IN THE SPOTLIGHT

Targeting ALK: The Ten Lives of a Tumor

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Summary: In this issue, Infarinato and colleagues report the results of preclinical testing of a novel ALK/ROS1 inhibitor, PF-06463922, in neuroblastoma. This small-molecule inhibitor was shown to efficiently inhibit the growth of patient-derived and established neuroblastoma xenograft models expressing mutated ALK. Although the *in vivo* data are impressive and the authors suggest that clinical trials are warranted, the presented data also suggest that it is as yet too early to welcome the new drug as a magic bullet. *Cancer Discov; 6(1); 20–1. ©2016 AACR.*

See related article by Infarinato et al., p. 96 (8).

Neuroblastomas are tumors of the peripheral adrenergic lineage that arise in young children. Although patients with low-risk tumors have an excellent outcome, those with high-risk disease have a survival probability of only 30% to 40%. Patients with high-risk disease typically attain complete remission after intensive multimodal treatment, but the majority experience relapse of fatal therapy-resistant lesions. Since the original identification of activating somatic *ALK* mutations in neuroblastoma in 2008, multiple large-scale sequencing studies have established a consensus mutation rate of approximately 8%, with amplification of *ALK* comprising another 4%. Studies on the prognostic impact of *ALK* mutations have been conflicting, whereas others have found that ALK overexpression supersedes *ALK* mutations in predicting outcome. Three types of kinase domain mutations are dominant—F1174L, R1275Q, and F1245C—all of which confer increased proliferation, growth factor independence, and activation of canonical downstream signaling pathways. These changes induce tumor development in nude mice, thus firmly establishing the oncogenic role of mutant ALK in neuroblastoma. The ALK^{F1174L} mutation has attracted much attention, primarily because of its cosegregation with *MYCN* amplification in human tumors and its capacity to enhance tumorigenicity in transgenic animals (1, 2).

As hardly any other mutated kinases had been identified in neuroblastoma, the discovery of *ALK* mutations in 2008 generated much hope for targeted therapy of this tumor, and enthusiasm was high for the immediate translation of this finding. This led to the rapid institution of a Children's Oncology Group (COG) phase I trial with the only clinically available inhibitor with activity against ALK, crizotinib. This drug had shown remarkable activity in patients with non–small cell lung cancer (NSCLC) characterized by expression of oncogenic ALK fusion proteins. However, in preclinical studies in neuroblastoma, it became clear that although crizotinib inhibited growth and induced apoptosis in cells expressing ALK^{R1275Q}, it failed to inhibit the growth of ALK^{F1174L}-positive cells (3). Further, F1174L was one of the resistance mutations that arose in adult cancer patients treated with crizotinib as a single agent (4). This deficiency was illustrated in the COG trial of crizotinib, where patients with neuroblastoma with point mutations in *ALK* had a less-than-favorable response. Only 1 of 11 patients with *ALK*-mutated tumors had an objective response, and in that case, the cells expressed the ALK^{R1275Q} mutation (5). These preclinical and clinical results question the rationale for further investigation of single-agent crizotinib in patients with neuroblastoma. Alternative strategies include combinations with other targeted agents, such as inhibitors of downstream signaling or evaluation of newer-generation ALK inhibitors.

PF-06463922 is an orally available, ATP-competitive small-molecule inhibitor of ALK and ROS1 with the ability to cross the blood–brain barrier (6). It leads to the induction of apoptosis in NSCLC cell lines at low nanomolar concentrations (100 times lower than crizotinib) and appears to be active against a wide range of mutations, including those that are refractory to crizotinib (7). Infarinato and colleagues now test PF-06463922 in neuroblastoma models expressing mutated *ALK*, and compare its activity against that of crizotinib (8). The inhibitor appears to be remarkably effective in xenograft and patient-derived xenograft (PDX) models of neuroblastoma expressing the more common *ALK* mutations. Four models were tested: two PDX models expressing F1174L and F1245C, respectively, and two established neuroblastoma cell line xenograft models expressing F1174L and R1275Q, all of which were treated for a minimum of 6 weeks. PF-06463922 induced a shrinkage of tumor volumes below palpable detection in all four models, starting from 2 to 3 weeks after the onset of treatment. Downregulation of ALK phosphorylation was shown only in the R1275Q xenograft model. In three models, the tumors remained undetectable during the full 6 to 9 weeks of treatment. In the fourth model (R1275Q), a small tumor emerged 7 to 8 weeks after the start of the treatment. Although this is a major improvement over responses obtained with crizotinib, the data also predict the limitations

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of the drug. Discontinuation of PF-06463922 resulted in regrowth of the tumors within 4 to 7 weeks in all 4 models, suggesting that in the clinical setting, a population of tumor cells will likely persist during treatment and ultimately give rise to relapse (8). The nature of the recurrent tumors was not investigated by Infarinato and colleagues. The tumors were followed by palpation only, which precludes an accurate estimate of the amount of viable tumor persisting during treatment. Additionally, in the *in vitro* studies, although the IC_{50} values were significantly better than those for crizotinib, PF-06463922 appeared to inhibit the growth of only a proportion of the cells, with as many as 25% to 50% remaining at maximum drug concentrations. Whether these remaining cells undergo growth arrest or senescence is not addressed by the data presented. It is possible that the drug leaves a residual subpopulation of inherently resistant cells that enter a slow cycling state only to rapidly proliferate after the drug stimulus is removed. This phenomenon of tumor cell plasticity in the presence of certain therapeutic agents (9) may well account for recurrences seen in the *in vivo* models described in this study. The fact that PF-06463922 causes complete growth inhibition of NSCLC cells expressing EML4–ALK and NIH3T3 cells transfected with the three neuroblastoma-associated *ALK* mutations further supports the premise that neuroblastoma tumors may contain a subpopulation of cells that are inherently resistant to PF-06463922.

The demonstration by Infarinato and colleagues of impressive tumor regression in both established cell line and PDX models, together with the lessons learned from phase I testing of crizotinib, provides a promising starting point for further preclinical characterization of PF-06463922. It will be especially important to elucidate the downstream signaling pathways that are disrupted by the compound's effect on mutant ALK as well as the mechanisms of cytotoxicity. A clarification of the cell fraction surviving *in vitro* and its relation to the recurrences seen *in vivo* seems urgent, as they clearly bear on the design of optimal clinical trials. The major clinical problem posed for neuroblastoma treatment is not the induction of complete remission, as this is readily achieved with standard therapeutic options in most patients. Rather, the greatest challenge lies in the prevention of recurrence, or in its eradication after it emerges. Whether PF-06463922 will be useful in this regard, or whether it will chiefly be an agent that efficiently kills tumor cells at diagnosis, remains to be seen. Interestingly, a recent sequencing effort directed to relapsed neuroblastoma suggests that MAPK pathway activation is prevalent in these tumors and has the potential to cause relapses (10). This finding is important because in a large fraction of relapsed tumors, the upregulation of MAPK signaling was due to *ALK* mutations, which makes elucidation of the basis for ALK inhibitor resistance even more important.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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