Mutations in ALK are a common mechanism of acquired resistance to small molecule ALK inhibitors in ALK-rearranged lung cancer. Different mutants exhibit differential sensitivity to ALK inhibitors. Matching the mutational profile of the tumor with the appropriate ALK inhibitor is likely to be important to maximize benefit for patients. *Clin Cancer Res; 20(22); 5576–8. ©2014 AACR.*

In this issue of *Clinical Cancer Research*, Katayama and colleagues describe the identification and characterization of two mutations that confer resistance to the novel small molecule ALK (anaplastic lymphoma kinase) inhibitor, alectinib (1). Since the discovery of rearrangements in the ALK gene in non–small cell lung cancers (NSCLC) in 2007, there has been rapid clinical development of small molecule ALK inhibitors for the 3% to 5% of NSCLCs driven by an ALK fusion oncogene (2). Crizotinib was the first ALK inhibitor to receive FDA approval for patients with ALK-rearranged (ALK⁺) NSCLC. Although the majority of patients with ALK⁺ NSCLC who are treated with crizotinib achieve dramatic radiographic and/or clinical improvement (3), resistance inevitably develops, generally within 1 year of starting crizotinib. Resistance to crizotinib emerges by a variety of mechanisms (4). In ~30% of cases, point mutations in ALK or amplification of the fusion gene can be identified, suggesting that such tumors may still be dependent on ALK for their survival. Another third of crizotinib-resistant tumors exhibit activation of signaling pathways that bypass the requirement for ALK (via EGFR activation or KIT amplification). The mechanisms of resistance in the remaining ~30% of cases are unknown.

To counter ALK-dependent mechanisms of resistance to crizotinib, multiple next-generation ALK inhibitors have been identified and are currently in clinical development, with FDA approval granted to ceritinib in 2014 for the treatment of advanced ALK⁺ NSCLC previously treated with crizotinib (5). Encouraging activity has also been observed with the ALK inhibitors alectinib and AP26113, both currently being evaluated in registrational clinical trials (6, 7). As new agents receive FDA approval, clinicians will be faced with the challenge of deciding how to choose initial therapy and sequence subsequent therapies to maximize benefit for their patients. Knowledge of the common and unique mechanisms of resistance to the different agents will be critical to inform these decisions.

Eight different mutations in the ALK tyrosine kinase (TK) domain have been described in crizotinib-resistant NSCLCs, with the L1196M "gatekeeper" and G1269A mutations being the most common (gatekeeper residues are found in multiple kinases and play a role in binding of ATP-competitive inhibitors; mutations at these residues are frequently causes of resistance to these drugs, e.g., EGFR<sup>T790M</sup> and BCR-ABL<sup>T315I</sup>; ref. 8). Ceritinib, alectinib, and AP-26113 are potent ALK inhibitors that have lower IC₅₀ than crizotinib for ALK and additionally suppress the kinase activity of several mutations associated with crizotinib-resistance including L1196M and G1296A. *In vitro* studies have demonstrated that some crizotinib-resistant mutants are cross-resistant to ceritinib (e.g., C1156Y, G1202R, 1151T-ins, and F1174C) and/or alectinib (G1202R). Indeed, analysis of ceritinib-resistant tumors from 10 patients revealed the presence of either the F1174C or G1202R mutations in 4 cases; in 2 of the cases, these mutations replaced either G1269A or S1206Y point mutations in ALK that had been identified following crizotinib resistance (9).

A limited number of studies to date have been conducted to understand mechanisms of resistance to alectinib. Molecular analysis from one alectinib-resistant tumor has been reported identifying the G1202R mutation (10). In this issue of *Clinical Cancer Research*, Katayama and colleagues integrate evidence from ALK-rearranged cell lines cultured long-term in alectinib and analysis of an alectinib-resistant tumor from a patient to identify two novel ALK mutations (V1180L and I1171T) that confer resistance to alectinib. Interestingly, the V1180L mutation—identified in the H3122 cell line following exposure to alectinib—is near the L196 gatekeeper residue, and the authors predict that the methyl group present on the leucine interferes with alectinib binding. Although, to date, the V1180L mutation...
has not been identified in crizotinib-resistant tumors, the authors demonstrate that this mutation does confer resistance to crizotinib. Surprisingly, however, the V1180L mutant retains sensitivity to ceritinib and AP26113, highlighting potential therapeutic avenues should this mutation eventually be identified in patient samples. The I1171T mutation was identified in a liver biopsy specimen from a patient with crizotinib-resistant disease treated with alectinib. This patient initially responded to alectinib, and disease progression was noted after 4 months of therapy. Molecular modeling of the mutation suggests that it causes a shift in the position of the C-helix and disrupts the formation of a hydrogen bond between E1167 of ALK and alectinib, leading to a reduced affinity of the mutant kinase for alectinib. It is possible that the I1171T mutation was present at low frequency, upon crizotinib resistance, because the same mutation has been found in crizotinib-resistant ALK+ neuroblastomas and was isolated in vitro in mutagenesis screens for mutations that confer crizotinib resistance (11, 12). Similar to the V1180L mutant, the I1171T mutation was sensitive to ceritinib (and partially to AP26113) in cell line experiments. Further confirming these observations, the patient described in this article exhibited a partial response to ceritinib following alectinib resistance.

Results from studies like those described by Katayama and colleagues (1) suggest that the spectrum of resistance-conferring ALK mutations is different for each ALK inhibitor, although some of the mutations confer resistance to one or more agents. Moreover, the findings indicate that multiple distinct mutations can emerge, even after exposure to the most potent ALK inhibitors. Finally, data from studies of crizotinib and ceritinib (9) indicate that ALK inhibitor-resistant tumors are heterogeneous, with several resistance mutations being present in individual tumors (although one may dominate). Depending on which ALK inhibitor is
trials investigating next-generation ALK inhibitors in both the first-line and refractory settings will be critical to establish whether use of more potent ALK inhibitors at diagnosis is superior to sequencing of therapies as resistance emerges. Progress in the treatment and development of therapies for ALK-rearranged lung cancer has been exponential in the past 5 years. Studies, like the one presented here, that define biomarkers predictive of response to respective ALK inhibitors and other potential therapies will be essential to refine treatment paradigms for patients with ALK-rearranged lung cancer.

Disclosure of Potential Conflicts of Interest
K. Politi is a consultant/advisory board member for Takeda and has a patent relating to EGFR T790M mutation testing that was licensed to Memorial Sloan Kettering Cancer Center to MolecularMD. S. Gettinger is a consultant/advisory board member for ARIAD Pharmaceuticals. No other potential conflicts of interest were disclosed.

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Grant Support
This work was supported by the NIH/NCI grants R01CA120247 (K. Politi) and R01CA121210 (K. Politi), the Lung Cancer Research Foundation (K. Politi), the Department of Defense Lung Cancer Research Program (K. Politi), and the Yale Cancer Center (K. Politi and S. Gettinger).

Received September 7, 2014; accepted September 9, 2014; published OnlineFirst September 16, 2014.

References
Perfect ALKemy: Optimizing the Use of ALK-Directed Therapies in Lung Cancer

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