

# Use of Hypomethylating Agents in Myelodysplastic Syndromes

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**Abstract:** Aberrant DNA methylation is one of the molecular hallmarks of cancer and leukemia. By repressing gene expression, it is considered a functional equivalent to the physical inactivation of tumor suppressor genes by deletions or mutations. To clinically exploit this process, compounds with DNA hypomethylating properties have been evaluated both in the laboratory and the clinic. Two such agents, 5-azacytidine and 5-aza-2'-deoxycytidine, are currently approved by the US Food and Drug Administration for the treatment of patients with myelodysplastic syndromes. Ongoing studies are evaluating alternative dosing schedules for these drugs and the activity and safety of this class of agent in combination with histone deacetylase inhibitors. Here we summarize the experience of hypomethylating agents in myelodysplastic syndromes.

Genetic and epigenetic alterations are the molecular hallmarks of cancer. Genetic alterations include mutations or deletions that alter the primary sequence of DNA. In contrast, epigenetic alterations result from biochemical modification of the composition of chromatin. DNA methylation and alteration of the histone code are two epigenetic changes that can result in transcriptional deregulation and gene silencing. Reversal of this process with hypomethylating agents results not only in gene expression reactivation but in killing of leukemic cells, a phenomenon that has been widely exploited in human clinical trials.<sup>1-4</sup> Hypomethylating agents are either nucleoside analogs (eg, 5-azacytidine,<sup>2</sup> 5-aza-2'-deoxycytidine,<sup>2</sup> 5-fluoro-2'-deoxycytidine,<sup>2</sup> 5,6-dihydro-5-azacytidine,<sup>2</sup> 1-β-D-arabino-furanosyl-5-azacytosine [fazarabine],<sup>5</sup> and 1-[β-D-ribofuranosyl]-1,2-dihydropyrimidin-2-one [zebularine]<sup>6</sup>) or nonnucleoside analogs (eg, MG98,<sup>7</sup> RG108,<sup>8</sup> green tea polyphenol [-]-epigallocatechin-3-gallate,<sup>9</sup> hydralazine,<sup>10</sup> procainamide,<sup>11</sup> psammaphin<sup>12</sup>). Currently, two nucleoside analog hypomethylating agents, 5-azacytidine (azacitidine; Vidaza, Pharmion) and 5-aza-2'-deoxycytidine (decitabine; Dacogen, MGI Pharma), are approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndromes (MDS). Both agents were initially developed as cytarabine derivatives with initially disappointing results, especially at higher concentrations. However, in follow-up studies and with lower-dose schedules, both drugs have shown efficacy in

## Keywords

5-azacytidine, 5-aza-2'-deoxycytidine, DNA methylation, myelodysplastic syndromes

MDS. The following is a review of the therapeutic role of hypomethylating agents in MDS.

### DNA Methylation in Cancer

DNA methylation refers to the addition of a methyl group to a cytosine. This process is mediated by the enzyme DNA methyltransferase and occurs only when the cytosine precedes a guanine (CpG dinucleotide). These CpG pairs are found at a less-than-expected frequency in human DNA, except in areas known as CpG islands. CpG islands are commonly found in proximity to the promoter region.<sup>13</sup> DNA methylation of promoter-associated CpG islands is associated with silencing of the corresponding gene.<sup>14</sup> In general, promoter-associated CpG islands are not methylated in nonmalignant cells. The exceptions are imprinted genes<sup>15</sup> and genes located on the inactivated X chromosome.<sup>16</sup> Aberrant DNA methylation is considered a functional equivalent to the physical inactivation of tumor suppressor genes by deletions or mutations.<sup>17</sup> To exploit this mechanism, compounds with hypomethylating properties have been evaluated both in the laboratory and the clinic. The two currently clinically available hypomethylating agents, 5-azacytidine and 5-aza-2'-deoxycytidine, are cytosine derivatives. The hypomethylating properties of cytosine derivatives was first noted by Jones and Taylor.<sup>2</sup> In their initial study, cytidine analogs containing a modification at the 5 position were able to induce myocyte differentiation in mouse embryo cells. However, this effect was not seen with other derivatives. Two important observations were noted. First, maximal hypomethylation was induced at a narrow drug concentration window, with effect on DNA methylation lost below or above this range. This observation has been reproduced more recently in leukemia patients treated with 5-aza-2'-deoxycytidine, in which DNA hypomethylation increased linearly at doses between 5 and 20 mg/m<sup>2</sup> per day, with no further increase above that.<sup>4</sup> The second observation was that the cell differentiation effect required several rounds of cell division. Interestingly, it is well accepted that clinical responses to both 5-azacytidine and 5-aza-2'-deoxycytidine may also require several courses of therapy, thus recapitulating this laboratory observation.

DNA hypomethylating agents lead to induction of global<sup>18</sup> and gene-specific DNA hypomethylation, both in vivo and in vitro. This in turn may have significant effects on cell differentiation, angiogenesis,<sup>19</sup> cell proliferation,<sup>1,3,4</sup> and apoptosis.<sup>20</sup> In addition, hypomethylating agents may have immunomodulatory effects mediated by the re-expression of tumor-associated antigens and promotion of immune recognition,<sup>21</sup> a mechanism that has been exploited together with immunotherapy in the treatment of cancer.<sup>22</sup>

### Chemistry

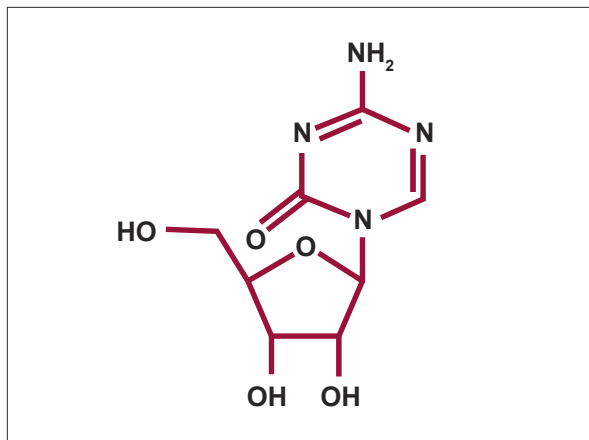
Both 5-azacytidine<sup>23</sup> and 5-aza-2'-deoxycytidine<sup>24</sup> were synthesized in the 1960s, and their antileukemic effect was first noted in 1968.<sup>25,26</sup> The chemical name for 5-azacytidine is 4-amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one and the chemical name for 5-aza-2'-deoxycytidine is 4-amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H). 5-Azacytidine and 5-aza-2'-deoxycytidine are both cytosine derivatives. Figures 1 and 2 show their structures.

### Pharmacology

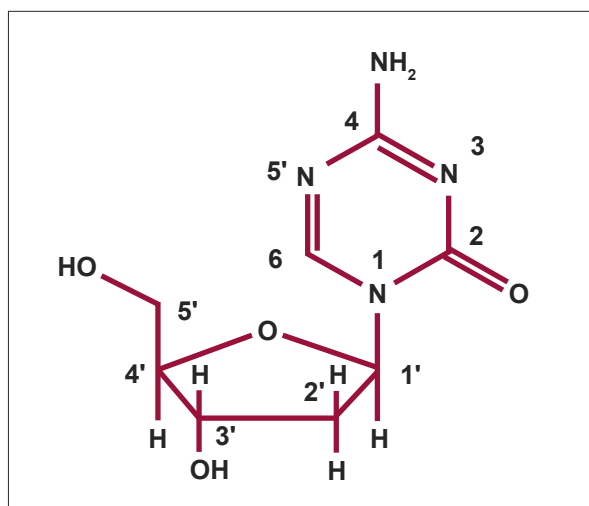
5-Azacytidine and 5-aza-2'-deoxycytidine are introduced into the cell by a nucleotide-specific transport system and activated by sequential phosphorylation to cytidine triphosphate (CTP) and deoxycytidine triphosphate (dCTP), respectively.<sup>27</sup> CTP is incorporated into RNA only, whereas dCTP is incorporated into DNA only. They form irreversible covalent adducts with DNA methyltransferase. The active compound is degraded by cytidine deaminase.<sup>28</sup> It is postulated that hypomethylating agents have two mechanisms of action: at high doses the DNA-DNA methyltransferase adducts trigger apoptosis and cell death<sup>29</sup>; however, at lower doses DNA hypomethylation is induced, a process dependent on cell division, leading to gene re-expression.<sup>2,30,31</sup>

Because of the lack of a reproducible methodology until recently, few pharmacokinetic studies of 5-azacytidine or 5-aza-2'-deoxycytidine are available.<sup>32-35</sup> In one study, 12 patients with MDS or acute myelogenous leukemia (AML) received 3-hour infusions of 5-aza-2'-deoxycytidine 15 mg/m<sup>2</sup> every 8 hours for 3 days with a second cycle 6 weeks later. Peak plasma concentrations were 49.0±22.2 ng/μL and 62.7±45.2 ng/μL in the first and second cycles, respectively, with no change in pharmacokinetics between the first and second cycle.<sup>33</sup> In another phase I study, 21 patients with advanced solid tumors received three 1-hour infusions of 5-aza-2'-deoxycytidine 25–100 mg/m<sup>2</sup> separated by 7-hour intervals. There were no detectable serum levels in most patients receiving doses between 25 and 60 mg/m<sup>2</sup>; however, between 75 and 100 mg/m<sup>2</sup> the peak plasma concentrations were 0.93 μM and 2.01 μM, respectively. The mean distribution half-life was 7 minutes and mean elimination half-life was 35 minutes.<sup>35</sup> 5-Aza-2'-deoxycytidine is rapidly metabolized in the liver with less than 1% urinary excretion<sup>36</sup>; it effectively crosses the blood-brain barrier, with a cerebrospinal fluid concentration of 58% of the plateau plasma level in dogs.<sup>37</sup>

The pharmacokinetic characteristics of intravenous (IV) and subcutaneous (SC) 5-azacytidine have also been



**Figure 1.** Chemical structure of 5-azacytidine.



**Figure 2.** Chemical structure of 5-aza-2'-deoxycytidine.

evaluated. The bioavailability of the SC route was 89% of the IV route. The median half-lives were  $0.36 \pm 0.02$  and  $0.69 \pm 0.14$  hours for the SC and IV routes, respectively. Interestingly, 5-azacytidine clearance exceeded the glomerular filtration rate and total renal blood flow, suggesting other nonrenal elimination pathways.<sup>38</sup> In a study using radioactive 5-azacytidine, 73–98% was excreted in urine with less than 1% excretion in feces.<sup>39</sup>

### Clinical Experience With 5-Azacytidine

5-Azacytidine was the first of the two drugs to be approved by the FDA, based on two phase II studies (protocols 8421 and 8921) and a randomized phase III trial (proto-

col 9221) conducted by the Cancer and Leukemia Group B (CALGB).<sup>42</sup> In these trials 5-azacytidine 75 mg/m<sup>2</sup> was administered daily for 7 days every 28 days by continuous IV infusion (CALGB 8421) or SC (CALGB 8921 and 9221). Because the classification of MDS has changed since those trials were published, the authors have recently reanalyzed the data using the World Health Organization (WHO)<sup>40</sup> classification and the International Working Group (IWG) criteria<sup>41</sup> for response (Table 1). A total of 118 patients were enrolled in the phase II studies (48 with IV and 70 with SC administration).<sup>42</sup> In the IV study, all patients had either refractory anemia (RA) with excess blasts (RAEB) or RAEB in transformation, whereas in the SC study, 16% of patients had either RA or RA with ringed sideroblasts. Response rates were comparable in both studies. In CALGB 8421 with IV 5-azacytidine, the complete response (CR), partial response (PR), and hematologic improvement (HI) rates were 15%, 2%, and 27%, respectively. Similarly, in CALGB 8921 with SC 5-azacytidine, the CR, PR, and HI rates were 17%, 0%, and 23%, respectively.

After the encouraging results seen in the phase II studies, the CALGB initiated a randomized phase III trial. A total of 191 patients were randomized to SC 5-azacytidine versus best supportive care in a crossover design.<sup>43</sup> The median time to AML transformation or death for patients receiving supportive care was 12 months, versus 21 months with 5-azacytidine ( $P=.007$ ). When the data were reanalyzed using the IWG criteria, the overall response rates were 47% (CR 10%, PR 1%, HI 36%) with 5-azacytidine versus 17% HI in patients who received supportive care (with no crossover to 5-azacytidine) only. The median number of cycles to response was three, and the median duration of response was five cycles. Of the 65 transfusion-dependent patients, 29 (45%) became transfusion-independent for a median duration of 9 months. When results from both phase II and III studies with SC 5-azacytidine were combined, the overall response rate was 44% (CR 13%, PR 1%, HI 31%) in 169 patients. The most common nonhematologic toxicity was nausea/vomiting, which occurred in 4% of patients.<sup>42</sup> Induction mortality is extremely rare with 5-azacytidine-based therapy.

Because the study had a crossover design, it was difficult to evaluate the effects of 5-azacytidine administration on overall survival. A landmark analysis at 6 months showed median survival of 18 months for 5-azacytidine and 11 months for supportive care ( $P=.03$ ).<sup>43,44</sup> In addition, patients on the 5-azacytidine arm experienced statistically significantly greater improvement in fatigue, dyspnea, physical functioning, positive affect, and psychologic distress than those in the supportive care arm.<sup>45</sup> At the 2005 American Society of Hematology (ASH) meeting,

**Table 1.** Phase II and III Studies of 5-Azacytidine

Study	n	Dose	CR n (%)	PR n (%)	HI n (%)	OR n (%)	MR, mo	MS, mo
CALGB 8421 <sup>42</sup> Phase II	48	75 mg/m <sup>2</sup> /d IV × 7d q 28 d	7 (15)	1 (2)	13 (27)	21 (44)	NA	NA
CALGB 8921 <sup>42</sup> Phase II	70	75 mg/m <sup>2</sup> /d SC × 7d q 28 d	12 (17)	0	16 (23)	28 (40)	NA	NA
CALGB 9221 <sup>42</sup> Phase III	99	75 mg/m <sup>2</sup> /d SC × 7d q 28 d	10 (10)	1 (1)	36 (36)	47 (47)	15	20

CALGB=Cancer and Leukemia Group B; CR=complete response; HI=hematologic improvement; MR=median response; MS=median survival; NA=not available; OR=overall response; PR=partial response; SC=subcutaneous.

Silverman and colleagues showed evidence that 5-azacytidine administration results in significant improvement of both time to AML transformation and overall survival in patients with high-risk MDS.<sup>46,47</sup> 5-Azacytidine has also been shown to benefit patients with AML. When data from the three CALGB trials were reanalyzed using the WHO criteria, the percentages of patients with AML were 52%, 37%, and 27% in protocols 8421, 8921, and 9221 (5-azacytidine arm), respectively. Response rates (CR + PR) ranged from 7% to 16% according to the IWG MDS criteria.<sup>41</sup> This benefit was also seen in a retrospective review by Sudan and associates. Of 20 AML patients who received 5-azacytidine, 9 (45%) achieved a response (4 CR, 5 PR) according to IWG AML criteria.<sup>48</sup> The median response duration was 8 months and overall survival was significantly longer in responders (15+ vs 2.5 months).<sup>49</sup> Although both studies were retrospective evaluations, they suggest that some patients with AML who cannot tolerate intensive chemotherapy may benefit from 5-azacytidine therapy. The clinical activity of 5-azacytidine has also been investigated outside the setting of the CALGB. Recently, French investigators have shown very similar results to those of the CALGB using the conventional SC 7-day schedule of 5-azacytidine.<sup>50</sup>

One of the major logistic problems with 5-azacytidine administration is the 7-day dosing schedule, which requires weekend injections. Lyons and coworkers recently reported initial results from a randomized phase II study of three different dose schedules of 5-azacytidine: 106 patients were randomized to 5, 7, or 10 days of 5-azacytidine without weekend injections. Of those patients, 42% had RA and 30% had RAEB. Responses were equivalent in the three arms, with 71% of the 38 transfusion-dependent evaluable patients achieving transfusion independence. Hematologic improvement was seen in 65%, 52%, and 55% of patients in the 5-, 7-, and 10-day arms, respectively, suggesting that the 5-day schedule is as

effective as the other two schedules.<sup>51</sup> Furthermore, the 5-day schedule was associated with less myelosuppression than the 7- or 10-day schedules.

One of the other critical issues with 5-azacytidine therapy is the use of growth factor support during therapy. It should be noted that most of the CALGB studies did not include that type of supportive care. This is in contrast with most recent studies with 5-aza-2'-deoxycytidine (discussed below), where supportive care was an integral part of therapy administration. Rossetti and colleagues presented data at the last ASH meeting summarizing their experience using 5-azacytidine and growth factors with excellent response rates.<sup>52</sup>

Another issue with 5-azacytidine is the best route of administration. Initially, 5-azacytidine was approved by the FDA for SC administration based on the results of the CALGB 9221 study. As summarized above, earlier studies with IV 5-azacytidine also indicated that drug administration was safe and efficacious via this route. Based on this, the FDA recently approved the use of 5-azacytidine for IV administration. In addition, investigators at Pharmion have developed an oral form of 5-azacytidine, and phase I clinical trials in humans are beginning this year in the United States. The advent of an oral hypomethylating agent may have significant implications for the treatment not only of MDS but of cancer in general. Finally, a large-scale survival study with SC 5-azacytidine in MDS patients has just completed accrual. This study will be fundamental for our understanding of the impact of this drug on the natural history of patients with MDS.

### Clinical Experience With 5-Aza-2'-Deoxycytidine

The initial phase I studies defined the maximum tolerated dose (MTD) of 5-aza-2'-deoxycytidine as between 1,500 and 2,250 mg/m<sup>2</sup>.<sup>34,35,53,54</sup> To explore the possibility

**Table 2.** Phase II and III Studies of 5-Aza-2'-Deoxycytidine

Study	n	Dose	CR n (%)	PR n (%)	HI n (%)	OR n (%)	MR, mo	MS, mo
Wijerman <sup>57</sup> Phase II	29	50 mg/m <sup>2</sup> /d for 72 hr q 6 wk	8 (27)	5 (17)	2 (1)	15 (54)	8	12
Wijerman <sup>58</sup> Phase II	66	15 mg/m <sup>2</sup> over 4 hr q 8 hr × 3 d q 6 wk	13 (20)	3 (4)	16 (24)	32 (49)	8	15
Kantarjian <sup>61</sup> Phase III	89	15 mg/m <sup>2</sup> over 3 hr q 8hr × 3 d q 6 wks	8 (9)	7 (8)	12 (13)	27 (30)	10.3	14
Kantarjian <sup>62</sup> Phase II	95	20 mg/m <sup>2</sup> IV × 5 d q 4 wk 20 mg/m <sup>2</sup> SC × 5 d q 4 wk 10 mg/m <sup>2</sup> IV × 10 d q 4 wk	32 (34)	1 (1)	26(28)	69 (73)	NA	19

CR=complete response; IV=intravenous; HI=hematologic improvement; MR=median response; MS=median survival; NA=not available; OR=overall response; PR=partial response; SC=subcutaneous.

that a lower dose may be more efficacious in exploiting the hypomethylating effects of 5-aza-2'-deoxycytidine, Zagonel and colleagues evaluated two low-dose regimens in MDS patients: 45 mg/m<sup>2</sup> infused over 4 hours daily for 3 days and 50 mg/m<sup>2</sup> continuous IV infusion daily for 3 days.<sup>55</sup> Ten patients with advanced MDS were treated. The overall response rate was 50%, with 4 patients achieving CR. The median duration of CR was 11 months (range, 10–14+ months). Both regimens were well tolerated, with 50% of patients experiencing transient marrow hypoplasia.<sup>55</sup> A phase I study by Issa and colleagues attempted to identify the lowest effective biologic dose.<sup>56</sup> 5-Aza-2'-deoxycytidine was administered in doses ranging from 5 to 20 mg/m<sup>2</sup> over 1 hour. The duration of therapy ranged from 10 to 20 days with total dose per course ranging from 50 to 300 mg/m<sup>2</sup>. Fifty patients were enrolled, 35 with AML, 7 with MDS, 5 with chronic myelogenous leukemia, and 1 with acute lymphocytic leukemia. The overall response rate was 32%. Responses were observed in 11 of 17 patients (67%) receiving a dose of 15 mg/m<sup>2</sup> for 10 days. Of interest, response rates dropped at higher or lower doses, indicating a narrow dose range of activity.<sup>56</sup>

In a small phase II study by Wijermans and associates, 5-aza-2'-deoxycytidine was administered at a dose of 50 mg/m<sup>2</sup> per day for 3 days every 6 weeks in 29 elderly patients with high-risk MDS.<sup>57</sup> The overall response rate was 54% (CR 28%, PR 26%) with a median response duration of 31 weeks.<sup>57</sup> This research led to a larger multicenter phase II trial.<sup>58</sup> In that study, 66 patients with MDS were treated with IV 5-aza-2'-deoxycytidine 45 mg/m<sup>2</sup> daily for 3 days every 6 weeks. The overall response rate was 49% (CR 20%, PR 4%, HI 24%; Table 2). The induction mortality rate was 8%.<sup>58</sup> A major cytogenetic response was observed in 31% of patients with

abnormal cytogenetics at initial presentation. Patients who achieved a complete cytogenetic remission had statistically significantly longer survival versus those who did not (24 vs 11 months; *P*=.02).<sup>59</sup> Platelet responses for the two consecutive phase II trials were reported separately. Of the 126 thrombocytopenic patients, 58% (47% major HI and 11% minor HI) showed a response after one cycle of therapy. The median survival for patients with either stable or rising platelet counts (13 and 25 months, respectively) was better than for patients with decreasing counts (4 months).<sup>60</sup>

The encouraging phase II results described above led to a multicenter randomized phase III trial in the United States.<sup>61</sup> 5-Aza-2'-deoxycytidine was administered at a dose of 15 mg/m<sup>2</sup> IV over 3 hours every 8 hours daily for 3 days every 6 weeks (Table 2). A total of 170 patients were randomized to 5-aza-2'-deoxycytidine plus best supportive care versus best supportive care only. Of the patients on the 5-aza-2'-deoxycytidine arm, 61 (69%) had International Prognostic Scoring System (IPSS) intermediate-2-/high-risk disease, and 74% were transfusion-dependent. The median number of cycles administered was three (range, 0–9) and the overall response rate was 30% (CR 9%, PR 8%, HI 13%). The median time to AML or death was not statistically different for the 5-aza-2'-deoxycytidine and supportive care-only arms (12.1 vs 7.8 months, respectively); however, 5-aza-2'-deoxycytidine treatment was associated with a longer median time to AML transformation or death in patients with de novo MDS (12.6 vs 9.4 months; *P*=.04), patients with high-risk MDS (9.3 vs 2.8 months; *P*=.01), and treatment-naive patients (12.3 vs 7.3 months; *P*=.08) compared to supportive care only.<sup>61</sup> In addition, responding patients had a longer median time to AML progression or death

**Table 3.** Clinical Activity of the Combination of 5-Aza-2'-Deoxycytidine and Valproic Acid (VPA) by VPA Dose<sup>72</sup>

VPA dose daily × 10 days	n	CR	CRp	OR, %
20 mg/kg	3	1	0	33
35 mg/kg	9	1	0	11
50 mg/kg	41	8	2	23
Total	53	10	2	22
Untreated acute myeloid leukemia/ myelodysplastic syndromes	10	4	1	50

N=number of patients; CR=complete remission; CRp=complete remission without complete platelet recovery; OR=overall response.

\*All patients received fixed dose 5-aza-2'-deoxycytidine 15 mg/m<sup>2</sup> daily × 10 days with escalating doses of VPA.

(17.5 vs 9.8 months; *P*=.01). The incidence of death was lower on the 5-aza-2'-deoxycytidine arm compared to the supportive care arm (14% vs 22%). Therapy was very well tolerated, with myelosuppression being the most common side effect. Grade III or IV hematologic adverse events were neutropenia 87%, thrombocytopenia 85%, febrile neutropenia 23%, and leukopenia 22%, with a decreasing incidence over the first four cycles. Grade III or IV nonhematologic toxicities included hyperbilirubinemia 6%, pneumonia 15%, and constipation 2%.<sup>61</sup> This study led to FDA approval of 5-aza-2'-deoxycytidine.

Based on the initial phase I studies of low-dose 5-aza-2'-deoxycytidine in advanced leukemia,<sup>56</sup> a phase II study of different low-dose schedules of 5-aza-2'-deoxycytidine was conducted at The University of Texas M. D. Anderson Cancer Center. Eligible patients were randomized following a Bayesian adaptive design to one of three arms: 1) 20 mg/m<sup>2</sup> IV over 1 hour daily for 5 days; 2) 20 mg/m<sup>2</sup> daily given in two SC doses for 5 days, or 3) 10 mg/m<sup>2</sup> IV over 1 hour 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 median number of cycles was six (range, 1–18). The overall response rate by the modified IWG criteria was 73%, with 32 patients (34%) achieving CR. Patients randomized to 20 mg/m<sup>2</sup> 1-hour IV daily for 5 days—the most dose-intensive arm—had the best response rate, with 39% CR. Four patients died from myelosuppression-related complications. No patient died from complications directly attributable to 5-aza-2'-deoxycytidine. Clinically insignificant transient transaminase elevations occurred in 4% of patients. Hospitalization was necessary in 110 of 622 courses (18%) for myelosuppression-related symptoms; however, only 34% of patients did not require hospitalization.<sup>62</sup> Responses have also been observed on re-treatment with 5-aza-2'-deoxycytidine in patients who had previously responded to the drug.<sup>63</sup>

### Combinations of DNA Methylation Inhibitors With Histone Deacetylase Inhibitors

Histone acetylation leads to an open chromosome configuration and consequently to gene transcription and cell differentiation. Several enzymatic activities control this process of histone acetylation/deacetylation.<sup>64</sup> Currently several compounds with histone deacetylation (HDAC) inhibitor properties are undergoing clinical evaluation in the treatment of MDS.<sup>65-69</sup> A synergistic effect of demethylation and HDAC inhibition in re-expression of genes was first reported by Cameron and coworkers using trichostatin and 5-aza-2'-deoxycytidine.<sup>70</sup> A similar in vitro synergistic effect with 5-aza-2'-deoxycytidine and valproic acid was observed.<sup>71</sup> A phase I/II study to evaluate the MTD and efficacy of 5-aza-2'-deoxycytidine and valproic acid in patients with AML/MDS has been conducted. Patients on the phase I part of the study received fixed-dose 5-aza-2'-deoxycytidine 15 mg/m<sup>2</sup> as a 1-hour IV infusion daily for 10 days with concomitant escalating doses of valproic acid 20, 35, and 50 mg/kg daily. The MTD of valproic acid was 50 mg/kg. Of 53 evaluable patients, 12 patients responded, with 10 patients achieving CR and 2 patients CR with incomplete platelet recovery. Of the previously untreated patients, 50% responded (5 of 10 patients). The median remission duration was 7.2 months (range, 1.3–12.6 months; Table 3).<sup>72</sup>

The combination of 5-azacytidine, valproic acid, and all-*trans*-retinoic acid (ATRA) in patients with high-risk MDS (>10% blasts), with relapsed/refractory AML, or over age 60 with untreated AML has also been evaluated.<sup>73</sup> A fixed dose of 5-azacytidine (75 mg/m<sup>2</sup> SC daily for 7 days) was used. ATRA 45 mg/m<sup>2</sup> daily was given orally for 5 days starting on day 3 of 5-azacytidine, with dose escalation of the valproic acid. The MTD of valproic acid was 50 mg/kg daily for 7 days. In an interim report of 31 patients, 9 achieved CR (absolute neutrophil count

**Table 4.** Clinical Activity of the Combination of 5-Azacytidine and Valproic Acid (VPA) by VPA Dose<sup>72</sup>

VPA (mg/kg)	n	CR	CRp	OR, %
50	18	7	2	50
62.5	7	1	0	14
75	6	1	1	33
Total	31	9	3	39
Untreated acute myeloid leukemia/myelodysplastic syndromes >60 years	16	7	2	56

N=number of patients; CR=complete remission; CRp=complete remission without complete platelet recovery; OR=overall response.

\*All patients received a fixed-dose of 5-azacytidine 75 mg/m<sup>2</sup> subcutaneously daily × 7 days plus all-*trans*-retinoic acid 45 mg/m<sup>2</sup> orally daily × 5 days starting on day 3 of 5-azacytidine with escalating doses of VPA.

10<sup>9</sup>/L, platelets 100 × 10<sup>9</sup>/L, and marrow blasts <5%) and 3 a CR without complete platelet recovery, with an overall response of 39% (Table 4). Of the 18 patients treated at the MTD, nine responses (50%) were observed. Histone acetylation and transient global hypomethylation were observed, but no correlation was seen between degree of hypomethylation and response. Higher levels of valproic acid were found in responders. Other forms of combination therapy with a hypomethylating agent and an HDAC inhibitor have been reported. In a study by Gore and colleagues, 32 patients with MDS or AML were treated with 5-azacytidine followed by phenylbutyrate. Of 29 evaluable patients, 11 responded (4 CR, 1 PR, 4 major HI). All responding patients showed evidence of p15 demethylation, while none of the nonresponders had any demethylation. In addition, 5-azacytidine induced histone deacetylation, which has not been shown with 5-aza-2'-deoxycytidine.<sup>74</sup> Other HDAC inhibitors currently being evaluated in combination with either 5-azacytidine or 5-aza-2'-deoxycytidine include MGCD0103,<sup>75</sup> vorinostat (Zolinza, Merck),<sup>76</sup> MS-275,<sup>77</sup> and LBH589.<sup>78</sup>

## Conclusion

Hypomethylating agents are presently at the forefront of the therapy and research for patients with MDS. Currently, two of the three FDA-approved drugs for MDS are hypomethylating agents. Both drugs, 5-azacytidine and 5-aza-2'-deoxycytidine, are efficacious and should be recommended for patients with MDS. Several questions remain. These include agent selection, dose/schedule/route of administration, and the role of combination therapy. In terms of drug selection, it is difficult if not impossible to recommend one agent over the other. Technically, 5-aza-2'-deoxycytidine is not approved for patients with low-risk MDS by the IPSS score. Beyond that, response rates with the approved dosing schedules of 5-azacytidine and 5-aza-2'-deoxycytidine are similar.

In lieu of a randomized comparative study of the two agents, the individual physician will have to select the appropriate agent based on his or her experience and practice setting. As for the schedule of administration, the newer lower-dose schedule of 5-aza-2'-deoxycytidine may be more active than the traditional schedule but perhaps at the expense of more myelosuppression; the newer 5-day schedule of 5-azacytidine appears equally efficacious, with less toxicity when compared with the traditional 7-day schedule. No clear benefit can be related now to the route of administration or the metabolism of each drug. Finally, studies of additional dosing schedules and combination therapy are ongoing and may increase our armamentarium against MDS.

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## References

1. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. *Blood*. 2002;100:2957-2964.
2. Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell*. 1980;20:85-93.
3. Uchida T, Kinoshita T, Nagai H, et al. Hypermethylation of the p15INK4B gene in myelodysplastic syndromes. *Blood*. 1997;90:1403-1409.
4. Yang AS, Doshi KD, Choi SW, et al. DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia. *Cancer Res*. 2006;66:5495-5503.
5. Glazer RI, Knode MC. 1-beta-D-arabinosyl-5-azacytosine. Cytocidal activity and effects on the synthesis and methylation of DNA in human colon carcinoma cells. *Mol Pharmacol*. 1984;26:381-387.
6. Cheng JC, Matsen CB, Gonzales FA, et al. Inhibition of DNA methylation and reactivation of silenced genes by zebularine. *J Natl Cancer Inst*. 2003;95:399-409.
7. Beaulieu N, Fournel M, MacLeod AR. Antitumor activity of MG98, an antisense oligodeoxynucleotide targeting DNA methyltransferase 1 (DNMT1) [abstract]. *Clin Cancer Res*. 2001;7:3800S.

8. Brueckner B, Garcia Boy R, Siedlecki P, et al. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res.* 2005;65:6305-6311.
9. Fang MZ, Wang Y, Ai N, et al. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits dna methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 2003;63:7563-7570.
10. Segura-Pacheco B, Trejo-Becerril C, Perez-Cardenas E, et al. Reactivation of tumor suppressor genes by the cardiovascular drugs hydralazine and procainamide and their potential use in cancer therapy. *Clin Cancer Res.* 2003;9:1596-1603.
11. Lee BH, Yegnasubramanian S, Lin X, Nelson WG. Procainamide is a specific inhibitor of DNA methyltransferase 1. *J Biol Chem.* 2005;280:40749-40756.
12. Pina IC, Gautschi JT, Wang GY, et al. Psammaphins from the sponge Pseudoceratina purpurea: inhibition of both histone deacetylase and DNA methyltransferase. *J Org Chem.* 2003;68:3866-3873.
13. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med.* 2003;349:2042-2054.
14. Herman JG. Hypermethylation of tumor suppressor genes in cancer. *Semin Cancer Biol.* 1999;9:359-367.
15. Barlow DP. Gametic imprinting in mammals. *Science.* 1995;270:1610-1613.
16. Goto T, Monk M. Regulation of X-chromosome inactivation in development in mice and humans. *Microbiol Mol Biol Rev.* 1998;62:362-378.
17. Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. *Ann Intern Med.* 2001;134:573-586.
18. Mund C, Hackanson B, Stresemann C, Lubbert M, Lyko F. Characterization of DNA demethylation effects induced by 5-Aza-2'-deoxycytidine in patients with myelodysplastic syndrome. *Cancer Res.* 2005;65:7086-7090.
19. Hellebrekers DM, Jair KW, Vire E, et al. Angiostatic activity of DNA methyltransferase inhibitors. *Mol Cancer Ther.* 2006;5:467-475.
20. Schmelz K, Wagner M, Dorken B, Tamm I. 5-Aza-2'-deoxycytidine induces p21WAF expression by demethylation of p73 leading to p53-independent apoptosis in myeloid leukemia. *Int J Cancer.* 2005;114:683-695.
21. Maio M, Coral S, Fratta E, Altomonte M, Sigalotti L. Epigenetic targets for immune intervention in human malignancies. *Oncogene.* 2003;22:6484-6488.
22. Gollob JA, Sciambi CJ, Peterson BL et al. Phase I trial of sequential low-dose 5-aza-2'-deoxycytidine plus high-dose intravenous bolus interleukin-2 in patients with melanoma or renal cell carcinoma. *Clin Cancer Res.* 2006;12:4619-4627.
23. Sorm F, Piskala A, Cihak A, Vesely J. 5-Azacytidine, a new, highly effective cancerostatic. *Experientia.* 1964;20:202-203.
24. Pliml J, Sorm F. Synthesis of 2-deoxy-D-ribofuranosyl-5-azacytosine. *Collection of Czechoslovak Chemical Communications.* 1964;29:2576-2577.
25. Sorm F, Vesely J. The activity of a new antimetabolite, 5-azacytidine, against lymphoid leukaemia in AK mice. *Neoplasma.* 1964;11:123-130.
26. Sorm F, Vesely J. Effect of 5-aza-2'-deoxycytidine against leukemic and hemopoietic tissues in AKR mice. *Neoplasma.* 1968;15:339-343.
27. Momparler RL. Pharmacology of 5-Aza-2'-deoxycytidine (decitabine). *Semin Hematol.* 2005;42:S9-16.
28. Momparler RL, Rossi M, Bouchard J, et al. Kinetic interaction of 5-AZA-2'-deoxycytidine-5'-monophosphate and its 5'-triphosphate with deoxycytidylate deaminase. *Mol Pharmacol.* 1984;25:436-440.
29. Juttermann R, Li E, Jaenisch R. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc Natl Acad Sci USA.* 1994;91:11797-11801.
30. Taylor SM, Jones PA. Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. *Cell.* 1979;17:771-779.
31. Taylor SM, Constantinides PA, Jones PA. 5-Azacytidine, DNA methylation, and differentiation. *Curr Top Microbiol Immunol.* 1984;108:115-127.
32. Aparicio A, Eads CA, Leong LA, et al. Phase I trial of continuous infusion 5-aza-2'-deoxycytidine. *Cancer Chemother Pharmacol.* 2003;51:231-239.
33. Cashen A, Shah A, Helget A, et al. A phase I pharmacokinetic trial of decitabine administered as a 3-hour infusion to patients with acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS). *ASH Annual Meeting Abstracts.* 2005;106:1854.
34. Rivard GE, Momparler RL, Demers J, et al. Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia. *Leuk Res.* 1981;5:453-462.
35. van Groeningen CJ, Leyva A, O'Brien AM, Gall HE, Pinedo HM. Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients. *Cancer Res.* 1986;46:4831-4836.
36. Chabot GG, Bouchard J, Momparler RL. Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deaza-uridine, thymidine or uracil arabinoside. *Biochem Pharmacol.* 1983;32:1327-1328.
37. Chabot GG, Rivard GE, Momparler RL. Plasma and cerebrospinal fluid pharmacokinetics of 5-Aza-2'-deoxycytidine in rabbits and dogs. *Cancer Res.* 1983;43:592-597.
38. Marcucci G, Silverman L, Eller M, Lintz L, Beach CL. Bioavailability of azacitidine subcutaneous versus intravenous in patients with the myelodysplastic syndromes. *J Clin Pharmacol.* 2005;45:597-602.
39. Israili ZH, Vogler WR, Mingoli ES, et al. The disposition and pharmacokinetics in humans of 5-azacytidine administered intravenously as a bolus or by continuous infusion. *Cancer Res.* 1976;36:1453-1461.
40. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood.* 2002;100:2292-2302.
41. Cheson BD, Bennett JM, Kantarjian H, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood.* 2000;96:3671-3674.
42. Silverman LR, McKenzie DR, Peterson BL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. *J Clin Oncol.* 2006;24:3895-3903.
43. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol.* 2002;20:2429-2440.
44. Silverman LR, McKenzie DR, Peterson BL, et al. Response rates using International Working Group (IWG) criteria in patients with myelodysplastic syndromes (MDS) treated with azacitidine. *ASH Annual Meeting Abstracts.* 2005;106:2526.
45. Kornblith AB, Herndon JE, Silverman LR, et al. Impact of azacitidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2002;20:2441-2452.
46. Silverman LR, McKenzie DR, Peterson BL, et al. Analysis of survival, AML transformation, and transfusion independence in patients with high-risk myelodysplastic syndromes (MDS) receiving azacitidine determined using a prognostic model. *ASH Annual Meeting Abstracts.* 2005;106:2523.
47. Silverman LR, McKenzie DR, Peterson BL, et al. Azacitidine prolongs survival and time to AML transformation in high-risk myelodysplastic syndrome (MDS) patients >= 65 years of age. *ASH Annual Meeting Abstracts.* 2005;106:2524.
48. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol.* 2003;21:4642-4649.
49. Sudan N, Rossetti JM, Shaddock RK, et al. Treatment of acute myelogenous leukemia with outpatient azacitidine. *Cancer.* 2006;107:1839-1843.
50. Fabre C, Chermat F, Legros L, et al. Treatment of high risk MDS and AML post-MDS with azacytidine (AZA): preliminary results of the French ATU program. *ASH Annual Meeting Abstracts.* 2006;108:2664.
51. Lyons RM, Cosgriff T, Modi S, et al. Hematologic improvement, transfusion independence, and safety assessed using three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. *ASH Annual Meeting Abstracts.* 2006;108:2662.
52. Rossetti JM, Falke E, Shaddock RK, et al. G-CSF Increases hematological response among patients with myelodysplasia treated with azacitidine. *ASH Annual Meeting Abstracts.* 2006;108:4868.
53. Momparler RL, Bouffard DY, Momparler LF, et al. Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer. *Anti-cancer Drugs.* 1997;8:358-368.
54. Schwartzmann G, Schunemann H, Gorini CN, et al. A phase I trial of cisplatin plus decitabine, a new DNA-hypomethylating agent, in patients with advanced solid tumors and a follow-up early phase II evaluation in patients with inoperable non-small cell lung cancer. *Invest New Drugs.* 2000;18:83-91.
55. Zagonel V, Lo RG, Marotta G, et al. 5-Aza-2'-deoxycytidine (Decitabine) induces trilineage response in unfavourable myelodysplastic syndromes. *Leukemia.* 1993;7(suppl 1):30-35.
56. Issa JP, Garcia-Manero G, Giles FJ, et al. Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood.* 2004;103:1635-1640.
57. Wijermans PW, Krulder JW, Huijgens PC, Neve P. Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome. *Leukemia.* 1997;11(suppl 1):S19-S23.
58. Wijermans P, Lubbert M, Verhoef G, et al. Low-dose 5-aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. *J Clin Oncol.* 2000;18:956-962.

59. Lubbert M, Wijermans P, Kunzmann R, et al. Cytogenetic responses in high-risk myelodysplastic syndrome following low-dose treatment with the DNA methylation inhibitor 5-aza-2'-deoxycytidine. *Br J Haematol.* 2001;114:349-357.

60. van den BJ, Lubbert M, Verhoef G, Wijermans PW. The effects of 5-aza-2'-deoxycytidine (Decitabine) on the platelet count in patients with intermediate and high-risk myelodysplastic syndromes. *Leuk Res.* 2004;28:785-790.

61. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer.* 2006;106:1794-1803.

62. Kantarjian H, Oki Y, Garcia-Manero G, et al. Results of a randomized study of three schedules of low-dose decitabine in higher risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood.* 2007;109:52-57.

63. Ruter B, Wijermans PW, Lubbert M. Superiority of prolonged low-dose azanucleoside administration? Results of 5-aza-2'-deoxycytidine retreatment in high-risk myelodysplasia patients. *Cancer.* 2006;106:1744-1750.

64. Garcia-Manero G, Issa JP. Histone deacetylase inhibitors: a review of their clinical status as antineoplastic agents. *Cancer Invest.* 2005;23:635-642.

65. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst.* 2000;92:1210-1216.

66. Gore SD, Weng LJ, Figg WD, et al. Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. *Clin Cancer Res.* 2002;8:963-970.

67. Kuendgen A, Strupp C, Aivado M, et al. Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. *Blood.* 2004;104:1266-1269.

68. Pilatino C, Cilloni D, Messa E, et al. Increase in platelet count in older, poor-risk patients with acute myeloid leukemia or myelodysplastic syndrome treated with valproic acid and all-trans retinoic acid. *Cancer.* 2005;104:101-109.

69. Kuendgen A, Schmid M, Knipp S, et al. Valproic acid (VPA) achieves high response rates in patients with low-risk myelodysplastic syndromes. *ASH Annual Meeting Abstracts.* 2005;106:789.

70. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet.* 1999;21:103-107.

71. Yang H, Hoshino K, Sanchez-Gonzalez B, Kantarjian H, Garcia-Manero G. Antileukemia activity of the combination of 5-aza-2'-deoxycytidine with valproic acid. *Leuk Res.* 2005;29:739-748.

72. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. *Blood.* 2006;108:3271-3279.

73. Soriano AO, Yang H, Tong W, et al. Significant clinical activity of the combination of 5-azacytidine, valproic acid and all-trans retinoic (ATRA) acid in leukemia: results of a phase I/II study. *ASH Annual Meeting Abstracts.* 2006;108:160.

74. Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res.* 2006;66:6361-6369.

75. Garcia-Manero G, Yang AS, Giles F, et al. Phase I/II study of the oral iso-type-selective histone deacetylase (HDAC) inhibitor MGCD0103 in combination with azacitidine in patients (pts) with high-risk myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). *ASH Annual Meeting Abstracts.* 2006;108:1954.

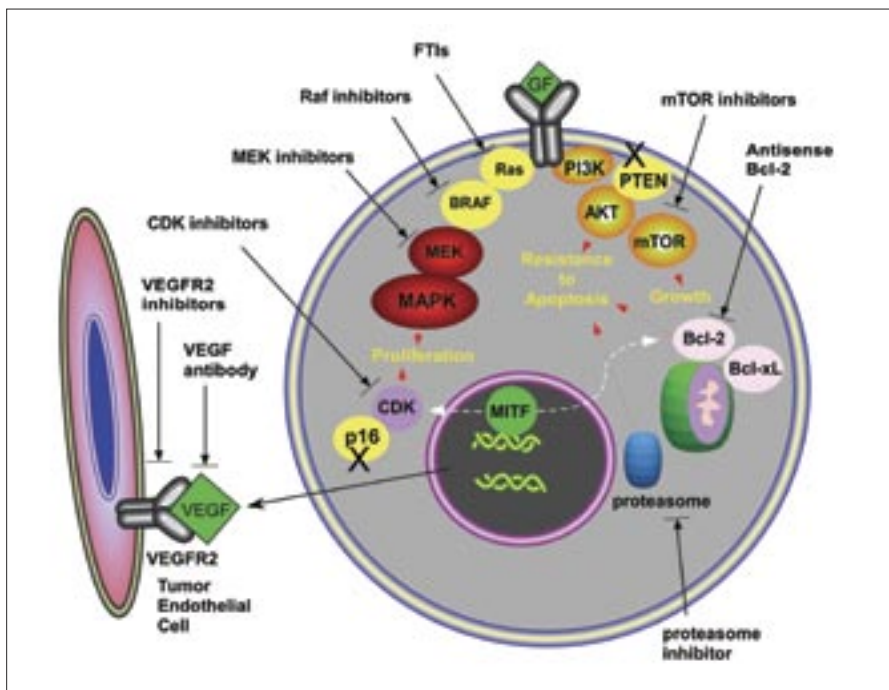
76. Garcia-Manero G, Yang H, Sanchez-Gonzalez B, et al. Final results of a phase I study of the histone deacetylase inhibitor vorinostat (suberoyanilide hydroxamic acid, SAHA), in patients with leukemia and myelodysplastic syndrome. *ASH Annual Meeting Abstracts.* 2005;106:2801.

77. Gore SD, Jiemjit A, Silverman LB, et al. Combined methyltransferase/histone deacetylase inhibition with 5-azacitidine and MS-275 in patients with MDS, CMMoL and AML: clinical response, histone acetylation and DNA damage. *ASH Annual Meeting Abstracts.* 2006;108:517.

78. Giles FJ, Fischer T, Cortes J, et al. A phase I/II study of intravenous LBH589, a novel histone deacetylase (HDAC) inhibitor, in patients (pts) with advanced hematologic malignancies. *ASH Annual Meeting Abstracts.* 2004;104:1802.

**Erratum**

Due to an editing error, mistakes appeared in Figure 1 in "Targeted Therapy for Metastatic Melanoma" by Drs. Ravi K. Amaravadi and Keith T. Flaherty (*Clin Adv Hematol Oncol.* 2007;5:386-394). The corrected figure appears below. *Clinical Advances in Hematology & Oncology* regrets the error.



**Figure 1.** Targeted therapy currently being evaluated for melanoma. A melanoma cell and tumor endothelial cell are shown. Yellow indicates targets with known recurring somatic or germline mutations in patients with melanoma; X: inactivating mutations or deletions have been described.

GF=growth factor, FTIs=farnesyl transferase inhibitors.