**Leukemia**

**Concept:** RUNX1 binding promotes eviction of BRG1 and binding of RING1B at MYC enhancers to repress MYC.

**Impact:** CBFβ–SMMHC may represent a potential therapeutic target to inactivate MYC in patients with inv(16) AML.

**CBFβ–SMMHC ACTIVATES MYC ENHANCERS TO PROMOTE LEUKEMOGENESIS**

In approximately 8% of patients with acute myeloid leukemia (AML), a chromosomal inversion, inv(16)(p13q22), results in expression of the CBFβ–SMMHC fusion oncoprotein. CBFβ is a stabilizing subunit that interacts with the transcription factor RUNX1, and the CBFβ–SMMHC fusion has a dominant-negative effect on RUNX1 activity. However, the role of CBFβ–SMMHC in oncogenesis remain poorly understood. Pulikkan and colleagues used a bivalent inhibitor of the CBFβ–SMMHC–RUNX1 interaction, Al-10-49, to elucidate the mechanism by which CBFβ–SMMHC inhibition induced apoptosis of inv(16) AML cells. RNA sequencing revealed that Al-10-49 treatment reduced MYC expression, resulting in apoptosis of inv(16) AML cells in vitro and in vivo. Mechanistically, Al-10-49 blocked the interaction between CBFβ–SMMHC and RUNX1, thereby freeing RUNX1 to globally enhance its DNA binding. Specifically, Al-10-49 treatment increased RUNX1 binding to three MYC enhancers, indicating RUNX1-mediated repression of MYC transcription. RUNX1 occupancy resulted in displacement of the SWI/SNF activating complex component BRG1 from MYC enhancers, replacing it with the polycomb repressive complex component RING1B, providing a mechanism for RUNX1-mediated MYC repression without disruption of the enhancer or promoter architecture. Further, Al-10-49 cooperated with the bromodomain inhibitor JQ1 to reduce inv(16) leukemogenesis in vitro and in vivo. Taken together, these findings elucidate a mechanism by which RUNX1 suppresses MYC by promoting SWI/SNF eviction and RING1B bindings. CBFβ–SMMHC restrains RUNX1 activity to promote leukemogenesis, and may be a potential therapeutic target to suppress MYC expression in patients with inv(16) AML.

**Drug Resistance**

**Major finding:** Two patients with IDH2R140Q AML acquired resistance to an IDH2 inhibitor via secondary IDH2 mutations.

**Concept:** IDH2 Q316E or I319M mutations blocked enasidenib binding, cooperating with IDH2R140Q to reduce 2HG levels.

**Impact:** Acquired Q316E or I319M mutations may underlie enasidenib resistance in patients with IDH2R140Q AML.

**SECOND-SITE IDH2 MUTATIONS CONFER RESISTANCE TO ENASIDENIB IN TRANS**

Mutations in IDH2 result in production of the oncometabolite 2-hydroxylutarate (2HG) to promote disease progression in acute myeloid leukemia (AML). Thus, an allosteric IDH2 inhibitor, enasidenib (AG-221), which binds to the IDH2 dimer interface to block the production of 2HG by mutant IDH2, was evaluated in a phase I/II trial of patients with IDH2-mutant AML. Enasidenib induced clinical responses, and Intlekofer, Shih, and colleagues investigated mechanisms of resistance in two patients who acquired resistance to enasidenib and continued to disease progression. These patients initially harbored the oncogenic IDH2R140Q mutation, and, at the time of resistance, each patient had acquired a second IDH2 missense mutation (Q316E or I319M). These acquired mutations occurred in trans, on the allele that was not affected by the R140Q mutation. Both the Q316E and I319M mutations occur at the interface where enasidenib binds the IDH2 dimer, blocking the drug interaction. Enasidenib reduced 2HG levels in cells harboring the R140Q mutation, and a secondary Q316E or I319M mutation, in cis or in trans, relieved this suppression of 2HG production, enhancing mutant IDH2 enzymatic activity. However, Q316E or I319M mutations had no effect on 2HG levels when the cooperating R140Q mutation was not present. The second site Q316E or I319M mutations conferred resistance to enasidenib in vitro and in vivo. A patient with acquired resistance to an IDH1 inhibitor who acquired an in cis mutation in IDH1 was also identified. The IDH1 S280F mutation occurred at the at the analogous residue to I319 and was associated with increased 2HG, suggesting that resistance to either IDH2 or IDH1 targeted therapies can occur through second-site mutations in cis or in trans. Taken together, these findings elucidate a mechanism by which second-site IDH2 mutations can promote acquired resistance to enasidenib in patients with AML, and further demonstrate the importance of elevated 2HG production in the IDH2-mutant tumorigenesis.

**Impact:** Acquired Q316E or I319M mutations may underlie enasidenib resistance in patients with IDH2R140Q AML.

**Concept:** IDH2 Q316E or I319M mutations blocked enasidenib binding, cooperating with IDH2R140Q to reduce 2HG levels.

**Impact:** Acquired Q316E or I319M mutations may underlie enasidenib resistance in patients with IDH2R140Q AML.

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## Second-Site IDH2 Mutations Confer Resistance to Enasidenib In Trans

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