Editorial Commentary

FLT3-IRAK dual targeting: an exciting new therapeutic option guided by adaptive activation of immune response pathways

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With the development of small molecule inhibitors that target oncogenic pathways in cancer therapy, mechanisms of resistance have been increasingly identified. Adaptive signaling responses to small molecule targeted therapy have been well-established as a consequence of oncogene pathway inhibition, and serve to inform translational science about possible mechanisms of long-term acquired resistance, as well as to suggest targets for combination therapy. Adaptive signaling responses that result from loss of negative feedback inhibition were first reported in response to PI3K-AKT and MEK-ERK inhibition (1-5). Reactivation of other nodes in critical mitogenic pathways is often noted, and in many cases, the response to intracellular signaling pathway inhibition involves upregulation of receptor tyrosine kinases (5-7).

Small molecule inhibitors of FLT3 have demonstrated some success in the management of patients with FLT3-mutated acute myeloid leukemia (AML). Two such agents are currently FDA-approved for treatment of FLT3-ITD (internal tandem duplication) AML—midostaurin (for newly diagnosed) and gilteritinib (for relapsed/refractory) (8). Results of the most recent pediatric study in patients with FLT3-ITD AML demonstrated significant benefit in both event free survival (EFS) and overall survival (OS) with the addition of sorafenib to standard chemotherapy (9). Despite this improvement, many patients do not have durable responses, and some patients do not respond at all (10-12). Given the poor overall prognosis associated with FLT3-ITD AML, there is an unmet need to improve upon currently

available targeted therapies, either through development of more potent inhibitors or through rationally designed combination therapy. Several reports have addressed the possible mechanisms underlying the adaptive response and limitations to therapy—these include the emergence of acquired resistance mutations in the FLT3 kinase domain (13), activation of parallel signaling pathways (14), upregulation of FLT3 ligand (15), and bone marrow stromal cell mediated activation of ERK signaling (16). Further, our group reported the finding of ERK signaling reactivation upon FLT3 inhibition, suggesting the role for combined FLT3/MEK inhibition in the treatment of FLT3-ITD AML (17). Finally, preclinical work combining FLT3 with other small molecule inhibitors has shown some promise in this challenging disease (15).

Melgar and colleagues sought to further investigate mechanisms of adaptive resistance to FLT3 inhibition in FLT3-ITD AML, in order to develop therapies that concomitantly target the primary oncogenic signaling pathway and the relevant adaptive resistance mechanism and therefore exert more potent therapeutic effects. The authors found that the primary adaptive resistance to FLT3 inhibition is mediated by a non-FLT3-mediated cell-intrinsic mechanism, rather than acquired mutations in FLT3. They examined in-cell kinase activity using serine-threonine kinase (STK) PamChip arrays and gene regulatory networks using RNA sequencing, respectively, and results of both investigations suggested that compensatory activation of innate immune stress pathways is implicated in adaptive

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resistance to FLT3 inhibitor. The authors further observed increased phosphorylated IRAK1/4 in response to FLT3 inhibition (quizartinib or gilteritinib) in FLT3-ITD AML cells both *in vitro* and *in vivo*. A potential mechanism of IRAK1/4 activation in adaptively resistant FLT3-ITD AML cells was identified, resulting from increased expression of upstream toll-like receptors (TLRs), such as TLR9, such as TLR9, that may account for the activation of innate immune pathways. The combination of FLT3 inhibition and IRAK1/4 inhibition exhibited synergy in cell growth inhibition. Functional studies with IRAK4 knock down and over-expression experiments provided confirmation that IRAK1/4 is required for adaptive resistance of FLT3-ITD AML to FLT3 inhibition. The authors expertly developed a series of dual FLT3/IRAK inhibitors, and identified a lead compound, NCGC1481. This compound was more effective at preventing compensatory activation of IRAK1/4, compared to combined inhibition of FLT3 and IRAK1/4 using two molecules. Crystal structures of the NCGC1481-IRAK4 complex and the NCGC1481-FLT3 complex showed that NCGC1481 competes with ATP binding in both IRAK4 and FLT3. Functional studies performed by the authors showed that NCGC1481 inhibits IRAK1/4 and compensatory innate immune signaling, prevents adaptive resistance *in vitro*, and effectively targets resistant FLT3-ITD AML xenografts.

Derangements in IRAK1/4 signaling have been implicated not only in cancer, but in cardiovascular and inflammatory diseases, indicating their physiologic importance (18). A phase 1 clinical trial of the selective IRAK4 inhibitor CA-4948 in patients with relapsed and refractory non-Hodgkin lymphoma (NHL) and AML is ongoing (NCT03328078). In addition, the kinase inhibitor pacratinib showed nanomolar inhibition of IRAK1/4 (19). Potent and effective clinically therapeutic FLT3 inhibition, while improved with the next generation inhibitors gilteritinib and quizartinib, remains challenging, with up to 20% of patients developing additional mutations while receiving FLT3 inhibitors (20). Identification of additional adaptive response pathways in models of FLT3-ITD [15], including Ras/MAPK and PI3K/AKT signaling cascades, lends support to dual inhibition of these pathways as an appealing therapeutic option. The downstream pathways of IRAK1/4 appear to be effectively inhibited by NCGC1481, suggesting that concomitant targeting of IRAK1/4 and FLT3 may yield more durable inhibition of bypass signaling and may be the most effective means to overcome adaptive resistance to FLT3 inhibition, though additional pre-clinical studies would be needed to confirm this hypothesis.

However, we would be remiss not to comment on the difficulties associated with tyrosine kinase inhibitors clinically. As the authors have pointed out, the promiscuity of even the more specific tyrosine kinase inhibitors (TKI) makes it difficult to know which signaling molecules are most important for inhibition, particularly in this adaptive response model. Using the KiNactiv profile, it is evident that IRAK1/4 and FLT3 are not the only targets of NCGC1481. Other kinases within a similar IC_{50} range include ABL and LYN. ABL fusions have been targeted in leukemia, both in the chronic myeloid leukemia [CML, t(9;22)] and in Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) (21,22). LYN activation is also seen in Ph-like ALL (23). In addition, inherited IRAK4 deficiency results in impaired immunity and increased susceptibility to infections (24); it is not clear whether infectious complications would be attributable to IRAK inhibition, but increased incidence of invasive infection would potentially be a dose-limiting toxicity, particularly when FLT3-IRAK1/4 inhibitors are combined with cytotoxic chemotherapy.

We commend the authors on their work to identify and validate the role of IRAK1/4 as an adaptive response mechanism in FLT3-ITD AML treated with FLT3 inhibitors; it remains to be seen whether this finding will have clinical and therapeutic implications.

Acknowledgments

Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011;19:575-86.

2. Chandarlapaty S, Sawai A, Scaltriti M, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 2011;19:58-71.

3. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500-8.

4. Pratilas CA, Taylor BS, Ye Q, et al. (V600E)BRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional output of the pathway. Proc Natl Acad Sci U S A 2009;106:4519-24.

5. Lito P, Pratilas CA, Joseph EW, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell 2012;22:668-82.

6. Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov 2012;2:227-35.

7. Montero-Conde C, Ruiz-Llorente S, Dominguez JM, et al. Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. Cancer Discov 2013;3:520-33.

8. Daver N, Schlenk RF, Russell NH, et al. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia 2019;33:299-312.

9. Pollard JA, Alonzo TA, Brown PA, et al. Sorafenib in Combination with Standard Chemotherapy for Children with High Allelic Ratio FLT3/ITD+ AML Improves Event-Free Survival and Reduces Relapse Risk: A Report from the Children's Oncology Group Protocol AAML1031. American Society of Hematology Washington, DC; 2019.

10. Konig H, Levis M. Targeting FLT3 to treat leukemia. Expert Opin Ther Targets 2015;19:37-54.

11. Grunwald MR, Levis MJ. FLT3 inhibitors for acute myeloid leukemia: a review of their efficacy and mechanisms of resistance. Int J Hematol 2013;97:683-94.

12. Grunwald MR, Levis MJ. FLT3 Tyrosine Kinase Inhibition as a Paradigm for Targeted Drug Development in Acute Myeloid Leukemia. Semin Hematol 2015;52:193-9.

13. Smith CC, Wang Q, Chin CS, et al. Validation of ITD mutations in FLT3 as a therapeutic target in human acute myeloid leukaemia. Nature 2012;485:260-3.

14. Piloto O, Wright M, Brown P, et al. Prolonged exposure to FLT3 inhibitors leads to resistance via activation of parallel signaling pathways. Blood 2007;109:1643-52.

15. Sato T, Yang X, Knapper S, et al. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. Blood 2011;117:3286-93.

16. Yang X, Sexauer A, Levis M. Bone marrow stroma-mediated resistance to FLT3 inhibitors in FLT3-ITD AML is mediated by persistent activation of extracellular regulated kinase. Br J Haematol 2014;164:61-72.

17. Bruner JK, Ma HS, Li L, et al. Adaptation to TKI Treatment Reactivates ERK Signaling in Tyrosine Kinase-Driven Leukemias and Other Malignancies. Cancer Res 2017;77:5554-63.

18. Singer JW, Fleischman A, Al-Fayoumi S, et al. Inhibition of interleukin-1 receptor-associated kinase 1 (IRAK1) as a therapeutic strategy. Oncotarget 2018;9:33416-39.

19. Singer JW, Al-Fayoumi S, Ma H, et al. Comprehensive kinase profile of pacritinib, a nonmyelosuppressive Janus kinase 2 inhibitor. J Exp Pharmacol 2016;8:11-9.

20. Alvarado Y, Kantarjian HM, Luthra R, et al. Treatment with FLT3 inhibitor in patients with FLT3-mutated acute myeloid leukemia is associated with development of secondary FLT3-tyrosine kinase domain mutations. Cancer 2014;120:2142-9.

21. Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. J Clin Invest 2007;117:2067-74.

22. Roberts KG, Yang YL, Payne-Turner D, et al. Oncogenic role and therapeutic targeting of ABL-class and JAK-STAT activating kinase alterations in Ph-like ALL. Blood Adv 2017;1:1657-71.

23. Tasian SK, Loh ML, Hunger SP. Philadelphia chromosome-like acute lymphoblastic leukemia. Blood 2017;130:2064-72.

24. Picard C, von Bernuth H, Ghandil P, et al. Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency. Medicine (Baltimore) 2010;89:403-25.