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REVIEW

ALK: a tyrosine kinase target for cancer therapy

Vijaykumar R. Holla,[1] Yasir Y. Elamin,[2] Ann Marie Bailey,[1] Amber M. Johnson,[1] Beate C. Litzenburger,[1] Yekaterina B. Khotskaya,[1] Nora S. Sanchez,[1] Jia Zeng,[1] Md Abu Shufean,[1] Kenna R. Shaw,[1] John Mendelsohn,[1,3] Gordon B. Mills,[1,4] Funda Meric-Bernstam,[1,5,6] and George R. Simon[2]

[1]Sheikh Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA; [2]Department of Thoracic/Head and Neck, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA; [3]Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA; [4]Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA; [5]Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA; [6]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Abstract The anaplastic lymphoma kinase (*ALK*) gene plays an important physiologic role in the development of the brain and can be oncogenically altered in several malignancies, including non-small-cell lung cancer (NSCLC) and anaplastic large cell lymphomas (ALCL). Most prevalent *ALK* alterations are chromosomal rearrangements resulting in fusion genes, as seen in ALCL and NSCLC. In other tumors, *ALK* copy-number gains and activating *ALK* mutations have been described. Dramatic and often prolonged responses are seen in patients with *ALK* alterations when treated with ALK inhibitors. Three of these—crizotinib, ceritinib, and alectinib—are now FDA approved for the treatment of metastatic NSCLC positive for *ALK* fusions. However, the emergence of resistance is universal. Newer ALK inhibitors and other targeting strategies are being developed to counteract the newly emergent mechanism(s) of ALK inhibitor resistance. This review outlines the recent developments in our understanding and treatment of tumors with *ALK* alterations.

Corresponding author: gsimon@mdanderson.org

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INTRODUCTION

In 1994, a positional cloning strategy revealed a unique rearrangement resulting from the fusion of the nucleophosmin (*NPM1*) gene, located on 5q35, to a previously unidentified protein tyrosine kinase gene located on 2p23 in an anaplastic large-cell lymphoma (ALCL) cell line (Morris et al. 1994). This new protein, called anaplastic lymphoma kinase (ALK), is expressed normally in the brain, small intestine, and testis, but not in the normal lymphoid cells (Morris et al. 1994). *ALK* shows the greatest sequence similarity to the insulin receptor subfamily of transmembrane tyrosine kinases. ALK contains an extracellular domain that has a low-density lipoprotein receptor domain class A (LDLa) region sandwiched between two meprin, A-5 protein, multiple receptor protein-tyrosine phosphatase mu (MAM) regions, followed by a glycine-rich region, a transmembrane region, and an intracellular domain containing a tyrosine kinase region. ALK is considered an orphan receptor, even though pleiotrophin (PTN) and midkine (MDK), both secreted growth factors, are known to bind and activate ALK downstream signaling (Stoica et al. 2001, 2002; Lu et al. 2005). PTN binding

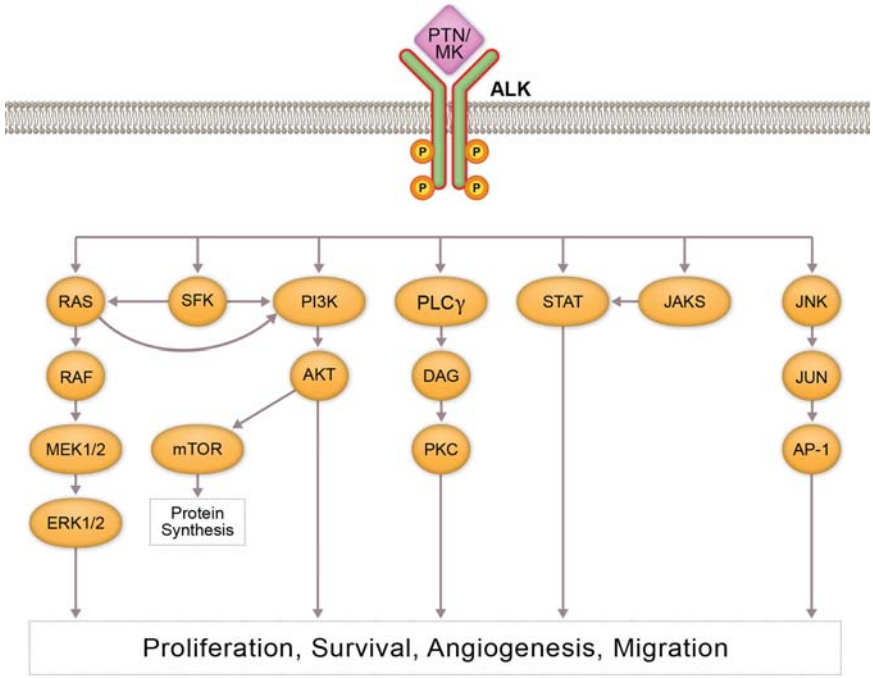

Figure 1. Anaplastic lymphoma kinase (ALK) signaling: Pathway figure that depicts normal activation of ALK through ligand binding. Receptor phosphorylation stimulates downstream signaling through the PI3K/AKT, MAPK/ERK, and STAT3 pathways. These signaling cascades can contribute to increased cancer cell growth, survival, and metastasis.

to ALK activates the mitogen-activated protein kinase (MAPK) pathway, whereas MDK binding to ALK induces insulin receptor substrate 1 (IRS1) phosphorylation, resulting in MAPK and phosphoinositide 3-kinase (PI3K) activation (Fig. 1; Bowden et al. 2002; Powers et al. 2002; Kuo et al. 2007; Palmer et al. 2009). However, studies that followed showed either no ligand binding activity of PTN and MDK to ALK (Moog-Lutz et al. 2005; Mathivet et al. 2007) or that PTN's effect on ALK is not due to direct binding (Perez-Pinera et al. 2007). Furthermore, a recent study shows longer heparin chains induce ALK dimerization, activation, and downstream signaling, indicating heparin serves as ALK's ligand or coligand (Murray et al. 2015).

ALK is thought to play a significant role in the development and function of the nervous system, where it controls the basic mechanisms of cell proliferation, survival, and differentiation in response to extracellular stimuli (Iwahara et al. 1997; Yao et al. 2013). The role of ALK in the development of model organisms, such as *Drosophila*, *Caenorhabditis elegans*, and zebrafish, has been well documented (Palmer et al. 2009). In *Drosophila*, binding of a secreted protein ligand jelly belly (Jeb) activates dALK during synaptogenesis and organization of the visceral musculature of the gut (Loren et al. 2003; Rohrbough and Broadie 2010). In *C. elegans*, ALK homolog, suppressor of constitutive dauer formation 2 (SCD-2) signals through the ligand hesitation behavior 1 (HEN-1) during neuromuscular junction development and regulates the dauer response to environmental stress (Liao et al. 2004; Reiner et al. 2008). In zebrafish, leukocyte tyrosine kinase (Ltk), closely related to ALK, is required for the establishment of iridophores and mutations in *ltk* show defects in pigmentation patterns (Lopes et al. 2008).

The most prevalent genomic *ALK* aberrations in human cancer are chromosomal rearrangements, resulting in fusion genes. *ALK* fusions arise from fusion of the 3′ half of ALK, derived from Chromosome 2 that retains its kinase catalytic domain, and the 5′ portion of a different gene that provides its promoter. Multiple different 5′ partners have been identified. Wild-type ALK is normally activated through binding of ligands to its extracellular domain, resulting in dimerization and autophosphorylation of the kinase domain. Structural studies show that fusion with multiple 5′ partners helps bypass this requirement and increase oncogenic potential of ALK, as evidenced by *NPM1-ALK* (Fujimoto et al. 1996) and *EML4-ALK* (Wang et al. 2015) in non-small-cell lung cancer (NSCLC). Increased copy number and the presence of activating point mutations that result in kinase activation are also linked to oncogenic activity of *ALK*. These genetic alterations are found in multiple malignancies, including, but not limited to, lung cancer, neuroblastoma, rhabdomyosarcoma, renal cell carcinoma, inflammatory myofibroblastic tumor (IMT), and inflammatory breast cancer (Webb et al. 2009; Kelleher and McDermott 2010). Additionally, a recent report describes an alternative transcription initiation site leads to the detection of an oncogenic *ALK* isoform (ALK^{ATI}) in 11% of melanomas and other tumor types (Wiesner et al. 2015). ALK^{ATI} arises independently of other *ALK* genomic alterations, and in vivo and in vitro studies show that ALK^{ATI}-driven tumors are sensitive to crizotinib. Although activating mutations and copy-number changes of the *ALK* gene are currently being investigated for their role in tumor development and treatment response, the main clinical therapeutic implications of ALK lie in targeting of *ALK* fusions with tyrosine kinase inhibitors. In general, *ALK* fusions are mutually exclusive with mutations in *EGFR, KRAS*, and *ERBB2* genes, indicative of these genes' signaling through similar downstream pathways (Takahashi et al. 2010).

ALK MUTATIONS

Gain-of-function mutations of *ALK* are described primarily in neuroblastoma. In addition, thyroid (Murugan and Xing 2011) and lung cancers (Wang et al. 2011) have been shown to carry activating *ALK* point mutations. Most of the mutations are located in the kinase domain, including two hotspot mutations: F1174 (mutated to C, I, L, S, or V) and R1275 (mutated to Q and L) (Franco et al. 2013). These two hotspot mutations represent 85% of all *ALK* mutations. All reported *ALK* mutations could be classified into three groups: ligand-independent mutations (F1174I, F1174S, F1174L, and R1275Q), ligand-dependent mutations (D1091N, T1151M, and A1234T), and a kinase-dead mutation (I1250T) (Hallberg and Palmer 2013). *ALK* mutations are frequently acquired within an *ALK* fusion gene as a result of crizotinib resistance (Choi et al. 2010), alectinib resistance (Kodama et al. 2014) in NSCLC, and lorlatinib resistance in ALCL (Mologni et al. 2015). Figure 2 and Table 1 describe functionally characterized *ALK* mutations in the literature.

ALK Mutations in Neuroblastoma

Gain-of-function mutations are reported in both familial and sporadic neuroblastoma patients (George et al. 2008; Janoueix-Lerosey et al. 2008). In familial neuroblastoma, *ALK* is a predisposition gene, and germline mutations have been found in 50% of familial neuroblastoma cases (Mosse et al. 2008). Most frequent gain-of-function germline mutations of *ALK* are G1128A, R1192P, and R1275Q (Janoueix-Lerosey et al. 2008; Mosse et al. 2008). However, in sporadic neuroblastoma, only 7% of cases show activating *ALK* mutations (Mosse et al. 2008). Two hotspot mutations, F1174L and R1275Q, lead to ALK autophosphorylation and cytokine-independent growth (Chen et al. 2008; Janoueix-Lerosey et al. 2008). Furthermore, F1174L, the most recurrent mutation, predominantly occurs in *MYCN*-amplified tumors and potentiates *MYCN* oncogenic activity in neuroblastoma

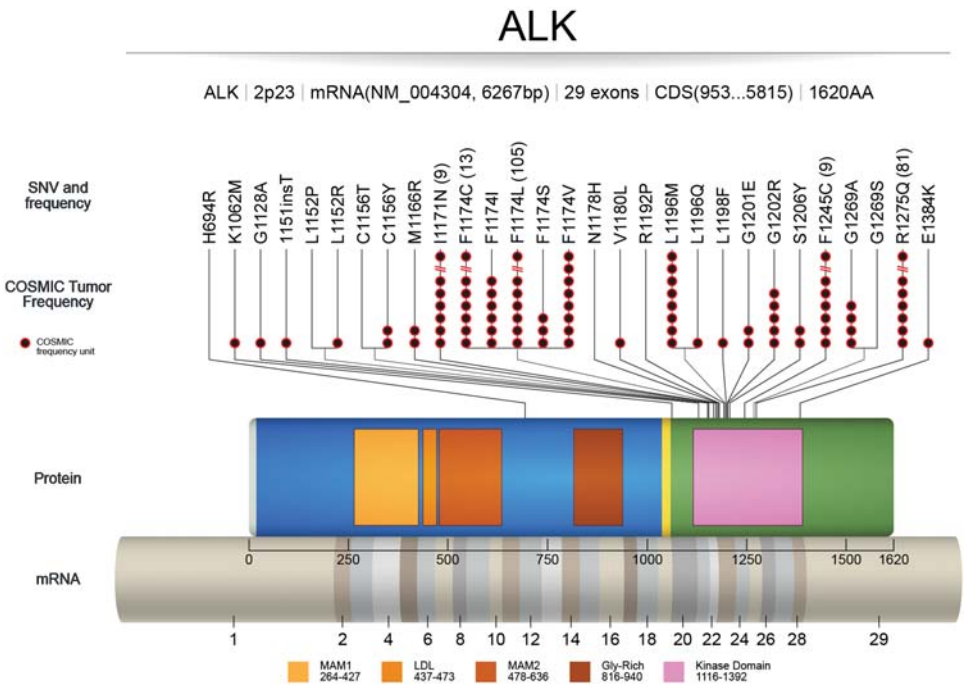

Figure 2. Activating mutations in *ALK:* COSMIC (tumor only) frequencies of *ALK* mutations with published literature on functional and/or therapeutic significance. mRNA sequence depicts full reference sequence of *ALK* (NM_004304) with exon numbers marked. ALK protein sequence (0–1620 amino acids) shows different functional domains (MAM1, LDL, MAM2, Gly-rich, and kinase domain) with starting and ending amino acid numbers (UniProt). COSMIC frequency units (black circles and numbers in parentheses) refer to the number of tumor samples with a particular single-nucleotide variant (SNV) found in COSMIC. The SNVs without the black circles are referenced in literature that is not recorded in COSMIC.

(De Brouwer et al. 2010; Berry et al. 2012). In vitro and in vivo studies with cell lines expressing *ALK* mutations show different sensitivity to ALK inhibitors. Tumors expressing the R1275Q mutation are sensitive to crizotinib and TAE684, whereas F1174L mutant cells exhibit sensitivity to these agents only at higher doses (George et al. 2008; Bresler et al. 2011).

ALK GENE REARRANGEMENTS

NPM1 was the first described fusion partner of *ALK* in 1994 in an ALCL cell line with a t(2;5) chromosomal rearrangement (Morris et al. 1994). Since then, several other *ALK* fusion partners have been described in multiple malignancies (Table 2). The role of *ALK* gene rearrangements as oncogenic drivers has been well established in preclinical models including transgenic mouse models (Solomon et al. 2009; Chen et al. 2010). In these models, ALK promotes the activation of downstream signaling pathways and other crucial aspects of malignant phenotypes like uncontrolled cellular proliferation and survival. The precise mechanisms that underlie the development of *ALK* gene rearrangements have yet to be elucidated. However, several steps are in play: the generation of double-strand DNA breaks, aberrant joining of the DNA ends, and selection of gene rearrangements that confer a survival advantage (Bunting and Nussenzweig 2013; Shaw and Engelman 2013). In general, different *ALK* fusion partners affect ALK homodimerization, as well as ALK signaling potential.

Table 1. List of *ALK* mutations known to be oncogenic

Alteration	Location	Functional significance	Tumor type	Reference(s)
H694R	Extracellular	Increased phosphorylation, promotes tumors in mice		Wang et al. 2011
K1062M	Juxtamembrane	Transforms cells, promotes tumors in mice	Neuroblastoma	Chen et al. 2008; Murugan and Xing 2011
G1128A	Kinase domain, glycine-rich region	Increased phosphorylation	Neuroblastoma	Mosse et al. 2008; Bossi et al. 2010; Schonherr et al. 2011
M1166R	Kinase domain	Increased phosphorylation	Neuroblastoma	Mosse et al. 2008; Chand et al. 2013
F1174I	Kinase domain	Increased phosphorylation	Neuroblastoma	Chand et al. 2013; Mologni et al. 2015
F1174L	Kinase domain	Disrupts auto-inhibitory function	Neuroblastoma	Chen et al. 2008; George et al. 2008; De Brouwer et al. 2010; Sasaki et al. 2010; Bresler et al. 2011; Heuckmann et al. 2011; Kodama et al. 2014; Infarinato et al. 2015; Zou et al. 2015
F1174S	Kinase domain	Ligand-independent activity	Neuroblastoma	Martinsson et al. 2011
L1198F	Kinase domain	Increased phosphorylation	Thyroid	Murugan and Xing 2011
G1201E	Kinase domain	Increased phosphorylation	Thyroid, Skin	Murugan and Xing 2011
F1245C	Kinase domain	Increased phosphorylation	Neuroblastoma	Schonherr et al. 2011; Infarinato et al. 2015
R1275Q	Kinase domain	Disrupts auto-inhibitory function	Neuroblastoma	Chen et al. 2008; George et al. 2008; De Brouwer et al. 2010; Bagci et al. 2012; Carpenter and Mosse 2012
E1384K	Kinase domain	Increased phosphorylation	Cervix	Wang et al. 2011

In fact, the comparison of a number of ALK fusion proteins has suggested differences in transforming and tumorigenic potential (Bridge et al. 2001; Cools et al. 2002).

ALK Gene Rearrangements in Different Tumor Types

In the majority of ALCL cases, ALK is activated through chromosomal rearrangement (Medeiros and Elenitoba-Johnson 2007). ALCL is a type of T-cell non-Hodgkin's lymphoma, and abnormalities of the *ALK* gene are common in this disease (Medeiros and Elenitoba-Johnson 2007). As many as 50% of all adult cases of ALCL are ALK-positive, and up to 90% of all pediatric ALCL patients are ALK-positive (Gustafson et al. 2009; Damm-Welk et al. 2015). The most frequent translocation in ALCL is *NPM1-ALK*, accounting for ~75%–80% of all ALK-positive ALCL (Pulford et al. 2004). Additionally, *TPM3-ALK*

Table 2. Known *ALK* chromosomal translocations that activate the tyrosine kinase domain

Fusion	Chromosomal aberration	Tumor types	Reference(s)
ATIC-ALK	Inv(2)(p23;q35)	ALCL	Colleoni et al. 2000; Armstrong et al. 2004
CAD-ALK	Inv(2)(p23;p22)	CRC	Amatu et al. 2015
CLTC-ALK	t(2;17)(p23;q23)	IMT, ALCL, BCL	Bridge et al. 2001; Cools et al. 2002; De Paepe et al. 2003; McManus et al. 2004; Cerchietti et al. 2011
DCTN1-ALK	t(2;12)(p23;q11)	IMT	Wang et al. 2012; Wiesner et al. 2014
EML4-ALK	inv(2)(p21;p23)	NSCLC	Soda et al. 2007; Choi et al. 2008; Lin et al. 2009; Li et al. 2011
FN1-ALK	t(2)(p23;q34)	Ovarian, IMT	Ren et al. 2012; Ouchi et al. 2015
HIP1-ALK	t(2;7)(p23;q11.23)	NSCLC	Fang et al. 2014; Hong et al. 2014; Ou et al. 2014
KIF5B-ALK	t(2;10)(p23;p11)	Lung	Takeuchi et al. 2009
KLC1-ALK	t(2;14)(p23;q32.3)	Lung	Togashi et al. 2012
NPM1-ALK	t(2;5)(p23;q35)	NHL, ALCL	Morris et al. 1994; Pulford et al. 2004; Palmer et al. 2009
RANBP2-ALK	Inv(2)(p23;q11–13)	IMT	Ma et al. 2003
SQSTM1-ALK	t(2;5)(p23;q35)	BCL	Takeuchi et al. 2011; d'Amore et al. 2013
STRN-ALK	t(2)(p23;p22.2)	Thyroid	Kelly et al. 2014
TFG-ALK	t(2;3)(p23;q21)	ALCL	Hernandez et al. 1999; Hernandez et al. 2002
TPM3-ALK	t(2;1)(p23;q25)	ALCL, IMT	Lamant et al. 1999; Lawrence et al. 2000; Armstrong et al. 2004
TPM4-ALK	t(2;19)(p23;q13.1)	ALCL, IMT	Lawrence et al. 2000; Meech et al. 2001

ALCL, anaplastic large-cell lymphoma; CRC, colorectal carcinoma; IMT, inflammatory myofibroblastic tumor; BCL, B-cell lymphoma; NSCLC, non-small-cell lung cancer; NHL, non-Hodgkin's lymphoma.

has been found in 12%–18% of ALCL. Other fusion proteins are found at much lower frequency (<2%), and include *TFG-ALK*, *CLTC1-ALK*, and *ATIC-ALK*. Importantly, 5-yr survival for ALK-positive ALCL patients is 70%–80%, as compared with 15%–45% for ALK-negative ALCL patients (Roskoski 2013). In lung cancer, the *EML4-ALK* fusion was identified in 2007 NSCLCs in patients within NSCLC (Soda et al. 2007) and occurs at a frequency of ~6.7% (Perner et al. 2008). This fusion is important diagnostically, as *EML4-ALK* is mutually exclusive with *EGFR* and *KRAS* mutations (Horn and Pao 2009). Moreover, *ALK* rearrangements with constitutive kinase activity occur in 2%–7% of all NSCLCs, and are associated with young age, male gender, and no or light smoking history (Shaw et al. 2009; Kwak et al. 2010). More than a dozen different variants of *EML4-ALK* have been identified in NSCLC. Other less frequent ALK fusions identified in lung cancer are *SEC31A-ALK*, *HIP1-ALK*, *KIF5B-ALK*, and *KLC1-ALK*.

Inflammatory myofibroblastic tumors (IMTs) are soft-tissue mesenchymal neoplasms. Fifty percent of IMTs show chromosomal translocations involving the 2p23 region, resulting in *TPM3/4-ALK* fusions (Griffin et al. 1999). Several other *ALK* fusion partners have been identified in IMT with <5% frequency, including *TPM4* (Griffin et al. 1999; Lawrence et al. 2000), *CLTC* (Bridge et al. 2001), *CARS* (Cools et al. 2002), and *RANBP2* (Ma et al. 2003). *ALK* fusions are associated with better prognosis in IMT (Chun et al. 2005). In thyroid cancer, translocations involving *ALK* are detected in 2.2% of papillary thyroid cancer (PTC) patients (Chou et al. 2015). Various *ALK* fusions (*EML4-ALK*, *GFPT1-ALK*, *TFG-ALK*, and *STRN-ALK*) are reported in thyroid cancer patient tumors (Kelly et al. 2014; Ji et al. 2015). Translocations

of *ALK* identified by fluorescence in situ hybridization (FISH) show strong immunohistochemistry (IHC) positivity in these tumors.

In addition, *ALK* rearrangements occur in 10% of spitzoid tumors with *DCTN1-ALK* and *TPM3-ALK* being the most common (Busam et al. 2014; Wiesner et al. 2014). IHC confirms the expression of chimeric ALK protein, followed by downstream activation of AKT, ERK, and S6 proteins. Crizotinib is able to block these *ALK* fusion-induced downstream activities.

ALK COPY-NUMBER VARIANTS

ALK gene amplification has been detected in variety of tumors (http://cancergenome.nih.gov/). In some reports, *ALK* gene amplification or increase in copy number due to polysomy of Chromosome 2 does not always correspond to the overexpression of ALK protein or increased downstream signaling. However, copy-number gain in NSCLC cell lines is associated with increased sensitivity to ALK inhibitors, such as crizotinib (Miyake et al. 2002).

ALK Copy-Number Variants in Different Tumor Types

Neuroblastoma cell lines and primary neuroblastoma tissues show *ALK* gene amplification that correlates with ALK protein overexpression and promotion of tumorigenesis in neuroblastoma (Miyake et al. 2002; Osajima-Hakomori et al. 2005). Constitutively active ALK, due to gene amplification, results in hyper-phosphorylation of downstream SHCC protein in neuroblastoma cell lines (Miyake et al. 2002). A follow-up in vitro study revealed phosphorylation of SHCC and other downstream activities (MAPK) were suppressed, which was associated with induction of apoptosis in neuroblastoma cell lines upon siRNA-mediated down-regulation of amplified *ALK*, suggesting targeting of ALK may be an appropriate therapeutic approach for tumors with *ALK* amplification (Osajima-Hakomori et al. 2005). Likewise, *ALK* gene amplification was detected in 9.4% (8/85) of primary neuroblastoma tissues (Osajima-Hakomori et al. 2005). FISH analysis showed *ALK* copy-number increase is a recurrent genetic event in neuroblastic tumors (39.1%, 96/245), however *ALK* gene amplification was seen at a lower frequency (1.2%, 3/246) in neuroblastomas (Wang et al. 2013). ALK protein expression by IHC (50.5%, 51/101) in these tumors is associated with a worsened patient prognosis.

A 2011 report revealed that a little more than 10% (11/107) of NSCLC patients exhibit *ALK* amplification and 63% (68/110) had copy-number gains, although it was observed in a small percentage of cells and was not associated with increased tissue expression of ALK, nor was it thought to be a significant tumor driving event for tumors (Salido et al. 2011). Another report characterized 191 patient samples and found 11% to have at least six copies of *ALK* (Khadija et al. 2012, *ASCO abstract #10556*). In the same study, >70% of NSCLC cell lines that gained three or four *ALK* copies also showed crizotinib sensitivity.

Amplification of *ALK* has been detected in ~11% of esophageal cancer (Schoppmann et al. 2013). However, there was no association of *ALK* amplification to either ALK protein expression or downstream phospho-STAT3 expression in these tumors. Copy-number gain of *ALK* was detected in 3.4% (26/756) (Bavi et al. 2013) and 37% (25/68) (Pietrantonio et al. 2014) of colorectal cancer patients. Copy-number increase does not correlate with protein expression in the above studies, but was associated with poor prognosis and may predict lack of benefit from anti-EGFR treatment in colorectal cancer patients. *ALK* copy number increases due to polysomy of Chromosome 2 or its gene amplification has been reported in multiple breast cancer studies and correlates with poor prognosis. However, in most of the cases this does not correlate with increased

protein expression as measured by IHC. Studies include amplification of *ALK* in 13.3% (130/980) of breast cancer patients (Siraj et al. 2015), copy-number gain of *ALK* in 62% (82/133) of breast cancer cases (Hanna et al. 2015), and copy-number gain of *ALK* in 47.2% (17/36) of inflammatory breast cancer (Kim et al. 2015). Mild increase in *ALK* copy number due to Chromosome 2 aneuploidy is reported in 64% (16/25) of inflammatory breast cancer cases (Krishnamurthy et al. 2013). However, there was no increased ALK mRNA or protein expression in these tumors. Increased copy number, correlating with high ALK protein expression, has been reported in 25% of rhabdomyosarcoma cases and is associated with poor prognosis in rhabdomyosarcoma patients (van Gaal et al. 2012; Yoshida et al. 2013; Lee et al. 2014). In vitro studies show antitumor activity with ALK inhibitors in ALK-positive rhabdomyosarcoma cell lines (van Gaal et al. 2013; Megiorni et al. 2015).

THERAPEUTIC IMPLICATIONS

Therapeutic implications for *ALK* gene alterations are predominantly associated with *ALK* gene fusions, which predict tumor response to ALK inhibitors. Crizotinib, ceritinib, and recently alectinib are FDA approved for the treatment of patients with metastatic NSCLC whose tumors are positive for *ALK* fusions. Early phase 1 studies showed crizotinib yielded sustained responses in *ALK*-fusion-positive metastatic NSCLC patients (Kwak et al. 2010; Shaw et al. 2011; Camidge et al. 2012). Two phase 3 studies, which led to FDA approval of crizotinib, further confirmed that crizotinib was superior to standard first-line pemetrexed + cisplatin chemotherapy in patients with previously untreated advanced *ALK*-rearranged NSCLC. In one study (PROFILE 1007), crizotinib showed overall response rate (ORR) of 65% as compared with 20% with either pemetrexed or docetaxel in patients who had failed one prior platinum-based regimen (Shaw et al. 2013). In another study (PROFILE 1014), progression-free survival (PFS) and ORR were significantly improved with crizotinib than with first-line therapy with platinum-pemetrexed (median, 10.9 mo vs. 7 mo) (Solomon et al. 2014). Furthermore, crizotinib was associated with disease control in *ALK*-fusion-positive NSCLC patients who had brain metastasis (Costa et al. 2015). In addition, crizotinib also showed therapeutic response in *ALK*-fusion-positive IMT patients (Butrynski et al. 2010) and pediatric patients with anaplastic large cell lymphoma and IMT (Mosse et al. 2013).

Although crizotinib has shown excellent activity in patients with NSCLC who are *ALK*-fusion-positive, durable responses remain uncommon, with a median PFS of 13 mo, because of the development of resistance that leads to disease progression. Although the mechanism of resistance is still being delineated, acquired secondary mutations in the ALK kinase domain (F1174L, F1174C, L1196M, I1171T, G1202R, S1206Y, G1269S, and G1269A) or *ALK* gene amplification (Choi et al. 2010; Sasaki et al. 2010; Heuckmann et al. 2011; Doebele et al. 2012b; Katayama et al. 2012) are known to be associated with resistance. Resistance can also be mediated by activation of alternative ALK-independent survival pathways that hamper the effectiveness of crizotinib, including the epidermal growth factor pathway, insulin-like growth factor pathway, RAS/SRC signaling, and AKT/mTOR signaling, among others (Doebele et al. 2012b; Katayama et al. 2012; Crystal et al. 2014; Ji et al. 2014; Mengoli et al. 2016). Tables 3 and 4 show *ALK* alterations that are either sensitive to ALK inhibitors (Table 3) or resistant to ALK inhibitors (Table 4).

Even though different resistance mechanisms are being discovered, most crizotinib-resistant tumors continue to depend on ALK signaling and are sensitive to more potent, structurally distinct, second-generation ALK inhibitors, such as ceritinib, alectinib,

Table 3. Therapy-sensitive *ALK* alterations

Drug (type)	Sensitive variant(s)	Tumor type	Level of evidence[a]	Reference(s)
Crizotinib (ALK TKI)	Fusion	NSCLC	1A	Kwak et al. 2010; Camidge et al. 2012; Shaw et al. 2013; Solomon et al. 2014
	RANBP2-ALK	IMT	3A	Butrynski et al. 2010
	NPM1-ALK	Neuroblastoma, lung	3B	Christensen et al. 2007
	EMLA-ALK			
	F1174L R1275Q	IMT	3B	Lovly et al. 2011; Di Paolo et al. 2015
Ceritinib (ALK TKI)	Fusion	Lung	1A	Shaw et al. 2014
	Fusion	Thyroid	3A	Godbert et al. 2015
	EML4-ALK/I1171T EML4-ALK/V1180L EML4-ALK/L1196M EML4-ALK/S1206Y EML4-ALK/G1269A		3B	Friboulet et al. 2014; Katayama et al. 2014
Alectinib (ALK TKI)	Fusion	Lung	1A	Seto et al. 2013; Gadgeel et al. 2014
	Amplification	Neuroblastoma	3B	Sakamoto et al. 2011
	L1196M G1269A C1156Y F1174L 1151Tins L1152R	NSCLC	3B	Sakamoto et al. 2011; Kodama et al. 2014; Yoshimura et al. 2016
	EML4-ALK/1151Tins EML4-ALK/L1152R EML4-ALK/C1156Y EML4-ALK/F1174L EML4-ALK/L1196M EML4-ALK/S1206Y EML4-ALK/G1269A	NSCLC	3B	Sakamoto et al. 2011; Kodama et al. 2014
Lorlatinib (ALK TKI)	NPM1-ALK/C1156F NPM1-ALK/I1171T NPM1-ALK/I1171N NPM1-ALK/F1174I NPM1-ALK/N1178H NPM1-ALK/E1201K NPM1-ALK/D1203N	ALCL	3B	Mologni et al. 2015
	F1174L F1245C R1275Q	Neuroblastoma	3B	Infarinato et al. 2015
	1151Tins C1156T L1196M G1202R G1269A		3B	Johnson et al. 2014; Zou et al. 2015
	EML4-ALK/L1196M EML4-ALK/G1269A	Lung	3B	Zou et al. 2015
Brigatinib (ALK TKI)	EML4-ALK/L1196M NPM1-ALK/L1196Q	NSCLC	3B	Katayama et al. 2011; Ceccon et al. 2013
ASP3026 (ALK TKI)	NPM1-ALK	ALCL	3B	George et al. 2014
	EML4-ALK/L1196M	NSCLC	3B	Mori et al. 2014

Continued

Table 3. *Continued*

Drug (type)	Sensitive variant(s)	Tumor type	Level of evidence[a]	Reference(s)
Entrectinib (ALK TKI)	EML4-ALK CAD-ALK	Colorectal	3B	Amatu et al. 2015, Lee et al. 2015
X-396 (ALK TKI)	EML4-ALK/C1156Y EML4-ALK/L1196M	Lung	3B	Lovly et al. 2011
	F1174L R1275Q	Neuroblastoma	3B	Di Paolo et al. 2015
Retaspimycin (HSP90 inhibitor)	EML4-ALK	NSCLC	3A	Sequist et al. 2010; Normant et al. 2011
Tanespimycin (HSP90 inhibitor)	EML4-ALK/V1180L	NSCLC	3B	Katayama et al. 2014
	NPM1-ALK	ALCL	3B	Bonvini et al. 2002
	TPR-ALK RANBP2-ALK/F1174L	IMT	3B	Sasaki et al. 2010

Only clinically available drugs are listed in the table.
TKI, tyrosine kinase inhibitor; NSCLC, non-small-cell lung cancer; NB, neuroblastoma; ALCL, anaplastic large-cell lymphoma; IMT, inflammatory myofibroblastic tumor; CRC, colorectal cancer.
[a]Definition of level of evidence based on Meric-Bernstam et al. 2015.

brigatinib, and lorlatinib. A preclinical study showed alectinib was able to block the resistant gatekeeper mutation (L1196M) in *ALK*-fusion-positive NSCLC cells (Sakamoto et al. 2011). Two phase 1/2 studies showed alectinib was well tolerated. The first study conducted in ALK inhibitor-naïve patients with *ALK*-rearranged NSCLC showed objective response of 93.5% (43 of 46) that included two patients with complete response and 41 patients with partial response (Seto et al. 2013). Another study that tested efficacy of alectinib in patients with crizotinib-resistant *ALK*-rearranged NSCLC showed objective response of 55% (24 of 44), with a confirmed complete response (CR) in one patient and confirmed partial response (PR) in 14 patients (Gadgeel et al. 2014). In a multicenter phase 2 study in crizotinib-resistant *ALK*-fusion-positive NSCLC patients, alectinib showed a 48% response rate with 33 of 69 patients demonstrating confirmed PRs (Shaw et al. 2016b).

In preclinical studies, ceritinib efficiently inhibits several ALK secondary mutations developed in the setting of crizotinib therapy (Friboulet et al. 2014). In a phase 1 study, 114 *ALK*-rearranged, crizotinib-naïve (80) and -resistant (34) NSCLC patients received ceritinib (Shaw et al. 2014). The overall response rate was 58%, with one patient achieving a CR, 65 patients a PR, and 25 patients with stable disease. This study also had six of seven patients with a confirmed response who carried *ALK* gene amplification or mutations (L1196M, S1206Y) after crizotinib therapy. In another phase 1 study with 20 *ALK*-rearranged patients (19 NSCLC and 1 IMT) ceritinib treatment resulted in an ORR of 55% (Nishio et al. 2015). A recent multicenter phase 1 study (ASCEND-1) tested the activity of ceritinib in 246 *ALK*-rearranged NSCLC patients (Kim et al. 2016). The ORR was 72% (60 of 83 ALK inhibitor [ALKi] naïve patients) and 56% (92 of 163 ALK inhibitor pretreated patients). A still ongoing phase 2 (ASCEND-2) study that evaluated ceritinib activity in 140 *ALK*-rearranged NSCLC patients who failed crizotinib in addition to other regimens showed investigator assessed RR of 38.6% (Crino et al. 2016).

Brain metastasis is a common problem in advanced NSCLC patients and 10%–40% NSCLC patients develop brain metastasis during the course of their disease (Huang and Ouyang 2013). Brain metastasis occurs in 30%–50% of *ALK*-positive NSCLC patients who

Table 4. Therapy resistant *ALK* alterations

Drug (type)	Resistant variant(s)	Tumor type	Level of evidence[a]	Reference(s)
Crizotinib (ALK TKI)	Amplification-EML4-ALK	NSCLC	3A	Doebele et al. 2012b
	1151insT L1152R C1156Y F1174V G1202R S1206Y G1269A	NSCLC	3A	Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015
	EML4-ALK/1151insT EML4-ALK/L1152R EML4-ALK/C1156Y EML4-ALK/L1196M EML4-ALK/L1198P EML4-ALK/D1203N		3B	Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015
	NPM1-ALK	ALCL	3B	Ceccon et al. 2013
	RANBP2-ALK	IMT	3A	Sasaki et al. 2010
Ceritinib (ALK TKI)	EML4-ALK/1151Tins EML4-ALK/L1152R EML4-ALK/C1156Y EML4-ALK/F1174C EML4-ALK/G1202R		3B	Friboulet et al. 2014
Alectinib (ALK TKI)	EML4-ALK/I1171Tins EML4-ALK/V1180L EML4-ALK/G1202R	NSCLC	3B	Katayama et al. 2014; Kodama et al. 2014

Only clinically available drugs are listed in the table.
TKI, tyrosine kinase inhibitor; NSCLC, non-small-cell lung cancer; NB, neuroblastoma; ALCL, anaplastic large-cell lymphoma; IMT, inflammatory myofibroblastic tumor; CRC, colorectal cancer.
[a]Definition of level of evidence based on Meric-Bernstam et al. 2015.

are naïve to ALK inhibitors (Doebele et al. 2012a; Simoff et al. 2013; Rangachari et al. 2015). Despite the inability of most of the chemotherapy regimens to cross the blood–brain barrier, pemetrexed treatment in NSCLC patients shows some effectiveness against brain metastasis (Bearz et al. 2010). Crizotinib has shown systemic and intracranial disease control in NSCLC patients that are *ALK*-positive (Costa et al. 2015). However, crizotinib-resistant patients develop new or show progression of preexisting intracranial lesions (Costa et al. 2015). Recent studies showed that second-generation agents ceritinib (Kim et al. 2014; Crino et al. 2016), alectinib (Gadgeel et al. 2014; Shaw et al. 2016b), and brigatinib (Kerstein et al. 2015) are active in intracranial diseases.

Clinical data with crizotinib, ceritinib, and alectinib showed that G1202R is a common ALK resistance mutation. However, there are other mutations that differ in sensitivity to the various anti-ALK kinase inhibitors (Table 4). Several next-generation ALK inhibitors are being developed to treat previously treated ALK inhibitor resistant mutations. The list includes brigatinib (Huang et al. 2016; Siaw et al. 2016), lorlatinib (Gainor et al. 2016; Shaw et al. 2016a), and X-396 (Di Paolo et al. 2015). Both brigatinib and lorlatinib inhibit several known resistant mutations, and lorlatinib showed effective inhibition against the G1202R mutation (Gainor et al. 2016). These results suggest that understanding the resistance mechanism and selecting the appropriate tyrosine kinase inhibitor (TKI) may be required to optimize response to therapy. Interestingly, a preclinical study showed

Competing Interest Statement

Funda Meric-Bernstam has had an honoraria role with Genentech and Roche Diagnostics; a consulting or advisory role for Genentech, Novartis, Roche, Inflection Biosciences, and Celgene; and research funding from Novartis, AstraZeneca, Taiho Pharmaceutical, Genentech, Calithera Biosciences, Debiopharm Group, Bayer, Aileron Therapeutics, PUMA Biotechnology, Verastem, and CytomX Therapeutics. G.B.M. has had a consulting role with Adventist Health, Allostery, AstraZeneca, Catena Pharmaceuticals, Critical Outcome Technologies, ImmunoMET, Isis Pharmaceuticals, Lilly, Medimmune, Novartis, Precision Medicine, Provista Diagnostics, Signalchem Lifesciences, Symphogen, Takeda/Millenium Pharmaceuticals, Tau Therapeutics, and Tarveda; stock options in Catena Pharmaceuticals, ImmunoMet, and Spindle Top Ventures; licensed technology in the HRD Assay, Myriad Genetics; and research funding from Adelson Medical Research Foundation, AstraZeneca, Breast Cancer Research Foundation, Critical Outcome Technologies, Illumina, Karus, Komen Research Foundation, Nanostring, and Takeda/Millenium Pharmaceuticals. J.M. has had a consulting role with MedImmune and Ziopharm; he serves on the board of directors and owns stock in Merrimack Pharmaceuticals and has patents/royalties in Imclone and Lilly.

Referees

Jonathan H. Schatz
Anonymous

that the inhibition of autophagy with chloroquine may restore crizotinib sensitivity (Ji et al. 2014).

In addition to targeting ALK directly, there are pharmacological strategies that allow for its indirect targeting. Specifically, there has been some success with inhibiting ALK indirectly by targeting heat-shock proteins, namely HSP90, in lung cancer. Inhibition of HSP90, a chaperone protein that stabilizes a wide variety of proteins, including ALK, has shown some preclinical efficacy in crizotinib-resistant *ALK* fusions (*EML4-ALK* and *NPM1-ALK*), including secondary resistant mutants in lung cancer models (Sang et al. 2013). In addition, several drug combinations, including ALK inhibitors and other receptor tyrosine kinase (RTK) inhibitors or HSP90 inhibitors, are being explored in preclinical/clinical studies: IGF1R (Lovly et al. 2014); MEK (Tanizaki et al. 2012; Crystal et al. 2014; Hrustanovic et al. 2015); and HSP90 (Sang et al. 2013). As recent preclinical data indicate, the immune checkpoint proteins are induced in ALK-positive NSCLC tumors (Ota et al. 2015; Hong et al. 2016); thus, combination therapies of checkpoint (PD-1/PD-L1, CTLA-4) and ALK inhibitors are being explored in the clinical setting for ALK-positive NSCLC patients (NCT02393625, NCT01998126).

Chemotherapy also remains a viable option in patients with *ALK* translocations. In terms of chemotherapy for NSCLC, pemetrexed-based chemotherapy may be more effective than other nonpemetrexed combinations (Camidge et al. 2011).

CONCLUSIONS

ALK rearranged tumors represent a specific subset of tumors that can be effectively targeted with currently available ALK inhibitors. Hence testing for *ALK* alterations in tumors known to have this molecular aberration is now an obligatory part of the diagnosis. FISH, next-generation sequencing (NGS) of tumor tissue, and sequencing of circulating tumor cells offer alternative and often complementary ways of detecting tumors with *ALK* alterations. The almost inevitable emergence of resistance during TKI therapy requires rebiopsy of the tumor at relapse to identify resistance mechanisms that could be targeted with newer ALK inhibitors and other novel therapeutic strategies, including HSP90 inhibitors and pemetrexed-based chemotherapy. Checkpoint inhibitors either as single agents or combination with ALK inhibitors are being evaluated in clinical trials.

ADDITIONAL INFORMATION

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