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Epigenetic Therapy of Leukemia: an update

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Abstract

Carcinogenesis is classically thought to result from genetic alterations in DNA sequence such as deletions, mutations, or chromosomal translocations. These in turn may lead to the activation of oncogenes, inactivation of tumor suppressor genes or formation of chimeric oncoproteins. Epigenetics, in contrast, refers to a number of biochemical modifications of chromatin, either to DNA directly or to its associated protein complexes, that affect gene expression without altering the primary sequence of DNA^{1,2}. A fundamental difference between genetic and epigenetic alterations is the irreversible nature of genetic lesions whereas epigenetic ones are potentially reversible, allowing for therapeutic intervention. In the last decade, it has become apparent that epigenetic changes play an important role in cancer, particularly in leukemia. Significant advances have been made in the elucidation of these processes as well as in translating this knowledge to the clinic, as in the development of new prognostic biomarkers or targeted therapies. In this review, we will focus on recent advances in epigenetic therapy in leukemia.

Keywords

DNA methylation; Histone deacetylase inhibitors; 5-azacitidine; 5-aza-2'-deoxycytidine; leukemia; Myelodysplastic syndrome

TARGETING ABERRANT DNA METHYLATION

DNA methylation refers to the addition of a methyl group to the C5 position of the pyrimidine ring of cytosine (C).¹ This reaction occurs only when the (C) is followed by guanine (G) (so called CpG dinucleotides), and is mediated by enzymes called DNA methyltransferases (DNMTs) where S-adenosyl-methionine serves as the methyl donor. CpG dinucleotides are found with a lower frequency in the human genome than expected assuming a statistically random distribution, but they are clustered. CpG islands are defined as regions of greater than 500 base pairs with a CG content greater than 55%. They may be located near the promoter and exonic regions of approximately 40% of the mammalian genes (promoter associated CpG islands) where they are generally not methylated. In contrast, CpG islands in intergenic regions are normally methylated. This may contribute to the transcriptionally inert state of non-coding DNA, while the unmethylated configuration of

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the promoter regions of CpG islands allows for gene expression. In cancer cells, these promoter associated CpG islands can be hypermethylated. The resulting aberrant gene silencing is functionally similar to inactivation by genomic alterations such as mutations or deletions.^{2,3} Induction of hypomethylation of these promoter associated CpG islands can lead to gene re-expression and therefore be of great clinical value. Currently, two hypomethylating agents are in clinical use: 5-azacitidine and 5-aza-2'-deoxycytidine (decitabine) (Figure 1).

5-AZACITIDINE

5-azacitidine is a nucleoside analogue with the capacity to induce DNA hypomethylation in vitro and in vivo. It was initially investigated at high doses (600–1500 mg/m² per course) as a cytotoxic agent for hematological malignancies in 1970s and 80s. These studies found high rates of toxicity, including severe and prolonged myelosuppression and thus high-dose 5-azacitidine was not pursued further.^{4–9} Lower doses of 5-azacitidine (75 mg/m² per day for 7 days every 28 days) were investigated by the Cancer and Leukemia Group B (CALGB) in patients with MDS (myelodysplastic syndromes). Two phase II studies were reported, one using intravenous (IV) 5-azacitidine (CALGB 8421)¹⁰ and another using a subcutaneous (SC) formulation (CALGB 8921).¹¹ Also, one phase III randomized study was conducted comparing SC 5-azacitidine with best supportive care (CALGB 9221).¹² The results of these 3 trials have been updated using the World Health Organization (WHO) classification of myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) as well as the International Working Group (IWG) criteria for disease response (Table 1).¹³ In CALGB 8421, 48 patients (52% of whom were reclassified with a diagnosis of AML by WHO classification) were treated with IV 5-azacitidine. A complete response (CR), partial response (PR) and hematological improvement (HI) of 15%, 2% and 21%, respectively, were reported.¹⁰ The overall response rate (ORR) was 44%. Similar response rates were observed in CALGB 8921 in which 5-azacitidine was administered by SC route.¹⁴

CALGB 9221 was a phase III randomized study of 191 patients with MDS. Patients were randomized to SC 5-azacitidine (n=99) or best supportive care (n=92).¹² After 4 months, patients in the best supportive care arm were allowed to cross over to the 5-azacitidine arm following evidence of disease progression. In the 5-azacitidine arm, CR, PR and HI were documented in 10%, 1% and 36% of patients compared to no CR or PR in the supportive care only arm (n=41). For patients (n=51) who crossed over to the 5-azacitidine arm, CR, PR and HI were documented in 6%, 4% and 25% of patients.¹³ Combining the data from both SC CALGB trials (8921 and 9221), 169 patients were treated with 5-azacitidine with an ORR of 44% (CR 13%, PR 1%, HI 31%).¹³ 5-azacitidine has also been evaluated by French investigators in patients with high risk MDS and secondary AML with an ORR of 62% (CR 16%, PR 25%, HI 21%).¹⁵

Impact on survival of 5-azacitidine in MDS—A trend towards improved survival was noted in the CALGB 9221 trial for patients treated with 5-azacitidine but statistical significance could not be achieved due to the cross-over design of the study. In a landmark analysis at 6 months, median survival for the 5-azacitidine arm was 18 months compared to 11 months for the supportive care arm.¹² This led to clinical trials focusing on the impact on survival of hypomethylating agents in MDS. At the 2007 American Society of Hematology (ASH) meeting, Fenaux and colleagues presented results of a phase III international multicenter randomized study comparing 5-azacitidine (n=179) to one of the three conventional care regimens (n=179) [(i) best supportive care (use of transfusions, antibiotics and G-CSF for neutropenic infections); (ii) low dose cytarabine (20mg/m²/day for 14 days every 28 days); (iii) conventional induction/consolidation chemotherapy] in patients with intermediate-2 or higher risk MDS.¹⁶ This trial did not allow erythropoietin. The use of 5-

azacitidine led to significantly improved overall survival compared to the conventional care regimens (24.4 months vs. 15 months, $p=0.0001$). This is the first therapy to ever show an improvement in overall survival in MDS.¹⁶ The survival advantage seen with 5-azacitidine was irrespective of age, sex, WHO classification, karyotype, or international prognostic scoring system (IPSS) group. At 2 years, 51% of 5-azacitidine treated patients were alive, compared to 26% in the conventional care regimens.¹⁶

Risk of AML transformation with 5-azacitidine in patients with MDS—5-azacitidine has been shown to reduce the risk of AML transformation in patients with MDS. In CALGB 9221, median time to AML or death was 21 months for 5-azacitidine group compared to 12 months for best supportive care ($p=0.007$).¹² Similarly the preliminary results by Fenaux et al, in intermediate-2 and high risk MDS patients, showed a median time to AML or death of 13 months in the 5-azacitidine group vs. 7.6 months in the conventional care regimens.¹⁶

Impact on quality of life with 5-azacitidine—Quality of life in patient with MDS is improved by treatment with 5-azacitidine. In CALGB 9221, patients on the 5-azacitidine arm experienced significantly greater improvement in fatigue, dyspnea, physical functioning, and psychological distress over the course of the study period than those in the supportive care arm.¹⁷

Transfusion independence in CALGB studies—In CALGB 9221, 65 of the 99 patients treated with 5-azacitidine were transfusion dependent at baseline. Of these, 29 patients (45%) became transfusion independent during the course of treatment for a median of 9 months, a significant improvement over the supportive care group.^{13,18}

Time to response and duration of therapy with 5-azacitidine—Time to response is of particular importance given that several courses of 5-azacitidine may be required before any evidence of response is appreciated. Silverman et al investigated this issue in the CALGB studies. They found the median number of cycles with 5-azacitidine needed for any response was 3, with 90% of responders doing so by cycle 6 (range, 1–17 cycles).^{10,12–14} This supports the theory that the clinical effect of 5-azacitidine in this patient population is largely epigenetic rather than cytotoxic. It also underscores the importance of making patients aware of the relatively slow clinical outcome expected with this type of treatment. In addition, neutropenia should not delay treatment with hypomethylating agents, especially for the first 3 cycles.

Another important issue is the duration of treatment. While this has yet to be systematically studied, multiple investigators agree that therapy cessation is associated with earlier relapse. Indefinite treatment is therefore recommended.

Route and schedule of administration of 5-azacitidine—Based on results from CALGB studies 8921 and 9221, 5-azacitidine was approved by the FDA on May 2004 for the treatment of MDS, in both low-risk and high-risk patients.¹⁹ The approved dose is 75 mg/m²/day SC for 7 days every 28 days. In January 2007, an IV formulation of 5-azacitidine was also approved by the FDA.²⁰ The IV formulation boasts the advantage of being devoid of skin reactions often observed with the SC administration. Conventional dosing for 5-azacitidine is for 7 consecutive days. Because of the inconvenience of the weekend injections, a shorter 5-day schedule of 5-azacitidine has been studied. In the preliminary results by Lyons²¹, patients were randomized to 1 of 3 schedules of 5-azacitidine administered every 4 weeks: 5-2-2 (75 mg/m²/day $\times$ 5 days, followed by 2 days off and two additional days); 5-2-5 (50 mg/m²/day $\times$ 5 days, followed by 2 days off and 5 additional

days); or 5 days only ($75 \text{ mg/m}^2/\text{day} \times 5 \text{ days}$).²¹ All three treatment regimens showed similar outcomes in terms of hematologic improvement, red blood cell transfusion independence, and safety profile. These results indicate that the 5 day schedule may be an alternative to 7 or 10 days schedules, although survival data is lacking with the 5-day schedule. In addition, an oral formulation of 5-azacitidine is being developed which, if found clinically effective, would provide a convenient alternative to the SC or IV routes of administration.²²

Use of growth factors—CALGB studies prohibited the use of growth factors such as G-CSF and erythropoietin in order to avoid confounding variables in the assessment of 5-azacitidine activity. Rossetti et al reported excellent response rates in a retrospective study of 5-azacitidine with concurrent use of G-CSF and erythropoietin.²³ A prospective evaluation of this approach is warranted.

5-AZA-2'-DEOXYCYTIDINE (DECITABINE)

Decitabine is another nucleoside analogue, structurally related to 5-azacitidine. Decitabine is a derivative of 5-azacitidine and a more potent hypomethylating agent on a molar basis. Like 5-azacitidine, decitabine was initially explored as a cytotoxic agent at doses of 1500–2500 mg/m^2 per course and was not pursued further due to prolonged myelosuppression.^{24,25} Zagonel et al explored two low-dose schedules of decitabine ($45 \text{ mg/m}^2/\text{day}$ over 4 hr for 3 days and $50 \text{ mg/m}^2/\text{day}$ continuous daily infusion for 3 days) in 10 patients with MDS. ORR was 50% with 4 patients achieving CR. A phase II trial was initiated in Europe in the early 1990s exploring this low dose schedule of decitabine. Twenty-nine patients (MDS 20, AML 9) were treated with decitabine ($50 \text{ mg/m}^2/\text{d}$ continuous intravenous infusion for 3 days every 6 weeks).²⁶ Overall response was observed in 54% of patients (CR 29%, PR 18%, HI 7%) with a median response duration of 31 weeks. This trial led to a larger phase II trial by Wijermans and colleagues in which a slightly different dosing schedule was explored.²⁷ Decitabine was given at 15 mg/m^2 every 8hr ($45 \text{ mg/m}^2/\text{day}$) IV daily for 3 days every 6 weeks. ORR of 49% was observed with 20% CR. A pooled analysis of the phase II studies from Europe has also been published.²⁸ In this analysis, a total of 177 patients received decitabine $40\text{--}50 \text{ mg/m}^2/\text{day}$ for 3 days every 6 weeks and a 49% ORR was reported (CR 24%, PR 10%, HI 14%). Median response duration was 36 weeks and median survival for the whole group was 15 months. These encouraging results led to a phase III randomized trial in the United States where patients were randomized to decitabine (15 mg/m^2 every 8hr ($n=89$) IV daily for 3 days every 6 weeks) plus supportive care vs. supportive care alone ($n=81$).²⁹ Of the patients on the decitabine arm, 69% had IPSS intermediate-2 or higher and 74% were transfusion dependent. ORR was 30% (CR 9%, PR 8%, HI 13%). Median time to response was 3.3 months. Patients treated with decitabine had a trend toward a longer median time to AML or death compared with patients treated with supportive care alone (12.1 mo vs. 7.8 mo, $p=0.16$). In the subgroup analysis, patients on the decitabine arm experienced a longer median time to AML or death than those who received supportive care, if they were treatment-naïve (12.3 mo vs. 7.3 mo, $p=0.08$), had an IPSS score of intermediate-2/high risk (12.0 mo vs. 6.8 mo, $p=0.03$), were classified based on IPSS score as high risk (9.3 mo vs. 2.8 mo, $p=0.01$), or had de novo MDS (12.6 mo vs. 9.4 mo, $p=0.04$).²⁹ A total of 43 patients (48%) received 2 cycles of decitabine which might have contributed to the low response rate in this phase III trial compared to previous reported phase II studies with decitabine.^{26–28} Based on this trial, the FDA approved decitabine for MDS (Intermediate-1 or higher IPSS class) in May, 2006.

Even lower doses of decitabine have been explored in the clinical setting, based on a number of observations. First, a low dose of decitabine (0.015 mg/kg daily for 10 days) was found to have biologic efficacy in reactivating hemoglobin F in patients with sickle cell disease.³⁰

Also, the drug has a short half-life, and an absolute requirement for DNA synthesis for activity. In addition, a phase I trial was reported showing efficacy of lower doses of decitabine in hematopoietic malignancies.³¹ Kantarjian et al reported results of a phase II study in which the total dose of decitabine was decreased to 100 mg/m²/course (from 135 mg/m²/course previously reported), and the schedule was increased to every 4 weeks instead of every 6 weeks.³² Patients were randomized following a Bayesian adaptive design to one of three arms: (1) 20 mg/m² IV over 1 hour daily for 5 days; (2) 20 mg/m² daily given in two SC doses for 5 days, or (3) 10 mg/m² IV daily for 10 days. Cycles were repeated every 4 weeks as long as there was evidence of residual marrow disease and no life-threatening complications. The ORR by the modified IWG criteria was 73%, with 32 patients (34%) achieving CR. Patients randomized to 20 mg/m² daily for 5 days (the most dose-intensive arm) had the best response rate with 39% CR.³² The median overall survival time for the entire group was 19 months. Based on these results, a dose/schedule of 20mg/m² IV daily for 5 days every 4 weeks is now considered the optimal schedule of decitabine. A phase II single arm study of decitabine (the ADOPT trial) was presented at the ASH 2007 conference, confirming the safety and activity of decitabine when used in this fashion.

DECITABINE VERSUS 5-AZACITIDINE

An important clinical question is whether one of the two FDA approved hypomethylating agents for MDS is better than the other. There has been no head to head comparison of the two agents. The phase III studies of the two agents comparing each to supportive care showed an overall response rate of 47% for the 5-azacitidine trial compared to 30% for the decitabine trial, whereas the complete response rate was similar (10% vs. 9%).^{13,29} One important difference between the two phase III trials is that in the 5-azacitidine trial median time to response was 3 treatment courses,¹³ while in the decitabine trial, 48% of patients had 2 cycles of treatment.²⁹ This might explain the low response rate with the latter. In addition, the optimal dosing of the two agents is still being established. Two survival studies, one with 5-azacitidine, the other with decitabine, have been completed. The first was presented at ASH 2007¹⁶ (described above) and the second's results are expected at ASH 2008. As previously discussed, 5-azacitidine use is associated with improved survival. A comparative study of 5-azacitidine versus optimal dose/schedule of decitabine is necessary to establish any superiority of one agent over the other.

TARGETING HISTONE ACETYLATION

Like DNA methylation, acetylation of histone proteins plays an important role in gene transcription regulation. Two units each of the 4 core histones, H2A, H2B, H3 and H4 form the nucleosome around which DNA is wrapped.³³ Core histones have NH₂-terminal tails, which are lysine-rich and can undergo post-translational covalent modifications to effect gene expression. One such important modification is acetylation and deacetylation of key lysine residues of histone H3 and H4.^{34,35} Acetylation of histones leads to open chromatin configuration and gene transcription. On the other hand, deacetylation leads to a repressive state. These changes are mediated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). The use of histone deacetylase inhibitors leads to a more permissive state and allows for gene expression. Several such agents are being explored in clinical studies (Table 3).

Sodium phenylbutyrate

Sodium phenylbutyrate (PB) is an aromatic fatty acid compound which was initially developed for treatment of urea cycle disorders and thalassemia.^{36,37} Based on their preclinical study indicating a role of PB in differentiation and inhibition of growth of primary leukemia cells,^{38,39} Gore et al. explored the use of single agent PB in patients with

MDS and AML. In the phase I trial, continuous 7 day infusion of PB was used (7/28 schedule: 7 days on, 21 days off schedule) in 11 patients with MDS and 16 patients with AML and a dose of 375 mg/kg/day was established as the maximally tolerated dose (MTD).⁴⁰ Dose limiting toxicities (DLT) were neurological complications (lethargy and confusion) and were reversible within 24–48 hours of stopping the infusion. In a follow-up trial, PB at the MTD dose was used in 2 more frequent dose schedules (7/14 schedule: continuous 7 day infusion with 7 day rest, 21/28: continuous 21 day infusion with one week rest) in 23 patients with MDS and AML.⁴¹ In both these studies with single agent PB, no CR or PR was observed and less than 10% had HI. HDAC inhibition was not measured in these trials as these were initiated prior to the knowledge of HDAC inhibition by PB.^{38,42}

Valproic acid

Valproic acid (VPA) is a short chain fatty acid used as an antiepileptic and mood stabilizer.⁴³ VPA has been shown to affect the growth of malignant cells in vitro,^{44,45} to prolong the G1 phase of the cell cycle,⁴⁶ and to have antiangiogenic activity in vitro.⁴⁷ It is possible that the antineoplastic effects of VPA are related to HDAC inhibitory activity.^{48–50}

In their first trial, Kuendgen et al used VPA alone and in combination with all-trans retinoic acid (ATRA) in patients with MDS and secondary AML.⁵¹ Use of ATRA was based on in vitro studies indicating synergism between VPA and ATRA in cellular differentiation and apoptosis induction in leukemia cell lines.^{48,52,53} VPA was administered to reach target serum concentrations between 50–100 µg/ml and ATRA was given at 80 mg/m² daily on a one-week-on one-week-off schedule. ATRA was planned for patients who did not respond to VPA or had relapsed after it. A total of 23 patients were treated (18 with VPA monotherapy, 5 with combination). In the VPA monotherapy group, 7 patients had hematological improvement and one patient had PR, for a response rate of 44% (8/18 patients) by IWG criteria. None of the 5 patients on combination therapy responded. Overall response rate was 35% for the entire study population and no patient achieved CR. All 3 patients with IPSS low risk MDS had a major hematological response whereas only 1 out of 4 patients with high risk MDS had a response (minor erythroid response). In this study, 3 of the 9 patients with elevated blast count had significant reduction in peripheral and bone marrow blast counts. This observation led to the expansion on this study to include more high risk MDS and AML patients. In the second report by Kuendgen, 75 patients were treated with a response rate of 30% in the MDS cohort (n=43) and 16% in the AML cohort (n=32).⁵⁴ However, most of the responses were HI and only one CR (high risk MDS patient) and one PR (intermediate-1 MDS patient) were noted. Patients with low risk MDS had the best response rate at 70%. There was no correlation between dose of VPA, VPA blood levels, and response. The benefit of ATRA was seen in patients who had relapsed on VPA. Of the 10 relapsed patients treated with ATRA, 4 had second remission with a median duration of 21 months (better than the median first remission of 4 months, =0.01). In another report from the same trial, the authors concluded that VPA monotherapy is of minimal activity in AML and that the addition of ATRA to VPA was not of benefit.⁵⁵ Several other groups have used the VPA and ATRA combinations either concomitantly or sequentially, in smaller number of patients with limited activity.^{56–59}

Depsipeptide (FK228)

This agent has been shown to have significant clinical activity in cutaneous lymphoma⁶⁰. Byrd et al. reported on the use of this agent in patients with AML (n=10) or CLL (n=10).⁶¹ Depsipeptide was given as an IV infusion at a dose of 13mg/m² on days 1, 8, and 15 of a 28 day cycle. Nausea, fatigue and other constitutional symptoms were seen in the majority of patients. No CR or PR was observed in either CLL or AML cohorts and further development of use of this agent in AML and CLL was stopped by this group. This agent was tested at

MD Anderson Cancer Center in combination with decitabine but significant cardiac toxicity was observed and the study was halted (Issa JP, personal communication).

MS-275

MS-275 has been shown to exhibit time and dose dependent growth inhibition of leukemia cell lines as well as primary leukemia blasts.^{62–64} Gojo et al. conducted a phase 1 trial of MS-275 (a synthetic benzamide derivative) in patients with AML and MDS.⁶⁵ Based on the study of MS-275 in solid tumors,⁶⁶ a starting dose of 4 mg/m² weekly for 2 or 4 consecutive weeks followed by 2-week washout was established. A total of 38 patients received the study drug at doses ranging from 4–8mg/m² weekly. Fatigue, nausea and vomiting were frequent non-hematological side effects noted. The MTD (maximally tolerated dose) of MS-275 was established as 8 mg/m² administered weekly for 4 weeks with 2 weeks washout. Responses were minimal with no patients achieving CR or PR. Three of the 34 evaluable patients were noted to have >50% reduction in bone marrow blasts. Histone acetylation was documented in all patients in either peripheral blood or bone marrow mononuclear cells. In addition, a synergistic action with fludarabine has been described in both AML and ALL cells⁶⁷.

LBH589

LBH 589 is a cinnamic hydroxamic acid analogue which has been shown to induce apoptosis and histone acetylation in acute leukemia cells. It is one of the most potent HDAC inhibitors currently available. Giles et al. treated 15 patients (13 AML, 1 MDS, 1 ALL) with LBH589 at doses ranging from 4.8 to 14.0 mg/m² IV daily for 7 days every 21 days⁶⁸. Reversible QTc prolongation was the DLT (dose limiting toxicity). The MTD (maximum tolerated dose) was not established, as 14mg/m² exceeded it and the lower dose cohort of 11.5 mg/m² could not be expanded given the concern for QTc prolongation. 27% of patients were also noted to have grade 3–4 hypokalemia, however no relation was noted between QTc prolongation and hypokalemia. Response was limited with no CR or PR. Reduction in peripheral blood blasts was seen in 8 of 11 patients whereas only 1 patient had reduction in bone marrow blasts.

MGCD0103

MGCD0103 is an isotype specific aminophenylbenzamide and has been shown to inhibit HDAC isotypes 1,2,3, and 11.⁶⁹ A dose escalation phase 1 study of oral MGCD0103 given 3 times a week in patients with AML and MDS has been conducted.⁷⁰ Doses of MGCD0103 ranged from 20–80 mg/m² orally. Twenty nine patients were treated (22 AML, 5 MDS, 1 ALL, 1 CML). Median age was 65 years and 83% of patients had received prior chemotherapy. Fatigue, nausea, diarrhea, vomiting were the most frequently reported adverse events. MTD was established at 60 mg/m² orally three times a week. No CR or PR was observed. Bone marrow response was seen in 3 of the 23 evaluable patients. One-third of the patients at MTD had induction of histone H3 acetylation. This agent is now being studied in combination trials with 5-azacitidine⁷¹.

Vorinostat (Suberoylanilide hydroxamic acid, SAHA)

Vorinostat is a small molecule HDAC inhibitor, which has been approved by the FDA for treatment of skin manifestations of cutaneous T-cell lymphoma.^{72–74} Vorinostat has been shown to promote cell cycle arrest and apoptosis and has shown in vitro activity against leukemia cells.^{75–78} A phase 1 study of single agent vorinostat in patients with advanced leukemia and MDS was recently conducted.⁷⁹ A total of 41 patients were treated (31 AML, 4 CLL, 3 MDS, 2 ALL, 1 CML) with a classical 3 + 3 dose escalation design. The starting dose was 100 mg orally three times daily for 2 weeks with 1 week washout. Both twice daily

and three times daily regimens were tested. DLTs included fatigue, nausea, vomiting, and diarrhea. The MTD was established as 200mg BID or 250mg TID daily for 14 days every 21 days. Of the 41 patients, 2 patients achieved CR and 2 CRi. Additionally, 7 patients had HI (>50% decrease in blast count). Median number of cycles to response/improvement was 2 (range, 1–8) and median response duration was 6 weeks. Transient acetylation of histone H3 was noted in all patients, irrespective of the dose level or response.

COMBINATION EPIGENETIC THERAPY

Despite activity in MDS and AML, use of hypomethylating agents is associated with relatively low CR and PR rates (20–35%) in this patient population when used as monotherapy. In recently reported studies of single-agent HDAC inhibitors, even lower response rates have been observed. Thus there is a need to develop effective combination regimens aimed at improving response rates, duration and eventually survival in patients with AML and MDS. One such approach is the use of a hypomethylating agent with an HDAC inhibitor. The rationale for this combination emerged from studies demonstrating synergistic effects in the reactivation of epigenetically silenced genes.^{80–83} Gene silencing associated with methylation of promoters is in part due to recruitment of transcriptional repression complexes such HDACs. These results also indicated that DNA methylation was the dominant alteration and DNA methylation followed by histone deacetylation resulted in maximal gene reactivation. These studies are summarized in table 4.

Decitabine and VPA

Based on a preclinical study evaluating the antileukemia effect of decitabine and VPA,⁸⁴ we evaluated the safety and activity of this combination in a phase 1/2 study.⁸⁵ The preclinical evaluation showed the growth inhibitory activity of these drugs to be independent of the sequence used.⁸⁴ For the clinical trials we selected a concomitant dosing schedule. Fifty four patients were treated (89% AML, 11% MDS)⁸⁵ : 20% of the patients were previously untreated and all were older than 60 years of age. In the phase 1 portion of the study, 22 patients were treated. Three dose levels of VPA were studied (20, 35 and 50 mg/kg orally daily for 10days). Decitabine was given as 15 mg/m² IV as a 1 hour infusion daily for 10 days. A dose of 50mg/kg daily of VPA was established as MTD and a total of 32 patients were treated in the phase 2 portion of the study. Nine of the 32 patients developed grade 3 non-hematological toxicity, mainly reversible neurotoxicity. CR was documented in 22% of patients (12 of 53 evaluable patients), including 4% with CRp (complete response with incomplete platelet recovery). Median time to response was 60 days (range 29–138 days) and median response duration was 5.6 months. Of note, induction mortality was extremely low at only 2%. Median survival was 15.3 months in responders and 4.9 months in non-responders. For previously untreated patients (n=10), 50% achieved CR + CRp. Higher VPA levels were associated with higher non-hematological toxicity, however no correlation between VPA levels and response was observed in whole group analysis. Interestingly, when only previously untreated patients were analyzed, responders did have significantly higher free VPA levels at day 10 compared to non-responders (32.4 mg/L vs. 14.0 mg/L, p=0.03). Histone H3 and H4 acetylation was documented in 35% of patients at 50 mg/kg VPA dose level. No correlation was observed between histone acetylation or induction of global hypomethylation and response. Currently, a randomized trial comparing the use decitabine with and without VPA is underway.

5-azacitidine, VPA and ATRA combination

Given the results of the above mentioned trials of VPA plus decitabine and VPA plus ATRA, the combination of VPA, 5-azacitidine and ATRA was investigated.⁸⁶ The dose of 5-azacitidine was 75mg/m² SC daily for 7 days (days 1–7) and ATRA was 45mg/m² orally

daily for 5 days (days 3–7). In the phase 1 portion of the study, 3 dose levels of VPA were tested (50, 62.5, 75 mg/kg orally daily for days 1–7). Nineteen patients were treated and a dose of 50 mg/kg orally daily for 7 days each course was established to be MTD for VPA. In the phase 2 part, 34 patients were treated at the MTD of VPA of which 8 had 3 grade nonhematologic toxicity (mainly reversible confusion and somnolence). Induction death was seen in 5% of patients. Overall 27% CR/CRp was observed (CR 22%, CRp 5%). In this study, bone marrow response (blasts <5% without meeting peripheral blood criteria for CR or CRp) was also documented and was seen in an additional 13% of patients. In the subgroup of previously untreated patients (n=33), 33% CR, 9% CRp and 9% bone marrow responses were noted. There was also no induction death observed in this subgroup. These were especially encouraging results, as all these patients were >60 years of age. In addition, 62% of the poor risk cytogenetics patients (-5,-7 chromosomal abnormality) responded (5/8 patients). Patients with increased VPA levels had a significantly increased rate of response (median bound VPA level on day 5 in responders was 132 µg/ml compared to 104 µg/ml in non responders, $p<0.005$), though there was no relation between VPA dose and response. Histone H3 and H4 acetylation was noted in 54% of patients, and a transient, global DNA hypomethylation was also seen. Again, there was no correlation between histone acetylation or DNA hypomethylation and clinical response.

An important observation from the 2 combination trials of VPA with 5-azacitidine or decitabine is that higher VPA levels are associated with higher response rates.^{85,86} This data has significant implications. First, if clinical responses are related to the HDAC inhibitory activity of this class of agents, substituting VPA with more potent HDAC inhibitors, such as vorinostat, MGCD0103 or LBH589, may result in effective combinations (below). Second, if clinicians are to use VPA, then higher doses (50 mg/kg/day, as done in these 2 studies) should be recommended.

5-azacitidine and PB

Two studies have been reported so far combining 5-azacitidine and PB in patients with AML and MDS. In the first, Gore et al. used varying doses of 5-azacitidine (25–75 mg/m²/day SC) for 5–14 days followed by 7 days continuous infusion of PB at 375mg/kg/day.⁸⁷ Of the 29 evaluable patients, 11 responded (5 CR, 1 PR, 5 HI). Reversible neurotoxicity secondary to PB occurred in 9 patients. In the second study Maslak et al. examined a fixed dose of 75 mg/m² SC daily of 5-azacitidine for 7 days, followed by PB at 200 mg/kg/day in 1–2 hour infusion for 5 days.⁸⁸ Of the 10 evaluable patients, 3 achieved PR. No CR was observed. Median time to response was 31 days and median duration of response was 45 days. Neurotoxicity related to PB (disorientation, somnolence) was seen in 80% of patients and was reversible upon cessation of therapy. Histone H4 acetylation was seen in all patients but there was no relation to clinical response. Neurotoxicity and the need for continuous infusion have hampered the development of PB as a therapeutic agent in myeloid malignancies.

5-azacitidine and MGCD0103

Based on the single agent activity of both 5-azacitidine and MGCD0103, the combination has been used in 37 patients in a phase 1/2 study.⁷¹ 5-azacitidine was administered at 75mg/m² SC daily for 7 days every 28 days. MGCD0103 was used in a dose escalation design with doses of 35, 60, 90, 110, and 135mg orally three times a week starting on day 5 of every cycle. In the preliminary analysis, ORR was 30% (CR 11%, CRi 14%, PR 5%). No induction mortality was observed. A randomized study is ready to be initiated comparing 5-azacitidine with or without MGCD0103.

5-azacitidine and MS-275

Gore et al. recently reported preliminary results of a phase 1/2 study of 5-azacitidine and MS-275.⁸⁹ 31 patients were treated with the combination of 5-azacitidine (dose 30, 40 or 50 mg/m² SC daily for 10 days every 28 days) and MS-275 (dose 2, 4, 6 or 8 mg/m² on days 3 and 10, every 28 days). Of the 27 evaluable patients, 2 achieved CR, 4 PR and 6 bilineage hematological improvements. An intergroup trial is currently comparing the combination of 5-azacitidine plus MS275 with 5-azacitidine alone.

Other combinations reported in preliminary fashion include vorinostat and decitabine^{90,91} and vorinostat and idarubicin^{92,93} in patients with MDS and AML.

CONCLUSIONS AND NEEDS

The field of epigenetic therapy is expanding rapidly. A large number of drugs with the capacity to alter the epigenetic structure of cancer cells are being used in clinical trials either as single agents or in combinations. Hypomethylating agents such as 5-azacitidine and decitabine are now established, approved therapies for MDS. Several issues related to hypomethylating treatments such as the optimal dose, schedule or route of administration are still being elucidated in clinical trials. The use of HDAC inhibitors as single agents has proved to be of limited clinical efficacy. Ongoing randomized trials exploring the use of HDAC inhibitors in combination with hypomethylation as well as other therapeutic agents will help elucidate the role of these agents in the treatment of MDS and leukemia.

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Figure 1.
Chemical structure of hypomethylating agents and related compounds.

Table 1

Clinical experience with 5-azacitidine

Study	Phase	N	% AML	Dose/ schedule	CR %	PR %	HI %	ORR
CALGB 8421 ¹⁰	II	48	52	75 mg/m ² /d IV × 7	15	2	27	44
CALGB 8921 ¹⁴	II	70	37	75 mg/m ² /d SC × 7d	17	0	23	40
CALGB 9221 ⁹⁴	III	99	27	75 mg/m ² /d SC × 7d	10	1	36	47

CALGB, Cancer and Leukemia Group B; N, number; AML, acute myelogenous leukemia ; CR, complete remission ; PR, partial response ; HI, hematologic improvement, ORR overall response rate ; IV, intravenous ; SC, subcutaneous ; d, days.

Table 2

Clinical experience with Decitabine

Study	Phase	N	% AML	Dose/ Schedule	CR %	PR %	HI %	ORR
Wijermans ²⁶	II	29	31	50 mg/m ² /d IV QD × 3	29	18	7	54
Wijermans ²⁷	II	66	30	15 mg/m ² q8hr IV QD × 3	20	5	24	49
Wijermans ²⁸	II	177	37	40-50 mg/m ² /d IV QD × 3	24	10	14	49
Kantarjian ²⁹	III	89	16	15 mg/m ² q8hr IV QD × 3	9	8	13	30
Kantarjian ³²	II	64	NR	20 mg/m ² IV QD × 5	39	NR	NR	NR
		14	NR	20 mg/m ² SC BID × 5 days	21	NR	NR	NR
		17	NR	10 mg/m ² IV × 10	24	NR	NR	NR
		95	6	All 3 groups together	34	1	28	73

N, number; AML, acute myelogenous leukemia; CR, complete remission; PR, partial response; HI, hematological improvement; ORR, overall response rate; d, day; QD, daily; IV, intravenous; SC, subcutaneous; BID, twice a day.

Table 3

Clinical experience with HDAC inhibitors

Drug	MS-275 ⁶⁵	Depsipeptide ⁶¹	Vorinostat ⁷⁹	MGC0103 ⁹⁵	LBH589 ⁶⁸	VPA ⁵¹
Phase	I	I	I	I	I	II
N	39	10	41	29	29	75
Toxicity	Neuro	Constitutional	GI	GI	Cardiac	Neuro
ORR	0	0	17%	10%	0	24%

VPA, valproic acid; N, number of patients; Neuro, neurotoxicity; GI, gastrointestinal; ORR, overall response rate

Table 4

Experience with combination epigenetic therapy

Study	AZA+PB⁸⁸	DAC+VPA⁸⁵	AZA+VPA⁸⁶	AZA+MGCD0103⁹⁶
Phase	I	I/II	I/II	I/II
N	36	54	53	52
Toxicity	Neuro	Neuro	Neuro	GI
ORR	30%	22%	42%	36%

AZA, 5-azacitidine; PB, phenylbutyrate; DAC, decitabine; VPA, valproic acid, N, number of patients; Neuro, neurotoxicity, GI, gastrointestinal; ORR, overall response rate