Editorial

Emerging resistance pathways in lung cancer: what has ROS-1 taught us?

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Comment on: Dziadziuszko R, Le AT, Wrona A, *et al.* An Activating KIT Mutation Induces Crizotinib Resistance in ROS1-Positive Lung Cancer. J Thorac Oncol 2016;11:1273-81.

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Molecular characterization of non-small cell lung cancer (NSCLC) and implications for treatment and prognosis are increasingly important. Selection of molecularly targeted systemic therapy is commonly based on clonal oncogenic drivers of which epidermal growth factor receptor (*EGFR*) and the fusion gene echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (*EMLA4-ALK*) are examples established in clinical practice. The former, found in approximately 10–15% of patients with NSCLC in the western world, is more prevalent in adenocarcinoma, non/light smokers, younger females, and patients of far eastern descent (1). Patients with EMLA4-ALK translocated tumors share similar clinical characteristics but are less prevalent (2). Several other rarer candidate driver mutations, including ROS proto-oncogene 1, receptor tyrosine kinase (*ROS-1*), are also implicated in NSCLC (3).

Crizotinib, a multi-targeted tyrosine kinase inhibitor, was initially awarded accelerated approval from the US Food and Drug Administration (FDA) in ALK driven pre-treated NSCLC in 2011. Approval was based on early phase studies (2,4) later supported by confirmatory phase 3 studies (5) and subsequently expanded to patients who were treatment naïve (6). Further generations of ALK inhibitors have since been developed with the aim of overcoming inevitable treatment failure due to tumor cell resistance (7,8).

ROS-1, a less frequent genomic event in NSCLC, is thought to be homologous with *ALK*. In patients who harbor this translocation response rates to crizotinib are 70–80% (9,10), which led to its FDA approval for this indication in 2016. Although crizotinib demonstrates excellent initial response rates in both these select groups, cancer progression is an almost certain event. Understanding the associated resistance mechanisms can help with next generation drug design to overcome these therapy resistant molecular changes. A clinically approved example of this approach is osimertinib, a tyrosine kinase inhibitor, which was developed to overcome treatment failure by targeting the *EGFR* resistance mutation *T790M*.

In the *Journal of Thoracic Oncology*, Dziadziuszko *et al.* report the identification of a novel resistance mechanism in NSCLC harboring a *ROS-1* translocation both in preclinical models and a patient data set. They initially describe a young smoking female patient with a ROS-1 translocated lung adenocarcinoma, who was treated with crizotinib. The tumor developed drug resistance at 15 months. *ROS-1* break-apart fluorescence *in situ* hybridization (FISH) analysis and DNA sequencing showed no evidence of gain or loss of *ROS-1* gene fusion copy number when comparing pre- and post-crizotinib tumor samples. Further molecular testing however revealed the development of a *KIT* gene mutation (*p.D816G*) in the crizotinib treated specimen. The authors examined three further patients, however two of these did not have

sufficient tissue for analysis and the third was shown not to harbor any *KIT* mutations. Despite the limited clinical data describing the same mutation, *in vitro* studies support the hypothesis that the *KIT* (*p.D816G*) mutation is part of a resistance pathway in crizotinib treated NSCLC cell lines. Furthermore, it was observed that crizotinib led to gradual loss of inhibition of ERK1/2, likely provoked by the emergence of the *KIT* mutation later overcome by ponatinib, an inhibitor of KIT (11).

Acquired drug resistance to targeted therapy is a common problem in advanced NSCLC. It largely takes two forms: first the development of further mutations in the target receptor rendering ineffective interaction with the originally effective drug, or second, the development of compensatory cross talk between intracellular pathways, overcoming the drug effects. A change in phenotype to small cell lung cancer, epithelial-to-mesenchymal transition, and immune evasion are also thought to play a role (12). In addition unselected therapies, such as chemotherapy, exert treatment pressure on cancers and may influence the molecular profile of emergent resistant clones (13).

It is well documented in ALK mutated lung cancer that resistance mutations develop within the tyrosine kinase domain, for example L1196M and C1156Y, following treatment with crizotinib (14). Although the development of second generation ALK inhibitors were designed to overcome crizotinib induced resistance mutations, studies have shown that their use promotes further mutations both within the tyrosine kinase domain and as separate oncogenic drivers (12,15).

Ceritinib, an inhibitor of ALK, is now used for patients with cancer progression post crizotinib (16). Encouragingly, it has also been shown to exhibit activity against ROS-1 driven disease, however this requires further clinical validation (17). Alectinib, an inhibitor of both ALK and the proto-oncogene RET, initially designed to overcome crizotinib resistance, has recently demonstrated superiority in treatment naïve patients compared to crizotinib, although its activity in ROS-1 driven disease remains uncertain (18). Similarly, *ROS-1* is associated with acquired missense mutations, such as G2032R and D2033N within the kinase domain, following treatment with crizotinib (19,20). Drugs such as cabozantinib are under investigation to overcome intrinsic mutations within *ROS-1* (21).

The reprogramming of signaling pathways through other molecules is well established (including activation of *EGFR* (12), *RAS* (22) and now the above described *KIT* mutation) playing a significant role in clinical drug resistance. Interestingly, *KIT* (*p.D816G*) is also a recognized resistance mutation in gastrointestinal stromal tumors (GIST) following treatment with imatinib, an inhibitor of KIT. Although the exact mechanism of resistance appears different in GIST tumors (23), *KIT* mutations in ALK translocated NSCLC have been shown to be managed with simultaneous crizotinib and imatinib treatment reversing its resistant phenotype in cell lines (24).

The ROS-1 tyrosine kinase receptor is thought to act through more than one intracellular pathway as downstream signaling through PI3K/AKT and MAPK are both recognized. The tyrosine protein kinase KIT shares similar signaling pathways following provocation with its natural ligand stem cell factor. The hypothesis that downregulation of these pathways through ROS-1 inhibition may lead to compensatory upregulation through the activation of KIT could conceivably lead to drug failure. Dual inhibition of ROS-1 and KIT could be explored to overcome the resistance pathway to crizotinib. This strategy of drug combination therapy is supported by the authors as ROS-1 and KIT can be constitutively active at the same time or ROS-1 activates KIT, through its inhibition. This approach seems worth evaluating as manipulation of neighboring pathways has been successful in MET amplified EGFR mutant NSCLC (25).

Emerging mechanisms of resistance in EGFR mutant lung cancer are now being tackled in the clinic. As described earlier, osimertinib, the first licensed tyrosine kinase inhibitor for this indication, targets the frequently identified *T790M* gatekeeper mutation within the EGFR tyrosine kinase domain. Particularly interesting is the superior efficacy of osimertinib over early generation primary inhibitors in patients who have never encountered EGFR inhibition. Recently, published studies have confirmed superiority in median progression free survival (PFS) and overall response rates (ORR) compared with standard of care treatment. At best, PFS was measured at 22.1 months and ORR almost 90%. Notably, it was shown that patients who developed progression in cancer in one of the studies demonstrated resistance pathways which did not include T790M (26,27).

Further work is still needed to develop robust methods of managing tumor resistance. Targeting these pathways may not always lead to reversing drug failure, as demonstrated by the authors. Variation in KIT behavior to inhibition with ponatinib was noted, indicating that certain cellular characteristics may dictate how KIT responds to

pharmacological suppression. In the absence of stromal tissue, an immune system and cellular heterogeneity, assessment of these mechanisms is more challenging and better tumor models are desperately needed to increase our understanding of why tumors behave in this way.

Complicating matters further, it is also important to consider both the temporal evolution and spatial heterogeneity of lung cancer (28). The increasing complexity of mapping a cancer's evolution through space and time requires repeated molecular characterization of new sites of disease to best understand the emerging mechanisms of resistance and tailor therapy for our patients. A personalized approach to cancer management remains an ethos commonly shared amongst cancer treating physicians, and this evidence provides further support for individualized molecular treatment against emerging validated targets. Future clinical trials concentrating on disease resistance to primary treatment, not just in NSCLC, remain hugely important.

Given its low prevalence and despite enthusiastic attempts to locate these patients, research into *ROS-1* mutations may be guided by ALK driven disease due to their shared homology. Current credible approaches to overcome treatment failure include deeper inhibition of ROS-1 and exploring the sequencing of targeted therapy tackling by-pass resistance mutations when they emerge. The balance of combination therapy and associated toxicity burden is as important as ever and these factors require attention when redesigning treatment pathways.

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Footnote

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