Targeting ALK: Precision Medicine Takes on Drug Resistance

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ABSTRACT Anaplastic lymphoma kinase (ALK) is a validated molecular target in several ALK-rearranged malignancies, including non–small cell lung cancer. However, the clinical benefit of targeting ALK using tyrosine kinase inhibitors (TKI) is almost universally limited by the emergence of drug resistance. Diverse mechanisms of resistance to ALK TKIs have now been discovered, and these basic mechanisms are informing the development of novel therapeutic strategies to overcome resistance in the clinic. In this review, we summarize the current successes and challenges of targeting ALK.

Significance: Effective long-term treatment of ALK-rearranged cancers requires a mechanistic understanding of resistance to ALK TKIs so that rational therapies can be selected to combat resistance. This review underscores the importance of serial biopsies in capturing the dynamic therapeutic vulnerabilities within a patient’s tumor and offers a perspective into the complexity of on-target and off-target ALK TKI resistance mechanisms. Therapeutic strategies that can successfully overcome, and potentially prevent, these resistance mechanisms will have the greatest impact on patient outcome.

INTRODUCTION

The discovery of anaplastic lymphoma kinase (ALK) dates back to 1994 when a chromosomal rearrangement t(2;5), resulting in a nucleophosmin (NPM1)–ALK fusion, was described in anaplastic large-cell lymphoma (ALCL; ref. 1). Subsequent work over the next two decades identified ALK fusion proteins as the oncogenic driver in numerous different malignancies. Perhaps most widely recognized is the echinoderm microtubule-associated protein-like 4 (EML4)–ALK fusion detected in non–small cell lung cancer (NSCLC) in 2007 (2). ALK-rearranged cancers have since emerged as a salient example of the paradigm of “oncogene addiction.”

In the span of less than a decade, collaborative efforts among basic and clinical scientists in academia and the pharmaceutical industry have led to the development of numerous ALK tyrosine kinase inhibitors (TKI). To date, three ALK TKIs (crizotinib, ceritinib, and alectinib) have received approval by the FDA for treatment of advanced “ALK-positive” NSCLC (i.e., NSCLC harboring an ALK rearrangement), with clinical trials demonstrating remarkable responses in this patient population (3–8). Yet, as with any targeted therapy, tumor cells evolve and invariably acquire resistance to ALK TKIs, leading to clinical relapse. Since the first demonstration of crizotinib’s activity in ALK-positive NSCLC, a multitude of studies have focused on elucidating mechanisms of resistance to ALK inhibition.

The rapid pace of ALK-targeted drug development, and the knowledge gained in parallel on resistance mechanisms, illustrate the power of an iterative, systematic discovery process—at the bench and the bedside—to advance cancer research and transform patients’ lives. This review provides an up-to-date, mechanistic framework for understanding how ALK, and the mechanisms of resistance to ALK TKIs, may be effectively targeted in order to extend and improve the lives of patients with ALK-driven cancers.

ALK BIOLOGY

The Function of Native ALK

ALK encodes a highly conserved receptor tyrosine kinase (RTK) within the insulin receptor superfamily, comprised of an extracellular domain, a single-pass transmembrane region, and an intracellular kinase domain (9). In adult humans, ALK expression is limited to the nervous system, testes, and small intestines (1). Activation of endogenous ALK requires ligand-dependent receptor dimerization and autophosphorylation. Recent work has established Augmentor α and β (FAM150) as the ligand for ALK (10–12). Although little is known about
the biological function of ALK in humans, insights may be gleaned from studying model systems.

In Drosophila melanogaster, Alk is activated by its ligand Jelly belly (jeb) and regulates the development of the gut musculature and neuronal circuitry within the visual system (13–16). In mice, Alk is expressed in the nervous system at the embryonic and neonatal stage, but minimally expressed in adults (9). Alk knockout mice notably achieve a normal life span, exhibiting mild abnormalities involving the frontal cortex and hippocampus and hypogonadotropic hypogonadism (17–19). Interestingly, visual disturbance and hypogonadism have been reported in patients treated with the first-generation, multitargeted ALK inhibitor crizotinib (3, 4). These toxicities, however, are unlikely to be ALK-related, given their decreased incidence with more selective ALK TKIs, such as ceritinib and alectinib (5–8).

**ALK Rearrangements in Cancer**

With the advent of next-generation sequencing (NGS)-based diagnostics, more than 20 different ALK fusion partner genes have been reported across multiple malignancies. In each cancer type, the full spectrum of chimeric ALK proteins and their individual frequency varies (Table 1). For example, in ALCL, where ALK rearrangements are seen in ~55% of adult patients (and nearly universally in pediatric patients), NPM1–ALK resulting from t(2;5)(p23;q35) is the most common fusion, accounting for up to 80% of cases (1, 20–29). Inflammatory myofibroblastic tumor (IMT) was the first solid tumor found to harbor ALK rearrangements, which occur in up to 50% of cases (30). In ALK-positive IMT, NPM1–ALK has not been reported, but a variety of other fusion partners not seen in ALCL have instead been identified (Table 1; refs. 30–37).

NSCLC was the second solid tumor in which oncogenic ALK fusions were detected. Soda and colleagues reported the identification of EML4–ALK in a small cohort of Japanese patients with NSCLC (2). Since then, ALK fusions have been detected in 3% to 7% of NSCLCs and have been associated with absence of smoking, younger age, and adenocarcinoma histology (38). Even though the relative proportion of NSCLCs harboring an ALK rearrangement is significantly lower than that of ALCL or IMT, patients with NSCLC constitute the largest subset of patients with an ALK-rearranged cancer due to the high incidence of lung cancer worldwide (39). Notably, studies in NSCLC have identified several additional ALK fusion proteins (40–45), which collectively occur less frequently than EML4–ALK. Furthermore, a number of breakpoint variants may be seen for a given fusion protein. The classic example is EML4–ALK, with over 10 distinct variants (46). In most cases, the breakpoint in ALK at intron 19, just preceding exon 20, is conserved.

At low frequency, ALK rearrangements have been detected in other cancers, including colorectal, breast, renal cell, esophageal, ovarian, and anaplastic thyroid carcinoma, and diffuse large B-cell lymphoma (Table 1). Several common themes have emerged based on the identified ALK rearrangements (47). First, in all ALK fusions, the entire ALK kinase domain is preserved. Second, the N-terminal partner contributes its promoter and oligomerization domain to the ALK fusion protein, leading to aberrant expression and constitutive activation of ALK. Consequently, the level of ALK fusion expression and the degree of signaling may vary depending on the partner gene. Indeed, in vitro studies using NIH3T3 cells have suggested differential effects of ALK fusion proteins on cell proliferation and invasion depending on the exact fusion (48). These findings have not yet been validated in patients, but could be clinically relevant if they translate into differential sensitivity of ALK fusions to TKIs in the clinic, as described below (49).

Lastly, ALK fusion proteins interact with a complex network of proteins and signal via multiple downstream pathways, including JAK/STAT, PI3K/AKT, and MEK/ERK, driving aberrant proliferation and survival (Fig. 1; refs. 50, 51). In the setting of chronic TKI exposure, dysregulation of these signaling nodes may enable acquired resistance to ALK inhibition (see the bypass signaling section below).

**TARGETING ONCOGENIC ALK**

Within months of the identification of EML4–ALK in NSCLC, preclinical studies established the transforming potential of this ALK fusion (2, 52). Transgenic mice expressing EML4–ALK in lung alveolar epithelial cells developed innumerable adenocarcinoma nodules in their lungs soon after birth (52). The dependency of ALK-rearranged lung cancer cells on ALK signaling for survival and growth (i.e., “addiction” to ALK) was demonstrated in cell lines and mouse models (52, 53), spurring the development of TKIs targeting ALK.

Although originally developed as a potent MET inhibitor, crizotinib was the first ALK-directed TKI to enter the clinic (53). In 2011, crizotinib was granted accelerated approval by the FDA based on the results of phase I/II studies demonstrating robust clinical activity in advanced ALK-rearranged NSCLC (54). Soon thereafter, two phase III trials showed that crizotinib was superior to first- and second-line cytotoxic chemotherapy in advanced ALK-rearranged NSCLC (3, 4). Across all crizotinib trials, the objective response rate (ORR) in patients with ALK-positive NSCLC ranged from 60% to 74%, with median progression-free survival (PFS) of 8 to 11 months. Subsequently, two second-generation ALK inhibitors, ceritinib and alectinib, received accelerated approval for patients with ALK-positive NSCLC previously treated with crizotinib. In the phase I ASCEND-1 trial, ceritinib demonstrated an ORR of 56% and median PFS of 6.9 months in crizotinib-pretreated patients with ALK-rearranged NSCLC (5, 6). In two phase II studies, alectinib induced objective responses in ~50% of crizotinib-resistant ALK-rearranged NSCLCs (7, 8); median PFS in both studies was 8 to 9 months.
### Table 1. ALK rearrangements in cancer

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Frequency of ALK rearrangements</th>
<th>ALK fusion partner gene</th>
<th>Location of fusion partner</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>NSCLC</td>
<td>3–7%</td>
<td>TPR</td>
<td>1q31.1</td>
<td>(2, 40–45)</td>
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<tr>
<td></td>
<td></td>
<td>CRIM1</td>
<td>2p22.2</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>EML4*</td>
<td>2p21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>STRN</td>
<td>2p22.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TFG</td>
<td>3q12.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIP1</td>
<td>7q11.23</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>PTPN3</td>
<td>9q31</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>KIF5B</td>
<td>10p11.22</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>KLC1</td>
<td>14q32.3</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CL TC</td>
<td>17q23.1</td>
<td></td>
</tr>
<tr>
<td>ALCL</td>
<td>~55% (in adults)</td>
<td>TPM3</td>
<td>1q21.3</td>
<td>(1, 21–29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATIC</td>
<td>2q35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TFG</td>
<td>3q12.2</td>
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<tr>
<td></td>
<td></td>
<td>NPM1*</td>
<td>5q35.1</td>
<td></td>
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<td></td>
<td></td>
<td>TRAF1</td>
<td>9q33.2</td>
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<td></td>
<td></td>
<td>CLTC</td>
<td>17q23.1</td>
<td></td>
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<td></td>
<td>RNF213</td>
<td>17q25.3</td>
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<td></td>
<td></td>
<td>TPM4</td>
<td>1p13.1</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>MYH9</td>
<td>22q12.3</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>MSN</td>
<td>Xq12</td>
<td></td>
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<tr>
<td>IMT</td>
<td>Up to 50%</td>
<td>TPM3</td>
<td>1q21.3</td>
<td>(30–37)</td>
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<tr>
<td></td>
<td></td>
<td>RANBP2</td>
<td>2q13</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>ATIC</td>
<td>2q35</td>
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<tr>
<td></td>
<td></td>
<td>SEC31A</td>
<td>4q21.22</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CARS</td>
<td>11p15.4</td>
<td></td>
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<td></td>
<td></td>
<td>PPFIBP1</td>
<td>12p11</td>
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<tr>
<td></td>
<td></td>
<td>CLTC</td>
<td>17q23.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TPM4</td>
<td>1p13.1</td>
<td></td>
</tr>
<tr>
<td>DLBCL</td>
<td>&lt;1%</td>
<td>RANBP2</td>
<td>2q13</td>
<td>(164–171)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EML4</td>
<td>2p21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEC31A</td>
<td>4q21.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SQSTM1</td>
<td>5q35</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>NPM1</td>
<td>5q35.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CLTC</td>
<td>17q23.1</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>&lt;1%</td>
<td>EML4</td>
<td>2p21</td>
<td>(172–175)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>N.D.</td>
<td>EML4</td>
<td>2p21</td>
<td>(173)</td>
</tr>
<tr>
<td>RCC</td>
<td>&lt;1%</td>
<td>TPM3</td>
<td>1q21.2</td>
<td>(176–179)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EML4</td>
<td>2p21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>STRN</td>
<td>2p22.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCL</td>
<td>10q22.2</td>
<td>(180)</td>
</tr>
<tr>
<td>RMC</td>
<td>N.D.</td>
<td>VCL</td>
<td>10q22.2</td>
<td>(180)</td>
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<tr>
<td>Esophageal cancer</td>
<td>N.D.</td>
<td>TPM4</td>
<td>1p13.1</td>
<td>(181, 182)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>N.D.</td>
<td>FN1</td>
<td>2q35</td>
<td>(183)</td>
</tr>
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</table>

Abbreviations: TPR, translocated promoter region; CRIM1, cysteine rich transmembrane BMP regulator 1; EML4, echinoderm microtubule associated protein like 4; STRN, striatin; TFG, TRK-fused gene; HIP1, huntingtin interacting protein 1; PTPN3, protein tyrosine phosphatase, nonreceptor type 3; KIF5B, kinesin family member 5B; KLC1, kinesin light chain 1; CLTC, clathrin heavy chain; ALCL, anaplastic large cell lymphoma; TPM3, tropomyosin 3; NPM1, nucleophosmin; TRAF1, TNF receptor associated factor 1; RNF213, ring finger protein 213; TPM4, tropomyosin 4; MYH9, myosin, heavy chain 9, nonmuscle; MSN, moesin; IMT, inflammatory myofibroblastic tumor; RANBP2, RAN binding protein 2; SEC31A, SEC31 homolog A; CARS, cysteinyl-tRNA synthetase; PPFIBP1, PTPRF interacting protein; binding protein 1; DLBC, diffuse large B-cell lymphoma; SQSTM1, sequestosome 1; WDCP, WD repeat and coiled coil containing; N.D., not determined; RCC, renal cell carcinoma; VCL, vinculin; RMC, renal medullary carcinoma; FN1, fibronectin 1. *EML4 and NPM1 are the most common fusion partner genes in NSCLC and ALCL, respectively. 
Another second-generation inhibitor, brigatinib, has been associated with a confirmed ORR of 62% and a median PFS of 13.2 months in crizotinib-pretreated patients in one phase I/II study (55). These second-generation inhibitors are more potent than crizotinib, can overcome the most common crizotinib-resistant mutations including the L1196M gatekeeper mutation, are active in crizotinib-resistant tumors without ALK resistance mutations, and are more effective than crizotinib against central nervous system (CNS) metastases (5–8).

ALK inhibitors have not yet been approved for use in other, non-lung ALK-driven cancers, although reports have been published of patients’ responses to ALK TKIs in ALK-positive ALCL and IMT (56–58). Multiple clinical trials are under way evaluating the activity of ALK TKIs across different tumors. In particular, the NCI-Molecular Analysis for Therapy Choice (NCI-MATCH) trial is enrolling adult patients with any advanced solid tumors and lymphomas refractory to standard therapy (NCT02465060); those patients found to have ALK-rearranged cancers (other than NSCLC and ALCL) will be eligible to receive treatment with crizotinib. Hence, the NCI-MATCH protocol will allow for systematic analysis of response to ALK inhibition in patients with ALK-rearranged cancers, irrespective of the cancer type.

Despite initial responses, patients treated with ALK TKIs inevitably progress within 1 to 2 years due to acquired resistance (3–8). This resistance is an expected consequence of tumor evolution (59) and is similar in principle to what has been
observed with other targeted therapies [e.g., KIT inhibitors in gastrointestinal stromal tumors (GIST) and EGFR inhibitors in EGFR-mutant NSCLC].

**MECHANISMS OF RESISTANCE TO ALK TKIs**

**Current Approaches to Study TKI Resistance**

Numerous ALK TKI resistance mechanisms have been identified since the discovery of ALK as a therapeutic target in NSCLC. These discoveries have relied on the use of diverse experimental systems and approaches (Fig. 2). Traditionally, sequencing analyses of pre-TKI and post-TKI tumor biopsies, followed by functional validation of candidate resistance mechanisms, have been a common approach (60–63). As an example, many of the secondary resistance mutations within the ALK tyrosine kinase domain were discovered in this manner.

To overcome the inherent limitations in biopsy specimens with regard to tumor quantity and quality, more recent studies have used patient-derived cell lines and xenograft models (62, 64). These models can serve as a platform for systematic loss-of-function (e.g., CRISPR, shRNA, or pharmacologic) and gain-of-function [e.g., open reading frame (ORF) library] screens. For example, compound drug screens in patient-derived cell lines led to the identification of MEK and SRC activation as novel ALK TKI resistance mechanisms (further discussed below; ref. 64). Liquid biopsies can also overcome some of the challenges of tumor biopsies (discussed below).

Together with traditional in vitro-generated cell line models (63, 65) and random mutagenesis screens (66, 67), these experimental approaches have led to the identification of two major classes of ALK TKI resistance mechanisms: (i) ALK-dependent, “on-target” mechanisms including ALK secondary resistance mutations or amplification, where the tumor cell dependency on ALK signaling persists; and (ii) ALK-independent, “off-target” mechanisms including activation of bypass tracks and lineage changes, where the tumor cells effectively escape dependency on ALK. Although pharmacologic properties of TKIs can also limit ALK TKI efficacy, particularly for CNS disease, these issues are beyond the scope of this review and have been discussed elsewhere (68). Below, we explore the biological mechanisms of acquired ALK TKI resistance primarily based on data obtained from studies of ALK-positive NSCLC.
ALK-Dependent Resistance: Secondary Mutations in the ALK Tyrosine Kinase Domain

In general, secondary mutations within the target kinase cause drug resistance by reinducing kinase activation and signaling despite the presence of the TKI. These resistance mutations can directly hinder TKI binding to the target kinase, alter the kinase’s conformation, and/or alter the ATP-binding affinity of the kinase. Unlike in EGFR-mutant NSCLC, where the T790M gatekeeper mutation is the predominant, clinically observed EGFR mutation causing resistance to first- and second-generation EGFR TKIs, a much broader spectrum of on-target mutations has been identified in ALK-positive NSCLC treated with ALK TKIs (Fig. 3; ref. 69). This situation is reminiscent of the wide array of resistance mutations observed after treatment with the ABL inhibitor imatinib in patients with chronic myelogenous leukemia (CML). The difference in spectrum of resistance mutations may be attributable to the genetic mechanism of oncogene activation (i.e., gene rearrangements involving ALK or ABL, versus activating point mutations within the EGFR kinase domain) and/or the mode of TKI binding (i.e., to the inactive kinase conformation for imatinib and crizotinib, versus the active conformation for EGFR TKIs erlotinib and gefitinib; refs. 70, 71). Both of these factors may influence the spectrum of drug-resistant mutations that arise in TKI-resistant patients.

The first ALK resistance mutation reported was the L1196M gatekeeper mutation, analogous to EGFR T790M (61). This mutation, also identified in a cell line model of crizotinib resistance (65), alters the gatekeeper residue at the bottom of the ATP-binding pocket and impairs TKI binding. The G1269A mutation also lies in the ATP-binding pocket and hinders crizotinib binding (72). Other resistance mutations affect residues adjacent to the N-terminus (C1156Y, L1152R, and I1151Tins) and C-terminus of the αC helix (F1174L/S, I1171T/N/S, C1156Y, 1151Tins) versus activating point mutations within the EGFR kinase domain and/or the mode of TKI binding (i.e., to the inactive kinase conformation for imatinib and crizotinib, versus the active conformation for EGFR TKIs erlotinib and gefitinib; refs. 70, 71). Both of these factors may influence the spectrum of drug-resistant mutations that arise in TKI-resistant patients.

Figure 3. Acquired resistance mechanisms to ALK TKIs. A, The reported frequency of each secondary resistance mutation in the ALK kinase domain is depicted for post-crizotinib (n = 55), post-ceritinib (n = 24), post-alectinib (n = 17), and post-brigatinib (n = 7) cases, based on ref. 78. The absence of a colored bar representing an ALK TKI (e.g., a blue bar representing brigatinib) indicates that the particular ALK mutation was not detected in the tested cases progressing after that specific ALK TKI (78). In B and C, the differential frequency of ALK secondary mutations in crizotinib-resistant versus second-generation ALK TKI (e.g., ceritinib, alectinib, and brigatinib)-resistant cases (20%–30% vs. 50%–70%, respectively) is highlighted. Notably, the frequency of the G1202R mutation is significantly higher after relapse on a second-generation ALK TKI compared with crizotinib. ALK amplification appears to be an infrequent mechanism of resistance to second-generation ALK TKIs, although the exact frequency has not been determined (N.D.). WT, wild-type.
its enzymatic activity (63, 74). The I1171T/N/S mutation may distort the αC helix to interfere with TKI binding (75–78). Solvent-front mutations (G1202R, G1202del, D1203N, and S1206Y/C) represent another class of ALK resistance mutations that impair drug binding likely through steric hindrance (63, 73, 76–78). Numerous studies have revealed that G1202R in particular confers high-level resistance to first- and second-generation ALK TKIs (63, 76, 78). It remains to be seen whether the same spectrum of mutations will be detected in other ALK-positive cancers treated with ALK TKIs; to date, several of the same mutations have been identified in ACLL cell lines (e.g., I1171T/N, F1174C, and L1196Q) and in a patient with IMT post-crizotinib (e.g., F1174L; refs. 79–81).

Given the structural differences among the available ALK TKIs, it is perhaps not surprising that each ALK TKI appears to be associated with a specific profile of secondary ALK resistance mutations (Fig. 3A). A notable example involves the general difference in resistance mutations that arise on the first-generation ALK TKI crizotinib versus second-generation ALK TKIs (e.g., ceritinib, alectinib, and brigatinib; Fig. 3). Recently, over 100 repeat biopsies from patients with ALK-positive NSCLC progressing on first- and second-generation AKI TKIs were analyzed (78). Secondary ALK mutations were observed in 20% to 30% of patients progressing on crizotinib, versus 56% of patients progressing on second-generation ALK TKIs (Fig. 3B and C; refs. 63, 72, 78). In line with prior studies, L1196M and G1269A were the most common resistance mutations detected in post-crizotinib samples. Interestingly, G1202R was found in only 2% of post-crizotinib samples, whereas it was the predominant resistance mechanism post-ceritinib, post-alectinib, and post-brigatinib (frequency ranging between 21% and 43%; ref. 78). This distinction likely reflects the greater potency of second-generation ALK TKIs versus crizotinib, resulting in the suppression of other, less potent resistance mutations that are seen with crizotinib. Of note, the third-generation pan-inhibitory ALK TKI lorlatinib has been shown to effectively inhibit G1202R in cell lines and in patients (78, 82).

Several other ALK resistance mutations also show differential sensitivity/resistance to distinct ALK TKIs. For example, I1171 mutations are frequently identified in alectinib-resistant specimens, but not in ceritinib-resistant cases (75–78). Cell lines harboring I1171 mutations are resistant to alectinib, but sensitive to ceritinib. Conversely, F1174 mutations confer resistance to ceritinib, but are sensitive to alectinib, in both preclinical and clinical studies (73, 76, 78). Defining the resistance mutation profiles associated with each ALK TKI will be critical to the rational sequencing of ALK TKIs in patients.

In the setting of failure of a second-generation ALK TKI, the development of a secondary ALK resistance mutation implies that ALK may still be functioning as the oncogenic driver. In patient-derived NSCLC cell lines with resistance to second-generation ALK TKIs, the third-generation ALK TKI lorlatinib could inhibit the growth of cell lines harboring ALK resistance mutations, but was inactive against those lines without ALK resistance mutations (78). This preclinical work suggests that defining ALK status is particularly crucial in patients who have progressed on a second-generation ALK TKI. In this working model (Fig. 4), those patients whose ALK TKI-resistant tumors harbor secondary mutations should be treated with another ALK TKI tailored to that resistance mutation, whereas those patients whose resistant tumors do not harbor an ALK resistance mutation should consider ALK-based combinatorial strategies (discussed below), rather than monotherapy with another ALK TKI.

As patients are treated with sequential ALK TKIs, compound resistance mutations can emerge, which adds another layer of complexity. Examples include C1156Y/I1171N after progression on sequential crizotinib, ceritinib, and alectinib, and E1210K/D1203N after sequential crizotinib and brigatinib (78). Functional studies in Ba/F3 cells suggest that compound mutations generally confer increased resistance to ALK TKIs (78). Similar examples exist in other oncogene-driven cancers treated using sequential TKIs. For example, in EGFR-mutant NSCLC treated with a first-generation followed by a third-generation EGFR TKI, compound T790M/C797S mutations arise, and when in cis, confer resistance to all available EGFR TKIs (83, 84). In the case of ALK TKI-resistant cells with compound mutations, however, in vitro studies suggest that the highly potent TKI lorlatinib may remain a potentially effective option (78).

It should be noted that the presence of compound mutations does not always translate into increased drug resistance. As an illustrative example, a compound C1156Y/L1198F mutation was recently discovered in a patient with ALK-positive NSCLC who had relapsed on crizotinib due to C1156Y, followed by sequential ceritinib and lorlatinib (74). Although C1156Y is sensitive to lorlatinib, the addition of L1198F disrupts binding of the drug and leads to lorlatinib resistance. Interestingly, biochemical and cellular assays demonstrated that the L1198F mutation paradoxically leads to resensitization to the less potent and selective inhibitor crizotinib. Based on the in vitro findings, the patient was retreated with crizotinib and had a durable response (74). This case underscores the emerging complexity of ALK resistance mutations and the importance of serial biopsies. It also serves as a proof of principle that timely collaborative research at the bench and the bedside can reveal important biological insights into tumor evolution and translate into immediate clinical benefit for patients.

**ALK-Dependent Resistance: Amplification of ALK**

ALK amplification occurs less frequently than secondary mutations, but it is a recognized cause of acquired resistance to crizotinib. In one series of crizotinib-resistant NSCLC cases, high-level ALK amplification was identified in 1 of 15 (6.7%), without an accompanying ALK mutation (63). Another series found new ALK copy-number gain in 2 of 11 post-crizotinib cases (18.2%), although one of these also had a concomitant ALK resistance mutation (72). Notably, a study using an ALK-positive cell line made resistant to crizotinib in vitro demonstrated that ALK amplification resulted in partial resistance, whereas the addition of the L1196M gatekeeper mutation resulted in high-level resistance (65). ALK amplification has not yet been detected as a resistance mechanism after next-generation ALK TKIs (78) and therefore may not be a clinically relevant resistance mechanism in the face of more potent ALK inhibition.
ALK-Independent Resistance: Activation of Bypass Signaling Pathways

Secondary ALK mutations and/or amplification account for ~30% of crizotinib resistance in ALK-positive NSCLC, yet most crizotinib-resistant tumors—including those without an identifiable on-target mechanism—remain ALK dependent with sensitivity to next-generation ALK TKIs (78). In contrast, 40% to 50% of cases resistant to second-generation ALK TKIs do not harbor on-target resistance mechanisms (Fig. 3B and C), and these are no longer ALK dependent based on studies described above (78, 85). One important category of ALK-independent, or off-target, resistance mechanism is the activation of bypass signaling track(s) through genetic alterations, autocrine signaling, or dysregulation of feedback signaling, resulting in the reactivation of downstream effectors required for tumor cell growth and survival.

Numerous examples of bypass signaling activation have been discovered as a cause of ALK TKI resistance (Fig. 1). EGFR activation was the first identified bypass mechanism (62, 63, 86). Phospho-RTK array analysis of ALK-rearranged lung cancer cell lines revealed increased EGFR phosphorylation in crizotinib-resistant cell lines lacking secondary ALK alterations, compared with parental crizotinib-sensitive cells (63). This was associated with higher EGFR mRNA expression and persistent activation of downstream ERK and AKT signaling. Interestingly, these cells did not harbor any EGFR mutations or amplification, suggesting that EGFR activation may result from receptor and/or ligand upregulation (63, 86). In patients, assessment of paired pre- and post-crizotinib biopsy samples identified increased EGFR activation in 4 of 9 cases (44.4%; ref. 63).

Subsequent work has implicated additional members of the HER receptor family in ALK TKI resistance (63, 87, 88). In a lentiviral ORF library screen designed to identify mediators of ALK TKI resistance, neuregulin-1 (NRG1), the ligand for ERBB3 (HER3) and ERBB4 (HER4) tyrosine kinases, emerged as the strongest driver of resistance (87). The resistant

![Figure 4](image-url)
phenotype induced by NRG1 overexpression was abrogated by combined inhibition of ALK and HER2, the dimerization partner for HER3. Consistent with these findings, gene expression profiling of crizotinib-resistant versus crizotinib-naive NSCLC tumor samples using RNA sequencing (RNA-seq) followed by single-sample gene set enrichment analysis (ssGSEA) identified EGFR and HER2 signatures as two of the most enriched gene expression signatures in resistant tumors (87). It is unknown whether dual blockade of EGFR/HER2 and ALK could be effective in treating patients with acquired resistance to ALK TKIs, but toxicities of the combination may be a major hurdle (further discussed below).

**MET** amplification is an example of bypass signaling that highlights the importance of considering the full spectrum of kinase targets for a given TKI in anticipating resistance mechanisms. MET activation is a known bypass signaling track in EGFR-mutant NSCLC (89, 90), but does not cause resistance to crizotinib, a potent ALK and MET inhibitor, in ALK-positive lung cancer. However, most next-generation ALK TKIs in development do not have anti-MET activity and, indeed, MET has recently been reported as a bypass mechanism in patients who have failed second-generation inhibitors like alectinib. In one case report, a post-alectinib biopsy did not reveal genetic alterations in **ALK** or **EGFR**, but demonstrated **MET** amplification by FISH. This patient subsequently responded to crizotinib (91).

Direct reactivation of downstream effector proteins can also enable acquired resistance. MEK reactivation is a key example of this resistance mechanism (64, 92). In a recent study using a patient-derived **ALK**-rearranged lung cancer cell line post-ceritinib, a compound drug screen identified the MEK inhibitor selumetinib (AZD6244) as a potent hit combined with ceritinib (64). Subsequent NGS of this cell line revealed a MAP2K1<sup>E675K</sup> activating mutation as the underlying genetic alteration leading to MEK activation. More importantly, a separate study demonstrated that ALK/MEK dual blockade may be effective not only in overcoming but also in delaying ALK TKI resistance (92). In ALK-positive lung adenocarcinoma cell lines and mouse xenograft models, the RAS-MEK pathway was found to be the critical downstream effector of EML4-ALK. Up-front combination therapy with ALK and MEK inhibitors led to an increase in the magnitude and duration of treatment responses and suppression of TKI resistance. Based on these findings, multiple combination regimens of ALK and MEK inhibitors may soon enter early-phase clinical testing.

Other examples of bypass signaling tracks clinically implicated in ALK TKI resistance include **PIK3CA** mutations [1 of 27 samples (3.7%), post-ceritinib; a case post-ceritinib; refs. 64, 78], **KIT** amplification [1 of 6 samples (16.7%), post-crizotinib; ref. 63], **IGF1R** activation [4 of 5 samples (80%), post-crizotinib; ref. 93], and **SRC** activation (ref. 64; Fig. 1). Parallel use of different experimental platforms has been pivotal to the discovery and validation of off-target resistance mechanisms. With the growing use of genomic-sequencing, proteomic, and phospho-proteomic technologies, more candidate off-target mechanisms will likely be discovered. In one series utilizing targeted NGS, mutations were detected post-second-generation ALK TKI in **TP53** (in 56% of 27 specimens examined), **DDR2**, **BRAF**, **FGFR2**, **MET**, **NRAS**, and **PIK3CA** (each in 1 specimen; ref. 78). Systematic assessment of pre- and post-TKI paired biopsies together with rigorous functional validation of candidate resistance mechanisms will be critical to identify those bypass tracks that represent therapeutic targets in resistant ALK-positive patients.

### ALK-Independent Resistance: Lineage Changes

Phenotypic changes such as epithelial-to-mesenchymal transition (EMT) and small cell lung cancer (SCLC) transformation can contribute to the development of ALK TKI resistance. With EMT, tumor cells acquire a mesenchymal morphology along with migratory and invasive capacities. At the molecular level, they gain the expression of mesenchymal markers (e.g., vimentin) and lose epithelial markers (e.g., E-cadherin). This phenomenon has been reported in ALK TKI-resistant cell lines and tumor samples (78, 94, 95). In one series, IHC staining on ceritinib-resistant biopsies revealed evidence of EMT in 5 of 12 cases, although in 3 of these concurrent ALK resistance mutations were detected. Therefore, the relative contribution of EMT to the ALK TKI-resistant phenotype remains to be established. The molecular mechanism by which EMT mediates ALK TKI resistance is also not known. At least in **EGFR**-mutant NSCLCs that acquire EGFR TKI resistance via EMT, preliminary studies suggest a role for AXL and IGF1R activation (96, 97). In another study, the SRC/FAK pathway emerged as a key signaling node in mesenchymal EGFR TKI–resistant cancer cells, whose growth was inhibited by the multitargeted SRC inhibitor dasatinib (98).

Histologic change of tumor cells from adenocarcinoma to SCLC has been observed in 3% to 10% of EGFR TKI–resistant NSCLCs (90), and a small number of recent case reports have now described resistance to crizotinib and alectinib mediated by this mechanism (99–102). Conceptually, this lineage change may contribute to ALK TKI resistance in a manner similar to EGFR TKI resistance, which has been associated with the acquisition of **BB** loss and genetic or epigenetic features of classic SCLC (103). As successful strategies are developed to target ALK resistance mutations and bypass signaling tracks (see below), lineage changes may emerge as an increasingly important mechanism of TKI resistance. Therefore, a better biological understanding of this process is needed.

### ALK-Independent Resistance: Drug Efflux Pump

P-glycoprotein (P-gp) is a highly conserved ATP-dependent efflux pump encoded by the multidrug resistance 1 (**MDR1**) gene, also known as ATP-binding cassette sub-family B member 1 (**ABCB1**; ref. 104). Recent work has identified P-gp overexpression as a potential resistance mechanism in 3 of 11 patients with ALK-positive, crizotinib- or ceritinib-resistant NSCLC (105). In a patient-derived cell line overexpressing P-gp, shRNA-mediated knockdown of **ABCB1** or pharmacologic inhibition of P-gp using verapamil reestablished the resistant cells to crizotinib and ceritinib (105). These findings need to be validated in larger patient cohorts. Importantly, P-gp can limit the CNS penetration of crizotinib and ceritinib (106, 107); alectinib, by comparison, is not a P-gp substrate and is able to achieve higher CNS levels (105, 108). Rational design of novel TKIs...
that are not substrates of P-gp or other ABC efflux transporters may help enhance penetration into the brain.

Primary ALK TKI Resistance

In addition to secondary (acquired) resistance, primary (intrinsic) resistance—defined as de novo lack of treatment response—can be seen after treatment with a TKI. For example, a small number (~5%) of patients with ALK-positive NSCLC treated with first-crizotinib have progressive disease as their best response (4). Mechanisms of intrinsic resistance are poorly understood, and this represents an important gap in the field of ALK TKI resistance. In theory, any of the acquired resistance mechanisms outlined above could cause primary resistance if they were preexisting in TKI-naive tumors. Indeed, in the case of EGFR-mutant lung cancers, primary resistance can arise due to a preexisting T790M gatekeeper mutation (109, 110). However, the occurrence of de novo ALK resistance mutations appears to be rare in ALK-positive lung cancers (111). Similarly, preexisting genetic alterations affecting bypass signaling could cause primary resistance (112), but have not yet been reported in ALK-positive NSCLC.

A number of studies have examined whether the specific ALK fusion variant may influence de novo sensitivity to ALK inhibitors. Differential ALK TKI sensitivity of EML4–ALK fusion variants was initially suggested by an in vitro study using a Ba/F3 cell line, in which variant v3a showed the least sensitivity to crizotinib and the tool compound TAE684, followed by v1 and v3b with intermediate sensitivity, and v2 with the greatest sensitivity (113). Drug sensitivity was found to correlate with the overall protein stability of each fusion variant. More recently, a retrospective analysis of 55 patients with EML4–ALK-positive NSCLC treated with crizotinib demonstrated a higher disease control rate and longer median PFS among those with EML4–ALK variant 1 compared with those with non-variant 1, suggesting that the precise ALK fusion variant may be clinically relevant (114). However, given the small number of patients in this retrospective analysis, these findings will need to be validated in larger studies (49).

It should be noted that a fraction of primary resistant cases may in fact be due to false-positive genotyping. The registration trials of the currently approved ALK TKIs required ALK positivity by the break-apart FISH assay, which can yield false-positive results due to technical issues and variability in pathologist interpretation (115). On the other hand, ALK FISH may also yield false-negative results. The alternative FDA-approved diagnostic, IHC, has several advantages over FISH, but also has imperfect sensitivity and specificity, highlighting the importance of using multiple diagnostic methods, including targeted NGS when clinically warranted (116, 117).

TUMOR HETEROGENEITY AND RESISTANCE

Polyclonal Resistance

A number of studies have demonstrated that heterogeneous resistance mechanisms may exist in tumor sites within one patient, or in tumor cells within one site, complicating efforts to therapeutically target resistant tumors. For example, ALK kinase domain resistance mutations have been reported to co-occur with ALK amplification (72) and with bypassing signaling activation such as c-KIT amplification (63). In another study, sequencing of 11 resistant tumor lesions acquired at autopsy from a patient with ALK-positive, ceritinib-resistant NSCLC revealed a MAP2K1-activating mutation and a PIK3CA-activating mutation in separate tumor sites (64). These findings collectively demonstrate the phenomenon of “polyclonal resistance,” whereby tumors acquire drug resistance through the simultaneous development of multiple resistance mechanisms. Over time, with exposure to the selective pressures of different TKIs, the clonal composition of these tumors can continue to evolve (68, 74, 118).

Liquid Biopsies

The ability to detect and characterize polyclonal resistance in patients is critical, as targeting only one subclonal cell population can potentially lead to therapy failures (118). As multiple tissue biopsies are usually not feasible, noninvasive liquid biopsies and analyses of circulating tumor DNA (ctDNA) represent a promising avenue to identify polyclonal resistance. The potential of liquid biopsies was recently highlighted in the context of colorectal cancer. In a patient progressing on the anti-EGFR monoclonal antibody cetuximab, discrete metastatic lesions were found to harbor different resistance mechanisms (e.g., a MAP2K1 mutation and a KRAS mutation), modulating treatment responses in a lesion-specific manner. Although single-lesion tumor biopsies failed to capture this heterogeneity, both resistance mutations were detected in the patient’s serial plasma ctDNA (118).

In lung cancer, significant inroads have been made in using plasma-based assays to detect EGFR mutations, including the T790M gatekeeper mutation (119, 120). Indeed, a plasma-based EGFR mutation test was recently FDA-approved based on data from a phase IV single-arm study of gefitinib (121), serving as a proof of concept that this diagnostic approach can be clinically implemented. Studies are now assessing whether liquid biopsies can similarly detect ALK fusions and resistance mutations in patients treated with ALK TKIs. Preliminary results appear promising (122, 123). In one study, cell-free DNA (cfDNA) isolated from plasma could be used to monitor the emergence and temporal evolution of ALK resistance mutations, including G1202R, during treatment with alectinib (122). However, more data are required before plasma-based assays can be routinely used in the clinic for monitoring ALK-positive patients. First, the ability of liquid biopsies to detect oncogenic fusions in general needs to be optimized. Second, their utility in real-time treatment monitoring and therapeutic stratification also needs to be further demonstrated through prospective and clinical intervention studies; this is one goal of the NCI-sponsored ALK Master Protocol. Ultimately, liquid biopsies have the potential to become a powerful diagnostic tool that complements repeat tumor biopsies and, at the same time, allows for more precise understanding at the genetic level of the longitudinal evolution and global landscape of resistance mechanisms.

DEVELOPING STRATEGIES TO OVERCOME RESISTANCE

Our understanding of the molecular mechanisms of ALK TKI resistance continues to mature, informing the
Overcoming Resistance to ALK Inhibitors

The goal of developing new therapeutic strategies is to address the two-fold challenge of (i) effectively treating the relapsed disease by overcoming the dominant resistance mechanism and (ii) enhancing the depth and duration of tumor response up front by preventing the emergence of resistance. Although many efforts thus far have focused on the former, strategies to prevent resistance up front are likely to have greater impact in the clinic.

Novel ALK TKIs: Many Options

Given the wide array of resistance mutations that can arise, multiple structurally distinct ALK inhibitors are needed. ALK TKIs are currently available or being developed and are listed in Table 2. As with the approved inhibitors crizotinib, ceritinib, and alectinib, we anticipate that these emerging ALK TKIs will: (i) possess different potencies against resistant ALK mutants, (ii) differ in target kinase selectivity, and (iii) give rise to a distinct spectrum of ALK resistance mutations.

For example, brigatinib (AP26113) is a potent ALK inhibitor that inhibits most crizotinib-resistant ALK mutants (124–129). In the phase II ALTA trial, 222 patients with advanced ALK-positive NSCLC who progressed on crizotinib were randomized to two different dosing schedules of brigatinib. The confirmed ORR ranged from 45% to 54%, with median PFS of 9.2 to 12.9 months (127), similar to the phase II data seen with alectinib. A phase III trial (ALTA-1L; NCT02737501) is ongoing to assess the efficacy of brigatinib versus crizotinib in TKI-naive ALK-positive NSCLC. In the interim, brigatinib has received breakthrough therapy designation by the FDA for the treatment of crizotinib-resistant, ALK-rearranged NSCLC. Some preclinical studies of brigatinib suggest activity against the G1202R mutation in cell lines and mouse models (128, 130), and a confirmed response to brigatinib has been reported in a patient with G1202R-mutant NSCLC (129). However, G1202R has also been detected in 43% (3/7) of ALK-positive NSCLC biopsies post-brigatinib (78). More importantly, a patient who relapsed after brigatinib with documented ALK1202R in a repeat biopsy went on to have a durable response to lorlatinib (131), suggesting that although brigatinib may have some activity against G1202R, its potency is compromised by this mutation.

Entrectinib (RXDX-101) and ensartinib (X-396) are notable for their additional kinase targets. Entrectinib inhibits TRK in addition to ALK, ROS1, and AXL. Updated results from a phase I study of entrectinib showed significant responses in TKI-naive patients with NTRK-, ROS1-, and ALK1-rearranged solid tumors (confirmed ORR of 100%, 86%, and 57%, respectively; ref. 134). A phase II basket study of entrectinib is currently recruiting participants (NCT02568267). Similar to crizotinib, ensartinib has activity against ROS1 and MET in addition to ALK, and it also targets AXL (135, 136). Preliminary analysis of ensartinib’s activity in ALK-rearranged NSCLC demonstrated responses in 7 of 8 patients who were crizotinib-naive and in 11 of 19 patients who were previously treated with crizotinib (136). A phase III randomized trial (eXalt3; NCT02767804) will compare ensartinib with crizotinib in TKI-naive ALK-positive NSCLC.

Lastly, lorlatinib (PF-06463922) is a third-generation ALK TKI that offers several advantages over second-generation TKIs. Lorlatinib has activity against all of the known ALK resistance mutations, including G1202R, and is highly selective for ALK/ROS1 (78, 82, 137). In the phase I portion of an ongoing phase I/II study (NCT01970865), lorlatinib was associated with an ORR of approximately 50% in patients with ALK-positive NSCLC, many of whom had progressed after two or more ALK TKIs (85). Responses to lorlatinib have also been reported in patients with ALK1202R-positive NSCLC (78, 85, 131). Notably, lorlatinib was developed to evade P-gp-mediated efflux, and thus can achieve excellent CNS penetration (105). In the phase I study, the intracranial ORR to lorlatinib was 39% among all patients (85). Despite its promising activity, resistance to lorlatinib also emerges. A compound C1156Y/L1198F mutation was detected in a patient relapsing on lorlatinib (74). Structural studies suggest that this mutation causes steric hindrance to the binding of lorlatinib, and when superimposed on another ALK mutation, C1156Y, it confers high-level resistance to lorlatinib (74). Studies are ongoing to uncover additional lorlatinib resistance mechanisms.

Sequencing of ALK TKIs

The recent advances reviewed above underscore the critical need for repeat biopsies to guide therapeutic strategies. We strongly recommend pursuing repeat biopsies—when feasible and safe—in patients progressing on an ALK TKI. Particularly for patients who relapse on a second-generation ALK TKI, the detection of off-target versus on-target resistance mechanisms in repeat biopsy specimens is an essential factor in selecting the next therapy. The former generally indicates persistent ALK dependency and potential sensitivity to another ALK TKI, whereas the latter suggests the need to pursue an alternative approach (e.g., clinical trial of an ALK-based combination regimen or standard cytotoxic chemotherapy; Fig. 4). In addition, the detection of a particular ALK resistance mutation may inform the choice of the next ALK TKI, as discussed above, both in the crizotinib-resistant setting and after failure of a second-generation ALK TKI.

Although general guidelines are emerging for determining the optimal later-line ALK TKI, which TKI to use in the first-line setting remains an ongoing controversy. Currently, crizotinib is the only FDA-approved agent for use in TKI-naive ALK-positive NSCLC (4), and a second-generation ALK inhibitor (e.g., ceritinib, alectinib) can be used once patients relapse on, or are intolerant of, crizotinib. However, the up-front use of a more potent and selective second-generation ALK inhibitor may substantially delay disease progression through various mechanisms. First, next-generation ALK TKIs have activity against multiple crizotinib-resistant mutations, including the most common L1196M and G1269A mutations (82, 138), and may thus suppress the outgrowth of any preexisting clones that harbor these mutations and also prevent them from emerging de novo. Additionally, most second-generation TKIs have greater CNS activity than crizotinib and would hence delay the development of brain and leptomeningeal metastases, which are commonly seen in crizotinib-treated patients (7, 8, 139, 140).

The first reported results comparing a second-generation ALK TKI with crizotinib in the TKI-naive setting came from
Table 2. Pharmacologic properties of ALK inhibitors approved by the FDA or in clinical testing

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<td>MET</td>
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<td>Resistance mutations known to be targeted by TKI</td>
<td>L1198F</td>
<td>L1171T/N1196M</td>
<td>S1206C/Y1269A/S</td>
<td>L1152P/R C1156Y/T</td>
<td>L1152P/R C1156Y/T</td>
<td>C1156Y/T L1196M</td>
<td>C1156Y/T L1196M</td>
</tr>
<tr>
<td>Reported resistance mutations to the TKI</td>
<td>I1151Tins L1152P/R C1156Y/T</td>
<td>L1171T/N1196M</td>
<td>S1206C/Y1269A/S</td>
<td>I1151Tins C1156Y/T</td>
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<td>C1156Y/T L1196M</td>
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<td>Approved for crizotinib-pre-treated</td>
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Abbreviations: TKI, tyrosine kinase inhibitor; IGF1R, insulin-like growth factor 1 receptor; IR, insulin receptor; GAK, cyclin G-associated kinase; LTK, leukocyte receptor tyrosine kinase; RET, rearranged during transfection; TRK, tropomyosin receptor kinase; N.D., not determined; 1L, first-line; N/A, not applicable.

*Brigatinib has been reported to have some activity against the ALK**1202R** mutation (128-130), but G1202R has also been detected in biopsy specimens from patients with ALK-rearranged NSCLC who relapsed on brigatinib (78), suggesting that its potency may be compromised against this mutation.

**G1202R is a highly refractory mutation resistant to all first- and second-generation inhibitors, but targeted by lorlatinib (74, 78).**

***L1198F has been found to confer resistance to lorlatinib in the context of another mutation, C1156Y (74).***
Overcoming Resistance to ALK Inhibitors

the J-ALEX study, comparing alectinib with crizotinib in Japanese patients with ALK-positive NSCLC. Preliminary results suggest that first-line alectinib may be superior to crizotinib (141). The median PFS in the crizotinib arm was 10.2 months [95% confidence interval (CI), 6.2–12.0 months], whereas the median PFS was not reached in the alectinib arm (95% CI, 20.3 months—not estimated; ref. 141). Based on these results, alectinib was recently granted FDA breakthrough therapy designation for first-line treatment of ALK-positive NSCLC. It is worth noting that there are a number of important limitations to the J-ALEX study, including an imbalance of patients with baseline brain metastases and a higher than expected rate of toxicities with crizotinib. A similar but global phase III trial (ALEX) comparing first-line alectinib to crizotinib in ALK-positive NSCLC is ongoing (NCT02075840), and results of this study will likely be practice-changing. The ALEX trials have not allowed patient crossover at the time of progression. Therefore, they will not address the more relevant comparison of first-line alectinib versus sequential crizotinib followed by alectinib.

Combination Regimens

The knowledge of bypass signaling tracks that can foster ALK TKI resistance has fueled efforts to develop combinatorial approaches for use in patients who relapse on TKI therapy, or in the up-front setting to delay resistance and potentially enable more durable responses than those achieved using monotherapy alone. Similarly, the discovery that each ALK TKI is associated with a unique spectrum of ALK resistance mutations suggests that combinations of ALK TKIs could be beneficial in overcoming or preventing on-target resistance mechanisms. Different combinations of targeted agents, chemotherapies, and/or immunotherapies with ALK TKIs are currently being evaluated. However, many of the ongoing studies are not biomarker driven.

Clinical trials to test the efficacy of dual ALK and MEK blockade are being developed in light of the compelling preclinical data discussed above (64, 92). Combinations of ceritinib and LEE011, a CDK4/6 inhibitor, and of ceritinib and everolimus, an mTOR inhibitor, are in early-phase testing in NSCLC (NCT02292550 and NCT02321501, respectively); preclinical data for these approaches are limited. The combination of alectinib with bevacizumab, an antiangiogenesis agent targeting vascular endothelial growth factor (VEGF), is being tested in patients with ALK-positive NSCLC with at least one CNS target lesion (NCT02521051). The rationale is that bevacizumab may help augment the systemic and intracranial drug activity by modulating the tumor vasculature (142). Findings from the ALK TKI resistance studies described above would also support efforts to therapeutically cotarget ALK with MET, EGFR, KIT, or SRC (Fig. 1).

Several studies are investigating the efficacy and tolerability of an ALK TKI combined with immunotherapy in lung cancer [e.g., crizotinib with nivolumab or ipilimumab (NCT01998126) or pembrolizumab (NCT02511184); ceritinib with nivolumab (NCT02393625); alectinib with alsetolizumab (NCT02013219); lorlatinib with avelumab (NCT02584634)]. However, there are limited preclinical data thus far to support this combination strategy. Although PD-1/PD-L1 inhibitors have demonstrated durable activity in a subset of NSCLC, responses are limited to ~20% of patients, and have been associated with high PD-L1 expression, high tumor mutational load, and smoking history (143–150). Patients with ALK-positive NSCLC tend to be never-smokers with a low tumor mutational load (151). Moreover, a recent study demonstrated that ALK-positive NSCLCs tend to lack concurrent PD-L1 expression and CD8+ tumor-infiltrating lymphocytes in the tumor microenvironment—an important component of response to immunotherapy (152). Indeed, no responses were seen among 6 patients with ALK-positive NSCLC treated with checkpoint inhibitors in a small retrospective analysis (152), and in large randomized studies of previously treated NSCLC, subgroup analyses demonstrated no survival benefit to checkpoint inhibitors over chemotherapy in never-smokers and/or EGFR-mutant patients (144, 145). Therefore, the potential benefit of combining immunotherapies with ALK TKIs is unclear at this time, and the optimal sequencing and timing of such an approach warrants careful investigation. In addition, future studies will need to define the level of PD-L1 expression in a larger cohort of ALK-positive (and other oncogene-driven) NSCLCs and explore whether additional biomarkers predictive of response to immunotherapy may be present in a subset of these patients.

Ultimately, any successful combination regimen will need to have demonstrated not only superior efficacy compared to monotherapy, but also tolerability and feasible dosing in patients. Toxicities are often exacerbated when two drugs are given in combination, and even unanticipated toxicities may arise. For example, the combination of the third-generation EGFR TKI osimertinib plus the PD-L1 inhibitor durvalumab (MEDI4736) was found to cause interstitial lung disease in 38% of the patients, compared to 2% to 3% in patients receiving either osimertinib or durvalumab as monotherapy (153). Similarly, combined pan-HER and ALK/ROS1/MET inhibition using dacomitinib and crizotinib, respectively, led to grade 3 or 4 treatment-related adverse events in 43% of patients with NSCLC, the most common of which were diarrhea, rash, and fatigue (154). The design of alternative dosing schedules, such as the use of submaximal doses of each individual drug, or pulsed or intermittent dosing, may help mitigate toxicities.

Targeting Oligopersistent or Oligopersistent Disease

Clinically, the course and pace of disease progression on a TKI can be variable among patients. Although most patients develop systemic, multisite progression requiring a change in systemic therapy, a subset of patients develop progression limited to only one or a few anatomic sites, with the remaining disease sites continuing to be controlled by the TKI. This phenomenon has been termed “oligoprogression” (155, 156). In the case of oligopersistent disease, local ablative therapy (LAT) using surgery or radiation may serve as a strategy that offers several advantages (Fig. 4). First, LAT can eradicate the TKI-resistant clone(s) at the progressing sites and potentially delay the evolution of more heterogeneous resistant tumor cell populations. Second, it permits the continued, maximal use of the TKI that is otherwise active against sensitive tumor clones in sites that are not progressing.

The feasibility and utility of the LAT approach has been suggested by a limited number of single-institution, retrospective
studies. In one study including 25 EGFR-mutant and ALK-positive NSCLC patients with oligoprogressive disease on erlotinib and crizotinib, respectively, LAT followed by recommencement of the TKI led to an additional 6.2 months of median PFS (157). In an updated analysis of 14 patients with ALK-positive NSCLC with extracranial oligoprogression on crizotinib, LAT led to 6- and 12-month actuarial local lesion control rates of 100% and 86%, respectively. The median time to second progression was 5.5 months, and median duration on crizotinib was 28 months (158). These results provide the rationale for larger, prospective studies of the local ablative strategy.

Taken one step further, patients may achieve an overall tumor response to a TKI but continue to have a few sites of persistent, residual disease (“oligopersistent disease”; ref. 155). Similar to the oligoprogressive situation, these sites of oligopersistent disease may serve as a reservoir of residual, TKI-insensitive tumor cells that can eventually drive systemic therapy failure (159). In one randomized phase II study, the efficacy of adding local consolidative therapy after induction systemic therapy was compared with maintenance systemic therapy or surveillance in oligometastatic NSCLC (160). Among 49 patients including 8 with EGFR-mutant or ALK-positive NSCLC who achieved disease control after the induction systemic therapy (e.g., platinum-doublet chemotherapy, erlotinib, or crizotinib), the addition of local therapy versus no local therapy led to an improved median PFS (11.9 months vs. 3.9 months), suggesting that aggressive treatment of oligopersistent sites of disease may help improve patient outcomes. Another phase II trial is assessing the efficacy of treating up to 5 sites of oligopersistent disease using stereotactic body radiotherapy within 6 months of initiating TKI therapy, specifically in patients with EGFR-mutant, ALK-rearranged, or ROS1-rearranged NSCLC (NCT02314364).

**Targeting Persister Cells**

An emerging question in ALK-rearranged cancers involves the role of drug-tolerant persister cells in driving residual disease on TKIs and ultimately therapy failure. Recent work suggests that persister cells, which survive the initial TKI exposure through adaptive mechanisms, can eventually acquire overt genetic alterations, leading to full-fledged resistance and clinical relapse (161, 162).

In *EGFR*-mutant NSCLC, persister cells have been identified as an important nidus for the *de novo* development of heterogeneous EGFR TKI resistance mechanisms including EGFR*<sup>T790M</sup>* (162, 163). The persister cell–derived, *de novo* EGFR*<sup>T790M</sup>*-mutant cells emerge later than, and are biologically distinct from, the early-resistant, preexisting EGFR*<sup>T790M</sup>*-mutant cells (163). Compared with early-arising T790M-mutant cells, late-arising T790M-mutant cells exhibit diminished apoptotic response to the T790M-mutant-selective EGFR TKI WZ4002. However, this can be overcome by the addition of navitoclax, a BCL2 inhibitor. Based on these preclinical findings, the combination of osimertinib and navitoclax is now being evaluated in patients with *EGFR*-mutant lung cancer after progression on a first-generation EGFR TKI (NCT02520778).

A role for persister cells in mediating ALK TKI resistance has yet to be determined. It will be important to characterize the biology of persister cells and identify their therapeutic vulnerabilities, which could be targeted to prevent *de novo* resistance. More broadly, there is a growing need to focus research efforts on designing and validating strategies to eradicate residual disease in order to suppress the emergence of resistance up front. This anticipatory approach—rather than treating resistance once it has already developed—has the greatest potential to truly transform patient outcomes.

**CONCLUSIONS**

ALK is an established therapeutic target in lung cancer and several other hematologic and solid malignancies, including ALCL and IMT. Since its discovery as a fusion oncogene in 1994, much insight has been gained into the biology of both native and oncogenic ALK. In parallel, numerous ALK inhibitors have entered the clinic, and to date, three have become standard therapies for advanced ALK-positive lung cancer.

Despite the remarkable responses seen with ALK TKIs, patients invariably relapse due to acquired resistance, and therefore developing strategies to overcome or prevent resistance is an urgent priority. With the growing knowledge of resistance mechanisms, new treatment approaches can be rationally designed. These new approaches hold the promise of more effectively overcoming and suppressing drug resistance, translating into deeper and more prolonged responses in patients with ALK-driven cancers.

**Disclosure of Potential Conflicts of Interest**

G.J. Riely reports receiving commercial research grants from Novartis, Pfizer, Ariad, and Genentech/Roche and is a consultant/advisory board member for Novartis and Genentech/Roche. A.T. Shaw is a consultant/advisory board member for Pfizer, Novartis, Ignyta, Lexox, Roche, Genentech, Daiichi-Sankyo, Taiho, EMD Serono, Foundation Medicine, Blueprint Medicines, and KSQ Therapeutics. No potential conflicts of interest were disclosed by the other author.

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**REFERENCES**


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