Published in final edited form as:

Curr Opin Oncol. 2008 November ; 20(6): . doi:10.1097/CCO.0b013e328313699c.

DEMETHYLATING AGENTS IN MYELOID MALIGNANCIES

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Abstract

Purpose of Review—Two demethylating agents are approved in myelodysplastic syndromes (MDS): 5-azacitidine and 5-aza-2'-deoxycytidine (decitabine). These drugs are structurally related and induce DNA hypomethylation. Aberrant DNA methylation is associated with gene silencing. It is proposed that hypomethylating agents work by inducing re-expression of epigenetically silenced genes. Here, we provide an up-to-date summary of the clinical experience with these drugs.

Recent Findings—5-azacitidine and decitabine were approved in the US based on clinical responses but no effect on survival was documented. Recent results from a phase III study have indicated that treatment of patients with higher-risk MDS with 5-azacitidine results in significant improvement in overall survival. Results of a randomized survival study of decitabine should be available in 2007. Reports of combination epigenetic therapies (a hypomethylating agent with a histone deacetylase inhibitor) indicate that these have significant activity in patients with MDS/AML. Randomized studies are testing the concept that the combinations are superior to single agent therapy.

Summary—Demethylating agents are the standard of care for patients with higher-risk MDS and the only agents known to improve the natural history of MDS. Further work in new combination therapies may result in further advances in the care of patients with MDS.

Keywords

DNA methylation; leukemia; epigenetics

Introduction

The term demethylating agents refers to a group of chemotherapeutic agents with the capacity, both in vitro and in vivo, to induce transient DNA hypomethylation. DNA methylation refers to the addition of a methyl group to a CpG site¹. These sites cluster together in areas known as CpG islands and are frequently localized in the proximity of key gene regulatory regions such as gene promoters. DNA methylation, both aberrant and physiologic, of these areas can result in gene silencing and in the equivalent of the physical inactivation, due to either mutations or deletions, of tumor suppressor genes^{2*}. At the present time, two hypomethylating agents are approved in the U.S. and are widely used in Europe and the rest of the world: 5-azacitidine³ and 5-aza-2'-deoxycytidine⁴ (decitabine). These two agents have significant activity in patients with higher risk myelodysplastic

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syndromes (MDS). Recently, 5-azacitidine has been reported to improve survival in patients with higher risk MDS in a randomized phase III study^{5**}. In this manuscript, we will provide an up to date review of the results with these two agents in MDS and other diseases.

Single agent decitabine

Decitabine is a nucleoside analogue that inhibits DNA methyltransferase (DNMT) activity resulting in global and gene specific DNA hypomethylation⁶. The clinical development of this agent goes back several decades⁷. Initially, decitabine was used in several small clinical trials in AML and higher risk MDS and eventually chronic myelogenous leukemia (CML). At that time, the development of decitabine focused on its use at the highest possible dose, in a way similar to the use of cytarabine (ara-C), a drug to which is structurally related. It should be noted that ara-C has no hypomethylating activity. Initial clinical trials with decitabine did not show any significant superiority compared to ara-C and the development of decitabine was halted until several groups reported the importance of aberrant DNA methylation as a critical event in cancer, and that this process could be reversed by hypomethylating agents, particularly when using these drugs at lower concentrations⁸. This led to the reemergence of an interest in performing clinical trials with these compounds in MDS and AML⁹. The initial studies with decitabine were conducted by the EORTC in Europe. The schedule studied in those trials was a dose of decitabine of 15 mg/m² IV infused over four hours three times a day daily for 3 days with courses repeated every 6 weeks. The three times a day schedule was an effort to provide the closest to a continuous infusion. Continuous infusions with decitabine and 5-azacitidine are impractical due to the fact that the drugs decay in solution. The 6 weeks schedule was implemented due to a concern of potential prolonged myelosuppression with more frequent administration rates. Results of initial trials with this schedule of decitabine included an overall response rate (ORR) of 48% (Table 1). Based on this data, this schedule (that we are going to refer from here on as the “3-day schedule”) was studied in the U.S. in a randomized phase III trial that led to the approval of decitabine¹⁰. Results are also summarized in table 1. Decitabine administration resulted in more clinical responses compared to supportive care (30% versus 7%). 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 months vs. 7.8 months; $p=0.16$). Patients that were treatment naïve (12.3 months vs. 7.3 months; $p=.08$), or had an IPSS score of intermediate-2/high risk (12.0 months vs. 6.8 months; $p=.03$), or had de novo MDS (12.6 months vs. 9.4 months; $p=.04$) had a trend towards improved survival. No differences in toxicity could be observed between the decitabine arm and the control group.

Although the results of these initial studies are of great relevance, it was clear that alternative schedules that did not require hospital admission with more activity were needed. Because lower concentrations of decitabine are more efficient as hypomethylating agents (JP Issa, personal communication), a phase I study of decitabine was conducted studying a daily IV schedule¹². Dose escalation was performed not only on the daily dose but also on the number of days of drug administration. This study was open to both patients with acute and chronic leukemias. Doses of decitabine ranged from 5 mg/m² daily for 10 days to 15 mg/m² daily for 20 days. In this study, decitabine was infused IV over 1 hour. The ORR was 32% and the maximal tolerated dose was not defined. Of importance, an intermediate dose schedule (15 mg/m² daily $\times$ 10) was associated with the highest response rate (5 out of 6 patients treated at that dose, 83%). Indeed, clinical activity decreased at doses above and below this dose level. Because of these results, a second cohort of 11 patients was treated at the 15 mg/m² daily $\times$ 10 dose/schedule with an ORR of 55%. This confirmed the activity of this intermediate dose level of decitabine. Because of the clinical activity of this daily schedule of decitabine, a randomized phase II study of three different schedules of daily

decitabine was conducted^{11**}. The total dose of decitabine in each arm was 100 mg/m². The randomization favored the 20 mg/m² daily × 5 schedule as the most active. With this schedule of decitabine drug-related mortality was approx. 5%. Main toxicities were myelosuppression and its associated complications that resulted in admission to the hospital in 66 % of patients. The median overall survival (OS) was 19 months. Median number of courses administered was 6+ (1–18+). To assess the effect of these newer schedule of decitabine (5-day schedule herein), an analysis was performed comparing the survival of patients with MDS treated with decitabine in this study with that of a contemporary cohort of patients treated with an ara-C containing regimen at MDACC¹³. This study indicated that treatment with decitabine was associated with improved survival compared to prior experience with ara-C based programs. This was despite the fact the complete remission rates were lower with decitabine as compared with intensive chemotherapy programs. This is probably due in part to the very low induction mortality rate observed with decitabine¹³. This data represented a change in practice as we may accept a lower response rate if induction mortality is also significantly lower and translates into better survival. The results of this trial confirmed the activity of decitabine in MDS and indicated that the 5-day schedule is currently the optimal way to administer this agent. An update of the 5-day schedule from MDACC was presented at ASH 2007¹⁴. Overall 36 pts (39%) pts achieved a CR, and 75 (81%) had a response by IWG criteria. The median number of cycles administered was 8+. The median time to response was 2.3 months, and the median duration of CR was 14 months (range 3 to 16+ months). To confirm these results, a multicenter single arm phase II study was conducted in North America using the 5-day schedule. The ORR reported was 43% with a median of 5 courses of therapy administered¹⁵. Final results of this study are to be published but the data presented demonstrated the activity and safety of the 5-day schedule. Other important data not currently available are the results of a “survival study” conducted in Europe comparing decitabine versus best supportive care. The results of this study are expected during 2007.

Single-agent 5-azacitidine

5-azacitidine is another nucleoside analogue structurally related to decitabine¹⁶. In contrast to decitabine, 5-azacitidine is a ribose structure that is incorporated into RNA and requires the activity of ribonucleotide reductase (RNR) to be incorporated into DNA and to exert its hypomethylating effect. It should be noted that RNR is the target of hydroxiurea, and therefore the concomitant use of hydroxiurea and 5-azacitidine should be avoided¹⁷. Initial studies with 5-azacitidine were performed by the CALGB and demonstrated that with either an IV or subcutaneous (SC) route of administration for 7 days, 5-azacitidine had activity in MDS and AML^{18*}. Based on the data from a SC study, a randomized phase III cross over study was conducted by the CALGB comparing 5-azacitidine (75 mg/m² SC daily × 7) versus supportive care³ in patients with MDS. Results are shown in Table 2. In summary, the study demonstrated an ORR of 60% with 5-azacitidine compared to 5% with supportive care. Patients in the cross over arm (those on the supportive care arm that then went to receive 5-azacitidine) had an ORR of 47%, thus confirming the results with 5-azacitidine. Median OS on the 5-azacitidine was 20 months compared to 14 for the supportive care arm. This was not significant due to the cross over design of the study. This study (CALGB 9221) lead to the approval of 5-azacitidine in the US for patients with MDS³. Because this study failed to demonstrate a survival effect⁵, a second randomized study was performed to study the effect of 5-azacitidine on survival. The results of this study were reported at the ASH 2007 meeting^{5**}. In that study, 358 patients with higher risk MDS (int-2 and high risk by IPSS) received either 5-azacitidine or “conventional care”. Conventional care could include supportive care, low dose ara-C or a traditional “7+3” induction program. Response rates with 5-azacitidine are shown in table 2. Importantly, a significant effect was observed on survival in patients that received 5-azacitidine versus the other interventions (HR 0.58; 95%

CI 0.43,0.77). This study still needs to be presented in manuscript form, but one of the key issues was the fact that patients with alterations of chromosome 7 were one of the groups that derived the most benefit from therapy. This data demonstrates the capacity of 5-azacitidine to alter the natural history of patients with MDS. This is demonstrated by the capacity of 5-azacitidine to potentially double the survival at 2 years in patients with higher risk disease (24.4 months on 5-azacitidine versus 15 months on conventional care) and establishes the role of 5-azacitidine as standard of care in this patient population.

Combination strategies

Traditionally, achievement of complete remission has been a prerequisite for improved survival. From that perspective, the use of hypomethylating agents has been associated with a relative low rate of CR rates (tables 1 and 2). Several groups have attempted to increase the response rate with hypomethylating based therapy by developing combinations. Because epigenetic manipulation with hypomethylating agents can lead to reactivation of aberrantly silenced genes, and therefore targets, multiple rational sequential combinations are possible. From a mechanistic perspective, the most widely study combination is that of a hypomethylating agent and a histone deacetylase inhibitor^{19,20}. Initial studies with trichostatin, and phenylbutyrate lead to combinations with valproic acid, once this drug was discovered to have HDAC inhibitory activity^{21,22}. Valproic acid is a fatty acid chain derivative traditionally used to treated epilepsy and other neurological disorders²³. Studies performed in Germany indicated that single agent valproic acid has activity in lower risk MDS²⁴. Based on these at least three studies have been reported with the combination of 5-azacitidine or decitabine with valproic acid^{25–27} (Table 3). These studies with valproic acid have been mainly phase I early phase II trials in patients with AML and higher risk MDS. Reported ORR (CR/CRps) ranged from 20% to 50%. In patients with previously untreated disease, the response rate was approximately 50%. Of importance, time to response has been consistently one course (ranging from 1 to 3 courses of therapy) and appears to be faster than the 4 to 6 courses required with single agent 5-azacitidine for initial response (add 2 more courses for best response), at least with 5-azacitidine²⁸. Induction mortality has also been very low, generally less than 5%. Main toxicity has been neurological related to valproic acid use. An important issue has been the optimal dose/schedule of valproic acid. In vitro studies had indicated that doses of at least 1 mM of valproic acid are required for induction of histone acetylation and that induction of cell death is proportional to the concentration of the drug used²⁹. Indeed, in two studies of valproic acid at MDACC^{25,26*}, patients that responded to the combination of valproic acid with either decitabine or 5-azacitidine tended to have the higher valproic acid levels in blood, recapitulating the in vitro data and arguing for the use of short high intensity courses of valproic acid, instead of prolonged lower dose schedule proposed by other investigators²⁴. This is also supported by the fact that toxicity with valproic acid is transient and that there is no good correlation between dose and levels, and blood levels and toxicity^{26*}.

This phenomenon of dose response with valproic acid observed in the studies described above supports the notion that replacing valproic acid by more potent HDAC inhibitors²⁰ may result in very active clinical combinations. Such studies are ongoing with both 5-azacitidine and decitabine with several HDAC inhibitors. One such study is the combination of 5-azacitidine and MGCD0103³⁰. MGCD0103 is a class 1 specific HDAC inhibitor with activity in AML and potentially MDS³¹. For the combination study, patients with relapse/refractory or de novo MDS/AML were eligible. A dose of MGCD0103 of 90 mg oral three times a week was found to be well tolerated in combination with 5-azacitidine. Response rates were approx. 50% in the group of patients that had not received prior therapy. Median time to response was also 1 cycle (range 1 to 3). Importantly, median OS has not been reached in patients that responded to therapy. This study serves as the bases for an ongoing

randomized study comparing 5-azacitidine with or without MGCD0103. This type of study is crucial to demonstrate the concept that the combination is better than single agent. Several other studies (decitabine +/-valproic acid and 5-azacitidine +/-MS-275) are ongoing.

Other combinations include 5-azacitidine with lenalidomide³² or TNF- α modulation³³ and the combination of decitabine or 5-azacitidine with mylotarg³⁴. Although these are small early pilot studies and the data limited, these experiences are of significant interest. In particular, the combination with mylotarg is important in view of the early results with this combination (a CR rate of 75% in 13 patients with elderly AML) as well as the fact that mylotarg has activity as a single agent in AML. Sequence seems to be important for the mylotarg and it should be administered at the end of the administration of the hypomethylating agent. Other combinations include the use of priming with growth factor support and with the use of thrombomimetic agents.

Other indications

Hypomethylating agents may have activity in solid tumors malignancies, lymphomas and other leukemias. For instance, decitabine has activity in chronic myelogenous leukemia in all phases of the disease, either alone or in combination with imatinib³⁵. These studies were prior to the development of second generation tyrosine kinase inhibitors, such as nilotinib or dasatinib, but demonstrated that this type of epigenetic manipulation may have a role in patients with advanced phase CML. Hypomethylating agents are also being tested in chronic lymphocytic leukemia and acute lymphocytic leukemia. It is too early to say whether this will have activity in this setting. In view of the activity of hypomethylating agents in MDS, it will be expected that they will also be active in AML, especially in older patients with poor risk characteristics, such as poor cytogenetics, who are not usually candidates for intensive chemotherapy. Although there is data in this setting with both 5-azacitidine and decitabine, two studies with decitabine (one by the EORTC using the 3-day schedule) and the other randomizing against low-dose ara-C are ongoing and could result in a new indication for decitabine. Another approach is the use of hypomethylating agents as maintenance type of strategy in patients in remission after more intense type of therapy. Data from the Nordic group was presented at ASH 2007. In a group of patients with higher risk MDS or AML that received "7+3" type induction therapy followed by maintenance with 5-azacitidine the OS was 17 months in patients that received 5-azacitidine maintenance therapy³⁶. Other studies with both decitabine and 5-azacitidine are exploring this issue that may have significant implications for patients with AML.

Conclusion

The development of the hypomethylating agents have resulted in the first group of therapies to provide a clear and consistent benefit to patients with MDS. It is possible that this could also translate into a benefit in AML, other leukemias and potential solid tumors and lymphomas. Although the data with 5-azacitidine on survival^{5**} is probably one of the most important in MDS, it is obvious that we are far from the results obtained with the tyrosine kinase inhibitors in CML. It is expected that more active compounds with more clinical activity will translate into greater survival benefits. Therefore the development of new more potent hypomethylating agents is of importance. Oral formulations³⁷ are under development but their pharmacokinetic characteristics need to be fully understood. Perhaps the development of new more potent histone deacetylase inhibitors and their combinations could improve on the therapeutic activity of these forms of epigenetic therapy.

Acknowledgments

G G-M is supported by a Physician-Scientist Program from MD Anderson Cancer Center, the Leukemia and Lymphoma Society of America and also from NIH grant 5PO1 CA108631.

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Table 1

Results with single agent 5-aza-2'-deoxycytidine

<i>Study</i>	<i>Initial 3-day schedule⁹</i>	<i>Randomized 3-day¹⁰</i>	<i>5 day schedule^{11**}</i>
Phase	II	III	II
N	66	89	95
CR	13 (20)	8 (9)	32 (34)
PR	3 (4)	7 (8)	1 (1)
HI	16 (24)	12 (13)	13 (13)
OR	32 (48)	27 (30)	46 (48)

Table 2

Results with single agent 5-azacitidine

<i>Study</i>	<i>CALGB 9221³</i>	<i>Updated CALGB 9221^{18*}</i>	<i>Survival study^{5**}</i>
Phase	III	III	III
N	99	99	179
CR	7 (7)	10 (10)	30 (17)
PR	16 (16)	1 (1)	21 (12)
HI	37 (37)	36 (36)	87 (49)
OR	60 (60)	47 (47)	138 (78)

Table 3

Results of combination studies with valproic acid

<i>Study</i>	<i>Decitabine and valproic acid²⁵</i>	<i>Decitabine and valproic acid in untreated patients²⁵</i>	<i>5-azacitidine and valproic acid^{26*}</i>	<i>5-azacitidine and valproic acid in untreated patients^{26*}</i>
Phase	I/II	III	I/II	I/II
N	53	99	53	33
CR	10 (19)	10 (10)	12 (22)	11 (33)
PR	2 (4)	1 (1)	3 (6)	3 (9)
HI	NA	36 (36)	7 (13)	3 (9)
OR	12 (23)	47 (47)	22 (41)	17 (51)