How Epigenetic Therapy Beats Adverse Genetics in Monosomy Karyotype AML

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The study by Greve and colleagues, in this issue of *Cancer Research*, provides new molecular insights into the intriguing clinical activity of DNA hypomethylating agents (HMA) in patients with acute myeloid leukemia (AML) with monosomal karyotypes. Patients with AML with adverse monosomal karyotypes are known to benefit from HMAs, but not cytarabine, a cytidine analog without HMA activity, but the specific molecular mechanisms remain poorly understood. The authors investigated the mechanistic effects of HMAs on gene reactivation in AML in the context of the most common monosomal karyotypes, genetic deletion of chromosome 7q and 5q. They identified genes with tumor-suppressive properties, an endogenous retrovirus cooperatively repressed by DNA hypermethylation, and increased genetic losses on hemizygous chromosomal regions versus normal biallelic regions in AML cell models. Treatment with HMAs preferentially induced expression of these hemizygous genes to levels similar to those of genes in a biallelic state. In addition to CpG hypomethylation, decitabine treatment resulted in histone acetylation and an open chromatin configuration specifically at hemizygous loci. By using primary blood blasts isolated from patients with AML receiving decitabine and AML patient-derived xenograft models established from patients with either monosomal karyotypes or normal cytogenetics, Greve and colleagues both validated their findings in primary patient samples and demonstrated superior antileukemic activity of decitabine compared with chemotherapy with cytarabine. These mechanistic insights into how epigenetic therapy beats adverse genetics in monosomy karyotype AML will open new therapeutic opportunities for a difficult-to-treat patient group.

See related article by Greve et al., p. 834

In acute myeloid leukemia (AML), the karyotype of the leukemic cell is the most powerful predictor of treatment outcome. Approximately 30% of cases of AML have an unfavorable karyotype, and if treated with conventional chemotherapy, a complete response rate of approximately 50% and a 5-year overall survival of 10%–20% is expected. Of those in the unfavorable group, almost half will have a complex karyotype, which is associated with a poorer outcome, and 40% of those will have a monosomal karyotype (defined as two monosomies or one monosomy plus an additional structural abnormality), which carries an even worse prognosis. Furthermore, this category of patients has a very dismal prognosis if treated with conventional chemotherapy (Surveillance Epidemiology and End Results Program of the NCI). Intriguingly, the DNA hypomethylating agents (HMA), decitabine and azacytidine, have significant activity in this population of patients (1–3), but the molecular mechanism remains poorly understood.

Losses of part or all of chromosomes 5 or 7 are the most common cytogenetic changes in unfavorable risk AML. In the context of 7q and 5q genetic deletion in AML, Greve and colleagues (4) investigated molecular mechanisms of the clinical activity of HMAs in AML with monosomies by analyzing two cell lines, one with monosomy 7 (AML1) and another with combined del 5q and del 17p, but intact alleles of 7q (ELF). Cell lines were compared with myeloid cancer cell lines without these monosomies. Treatment with low-dose HMAs reduced cell proliferation, and RNA sequencing analysis after low-dose decitabine treatment revealed strikingly higher upregulation of certain transcripts of genes located on the remaining copy of chromosomes 7q, 5q, and 17p, respectively, in cells with monosomy (hemizygous expression) as compared with the cell line where both copies of the genes were present. Crucially, the authors then validated their gene expression results from the cell lines in sorted myeloid blasts from patients with AML treated with HMA with and without monosomies. The findings in patients receiving actual treatment were compelling, although the level of upregulation in the patient's cells was modest compared with the cell lines. The limited effects in patient samples were consistent with prior findings: decitabine effects in patients were never as impressive as when modeled in cell lines for several reasons, including low-dose schedules in the clinic, versus schedule optimization *in vitro*, and genetic/epigenetic heterogeneity of patients.

One particularly interesting study demonstrated strong preferential upregulation of the endogenous retrovirus group 3 member 1 (*ERV3-1*) and zinc finger protein 117 (*ZNF117*) transcripts on monosomic chromosome 7q by HMAs (1). This HMA-induced upregulation restored expression of *ERV3*-1 and *ZNF117* to levels comparable with those found in cells with biallelic expression, suggesting DNA methylation can cooperate with chromosomal deletion to silence gene expression. *ERV3-1* and *ZNF117* are known to be regulated as a single transcriptional unit, sharing a CpG-rich promoter region (but not an "official" CpG island). Greve and colleagues (4) epigenetically interrogated that CpG region using the two ideal cell models in the context of monosomal karyotype AML. DNA methylation analysis using MassARRAY showed baseline hypermethylation of the shared

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ERV3-1/ZNF117 promoter in AML1 and hypomethylation of this region in ELF. Furthermore, HMA treatment led to a significant reduction of *ERV3-1/ZNF117* promoter CpG methylation in AML1 (especially of the initially highly methylated CpGs), whereas no change in methylation levels of this region was detectable in ELF. Epigenetic regulation of the *ERV3-1/ZNF117* promoter was further investigated using chromatin immunoprecipitation-qPCR and ATAC sequencing for the activating histone modification H3K4me3 and an open chromatin configuration. HMA treatment increased H3K4me3 levels at the *ERV3-1/ZNF117* promoter in AML1, whereas the H3K4me3 occupancy did not change in ELF, which had much higher baseline H3K4me3 levels. Increased chromatin accessibility was also observed in the *ERV3-1/ZNF117* promoter region in AML1, but not ELF, following HMA treatment. Collectively, these observations suggest that hypomethylation, histone acetylation, and chromatin accessibility all contribute to the molecular mechanism of HMAs in monosomal karyotype AML.

The ability of decitabine to induce long terminal repeat (LTR) retrotransposons and upregulate viral defense genes (5, 6), such as *RIG-I* and *MDA5*, has been reported previously (7), prompting the authors to extend the search for decitabine-induced genes by interrogating multi-copy transposable elements (TE) broadly distributed over the entire genome. The authors observed that more TEs were induced by decitabine in AML1 compared with ELF, with LTRs being strongly enriched in the top TEs in both cell lines, further suggesting that HMAs induce expression of neoantigens, a phenomenon that has been recently described (5, 6). Given this general induction of LTR-containing TEs by decitabine, and thus, the potential increase of immunogenicity in the different AML cell lines (by dsRNA and neoantigens), the authors analyzed *RIG-I* and *IRF7*, two downstream targets of the antiviral immune response cascade (5–7). Upregulation of both was observed in cell lines, as well as in primary blood blasts serially isolated from patients receiving decitabine, suggesting that activation of an antiviral response may contribute to HMAs activity in monosomal karyotype patients.

Clinical trials in patients have shown that decitabine works better than cytarabine in monosomic AMLs (1–3). To confirm the preference for HMAs in this notoriously difficult patient group, the effect of decitabine versus cytarabine in patient-derived xenograft (PDX) mice developed from patients with monosomies and compared with patients without monosomies was examined. Survival extension of mice was higher with decitabine treatment compared with cytarabine, demonstrating that decitabine is superior to cytarabine in treatment of AML PDX with or without monosomal karyotype and *TP53* mutation. These findings provide additional *in vivo*, that is, PDX data to support previously published clinical results of the antileukemic activity of decitabine in cytogenetic cohorts (1–3).

HMA-induced viral mimicry (5, 6) enables cytokine production, attraction of activated CD8+ T lymphocytes to tumor sites, and antitumor responses (8). Given that AML derives from clonal expansion of immature myeloid cells in bone marrow and disrupts normal hematopoiesis, it will be important to study how decitabine treatment affects the bone marrow microenvironment (BME), leukemia stem cells, and immune and other supporting cells to facilitate treatment responses (9). Notably, as HMAs induce global transcriptional perturbation of innate immune signaling pathways, including IFN and inflammasome, which could affect the BME, it will also be important to study inflammasome responses *in vivo* (10). Therefore, studies in immunocompetent models are needed to determine whether the enhanced antiviral response changes the antitumor BME, further contributing to the success of decitabine treatment in monosomy 7 AML.

In summary, given the robust clinical observation of recurrent complete remissions in HMA-treated patients with AML/myelodysplastic syndromes (MDS) with monosomal karyotypes involving chromosomes 7, 5, and 17 (with a high prevalence of *TP53* mutations), the mechanistic discoveries and translational implications by Greve and colleagues (4) are of high significance for a very unfavorable risk group. The observations that low-dose decitabine treatment induces changes in cytokine expression and ERVs may even go beyond providing mechanistic explanation for how HMA therapy overcomes adverse genetics in monosomy karyotype AML. Greve and colleagues (4) provide mechanistic rationale for testing new epigenetic drug combinations, such as DNMTi and HDACi, as well as combing epigenetic therapy and immunotherapy, to positively impact survival of patients bearing monosomal karyotypes in myeloid neoplasias. The recent approval of oral decitabine (INQOVI, Astex Pharmaceuticals, Inc.) by the FDA for MDS provides yet another epigenetic tool for future use in the treatment of patients with AML/MDS with adverse genetics. Altogether, the mechanistic findings by Greve and colleagues (4) are the first to mechanistically characterize the important effect of hypomethylating drugs in a clinically high-risk group of patients with AML/MDS based on genetic deletion.

Authors' Disclosures

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