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Combination therapy with DNA methyltransferase inhibitors in hematologic malignancies

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Summary

A variety of epigenetic changes contribute to transcriptional dysregulation in myelodysplastic syndromes (MDSs) and acute myeloid leukemia (AML). DNA methyltransferase (DNMT) inhibitors—azacitidine and decitabine—have significant activity in the treatment of MDS. Despite marked activity in myeloid malignancy, monotherapy with DNMT inhibitors is limited by low complete and partial response rates (7–20%) and median response durations of 15 months. As with classical cytotoxic therapy, the targeting of biologic pathways and mechanisms may best be accomplished using a combination of agents offering complementary mechanisms and synergistic pharmacodynamic interactions. The goal of this approach is to improve response rates, quality, and duration, and to minimize adverse events. There are a number of new therapies under development for the management of MDS and AML. This review article touches on some of the more promising combination regimens in various phases of investigation. The treatment of MDS and AML is undergoing rapid evolution. Cytogenetic complete remission and prolonged survival represent important goals. Incremental improvements in disease state and quality-of-life issues are also important for patients. Given the overall failure of cytotoxic chemotherapy in the achievement of cures in MDS and MDS-related AML, the application of less toxic, biologically directed agents may represent a more promising approach to treatment. Combination therapies with DNMT inhibitors using optimal dosing regimens to focus on methylation reversal with lower doses over longer periods of time, rather than direct cytotoxic effects, are beginning to suggest promising results in MDS and AML.

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Keywords:

acute myeloid leukemia, azacitidine, decitabine, DNA methyltransferase inhibitors, myelodysplastic syndrome

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Introduction

As in many cancers, a variety of epigenetic changes contribute to transcriptional dysregulation in myelodysplastic syndromes (MDSs) and acute myeloid leukemia (AML); such changes are likely to contribute to the pathogenesis and/or maintenance of the malignant phenotype in these diseases. For MDS, one of the most common hematologic malignancies in the United States,[1] BEST SUPPORTIVE CARE has been the standard therapy and has involved blood transfusions and the use of recombinant growth factors.

The DNA methyltransferase (DNMT) inhibitors azacitidine (5-azacytidine; Vidaza®, Pharmion Corp., Boulder, CO, USA) and decitabine (Dacogen™, SuperGen Inc., Dublin, CA, USA, and MGI Pharma Inc., Bloomington, MN, USA) have shown significant activity in the treatment of MDS. As monotherapy, DNMT inhibitors produce an overall response (complete and partial remission plus hematologic improvement[2]) of about 50%.[3] Compared with best supportive care, DNMT inhibitors have significantly extended time to AML transformation from MDS and improved survival in some patients.[4] The management of AML has traditionally involved intensive chemotherapy; nonetheless, the majority of patients develop recurrent disease.[5] Very preliminary data with DNMT inhibitors in AML have been encouraging, with promising response rates of 23%; however, these data are limited because of the use of dose schedules that probably exceed DNMT inhibition and may have exceeded optimal clinical activity.[6]

Despite marked activity in myeloid malignancy, the use of DNMT inhibitors as monotherapy is limited by low complete and partial response rates (CR + PR = 23%) and median response durations of 15 months for azacitidine[3] and less for published decitabine regimens.[7,8] As with classical cytotoxic therapy, the targeting of biologic pathways and mechanisms may best be accomplished using combination strategies with agents offering complementary mechanisms and hopefully synergistic pharmacodynamic interactions. The goal of this approach is to improve response rates, quality of responses, and response duration and to take advantage of lower doses to minimize adverse events.

There are a number of new therapies under development for the management of MDS and AML. These include histone deacetylase (HDAC) inhibitors; lenalidomide (CC5013/Revlimid®, Celegene, Warren, NJ, USA); signal transduction inhibitors including bevacizumab, SCIO469, and PTK787; tumor necrosis factor (TNF) antagonists including etanercept and infliximab; farnesyl transferase inhibitors such as tipifarnib and lonafarnib; arsenic trioxide; retinoids; and the glutathione *S*-transferase P1-1 inhibitor TLK199. Recognizing the importance of developing combination strategies in these conditions, this review will examine agents under clinical

investigation in combination with DNMT inhibitors, and new compounds on the horizon that may be candidates for future investigation.

Agents being explored with DNA methyltransferase inhibitors

At present, there are two classes of agent under investigation in combination with DNMT inhibitors for AML and MDS. These include HDAC inhibitors and tumor necrosis factor receptor inhibitors. Current trials are shown in Table 1.

Disease	Regimen	Phase	Study site

AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; FHCRC, Fred Hutchinson Cancer Research Center; MDS, myelodysplastic syndrome; SLL, small lymphocytic lymphoma.

MDS, CML, AML	▪ MS-275 orally days 3 and 10 ▪ Azacitidine subcutaneously days 1–10 ▪ Cycle repeats every 28 days	I (open and recruiting patients)	Sidney Kimmel Cancer Center, Baltimore, MD, USA
AML, refractory CLL, SLL	▪ Decitabine intravenously days 1–10 ▪ Valproic acid orally three times a day, days 5–21 ▪ Cycle repeats every 28 days	I (open and recruiting patients)	Arthur G James Cancer Hospital and Richard J Solove Research Institute, Columbus, OH, USA
MDS (advanced)	▪ Azacitidine ▪ Etanercept (FHCRC protocol #1926)	I/II (open and recruiting patients)	Fred Hutchinson Cancer Research Center, Seattle, WA, USA

HDAC inhibitors

Epigenetic biology provides sound rationale for the sequential use of DNMT inhibitors and HDAC inhibitors. Silencing of genes associated with methylated promoters is due, at least in part, to the recruitment of transcriptional repression complexes, including HDACs, via specific methyl-binding proteins. The recruitment of HDACs leads to removal of acetyl groups from specific lysine residues in the tails of histones in nucleosomes associated with the methylated promoter. This interaction leads to a transcriptionally repressive state of chromatin (heterochromatin) associated with that gene. HDAC inhibitors by themselves cannot reactivate expression of heavily methylated genes. However, the addition of HDAC inhibitors after previous exposure to a suboptimal concentration of a DNMT inhibitor leads to additive or synergistic reactivation of gene expression in a variety of methylated genes in many malignancies (Figure 1).[9]

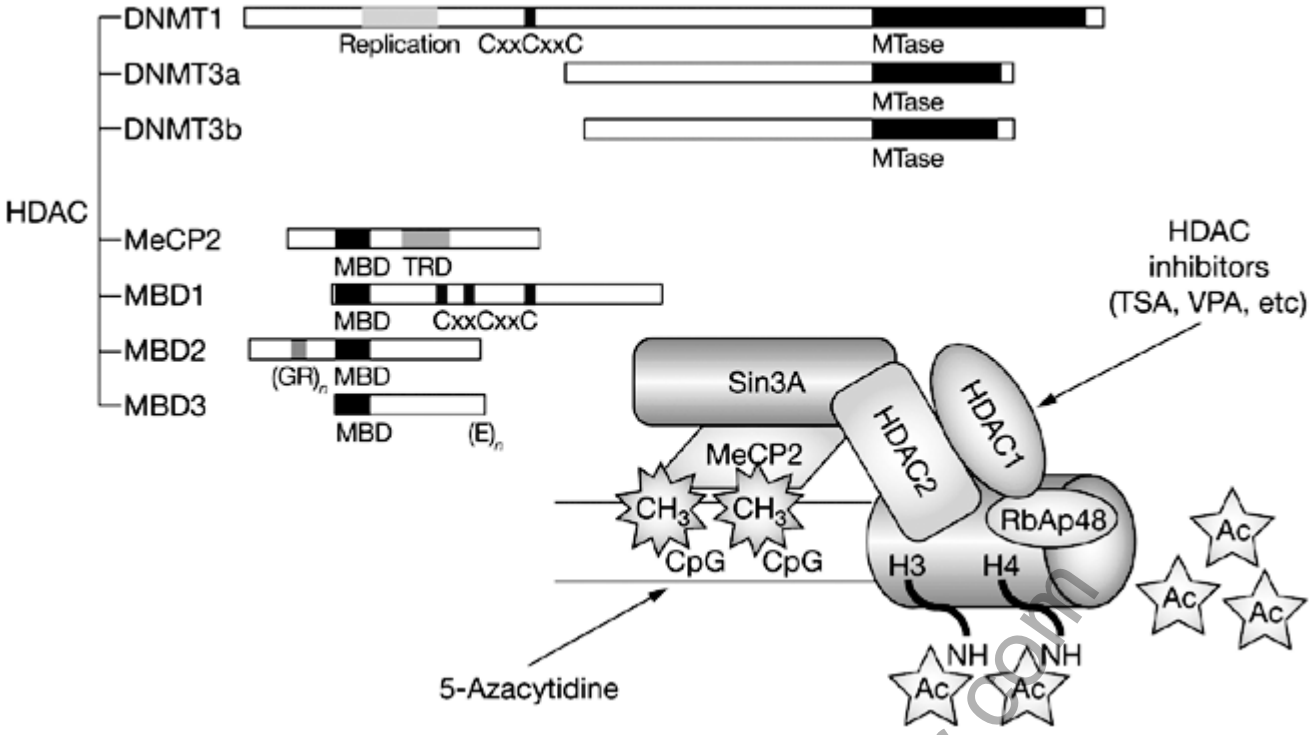

Figure 1. Effects of DNA methylation and chromatin structure on gene transcription in normal and tumor cells

Ac, acetyl; DNMT, DNA methyltransferase; H(3, 4), histone (3, 4); HDAC, histone deacetylase; MBD, MeCP2, methyl-CpG binding proteins; MTase, methyltransferase; TRD, transcriptional repression domain; TSA, trichostatin A; VPA, valproic acid. Reprinted with permission from Leone G *et al*. (2003) Inhibitors of DNA methylation in the treatment of hematological malignancies and MDS. Clinical Immunology 109: 89–102.

There are five main classes of HDAC inhibitor, of which four classes are currently being studied (Box 1). These include aliphatic acids or short-chain fatty acids, hydroxamic acids, tetrapeptides (both epoxyketone-containing and non-epoxyketone-containing), and benzamides. Valproic acid and phenylbutyrate are short-chain fatty acids that inhibit HDAC both *in vitro* and *in vivo*.

Histone deacetylase inhibitors in clinical trials for hematopoietic malignancies

Hydroxamic acid

- SAHA (suberoylanilide hydroxamic acid)
- LAQ-824
- PXD101
- CBHA
- LBH589

Aliphatic acid

- Valproic acid
- Phenylbutyrate

Cyclic tetrapeptide

- Depsipeptide

Benzamide

- MS-994
- CI-994

The *in vivo* relationship between the inhibition of DNMT1 by azacitidine and hematological improvement has just begun to be explored. One of the most promising investigations has been conducted by Gore and colleagues

at Johns Hopkins.[10] This group has evaluated various dosing regimens of azacitidine followed by a 7-day infusion of phenylbutyrate (375 mg/kg per day by intravenous continuous infusion) in patients with MDS and AML in a phase I study. Patients received azacitidine at the following dosages: 75 mg/m^2 per day for five doses, 50 mg/m^2 per day for five doses, 50 mg/m^2 per day for 10 doses; 50 mg/m^2 per day for 14 doses; or 25 mg/m^2 per day for 14 doses for a minimum of four cycles (every 28 days). Clinical response, including complete responses, was induced and in some cases was associated with reversal of gene methylation (SD Gore, S Baylin and J Herman, unpublished data).

In the phase I/II setting, Garcia-Manero *et al*.[11] evaluated decitabine plus valproic acid in 55 patients with leukemia. Patients received decitabine 15 mg/m^2 intravenously daily for 10 days plus valproic acid orally at 20, 30, or 50 mg/kg per day for 10 days. Of 41 evaluable patients, 8 had a complete remission; this included 3 of 4 patients with previously untreated MDS/AML. The overall response rate was 53%, with activity seen at all dose levels. Three patients (25%) who received doses greater than 20 mg/kg valproic acid showed evidence of histone acetylation.

Other HDAC inhibitors are being investigated in combination with methylation inhibitors (Table 1). MS-275 is an orally bioavailable benzamide HDAC inhibitor with a prolonged half-life allowing intermittent dosing.[12] A phase I study of 10-day schedules of azacitidine combined with MS-275 administered on days 3 and 10 in patients with AML and MDS is ongoing at Johns Hopkins. Suberoylanilide hydroxamic acid (SAHA) is a hydroxamic acid HDAC inhibitor with promising single-agent activity in patients with advanced leukemia and MDS.[13] There are currently two regimens combining SAHA with methylation inhibitors. The combination of azacitidine and SAHA is being investigated by LR Silverman, while decitabine is being evaluated with SAHA by G Garcia-Manero and colleagues. FK228 is a non-epoxyketone-containing tetrapeptide HDAC inhibitor that is being studied in combination with decitabine by D Schrump in primary thoracic malignancies at the US National Cancer Institute. Decitabine in combination with valproic acid is also being evaluated at the Arthur G James Cancer Hospital in patients with AML, refractory chronic lymphocytic leukemia, and small lymphocytic lymphoma.

TNF receptor inhibitors

The demonstration of dysregulated cytokine milieu, including TNF concentrations, in bone marrow of patients with MDS[14] led to investigations of TNFα blockade as treatment for these disorders. Initial studies have shown limited improvement in cytopenias in response to etanercept and infliximab. The failure of these studies to induce more dramatic responses suggests that TNFα is only one factor in the dysregulation associated with hematopoiesis in MDS. One current trial is evaluating the use of etanercept plus azacitidine in patients with advanced MDS (Table 1). This trial design is akin to combination chemotherapy trials: an empiric combination of different active agents, rather than the use of two agents based on complementary or synergistic pharmacodynamic mechanisms.

Candidates for future exploration

In addition to the ongoing clinical trials, there is much interest in combination therapy with other agents that have demonstrated efficacy in MDS or AML. Inevitably, a variety of compounds that have clinical activity in MDS and AML will be studied in empiric combinations with DNMT inhibitors. Many of these are under investigation as single-agent therapy; however, such therapies may provide complementary mechanisms to methyltranferase inhibitors suitable for further investigation in the clinic. Some agents may have a specific pharmacodynamic rationale, such as signal transduction inhibitors and retinoids.

Signal transduction inhibitors

Transcription of DNA methyltransferase appears to be dependent upon the Ras-MAPK (Ras mitogen-activated protein kinase) signaling pathway.[15] This underlying mechanism may present an opportunity for the study of farnesyl transferase inhibitors in combination with DNMT inhibitors. Farnesyl transferase inhibitors inactivate a variety of proteins in the Ras-MAPK pathway through the inhibition of prenylation. Both tipifarnib and lonafarnib have activity in MDS, with clinical response rates of 20–30%.[16, 17]

Retinoids

Retinoids play an important role in effecting cellular signals that involve cell growth, differentiation, and carcinogenesis. Esteller *et al*.[18] established the biological rationale for the combination of DNMT inhibitors and retinoids. Those authors evaluated the effects of retinol (vitamin A) on the cellular retinol-binding protein 1 (CRPB1) gene and retinoic acid receptor beta 2 (RARß2) in a study using 36 cell lines and 553 tumor samples *in vitro*. Testing confirmed that the loss of CRPB1 was correlated with methylation of the gene that encodes it. Treatment with decitabine reactivated CRPB1 expression. Both CRPB1 methylation and *RARß2* hypermethylation were found in primary human premalignant cells. Investigators observed that diets high in vitamin A were associated with a reduced frequency of methylation in the genes encoding CRPB1 and RARß2. Given the importance of retinoids in myeloid differentiation,[19] it is possible that DNMT inhibitors may further sensitize cells to the action of retinoids and increase the range of myeloid malignancies affected in response to retinoids beyond acute promyelocytic leukemia.

Other potential combinations

With the availability of a number of new therapeutic agents in the clinic, there are several candidates that may be worth exploring despite the lack of data supporting a specific pharmacodynamic rationale. These include lenalidomide, a thalidomide analog with a very high response rate in MDS associated with deletions of chromosome 5q, vascular endothelial growth factor (VEGF) receptor antagonists, and arsenic trioxide.

Antiangiogenic agents

VEGF has been shown to be an important angiogenic molecule in MDS and a potent growth factor for AML blasts. This has led to the investigation of bevacizumab (an anti-VEGF agent) as monotherapy in MDS[20] and in timed sequence following chemotherapy, which induced an increase VEGF levels in patients with AML.[21]

Recent studies with lenalidomide—a multifunctional angiogenic inhibitor and immune modulator with fewer neurotoxic and teratogenic effects than thalidomide—have shown important activity in MDS.[22] List *et al*.[22] evaluated the safety and hematological response of lenalidomide, 25 or 10 mg per day or 10 mg per day for 21 days of every 28-day cycle, in 43 patients with symptomatic anemia or transfusion-dependent MDS. Twenty-four patients (56%) had a favorable response. Of the 32 patients who were transfusion-dependent, 20 (63%) achieved transfusion-independence. Side effects included neutropenia (65%) and thrombocytopenia (74%). These side effects necessitated either a change in dose or interruption of therapy in 58% of the study patients. Despite the side effects, lenalidomide has been proposed as a viable alternative for those low-risk patients with MDS who do not respond to conventional therapy or erythropoietin.

Arsenic trioxide

Arsenic trioxide suppresses myeloblast elaboration of VEGF-A and has a direct cytotoxic effect on neovascular endothelium in MDS and AML.[4] It has been used successfully in the treatment of acute promyelocyctic leukemia (APL), credited with an 85% complete remission rate in relapsed patients. It has also been shown to be useful in the treatment of patients with MDS. Douer *et al*.[23] enrolled seven patients with non-APL AML in a preliminary study evaluating the combination of arsenic trioxide and ascorbic acid. Dosage regimens were based on successful phase I/II trials conducted by Bahlis *et al*.:[24] arsenic trioxide 0.25 mg/kg per day intravenously with 1 g of ascorbic acid (5 days on/2 days off) for 5 weeks, followed by 2 weeks of rest (25 days of treatment over a 35-day period constituted one cycle). Patients could receive up to four cycles. Of the three patients who had failed to respond to previous chemotherapy, none responded to this therapy. Of the four previously untreated patients, three experienced a response as the bone marrow blasts decreased from greater than 40% to less than 5%. However, only one patient had more than one cycle, but demonstrated an improvement in peripheral blood counts. In addition, one patient experienced symptoms similar to 'differentiation syndrome', indicated by shortness of breath and severe hypoxemia, which has been noted in some patients treated with arsenic trioxide.

Conclusion

The treatment of MDS and AML is evolving rapidly. Cytogenetic complete remission and prolonged survival are important goals. Incremental improvements in disease state and quality-of-life issues are also important for

patients. Given the overall failure of cytotoxic chemotherapy in the achievement of cures in MDS and MDS-related AML (AML with trilineage dysplasia and AML in the elderly), the application of less toxic, more biologically directed agents may be a more promising approach to treatment. Continued study of epigenetic abnormalities in clonal stem cell disorders will hopefully lead to the identification of silenced genes whose reactivation may stem the progression of the clinical disorders. Combination therapies with DNMT inhibitors, using optimal dosing regimens to focus on methylation reversal and relying on lower doses over longer periods of time, rather than on direct cytotoxic effects, are beginning to suggest promising results in MDS and AML.

Currently, much clinical interest is focused on combination with HDAC inhibitors, the compounds for which combination therapy has the greatest biologic rationale. Agents with other therapeutic targets present additional opportunities to explore the combinations with DNMT inhibitors as well as with other active compounds. Such strategies may lead to improvements in response rates, remission, and survival while offering greater tolerability. The research continues and much more needs to be accomplished before these goals will be achieved.

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Competing interests

Prof Steven D Gore is a consultant of Pharmion Corp. and Celgene Corp. and a member of their speakers' bureaus