Published in final edited form as:

Cancer Discov. 2017 May ; 7(5): 459–461. doi:10.1158/2159-8290.CD-17-0270.

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

Daniel Thomas¹ and Ravindra Majeti¹

¹Department of Medicine, Division of Hematology, Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine

Abstract

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 papers 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 pre-clinical activity in combination with a targeted FLT3 kinase inhibitor to eliminate *IDH2* mutant clones.

TEXT

The discovery of hot-spot mutations in the canonical Krebs's 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 the late 2000s (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 IDH2 R140Q, IDH2 R172K, or IDH1 R132H) consistently encode the substitution of an arginine residue located in the catalytic site for another similar but non-identical basic amino acid (1). Clonal stem cell studies have shown that *IDH* mutations, present in approximately 20% of patients with acute myeloid leukemia (AML) (2), can arise as early and stable drivers in the evolution of leukemia that persist in remission (3). *IDH* 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 R140Q and R172K and the first mutant IDH inhibitor to enter clinical trial (NCT01915498). Interim results from a phase I/II trial for patients with *IDH2*-mutant advanced hematologic malignancies, including relapsed or refractory AML, have been

Correspondence should be addressed to: Ravindra Majeti, MD, PhD, Stanford Institute for Stem Cell Biology and Regenerative Medicine, Lokey Stem Cell Building, 265 Campus Drive, Stanford, CA 94305, rmajeti@stanford.edu, Phone: 650-721-6376, fax: 650-736-2961.

Conflict of Interest Statement: R.M. is an equity holder, consultant, and serves on the Board of Directors of Forty Seven Inc. D.T. has no potential conflicts of interest.

encouraging with monotherapy inducing clinical responses, including some complete remissions, and reductions in 2-HG levels, both in patients with R140Q and R172K mutations (4). Intriguingly, absolute increases in granulocyte counts were observed in some patients indicating a pro-differentiation effect, but without a proportional decrease in the mutant allele frequency (4). A similar phenomenon resembling a differentiation-like syndrome akin to that often observed with all-trans retinoic acid therapy in acute promyelocytic leukemia was recently reported in three *IDH1*-mutant positive AML patients treated with AG-120, a mutant IDH1 inhibitor (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. This data raises 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 papers in this issue of *Cancer Discovery* from Agios Pharmaceuticals in collaboration with INSERM (Yen et al, 2017) (6), and from the laboratories of Ross Levine and Ari Melnick (Shih et al, 2017) (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 (Figure 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 et al (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 et al shows that AG-221 stabilizes the open conformation thereby inhibiting conversion of alpha-ketoglutarate to 2-HG and can bind both wildtype-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.

To examine the biological effects of decreasing 2-HG in *IDH2*-mutant leukemia, Yen et al 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 *IDH2* R140Q patient-derived xenografts, in vivo treatment for 10–20 days resulted in upregulation of mature myeloid markers CD11b, CD14, CD15, and CD24, and a decrease in progenitor marker CD117, and again differentiated cells retained the *IDH2* R140Q 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 which 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 leukemic 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 et al (7) use a murine model of *IDH2* R140Q 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 semi-targeted DNA methyltransferase inhibitor. Importantly, they note that both AG221 or 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 non-mutant 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 it will likely require combination therapies to eliminate disease cells. The study by Shih et al provides the pre-clinical 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 *IDH* 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.

References

1. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated *IDH1* mutations produce 2-hydroxyglutarate. *Nature*. 2009; 462:739–44. [PubMed: 19935646]

2. DiNardo CD, Ravandi F, Agresta S, Konopleva M, Takahashi K, Kadia T, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. *Am J Hematol*. 2015; 90:732–6. [PubMed: 26016821]
3. Corces-Zimmerman M, Hong W, Weissman I, Medeiros B, RM. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A*. 2014; 111:2548–53. [PubMed: 24550281]
4. Stein EM, DiNardo C, Altman JK, Collins R, DeAngelo DJ, Kantarjian HM, et al. Safety and Efficacy of AG-221, a Potent Inhibitor of Mutant IDH2 That Promotes Differentiation of Myeloid Cells in Patients with Advanced Hematologic Malignancies: Results of a Phase 1/2 Trial. *Blood*. 2015; 126:323.
5. Birendra KC, DiNardo CD. Evidence for Clinical Differentiation and Differentiation Syndrome in Patients With Acute Myeloid Leukemia and IDH1 Mutations Treated With the Targeted Mutant IDH1 Inhibitor, AG-120. *Clin Lymphoma Myeloma Leuk*. 2016; 16:460–5. [PubMed: 27245312]
6. Yen K, Travins J, Wang F, David MD, Artin E, Straley K, et al. AG-221, a First-in-Class Therapy Targeting Acute Myeloid Leukemia Harboring Oncogenic IDH2 Mutations. *Cancer Discovery*. 2017 this issue.
7. Shih AH, Meydan C, Shank K, Garrett-Bakelman FE, Ward PS, Intlekofer A, et al. Combination targeted therapy to disrupt oncogenic signaling and reverse epigenetic dysfunction in *IDH2*- and *TET2*-mutant acute myeloid leukemia. *Cancer Discovery*. 2017 this issue.
8. Kernytzsky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C, et al. IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood*. 2015; 125:296–303. [PubMed: 25398940]
9. Shih AH, Jiang Y, Meydan C, Shank K, Pandey S, BarreYRO L, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell*. 2015; 27:502–15. [PubMed: 25873173]
10. Stone RM, Mandrekar S, Sanford BL, Geyer S, Bloomfield CD, Dohner K, et al. The Multi-Kinase Inhibitor Midostaurin (M) Prolongs Survival Compared with Placebo (P) in Combination with Daunorubicin (D)/Cytarabine (C) Induction (ind), High-Dose C Consolidation (consol), and As Maintenance (maint) Therapy in Newly Diagnosed Acute Myeloid Leukemia (AML) Patients (pts) Age 18–60 with *FLT3* Mutations (mut): An International Prospective Randomized (rand) P-Controlled Double-Blind Trial (CALGB 10603/RATIFY [Alliance]). *Blood*. 2015; 126:6. [PubMed: 26138538]

Figure 1. Schematic of AG-221 Effects on *IDH2*-Mutant AML

In vivo and in vitro treatment of *IDH2* R140Q mutated leukemic 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 pre-clinical models and in some patients. In vivo studies in murine models of *IDH2*-mutated leukemia suggest AG-221 in combination with anti-proliferative strategies including cytarabine or FLT3 kinase inhibitors may be beneficial in achieving further eradication of *IDH2*-mutant positive cells.