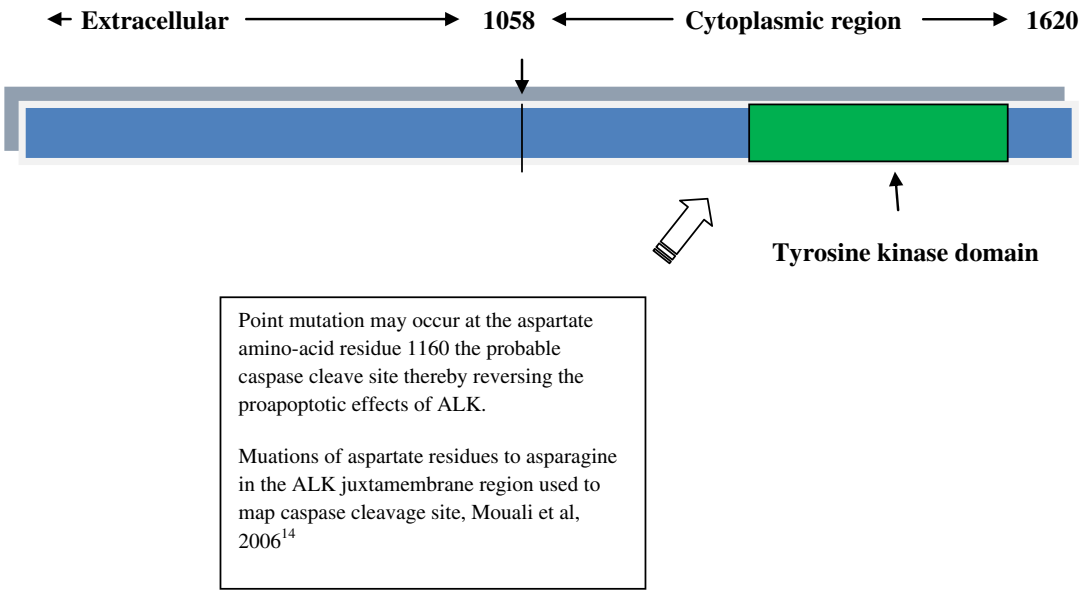

Fig. 2 – Representation of the anaplastic lymphoma kinase gene.[12] Adapted from Pulford et al.

error. This is especially relevant in cases of 'carcinoma of unknown primary'. Systemic anaplastic large cell lymphoma can be sub stratified into cases of ALK-positive and ALK-negative disease, an additional group, primary cutaneous ALCL is usually ALK negative.[16] Further research by Morris et al of St Jude's Children's Hospital later showed that a translocation t (2; 5)(p23; q35), which occurs in 50–75% of ALCL, fuses a gene NPM nucleolar phosphoprotein on chromosome 5q35 to ALK on chromosome 2p23.[17] NPM is a non-ribosomal nucleolar phosphoprotein involved in the assembly of preribosomal particles. This major therapeutic advance established a molecular explanation for ALK+ ALCL and may be a basis for emergent therapeutic strategies in this disease. This NPM–ALK fusion gene product is not the only molecular translocation in this disease and further translocations with their corresponding frequencies are t (1; 2) 17.5%, t (2; 3) 2.5%, inv (2) 2.5% t (2; 17) 2%.[18]

ALK positivity on immunophenotyping is prognostically important as patients with ALK-positive disease have a more favourable prognosis. Immunostaining with a p80 antibody to define ALK positivity found that 5-year survival was 79.8% for p80 (ALK+) disease and 32.9% for p80 (ALK-) disease.[19] Furthermore the International prognostic index may be used to sub stratify outcomes for ALK+ disease. Demographically ALK+ disease occurs particularly in younger patients with a male female ratio of 6.5–1.[20]

3. Neuroblastoma

Neuroblastoma is a childhood cancer that occurs most frequently in the first year of life and descends in frequency thereafter with a median age of diagnosis of nineteen months. Epidemiologically neuroblastoma has an age standardised incidence rate in Europe of 10.9 cases per million children (52.6 in infants) with an average overall survival of 59% in the period 1978–1997.[21] The molecular characteristics of neuroblastoma have been extensively investigated and some have been correlated with clinical outcome. The most frequently described molecular change is amplification of the MYCN oncogene, which occupies a locus on chromosome 2 near the ALK gene and is correlated with an adverse prognosis.[22] The first gene to be linked to familial neuroblastoma is PHOX2B, a sympatho-adrenal lineage specific developmental gene. Its impact is small however. In one study only 6.4% (3/47) of cases with a presumed genetic predisposition to neuroblastoma were found to have a HOX2B mutation and 0% (0/86) of sporadic cases had the mutation.[23]

The importance of ALK in familial and sporadic neuroblastoma was established in October 2008 in four separate publications. Using genome wide association studies ALK was found to be mutated in 6.1–12.4% of sporadic cases. Mutations occurred in mutation hotspots and amplification of the ALK was also implicated in the pathogenesis of neuroblastoma. *In vitro* studies using RNA inhibition and a small molecule inhibitor (TAE 684) of constitutive anaplastic lymphoma kinase activity showed that ALK inhibition is a promising therapeutic strategy. One of the studies found ALK to be a familial neuroblastoma predisposition gene by performing genome wide scans for approximately 6000 SNP's in 20 families with hereditary neuroblastoma of varying levels of certainty. The investigators found a significant linkage signal at chromosome 2p with a predisposition locus at chromosome band 2p23-p24 identified by mapping informative recombination events. Re-sequencing of regional candidate genes found three separate ALK gene mutations that segregated with the disease in eight separate families. They then found a frequency of 12.4% for somatic mutations in ALK when they sequenced 194 high-risk cases of neuroblastoma.[24]

Three further publications were structured such that ALK was shown to be of pathogenic significance that ALK had mutation hotspots and that ALK inhibition was of potential therapeutic benefit.[25–27] One study found a frequency of ALK gene mutations in 8% in primary neuroblastomas. Five non-synonomous gene mutations (three somatic and two

germline) were found in the kinase domain and the most frequent mutation F1174L was found in three differing neuroblastoma cell lines. A second study that used genome wide comparative genomic hybridisation found recurrent copy number increase at the ALK gene locus. By sequencing of cell lines and primary tumour DNA mutation hotspots were identified. Finally a study of 215 neuroblastomas found recurrent ALK copy number gain and gene amplification. DNA sequencing found ALK novel missense mutations in 6.1% of tumours and 33% of neuroblastoma derived cell lines.

The finding that ALK is implicated in neuroblastoma may have implications for screening for the disorder. At a minimum it may provide a partial resolution of the problem that has existed for decades. Historically it has been known that attempted screening for neuroblastoma can be performed with urinary catecholamine's (homovanillic and vanillymandelic acid) with or without abdominal ultrasonography. The disease has a heterogeneous biology and screening can increase the incidence rates without influencing the cumulative disease mortality. Effectively screening can increase the detection of indolent cancer that never will become clinically important either through spontaneous regression or through differentiation into ganglioneuromas but be ineffective in ameliorating the adverse impact of aggressive cases of neuroblastoma. Two studies, a Canadian study in Quebec of 500,000 patients with a comparative control group of 4.2 million patients and a study of 2.6 million infants in Germany of 1 year old infants with a comparator group of 2.1 million infants had negative findings that were consistent in their conclusions.[28,29] Conflicting studies have been conducted in Japan. These descriptive and observational Japanese studies led to an over diagnosis of clinically inconsequential neuroblastoma without evidence of a reduction of the death rate and in March 2004, the Japanese government discontinued their screening programme.[30] Identification of ALK mutations in index cases allows screening of ALK mutations in their relatives. In the future, population-based screening of infants for ALK mutations may predict asymptomatic children that require vigilant screening. ALK mutations may now guide clinical management in a manner analogous to a specific mutation in the RET proto-oncogene deciding patient management based on anticipated natural history of the disease depending on recognised RET gene mutatations.[31] The penetrance of ALK mutations (i.e. the proportion of patients with ALK mutations that develop neuroblastoma) remains to be conclusively determined. These preliminary studies suggest an averaged mutation penetrance of approximately 57%.

4. Inflammatory myofibroblastic tumour

Inflammatory myofibroblastic tumours are tumours of uncertain pathogenesis that comprise mesenchymal proliferations of myofibroblasts with inflammatory infiltrates of eosinophils, plasma cells and lymphocytes. They occur primarily in children and young adults. Cytogenesis banding studies have shown that approximately 50% of IMT have clonal rearrangements of chromosome 2 and recurrent involvement of 2p23 the locus for ALK occurs in inflammatory myofibroblastic tumours.[32] Tropomyosin TMP3 and TMP4 fusion oncoproteins have been recognised as the most frequent gene rearrangement partners with the anaplastic lymphoma kinase gene (ALK).

5. Non-small cell lung cancer

Lung cancer is the most common malignancy and consists of non-small cell lung cancer (NSCLC) and small cell lung cancer with frequencies of 85 and 15%, respectively. Treatment of non-small cell lung cancer with cytotoxic chemotherapy is of limited therapeutic benefit. EGFR inhibitors can be therapeutically beneficial in cases of NSCLC with activating mutations in the epidermal growth factor (EGFR) gene in exons 18, 19 and 21.[33,34] These account for approximately 8–9% of NSCLC and are more common in women, never smokers, people of Asian ethnicity and patients that have lung cancers of adenocarcinoma or bronchioloalveolar carcinoma subtype. In 2007, Soda et al. in Japan described an almost mutually exclusive subtype of NSCLC to that with EGFR mutations. This subtype of NSCLC has a fusion gene involving ALK and a fusion partner of emergent therapeutic significance. By creating a retroviral c DNA library from a resected non-small cell lung adenocarcinoma carcinoma from a 62-year-old male smoker, they identified a novel transforming fusion gene EML4–ALK (Echinoderm microtubule-associated protein 4-anaplastic lymphoma kinase). The methodology used was that they created a retroviral c DNA expression library and oncogenic activity was determined using a mouse 3T3 transformation assay. A c DNA fragment with oncogenic activity was found to have 5′ and 3′ ends that had nucleotide sequences that corresponded to the genes EML4 and ALK. EML4 and ALK are on chromosome 2p21 and 2p23, respectively, and are separated by 12 megabases with opposite orientations. Furthermore, variant isoforms depending on the cleavage site can exist and these isoforms have been confirmed and extended in their number by subsequent investigations. A clinical extension of the study that used reverse transcriptase PCR to determine the frequency of the EML4–ALK fusion gene in lung cancer specimens found that it occurred in 9.1% of cases ($n = 33$). A screening study of other solid and haematopoietic malignancies ($n = 261$) found that EML4–ALK appeared to be exclusive to NSCLC. Soda et al also performed RT-PCR on

Table 2 – 266 Resected primary non-small cell lung cancers, University of Hong Kong, Queen Mary hospital.

Variable	Total (%)	EML4–ALK positive (%)
Adenocarcinoma	76.8	84.6
Lymphoepithelioma-like carcinoma	4.1	0
Squamous cell carcinoma	12.8	0
Other histological subtypes	4.5	15.4
EGFR mutated	47	0
Wild	53	100
KRAS mutated	8.3	0
Wild	91.7	100

Bracketed percentages sample series 1st, EML4–ALK-positive subsample 2nd: adenocarcinomas lymphoepithelioma-like carcinoma (4.1), (0), squamous cell carcinomas and other histological subtypes of non-small cell lung cancer (4.5), (15.4).[40]

Table 3 – EML4–ALK variant fusion genes in studies of non-small cell lung cancer. Table adapted from: Leora Horn, William Pao. EML4–ALK: honing In on a new target in non-small-cell lung cancer. JCO 2009;27(26):4232–5.[44]

Reference	Ethnicity. Case number	EML4–ALK frequency	Research/clinical implications
D1 Fukuyoshi Y, Inoue H, Kita Y, et al. EML4–ALK fusion transcript is not found in gastrointestinal and breast cancers. Br J Cancer 2008;**98**(9):1536–9 [Epub 2008 Apr 15].	Japanese, 104	0.1%	104 Lung cancer cases were tested. There were 645 gastrointestinal (555) and breast cancer (90) samples. One of the lung cancer samples was positive for the EML4–ALK fusion transcript. 0 Were detected in the gastrointestinal and breast cancer cases. The EML4–ALK chimeric fusion gene was considered specific to NSCLC in this series
Shinmura K, Kageyama ST, Tao H, et al. EML4–ALK fusion transcripts, but no NPM–, TPM3–, CLTC–, ATIC–, or TFG–ALK fusion transcripts, in non-small cell lung carcinomas. Lung Cancer 2008;**61**:163–9	77	2.6%	Investigators examined 77 non-small cell lung carcinomas (NSCLCs) for EML4–, NPM–, TPM3–, CLTC–, ATIC–, and TFG–ALK fusion transcripts as these have been found in haematological malignancies. No expression of NPM–, TPM3–, CLTC–, ATIC–, or TFG–ALK fusion transcripts were detected in any of the cases. Expression of EML4–ALK fusion transcripts was detected in 2 (2.6%) of the 77 NSCLCs
Shaw AT, Beow Y. Yeap, Mari Mino-Kenudson, R, et al. JCO 2009;4247–53	US Australian study	13%	The clinical features and outcome of patients with NSCLC who harbour EML4–ALK was studied.141 tumours screened, 19 (13%) were EML4–ALK mutant, 31 (22%) were EGFR mutant, and 91 (65%) were wild type (WT/WT) for both ALK and EGFR. Compared with the EGFR mutant and WT/WT cohorts, patients with EML4–ALK mutant tumours were significantly younger ($P < .001$ and $P = .005$) and were more likely to be men ($P = .036$ and $P = .039$). Patients with EML4–ALK-positive tumours were more likely to be never/light smokers compared with patients in the WT/WT cohort ($P < .001$). 18/19 EML4–ALK tumours were adenocarcinomas, predominantly the signet ring cell subtype
Soda M, Choi, Enomoto M, et al. Identification of the transforming EML4–ALK fusion gene in non-small cell lung cancer. Nature 2007;**448**:561–6	75	6.7%	Landmark paper describing the initial identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. A small inversion within chromosome 2p results in the formation of a fusion gene (EML4–ALK) in (NSCLC) cells. Mouse 3T3 fibroblasts forced to express this fusion tyrosine kinase generated transformed foci in culture and caused subcutaneous tumours in nude mice
Martelli MP, Sozzi G, Hernandez L, Pettirossi Takeuchi K, Choi YL, Soda Makeuchi et al. Clin Cancer Res 2008;14(20):6618–24	European, (Italy and Spain) 120	8%	EML4–ALK rearrangement in non-small cell lung cancer and non-tumour lung tissues. 9 of 120 NSCLC samples (V1 and V3) had EML4–ALK fusion transcripts, but these were not specific for NSCLC as were also found in non-cancerous lung tissues taken far from the tumour. 0 Transcripts were detected in matching tumour samples from these patients. Concluded that EML4–ALK cannot be regarded as specific diagnostic tool for NSCLC
Takeuchi K, Choi YL, Soda Makeuchi et al. Clin Cancer Res 2008;**14**(20):6618–24	Japanese, 656 solid tumours of the lung (364) and 10 other organs	Lung adenocarcinomas (253 cases) 4.35%. 0 EML4–ALK in other types of lung cancer (0/111) or from 10 other organs (0/292)	A multiplex reverse transcription-PCR (RT-PCR) system that captures all in-frame fusions between the two genes in EML4–ALK was developed. Associated with adenocarcinoma

sputum samples from patients with the EML4–ALK subtype and found that it was a potentially useful screening procedure for patients with NSCLC and this molecular subtype. They also used a chemical inhibitor of ALK (WHI-P154) and inhibition of the tyrosine kinase phosphorylation of EML4–ALK occurred.[1,35]

Table 4 – Illustrative examples in cancer of chromosome abnormalities involving tyrosine kinases and transcription factors. Table adapted from: Frohling S, Dohner H. Chromosomal abnormalities in cancer. Cytogenetic abnormalities are a characteristic attribute of cancer cells. N Engl J Med 2008;359:722 [Review article].

Formation of chimeric fusion genes
Involving tyrosine kinases
*t (2; 5)(p23; q35). Gene fusion; ALK–NPM1, disease; Anaplastic large-cell lymphoma in inflammatory myofibroblastic
Inv(2)(p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis
Ma Z, Cools J, Marynen P, et al. 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 2000;**95**(6):2144–9
*TMP3–or TMP4–ALK t (1; 2), CLTC–ALK t (2; 17) and CARS–ALK t (2; 11) i.e. cysteinyl-tRNA synthetase (CARS) gene: in inflammatory myofibroblastic tumours
*EML4–ALK chimeric fusion gene. Inversion within chromosome 2p; non-small cell lung cancer
t (9; 22)(q34.1; q11.23) BCR-ABL1 chronic myeloid leukaemia, acute lymphoblastic (imatinib, dasatinib, nilotinib)
inv(2)(p22-p21p23)§ EML4–ALK non-small-cell lung cancer
t (4; 14)(p16.3; q32.33)§ WHSC1-IGHG1 multiple myeloma
del(4)(q12q12)§ FIP1L1-PDGFRA myeloid neoplasm associated with eosinophilia (imatinib)
t (5; 12)(q31-q32; p13) PDGFRB-ETV6 myeloid neoplasm associated with eosinophilia (imatinib)
episome(9q34.1)§ NUP214-ABL1 Acute lymphoblastic leukaemia (imatinib)
inv(10)(q11.2q11.2)§ RET-NCOA4 papillary thyroid cancer
t (12; 15)(p13; q25) ETV6-NTRK3 various cancers
inv(10)(q11.2q21) RET-CCDC6 papillary thyroid cancer
Involving transcription factors
Some tumour examples:
t (11; 22)(q24.1-q24.3; q12.2) FLI1-EWSR1 Ewing's sarcoma
t (2; 3)(q12-q14;p25) PAX8-PPARG follicular thyroid cancer
t (21; 22)(q22.3; q12.2) ERG-EWSR1 Ewing's sarcoma
1. Debelenko LV, Arthur DC, Pack SD, et al. Identification of CARS–ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumour. Lab Invest 2003;**83**(9):1255–65
2. Imatinib has not been approved for the treatment of myeloid neoplasms associated with eosinophilia and NUP214-ABL1 positive acute lymphoblastic leukaemia, but therapeutic efficacy is predicted on the basis of preclinical studies. The other drugs listed have been approved for treatment of the indicated tumour types. Data, 2008
3. The del(4)(q12q12)§ FIP1L1-PDGFRA chromosomal alteration is cytogenetically invisible
4. Targeted therapeutics are in brackets

This initial study was done in Japan and differing relative frequencies of EGFR and response rates to tyrosine kinase inhibition have been found between Asian and Western populations. Differing frequencies of EML4–ALK fusion gene variants in diverse populations are documented in Table 3. The possibility of ethnic variation of EML4–ALK fusion gene frequency has been systemically studied and large differences have not emerged. A large-scale study using RT-PCR for EML4–ALK mRNA found that it occurred with a frequency of 3.4% (5/149) in adenocarcinomas and a frequency of 0% (0/72) in other lung cancer subgroups. Of the 5 cases with an EML4–ALK fusion three were acinar adenocarcinomas ($P = 0.00018$) and two were mixed type adenocarcinoma histology.[36] A separate study in Hong Kong found a fusion gene frequency of 4.9%.[37] A study on a western population was conducted on specimens of NSCLC. EML4–ALK transcripts were found in lung tumours but also were detected in non-cancerous lung taken remote from the tumour in some patients who had EML4–ALK-negative lung cancers. This study concluded that EML4–ALK was not a specific diagnostic test for NSCLC.[38]

The predictive impact of differing ethnicities on the frequency of EMF4-ALK fusion gene variants has been studied in several populations. No significant ethnic variation in frequencies has emerged. The EML4–ALK fusion gene has been detected in approximately 7% of Japanese patients and 3% in a mixed Caucasian -Asian study.[39] Clinical correlation with adenocarcinomas and never/ light smokers and EML4–ALK do exist however. Some relevant studies and findings are documented below and in Table 2:

In a study from the University of Hong Kong Lung Cancer Study Group of ethnic Chinese patients, 266 resected primary NSCLC cases were investigated for EML4–ALK fusion variants using RT-RCP and sequencing. It was found that 13 cases (4.9%) had EML4–ALK transcription variants. The EML4–ALK fusion gene occurred in mutual exclusion to EGFR and KRAS mutations. Patients with EML4–ALK-positive adenocarcinomas had a young median age $P = (.018)$ and EML4–ALK was associated with non-smokers ($P = .009$) (mm). The following clinic pathological correlations with EML4–ALK fusion were observed.[40]

The derived opinion was that EML4–ALK may be predictive for therapeutic efficacy of ALK inhibitors in non-small cell lung cancer. A separate study of 103 Chinese patients found that 2.9% had the EML4–ALK fusion gene.[41]

In a mixed ethnicity study a US group examined the frequency of EML4–ALK using RT-PCR and exon array analysis in 138 American and 167 Korean patients' ($n = 305$) non-small

Table 5 – Molecular therapeutics and preclinical experiments relevant to ALK and cancer treatment.

PF02341066 (Prizer)	Inhibitor of c-Met/HGFR and the ALK receptor tyrosine kinase 31 NSCLC accrued to date with ALK rearrangement 65% experienced a response (19 PR, 1CR Not approved by FDA). Randomised phase 3 trial initiating in US Data derived from AACR-IASLC joint conference on molecular origins of lung cancer: proscpects for personalised prevention and therapy, Coronado, California, 15th; 2010.
GSK1838705A	Inhibits ALK, with an IC(50) od 0.5 nmol/L and causes complete regression of ALK-dependent tumours *in vivo*. It also is a kinase inhibitor of IGF-IR and the insulin receptor. Initial studies showed minimal effect on glucose homostasis (Ref. mol cancer ther) Note: Type I insulin-like growth factor (IGF-IR) and NPM–ALK were found to have an association and reciprocal functions in ALCL. In T-cell ALK+ anaplastic lymphoma cells IGF-IR interacts with the NPM–ALK oncogene Mol Cancer Ther 2009;**8**(10):2811–20 Sabbatini P, Korenchuk S, Rowand JL, et al. GSK1838705A inhibits the insulin-like growth factor-1 receptor and anaplastic lymphoma kinase and shows antitumour activity in experimental models of human cancers. Mol Cancer Ther 2009;**8**(10):2811–20
Experimental studies PI3K inhibitors wortmannin and LY294002 Role of Akt	PI3K complexes with NPM/ALK. Both PI3K and Akt kinase were permanently activated in NPM/ALK-transfected BaF3 murine hematopoietic cells and in NPM/ALK positive, but not in NPM/ALK-negative, patient-derived anaplastic large cell lymphoma cell lines. The PI3K inhibitors wortmannin and LY294002 induced apoptosis in NPM/ALK+ cells but exerted only minor effects on the control BaF3 parental cells and peripheral blood mononuclear cells stimulated by growth factors Retroviral infection of NPM/ALK+ BaF3 cells with a dominant-negative PI3K mutant (Δp85) or a dominant-negative Akt mutant (K179M) inhibited proliferation and clonogenic properties of the infected cells Conclusion: NPM/ALK constitutively activates the PI3K-Akt pathway Artur Slupianek, Margaret Nieborowska-Skorska, Grazyna Hoser, et al. Role of phosphatidylinositol 3-kinase-akt pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. Cancer Res 2001;**61**:2194–99
Therapeutic inhibition of DNA methyltransferase treatment with TNFα	*Preclinical data*: ALK+ T cell anaplastic lymphoma fails to express TNFα and frequently exhibits DNA methylation of the TNFα gene promoter 14 TNF alpha tissue samples demonstrated some extent of TNFα promoter methylation Methylation of the distal promot exerts more profound inhibitory effects and is more frequent than proximal methylation Zhang Q, Wang HY, Bhutani G, et al. Lack of TNFalpha expression protects anaplastic lymphoma kinase-positive T-cell lymphoma (ALK+ TCL) cells from apoptosis. Proc Natl Acad Sci USA 2009;**106**(37):15843–8
Human single-chain variable fragment (scFv) antibodies that target the ligand-binding domain in ALK	(ALK) and it's ligand PTN (the growth factor pleiotrophin) are both highly expressed during nervous system development and have been implicated in the malignant progression of different forms of cancer A single-chain variable fragment (scFv) antibodies that target the ligand-binding domain (LBD) in ALK was used in laboratory experiments. Conclusion: the emergent findings suggested a rate-limiting function of the PTN/ALK interaction that may be exploited therapeutically scFv and PTN competed to bind to ALK in cells Human glioblastoma cells U87MG invasion of an endothelial cell layer was inhibited by scFv Reversal of growth of tumour xenografts in mice post the induction of the conditional expression of the anti-ALK scFv Stylianou DC, Auf der Maur A, Kodack DP, et al. Effect of single-chain antibody targeting of the ligand-binding domain in the anaplastic lymphoma kinase receptor. Oncogene 2009;**28**(37):3296–306 [Epub 2009 July 27]

cell lung cancers and NSCLC cell lines. EML4–ALK variants were detected in 8 of 305 (3%) of tumours and 3 of 83(3.6%) of cell lines. All clinical cases were adenocarcinomas. One of three EML4–ALK cell lines had *in vitro* and *in vivo* sensitivity to TAE684 an ALK kinase inhibitor suggesting that ALK inhibition may be effective in EML4–ALK inversion NSCLC but only in a subset.[42]

In a US–Australian study 141 cases of NSCLC were enriched for EML4–ALK based on clinical characteristics. Identification of the chimeric EML4–ALK gene was by using fluorescent in situ hybridisation for ALK rearrangements and the ALK gene product was identified by immunohistochemistry. Of the cases examined, 13% were EML4–ALK mutant, 22% were EGFR mutant and 65% were wild type for ALK and EGFR. In a comparative analysis of the subdivided patient cohorts, patients with EML4–ALK mutant neoplasms were younger ($P < .001$ and $P = .005$), more likely to be men ($P = .036$ and $P = .039$) and cases that were EML4–ALK positive were more likely to be never/light smokers compared with patients in the WT/WT cohort ($P < .001$). Eighteen of the 19 EML4–ALK tumours were adenocarcinomas, predominantly the signet ring cell subtype.[43]

The EML4–ALK fusion gene appears to be unique to NSCLC. EML4–ALK has mutual exclusivity with KRAS and EGFR

mutations.[37] The chimeric fusion gene is an inversion in the short arm of chromosome 2 (chromosome 2p21 and chromosome 2p23). Seven variants have been identified V1–V7. All require the intracellular tyrosine kinase domain of ALK starting at a portion encoded by exon 20. The EML4 is variably truncated (portions encoded by exons 2,6,13,14,15,18 and 20.[44] EML4–ALK fusions have also been associated with adenocarcinomas with acinar histology.[36,42]

6. ALK-directed therapeutics

Therapeutics directed at the chimeric fusion gene: small molecules RNA inhibition and tumour vaccination.

ALK-directed therapeutics means that ALK inhibition may be used to effectively treat cancers emerging from differing tissues. In ALK+ anaplastic large cell lymphoma, three molecularly targeted treatment approaches emerge. Firstly, like the inhibition of BCR-ABL using imatinib mesylate in chronic myeloid leukaemia, small molecule inhibition of the fusion gene NPM–ALK can downregulate constitutive ALK activity. Secondly, RNA inhibition is a strategy that has been used by investigators *in vitro*. Targeting of the intracytoplasmic region of mouse ALK with short hairpin RNA caused reversion of the tumour phenotype. This also occurred using a lentiviral ALK knockdown model in human ALCL cells.[45] Finally ALK-positive lymphoma is a model disease system for a tumour vaccination strategy as ALK expression is mainly restricted to the immunoprivelaged nervous system and it expresses immunogenic epitopes that can be recognised by cytotoxic T lymphocytes.[46]

The recognition of the EML4–ALK genotype in non-small cell lung cancer and its mutual exclusivity from EGFR mutated non-small cell lung cancer are an important potential therapeutic advance. Soda et al in a mouse model for EML4–ALK-positive non-small cell lung cancer found that all transgenic mice that expressed EML4–ALK specifically in alveolar epithelial cells developed hundreds of adenocarcinoma nodules within weeks of birth. Treatment with an oral inhibitor of ALK activity caused rapid disappearance but not complete microscopic elimination of the tumours confirming the oncogenic activity of the fusion kinase arising from the recurrent chromosome inversion inv (2)(p21p23).[47] In neuroblastoma investigators have used short hairpin-mediated knockdown of ALK-mutated neuroblastoma cell lines.[3–5] ALK-mutated neuroblastoma cell lines were sensitive to treatment with the small molecule inhibitor TAE684 and ALK-mutated neuroblastoma is a potential therapeutic target for tyrosine kinase inhibitors. Molecular therapeutics and preclinical experiments relevant to ALK and cancer treatment are documented in Table 5.

7. Potential synergistic therapeutics

Therapeutic strategies directed against ALK but not specific for it have emergent possible therapeutic benefits in cancer treatment. These include IGF-IR interaction with NPM–ALK, GSK 1838705A, an inhibitor of IGF-IR, the insulin receptor and ALK and PF-02341066 an inhibitor of c-Met/HGFR and the ALK receptor tyrosine kinase. Also pre-clinical data suggest an expansion of ALK-directed therapeutics.

7.1. *IGF-IR/IGF-1: pre-clinical evidence*

In 2008 type I insulin-like growth factor (IGF-IR) and NPM–ALK were found to have an association and reciprocal functions in ALCL. Co-investigators at MD Anderson and the University of Alberta, Edmonton have found that in T-cell ALK+ anaplastic lymphoma cells IGF-IR interacts with the NPM–ALK oncogene.[48] A correlative example in chronic myeloid leukaemia is the CML blast crises as BCR-ABL induces autocrine IGF-1 signalling using Hck and Stat5b. Inhibition of Hck and Stat5b using shRNA or small molecule drugs decreased proliferation and enhanced apoptosis.[49] Synergistic therapeutics directed against ICF-IR/IGF-I and NPM–ALK are conceptually attractive based on preclinical evidence. IGF-IR and IGF-I are expressed in ALK+ALCL primary tumours. In the experiments by Shi et al. on 5 ALK+ ALCL cell lines (Kappa 299, SU-DHL, SUP-M2, SR-786 and DEL) only the cell line Kappa 299 lacked IGF-I protein expression.[48] Preliminary experimental evidence suggests that IGF-IR may also have a role in T cell lymphomas that lack expression of the chimeric oncogene NPM–ALK.

7.2. *GSK 1838705A, an inhibitor of IGF-IR, insulin receptor and ALK*

In experimental models of human cancer GSK 1838705A a small molecule kinase inhibitor of IGF-IR, the insulin receptor and ALK demonstrated experimental evidence of antitumour activity. GSK1838705A has been found to block the *in vitro* proliferation of cell lines derived from haematological malignancies, including multiple myeloma, solid tumours including Ewing's sarcoma, and retards the *in vivo* growth of human tumour xenografts. GSK1838705A inhibits ALK, with an IC(50) of 0.5 nmol/L, and causes complete regression of ALK-dependent tumours *in vivo* at well-tolerated doses. It is emerging as new molecular therapeutic advance for cancer treatment.[50]

7.3. *Phase 1 study: MET and ALK*

A new compound PF-02341066 is a selective, ATP-competitive, small molecule oral inhibitor of c-Met/HGFR and the ALK receptor tyrosine kinase. A Phase 1 dose escalating study was performed on patients with advanced cancer other than leukaemia. 37 patients were accrued into the dose escalation part of the study. 10 patients subsequently entered an enriched RP2D cohort of patient's with tumours harbouring c-Met amplification/gene mutation or ALK fusion genes. Of 10 NSCLC patients with EML4–ALK rearrangement, 1 patient had a partial response (PR), 2 patients have achieved unconfirmed PR and 4 patients have had stable disease.[44,51]

Further details of this rapidly expanding field are documented in the table attached.

8. 'ALKoma' a new member of haemato-oncologic molecular disease

Like other receptor tyrosine kinases, ALK may be activated by chromosomal translocations, genomic amplification or point mutations.[52] Chromosomal translocations appear to be the most frequent genomic abnormality involving the ALK gene.

Table 6 – ALK chromosomal fusion partners, chromosomal rearrangements or mutations in pathologic disorders.

ALK fusion/mutation	Neoplasm
1. NPM–ALK t (2; 5)(p23; q35). Gene fusion	Approx 80% of anaplastic large-cell lymphoma
2. ALK Inv(2)(p23q35)*; induces constitutive kinases anaplastic lymphoma kinase tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis	Potentially, 20% of anaplastic lymphoma i.e. those lacking NPM–ALK gene fusion
Ma Z, Cools J, Marynen P, et al. 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 2000;**95**(6):2144–9	
3. Tropomyosin (TMP3–ALK) t (1; 2)(p25; p23) crytic rearrangement	
4. Tropomyosin (TMP4–ALK) t (2; 19)(p23; p13.1) TMP3–ALK and TMP4–ALK oncogenes in inflammatory myofibroblastic tumours. Am J Pathol 2000;**157(**2):377–84	Inflammatory myofibroblastic tumours
5. CLTC–ALK t (2; 17)	
6. CARS–ALK t (2; 11) i.e. cysteinyl-tRNA synthetase (CARS) gene	
7. EML4–ALK chimeric fusion gene	Non-small cell lung cancer
8. ALK germline mutations Also some neuroblastomas occur with congenital malformations of neural crest and germline mutation of PHOX2B (note; developmental role of ALK in neural crest)	Hereditary neuroblastomas[24]
Allouche M. ALK is a novel dependence receptor: potential implications in development and cancer. Cell Cycle 2007;**6**(13):1533–8 [Epub 2007 May 14, Review]	
9. Activating somatically acquired Mutations in ALK,in one high-risk series 12.4% In a series of neuroblastomas ALK gene copy number was increased On sequence analysis of DNA from neuroblastomas ALK mutations were mainly clustered at 2 mutation hotspots at the kinase domain ALK locus is centromeric to the MYCN locus Myc amplification occurs frequently in neuroblastomas In a series 5 non-synonymous sequence variations were identified in the kinase domain of ALK, the most frequent mutation was F1174L	Neuroblastomas (linkage signal 2p23-24)[25–27]
Listing emphasises the main relevant principles and is not intended to be a full comprehensive review of all known possibilities which are extensive	

Fusion gene products in haematological disease have been recognised for decades with the finding of the Philadelphia chromosome by Nowell and Hungerford in 1960 and the description of the chromosome (9:22) BCR-ABL translocation by Rowley in 1973.[53,54] This became therapeutically important when in a comparator crossover design study (The IRIS Study) imatinib mesylate an inhibitor of constitutive BCR-ABL tyrosine kinase activity was compared to interferon and low dose cytarabine with respective rates of complete cytogenetic response of 76.2% and 14.5% after a median follow-up of 19 months.[55] Similarly acute myeloid leukaemia has translocations including t (8; 21) (q22; 22), t (15; 17) (q22; q12) and inv (16) (p13q22).[56] The apparent frequent description of translocations in haematological malignancies and their infrequent description in solid cancers do not mean that there are differing proportions. Mitelman et al have found that that there may not be tissue specific differences in fusion and rearranged genes. Epithelial tumours may be caused by numerous chromosomal translocations that are individually rare but cumulatively frequent in solid cancers. Furthermore, important recurring fusion genes in solid cancers may not have been found to date. They found a linear correlation between the number of fusion genes, balanced chromosomal translocations and rearranged genes with the number of reported cases of chromosomal abnormalities in different cancers.[57] This analysis may be confirmed as the list of solid malignancies with recognised fusion genes is lengthening. The TMPRSS2-ERG and TMPRSS2-ETV fusion genes have recently been recognised in prostate cancer.[58,59]

Solid tumours with fusion chromosomal translocations and corresponding molecular lesions include Ewing's sarcoma; t (11:22) EWS-FLI1, t (21; 22) EWS-ERG, t (7; 22), EWS-ETV1 and alveolar rhabdomyoscarcoma; t (2:13) PAX3-FKHR and t (1; 13) PAX7-FKHR.Other tumours with chromosomal rearrangements include synovial sarcoma t (X: 18) SYT-SSX1/SYT-SSX2 and malignant melanoma of soft parts t (12; 22) EWS-ATF1[60] (Table 6 also documents ALK chromosomal fusion partners). Soda et al.'s discovery of an EML4–ALK fusion gene in

non-small cell lung cancer adds a further fusion gene to the list. ALK abnormalities including fusion genes occur in disparate cancers lead to the recognition of the phenomenon ALKoma and the potential that differing cancers will be sensitive to treatment with ALK inhibitors.[61] Examples of chromosome abnormalities involving tyrosine kinase and transcription factors including the ALK gene are listed in Table 4.

Amplification of the ALK gene has been found to occur in neuroblastoma. This is similar to an internal tandem duplication of the tyrosine kinase gene Flt 3 that occurs in acute myeloid leukaemia. FLT3 mutations confer an adverse prognosis in AML. Similarly in a representative set of 491 sporadically occurring primary neuroblastomas, ALK amplification or gain was associated with an aggressive clinical phenotype including metastasis ($P < 0.0001$) and death ($P = 0.0003$).[2] Amplification of the Her-2 gene in a subset of breast cancers also confers a more aggressive natural history.

Finally, ALK may undergo auto-activating mutations that confer constitutive activity in the absence of a ligand. In neuroblastoma, this is most frequently phenylalanine 1174. Mutations occur in mutation hotspots and are most frequent in the kinase domain of the ALK gene. Neuroblastoma is now a member of a group of tumours that may arise from biallelic oncogene activation by activating mutations, or amplification of ALK. Similar oncogene activation occurs in papillary carcinoma of the kidney. The molecular sequence in that instance is that the MET gene undergoes auto activating amino acid substitutions possible duplication of chromosome 7 which contains the MET locus and later acquisition of additional mutations leading to papillary carcinoma of the kidney.[62] A further correlate would be the development of medullary thyroid cancer due to mutations in RET either a germline mutation in MEN/familial medullary thyroid cancer with a subsequent sporadic mutation or the sequential acquisition of bialleic sporadic mutations in sporadic cases.

It is to be remembered that increased cancer aetiologies are being recognised both in molecular pathogenesis and in environmental triggers. For example in a recent case controlled study a potential association between silicone breast implants and anaplastic large cell lymphoma was noted by de Jong et al.[63] of The Netherlands Cancer Institute, but a consensus of opinion is yet to emerge.[64]

The diseases that have ALK abnormalities are expanding and cancer treatment is moving from a tissue of origin-designated disorder to a molecular disorder. Reclassification of disease allows us to group once disparate cancers such as those described into disorders of shared molecular dysfunction such as 'ALKoma'. The therapeutic potential is of manifest possibilities that will benefit patients in years to come.

Conflict of interest statement

None declared.

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