ALK: a tyrosine kinase target for cancer therapy

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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.

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
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 (ALKATI) in 11% of melanomas and other tumor types (Wiesner et al. 2015). ALKATI arises independently of other ALK genomic alterations, and in vivo and in vitro studies show that ALKATI-driven tumors are sensitive to crizotinib. Although activating mutations and copy-number changes of the ALK gene are commonly 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) (Franco et al. 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...
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.
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*...
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

| 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.
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,
| Drug (type) | Sensitive variant(s) | Tumor type | Level of evidence | 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 | IMT | 3B | Lovly et al. 2011; Di Paolo et al. 2015 |
| | F1174L | | | |
| | R1275Q | | | |
| Centinib (ALK TKI) | Fusion | Lung | 1A | Shaw et al. 2014 |
| | Fusion | Thyroid | 3A | Godbert et al. 2015 |
| | EML4-ALK/I1171T | | 3B | Friboulet et al. 2014; Katayama et al. 2014 |
| | EML4-ALK/V1180L | | | |
| | EML4-ALK/L1196M | | | |
| | EML4-ALK/S1206Y | | | |
| | EML4-ALK/G1269A | | | |
| | Fusion | Lung | 3A | Sakamoto et al. 2011; Kodama et al. 2014; Yoshimura et al. 2016 |
| | Amplification | Neuroblastoma | 3B | Sakamoto et al. 2011 |
| | L1196M | NSCLC | 3B | Sakamoto et al. 2011; Kodama et al. 2014 |
| | G1269A | | | |
| | C1156Y | | | |
| | F1174L | | | |
| | 1151Tins | | | |
| | L1152R | NSCLC | 3B | Sakamoto et al. 2011; Kodama et al. 2014 |
| | EML4-ALK/I1171N | | | |
| | EML4-ALK/L1196M | | | |
| | EML4-ALK/S1206Y | | | |
| | EML4-ALK/G1269A | | | |
| Lorlatinib (ALK TKI) | NPM1-ALK/C1156F | ALCL | 3B | Mologni et al. 2015 |
| | NPM1-ALK/I1171T | | | |
| | NPM1-ALK/I1171N | | | |
| | NPM1-ALK/F1174I | | | |
| | NPM1-ALK/N1178H | | | |
| | NPM1-ALK/E1201K | | | |
| | NPM1-ALK/D1203N | | | |
| | F1174L | Neuroblastoma | 3B | Infarinato et al. 2015 |
| | F1245C | | 3B | Johnson et al. 2014; Zou et al. 2015 |
| | R1275Q | | | |
| | 1151Tins | | | |
| | C1156Y | | | |
| | L1196M | | | |
| | G1202R | Lung | 3B | Zou et al. 2015 |
| | G1269A | | | |
| | EML4-ALK/L1196M | NSCLC | 3B | Katayama et al. 2011; Ceccon et al. 2013 |
| | EML4-ALK/G1269A | | | |
| | Brigatinib (ALK TKI) | EML4-ALK/L1196M | NSCLC | 3B |
| | NPM1-ALK/L1196Q | | | |
| | ASP3026 (ALK TKI) | NPM1-ALK | ALCL | 3B | George et al. 2014 |
| | EML4-ALK/L1196M | NSCLC | 3B | Mori et al. 2014 |
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 3. Continued

| Drug (type)          | Sensitive variant(s) | Tumor type | Level of evidencea | 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      | Lung       | 3B                 | Lovly et al. 2011                 |
|                      | EML4-ALK/L1196M/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, NPM1-ALK, TPR-ALK, RANBP2-ALK/F1174L | NSCLC, ALCL, IMT | 3B | Katayama et al. 2014, Bonvini et al. 2002, 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.

aDefinition 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

| Drug (type)          | Resistant variant(s) | Tumor type | Level of evidencea | Reference(s) |
|----------------------|----------------------|------------|--------------------|--------------|
| Crizotinib (ALK TKI) | Amplification-EML4-ALK | NSCLC      | 3A                 | Doebele et al. 2012b |
|                      | 1151insT             | NSCLC      | 3A                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | L1152R               | NSCLC      | 3A                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | C1156Y               | NSCLC      | 3A                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | F1174V               | NSCLC      | 3A                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | G1202R               | NSCLC      | 3A                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | 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    | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/L1152R      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/C1156Y      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
| Ceritinib (ALK TKI)  | EML4-ALK/L1152R      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/L1156Y      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/L1196M      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/L1198P      | NSCLC      | 3B                 | Choi et al. 2010; Doebele et al. 2012b; Katayama et al. 2012; Ou et al. 2015; Zou et al. 2015 |
|                      | EML4-ALK/D1203N      | NSCLC      | 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                 | Cecon et al. 2013 |
|                      | RANBP2-ALK           | IMT        | 3A                 | Sasaki et al. 2010 |
| Alectinib (ALK TKI)  | EML4-ALK/1171Tins    | NSCLC      | 3B                 | Friboulet et al. 2014 |
|                      | EML4-ALK/V1180L      | NSCLC      | 3B                 | Friboulet et al. 2014 |
|                      | EML4-ALK/G1202R      | NSCLC      | 3B                 | Friboulet 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.

*Definition of level of evidence based on Meric-Bernstam et al. 2015.
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

Funding

This work was supported in part by 1U01 CA180964 from the Sheikh Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy; Cancer Prevention and Research Institute of Texas (CPRIT) grant RP150535, Precision Oncology Decision Support Core; National Center for Advancing Translational Sciences (NCATS) grant UL1 TR000371 (Center for Clinical and Translational Sciences); the Bosarge Foundation; and the MD Anderson Cancer Center Support grant (P30 CA016672).
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