Optimizing Next-Generation AML Therapy: Activity of Mutant IDH2 Inhibitor AG-221 in Preclinical Models

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Summary: AG-221 or enasidenib is a first-in-class selective inhibitor of mutated isocitrate dehydrogenase 2 (IDH2) with early demonstrated clinical efficacy in acute myeloid leukemia as a single agent, yet with persistence of mutant IDH2 clones. Two articles in this issue of Cancer Discovery provide further insight into the biological activity of AG-221 in promoting differentiation of IDH2-mutant cells and reversing aberrant DNA methylation over time, and demonstrating preclinical activity in combination with a targeted FLT3 kinase inhibitor to eliminate IDH2-mutant clones. Cancer Discov; 7(5); 459–61. © 2017 AACR.

See related article by Yen et al., p. 478 (6).
See related article by Shih et al., p. 494 (7).

The discovery of hotspot mutations in the canonical Krebs cycle enzymes isocitrate dehydrogenase 1 and 2 (IDH1/2) across multiple cancer types is arguably one of the most intriguing and potentially clinically actionable findings that has emerged from high-throughput sequencing efforts in recent years (1). By directly linking cell metabolism and epigenetics, IDH1/2 mutations have been an active focus of drug development since it was noted that recurring mutations (most commonly IDH2R140Q, IDH2R172K, or IDH1R132H) consistently encode the substitution of an arginine residue located in the catalytic site for another similar but nonidentical basic amino acid (1). Clonal stem cell studies have shown that IDH1/2 mutations, present in approximately 20% of patients with acute myeloid leukemia (AML; ref. 2), can arise as early and stable drivers in the evolution of leukemia that persist in remission (3). IDH1 mutations result in a neomorphic enzymatic activity that produces a measurable oncometabolite, 2-hydroxyglutarate (2-HG), from its substrate isocitrate instead of the normal product, alpha-ketoglutarate. 2-HG has been found to inhibit alpha-ketoglutarate-dependent dioxygenases, including histone and DNA demethylases, causing global changes in histone and DNA methylation. AG-221, or enasidenib, developed by Agios Pharmaceuticals in partnership with Celgene, is an oral selective inhibitor specific for IDH2-R132H (4). A crystal structure reported by Yen and colleagues shows that AG-221 binds allosterically to a deep pocket buried in the dimer interface between two IDH2 protein units and exhibits slow-on slow-off binding kinetics similar to AGI-6780, a sulfonamide inhibitor first reported to inhibit mutant IDH2 (5). These observations suggest that AG-221 works principally by promoting differentiation rather than by inducing cell death, and that IDH2-mutant cells may not be completely dependent on the oncometabolite 2-HG for cell growth and survival. These data raise many important questions: Can AG-221 reverse the DNA methylation changes associated with increased 2-HG? Why do some IDH2-mutant cells persist with extended AG-221 treatment? Can AG-221 be combined with other targeted therapy to eradicate persisting mutant clones? Two articles in this issue of Cancer Discovery from Agios Pharmaceuticals in collaboration with INSERM (6) and from the laboratories of Ross Levine and Ari Melnick (7) use patient-derived xenografts and genetically engineered mouse models to investigate how this first-in-class epigenetic targeted therapy may be harnessed to improve outcomes in AML (Fig. 1).

IDH2 forms an asymmetric homodimer and carries out its function through two hydrophilic active sites formed by both protein subunits. AG-221 was developed from a triazine core that was optimized for pharmacokinetic properties through hit-to-lead medicinal chemistry. Yen and colleagues (6) show that AG-221 binds allosterically to a deep pocket buried in the dimer interface between two IDH2 protein units and exhibits slow-on slow-off binding kinetics similar to AGI-6780, a sulfonamide inhibitor first reported to inhibit mutant IDH2 (8). A crystal structure reported by Yen and colleagues shows that AG-221 stabilizes the open conformation, thereby inhibiting conversion of alpha-ketoglutarate to 2-HG, and can bind both wild-type–mutant heterodimers and mutant homodimers. After oral administration of a single dose in mice bearing IDH2-mutant tumors, AG-221 decreased 2-HG levels by 93% in serum and 97% in tumor tissue at 12 hours.

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In vivo and in vitro treatment of IDH2R140K-mutated leukemia blasts with the IDH2 mutant–specific inhibitor AG-221 promotes differentiation in a significant proportion of cells. These changes include increased granulation and phagocytic activity and upregulation of mature myeloid markers with concomitant downregulation of global DNA hypermethylation and histone methylation. Consistent with early results from clinical trials, differentiated cells are IDH2 mutation–positive and persist in the bone marrow after blast reduction in preclinical models and in some patients. In vivo studies in murine models of IDH2-mutated leukemia suggest AG-221 in combination with antiproliferative strategies, including cytarabine or FLT3 kinase inhibitors, may be beneficial in achieving further eradication of IDH2 mutant–positive cells.

To examine the biological effects of decreasing 2-HG in IDH2-mutant leukemia, Yen and colleagues tested primary patient AML samples that had endogenous IDH2 mutations. Rather than causing apoptosis, treatment with AG-221 ex vivo induced moderate changes in some cells consistent with differentiation, including an increase in granulation and lobulated nuclei and enhanced phagocytosis. These partially differentiated myeloid cells were all IDH2-mutant positive, clearly indicating a differentiation-promoting effect. In four IDH2R140K mutant–positive cells treated with AG-221, the patient-derived xenograft, in vivo treatment for 10 to 20 days resulted in the upregulation of the mature myeloid markers CD11b, CD14, CD15, and CD24 and a decrease in the progenitor marker CD117, and again differentiated cells retained the IDH2R140K mutation. In an impressive experiment designed to mimic human disease, an IDH2-mutant patient sample was engrafted into immunodeficient mice, and treatment was initiated only once circulating blasts reached 10%, a level that almost certainly corresponds to a bone marrow blast count > 20% as required for the diagnosis of AML in human patients. Continuous treatment with various doses of AG-221 led to an overall survival benefit, with 100% survival of mice treated with the highest dose. Interestingly, even in these long-term survivors, blasts still persisted in the bone marrow and had a significant mutant IDH2 allele burden, similar to the results in patients (4). Thus, it appears that epigenetically targeted mutant IDH2 inhibition can cause differentiation of leukemia blasts, an overall survival benefit in animal models, and clinical responses, but is not able to eradicate the leukemic clone.

In the second report, Shih and colleagues (7) use a murine model of IDH2R140K combined with FLT3-ITD mutation to test the biological properties of AG-221. This new in vivo model is characterized by expansion of c-Kit–positive cells in the blood and replacement of the stem/progenitor compartment by a monomorphic population of CD48−CD150− multipotent progenitor cells resembling leukemia stem cells (9). They also compare and contrast their results with their previously described model combining TET2 deficiency with FLT3-ITD (9), but instead treat these leukemias with 5-azacitidine as a semitargeted DNA methyltransferase inhibitor. Importantly, they note that both AG-221 and 5-azacitidine alone had an in vivo benefit with induction of more mature myeloid cells coming from mutant leukemic progenitor-like blasts, evidence again for an epigenetically driven differentiation effect. Importantly, in both models, combination therapy with the FLT3 inhibitor AC220 (quizartinib, now in phase III clinical trials NCT02039726) led to decreased mutant cell burden with recovery of some normal hematopoiesis coming from nonmutant hematopoietic stem/progenitor cells. Nevertheless, mutant blasts were still retained in the bone marrow, and survival outcomes were not assessed in detail, although the leukemic stem cell compartment was reduced. As expected and consistent with the in vitro studies, DNA methylation changes linked to IDH2 or TET2 mutations were reverted by monotherapy, but this effect was more pronounced with combination therapy. Taken together, these studies suggest that epigenetic therapies can lead to differentiation effects with persistence of the mutant clone, and that combination therapies will likely be required to eliminate disease cells. The study by Shih and colleagues provides the preclinical basis for such combination trials that are eagerly awaited.

Both of these exciting studies are consistent with the preliminary clinical data: IDH-mutant inhibitors have robust biological activity in decreasing 2-HG levels and can promote differentiation of mutated blasts in vivo, but are unlikely to
eradicate mutant clones in the majority of cases when given as monotherapy; FLT3 kinase inhibitors have limited activity as monotherapy, but can provide significant clinical benefit in combination with conventional chemotherapy (10). In considering the implications of these studies, several important questions remain: Will similar responses be seen in solid organ cancers with IDH1/2 mutations (NCT02273739)? Can disease control with persistence of mutated cells, but with incomplete myeloid differentiation, translate into long-term clinical benefits? Are there other leukemogenic effects exerted by IDH2 mutations that are independent of 2-HG? Will other combinations also exhibit therapeutic effects in reducing mutant clones and leading to survival benefits? Can these models be used to help prioritize agents for future clinical trials and combinations? Clearly, it is an exciting time for targeted therapies in AML.

Disclosure of Potential Conflicts of Interest

R. Majeti has an ownership interest (including patents) in Forty Seven Inc. and is a consultant/advisory board member for the same. No potential conflicts of interest were disclosed by the other author.

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