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Epigenetic Therapies in MDS and AML

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Abstract

The use of low dose hypomethylating agents for patients with myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (AML) has had made a significant impact. In the past, therapies for these diseases were limited and patients who elected to receive treatment were subject to highly toxic, inpatient chemotherapeutics, which were often ineffective. In the era of hypomethylating agents (azacitidine and decitabine), a patient with high grade MDS or AML with multilineage dysplasia can be offered the alternative of outpatient, relatively low-toxicity therapy. Despite the fact that CR (CR) rates to such agents remain relatively low at 15–20%, a much larger percentage of patients will have clinically significant improvements in hemoglobin, platelet, and neutrophil counts while maintaining good outpatient quality of life. As our clinical experience with azanucleotides expands, questions regarding patient selection, optimal dosing strategy, latency to best response and optimal duration of therapy following disease progression remain, but there is no question that for some patients these agents offer, for a time, an almost miraculous clinical benefit. Ongoing clinical trials in combination and in sequence with conventional therapeutics, with other epigenetically active agents, or in conjunction with bone marrow transplantation continue to provide promise for optimization of these agents for patients with myeloid disease. Although the mechanism(s) responsible for the proven efficacy of these agents remain a matter of some controversy, activity is thought to stem from induction of DNA hypomethylation, direct DNA damage, or possibly even immune modulation; there is no question that they have become a permanent part of the armamentarium against myeloid neoplasms.

13.1 Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of malignant myeloid disorders characterized by peripheral blood cytopenias in association with bone marrow hypercellularity and dysplasia [1]. Patients with high grade MDS (int-2 or high by IPSS criteria, Fig. 13.1) have a high rate of transformation to acute myeloid leukemia (AML) and poor long-term survival with a life expectancy in the absence of treatment between 0.4 and 1.8 years [2]. The International Prognostic Scoring System (IPSS) was developed as a tool for stratifying patient outcomes based upon readily available clinical characteristics. Figure

13.1 details the components necessary for the generation of an IPSS score and the expected survival for each designation [2]. “Secondary” AMLs such as those arising in patients with an antecedent MDS diagnosis are generally resistant to traditional chemotherapeutics and the overall survival (OS) in this group of patients is universally poor [3–5]. Both MDS and AML are diseases of the elderly with a majority of patients diagnosed when they are older than 60 years [5]. Although a small minority of patients with MDS will present with mild cytopenias and low grade disease, a majority do not [2]. Patients with MDS associated with multilineage cytopenias (anemia, thrombocytopenia, and neutropenia), high bone marrow blast percentages, or characteristic adverse chromosomal features often progress rapidly to AML and in the absence of bone marrow transplantation, ultimately die of their disease [2].

For these patients, and for a large number of older people who present with putatively de novo myeloid leukemias, but with unrecognized low grade cytopenias and bone marrow dysplasia, conventional induction chemotherapeutics (IC, with daunorubicin and cytarabine) have been in large measure disappointing [6]. Furthermore many such patients are unfit for intensive treatment and are offered instead low dose cytarabine, clinical trials or supportive care [7]. In this group the OS rates at 2 and 5 years remain only 10% and 2% respectively [3, 4]. Patients who are fit to receive traditional IC require long periods of time (often 4–6 weeks) in the hospital, and this treatment offers a complete remission rate of only 20–30%, with median survivals ranging between 5 and 13 months [6, 8, 9]. In addition to induction failure and early relapse, even in those who achieve remission, prolonged hospitalization can have the side effect of physical deconditioning and the 3 or more weeks of neutropenia resulting from this treatment can result in resistant bacterial and fungal infections [6]. These burdens create patients who are unable to return to good quality of life and who become ineligible for salvage therapy or clinical trials upon relapse due to poor performance status, organ dysfunction or infection. Even in those who retain an excellent performance status following induction, primary refractory AML remains a significant quality of life problem, requiring frequent blood transfusions, extensive prophylactic antibiotic regimens, and regular hospital visits [9].

Until recently, toxic traditional IC was the only real option for fit patients with high grade MDS or AML with MDS related changes [1]. Recently however, the epigenetically active drugs azacitidine (Aza, Vidaza, Celgene, Concord OH) and decitabine (Dac, Dacogen, Esai Inc., Mars, PA) have been approved both in the United States and Europe for the treatment of MDS and low blast count (<30%) AML [7, 10]. These drugs, both of which are incorporated into DNA resulting in the depletion of the intracellular methyltransferases (DNMTs) when given at low dose, were the first epigenetically active therapy to be approved for cancer. They have resulted in a significant change in the approach to patients with MDS and required the development of the International Working Group (IWG) response criteria in MDS in order to measure meaningful improvements in cytopenias that did not fit into the traditional response assessment which designated only complete (CR) or partial (PR) responses as meaningful [11, 12]. A summary of the IWG response criteria in MDS are provided in Table 13.1. In particular, Aza has been shown to improve OS, delay the transformation to AML in high-grade MDS patients, and produce significant responses in patients with low blast count AML [7]. Although a statistically significant survival benefit has not been demonstrated following treatment with Dac, this drug has been shown to

produce both CRs and hematological improvements in both MDS and AML patients who receive it [10, 13]. Taken together these drugs offer an effective alternative to induction chemotherapy and have become the standard of care for patients with MDS as well as selected patients with AML.

As with conventional chemotherapeutic strategies for these patients, responses are usually limited to a year or two, but therapy is largely outpatient, with minimal end organ toxicity and few side effects [14]. Despite notable limitations, these drugs have made a significant impact upon quality of life for a large number of patients with high grade MDS and AML. Ongoing work to understand the mechanism responsible for the efficacy of these drugs and the ultimate loss of response observed clinically is ongoing. Furthermore, the development of novel dosing strategies, combinations, and the appropriate use of allogeneic transplantation provide hope for improving response duration and possibly even providing an opportunity for long-term remission to these unfortunate patients.

13.2 Single Agent “Hypomethylating” Therapy for MDS and AML

13.2.1 Azacitidine

Aza is a nucleoside analog of cytidine in which the carbon 5 position of the pyrimidine ring has been substituted with nitrogen (Fig. 13.2a) [15]. It is imported into cells by the action of nucleotide transporters, where it is activated by uridine–cytidine kinase and incorporated into RNA (Fig. 13.3) [15]. Sixty to 80% of the Aza dose given is incorporated into RNA and this has impacts upon protein synthesis and RNA metabolism [15]. Twenty to 40% of the dose is converted into the deoxyribonucleoside Dac by the action of ribonucleotide reductase [15]. This deoxyribonucleoside base is then phosphorylated and incorporated into DNA where it acts as a suicide substrate for DNMTs and induces DNA hypomethylation during cellular replication as well as DNA damage due to adduct formation [15]. Aza was first synthesized and tested in 1960s and 1970s [16, 17]. In early clinical trials as a traditional chemotherapeutic, it was demonstrated to be effective in myeloid malignancy, however its efficacy was limited by significant gastrointestinal toxicity and prolonged cytopenias [16–18]. Cytarabine or AraC was developed at about the same time. This drug, another nucleoside analog of cytidine whose activity is thought to result in chain termination, is among the most active drugs used for myeloid malignancy. Ultimately the toxicity of 5-Aza limited its further clinical development, and cytarabine became the nucleoside analog of choice in myeloid malignancy [17, 18].

In 1978, Peter Jones and colleagues demonstrated that treatment of mouse embryo cells in vitro with Aza and its deoxy analog 5-aza-2'-deoxycytidine (Dac) could induce differentiation into functional myotubes [19]. Jones and Taylor went on to show that this differentiation resulted from changes in DNA methylation elicited by treatment with azanucleosides [20, 21]. Further work, by Dr. Jones and others, identified methylation as a common event in many malignancies, including the pre-leukemic condition known as MDS, a disease for which no treatment was available [22]. Although initially used as a laboratory tool to test gene and chromosome specific methylation changes, the identification of methylation as a potentially reversible cancer specific event spurred interest in the

possibility that cancers treated with these drugs might be induced to differentiate and potentially to apoptose and die.

Ultimately in the 1990s, insights into methylation events common to MDS, specifically identification of recurrent methylation of tumor suppressor genes such as p15INK4B, resulted in the development of a number of phase I and II clinical trials of azanucleotides in this disease [23, 24]. Table 13.2 reviews the key published trials with single agent azanucleotides in MDS.

Among the first published trials with Aza for the treatment of MDS delivered the drug at 75 mg/m² as a continuous intravenous infusion for 7 days every 4 weeks [25]. This trial enrolled high grade MDS patients with symptomatic disease characterized by red cell and platelet transfusion dependence and poor life expectancy (refractory anemia with excess blasts (10–20%), or refractory anemia with excess blasts in transformation (20–30%). Forty three patients were evaluable and responses were seen in 21 (49%) of these patients [25]. Five patients (12%) achieved a CR, 11 (25%) achieved a partial response (PR), and 5 “improved” (a response characterized in the study as a 50% reduction in transfusion requirements, or improvement in platelets, hemoglobin or neutrophils) [25]. OS in these high risk patients was 13.3 months and for those achieving CR or PR was 14.7 months, and the chief toxicities were mild to moderate nausea [25]. A number of other clinical trials using this drug were published suggesting that Aza had significant activity in MDS and these results were sufficient to prompt two larger, randomized trials of Aza in MDS [26].

In 2004, the FDA approved Aza for the treatment of MDS based upon results from a single phase III clinical trial (described in detail below) [27]. A second trial demonstrating survival was required by European regulators, and this was published formally in 2009 [7]. These trials established Aza as the standard of care approach to patients with int-2 and high risk MDS by demonstrating a prolongation in the time to progression to AML, decreased transfusion requirements, improvements in neutropenia, and ultimately, improvements in OS.

13.2.1.1 CALGB 9221—The first phase III trial of Aza in patients with MDS was published by investigators from the CALGB [27]. CALGB 9221 enrolled 191 patients of median age 68 with French American British-defined MDS (reference for FAB classification), to receive either supportive care or Aza at a dose of 75 mg/m²/day subcutaneously for 7 of 28 days. Patients were maintained on their randomized arm for 4 months, after which patients who were deemed to have progressed on the supportive care arm could crossover to the Aza arm. Patient characteristics were distributed evenly across both arms with 59% of the patients overall having RAEB or RAEB-T by FAB criteria (46% Int-2 or high by IPSS) [27]. Sixty five percent of the enrolled patients were red blood cell transfusion dependent (69% Aza arm, 61% supportive care arm) [27].

Responses were evaluated in both arms. Among patients randomized to receive supportive care, 5% met criteria for improvement; no patients on this arm achieved a CR or PR. Of the 99 patients randomized to receive Aza, 60% ($n = 60$) achieved a response ($p < 0.0001$) [28]. Responses were classified as CR in 7% ($n = 7$), PR in 16% ($n = 16$), and improvement in

37% ($n = 37$). Of those patients demonstrating “improvement,” 35% had increases in three cell lines inadequate to qualify as a PR, 30% had improvement in two cell lines, and 35% had improvement in only one cell line. Responses did not depend upon MDS sub-classification. Forty nine patients crossed over to receive Aza, of these 47% ($n = 23$) responded and 10% ($n = 5$) achieved a CR [27]. Patients treated with Aza had a median time to progression to AML or death of 21 months vs. 12 months in those patients treated with supportive care alone, and this was statistically significant ($p = 0.007$), median OS in an intention to treat analysis was 20 months in the Aza treated patients vs. 14 months for those randomized to supportive care, although this difference was not statistically significant ($p = 0.10$) [27].

Due to the design of this study, the survival analysis was confounded by the 49 patients who crossed over to receive Aza. In order to eliminate this bias, a landmark analysis at the 6 month date was performed. Three subgroups were identified, the first included patients randomized to supportive care who did not crossover, or who crossed over after the six 6 month time point, the second were patients who were randomized to Aza, and the third were patients who crossed over after 4 months, but before 6 months [27]. This analysis excluded 36 patients who died before the landmark date. The median survival in these three groups was 11 (supportive care only), 14 (early crossover), and 18 (randomized to Aza) months respectively. A statistically significant difference in survival was observed between the Aza treated and supportive care groups ($p = 0.03$), but not between supportive care and early crossovers [27].

Transfusion requirements were tracked in both groups. In the Aza treated group transfusion needs increased during the first cycle, and thereafter declined, whereas in the supportive care arm transfusion requirements remained stable or increased. Of the 99 patients initially randomized to receive Aza, 51% had an improvement in hemoglobin, 45% (29) became RBC transfusion independent, and 6 (9%) had a reduction in transfusion dependence by at least 50%. Improved platelet counts were observed in 47%, and increased white cell counts were seen in 40% of the Aza treated patients [27].

In addition to objective improvements in transfusion requirements, white cell counts, survival and prolonged time to AML transformation, patients treated with Aza on this trial experienced significant improvements in quality of life. These were reported as improvements in fatigue, physical functioning, dyspnea, psychological distress, and positive effect, all of which demonstrated statistical significance when compared to patients treated with supportive care alone with a p value < 0.01 [27]. Similar results were observed in the patients who crossed over to Aza. Toxicities among the Aza treated patients were most frequently related to myelosuppression and were difficult to distinguish from the underlying disease. It was notable that treatment with Aza did not appear to increase the infection or bleeding rates above background, and furthermore only one treatment related death was reported on the study, emphasizing the safety of this therapy, even for older patients [27].

13.2.1.2 AZA-001—Although the data from CALGB 9221 was compelling, this study did not, in the final analysis, demonstrate a difference in OS between the patients randomized to receive Aza and those randomized to supportive care, likely as a result of the crossover trial

design. The AZA-001 study was designed to address the question of whether Aza provided an OS benefit for high grade MDS patients [7]. This cleverly conceived, international, randomized trial definitively demonstrated that Aza 75 mg/ m² given subcutaneously for 7 days of a 28 day schedule prolonged OS when compared with conventional care regimens (CCRs) as selected by the patients physician. The investigators aimed to provide at least six cycles of Aza to those patients randomized to the experimental arm. Conventional care was assigned by the patient's physician prior to randomization depending upon the patient's age, performance status co-morbidities and patient preference. CCR consisted of the three most common treatments for patients with int-2 or high risk MDS: IC including cytarabine 100–200 mg/m²/day × 7 days plus, daunorubicin 45–60 mg/m² × 3 days or idarubicin 9–12 mg/m²/day × 3 days or mitoxantrone 8–12 mg/m²/day, low dose cytarabine (LDAC) at a dose of 20 mg/m² for 14 days every 28 days, or best supportive care (BSC). All patients randomized received CCR as selected by their physician or Aza on trial. A total 358 patients were randomized. In this way a pre-specified subgroup analysis based upon physician assignment was possible and helped to eliminate differences in outcome based upon issues of performance status and patient fitness.

The primary OS endpoint of this study was met after a median follow-up of 21.1 months [7]. At this analysis the OS in the Aza treated patients was 24.5 months vs. 15 months for patients assigned to CCR and this result was found to be statistically significant ($p = 0.0001$). Two year OS also favored Aza, at 51% vs. 25% for CCRs ($p = 0.0001$) [7]. Predefined subgroup analysis was also done in order to compare Aza responses with each of the CCRs selected and within specific cytogenetic and IPSS risk groups. There were significant differences between Aza and BSC with an OS benefit for azacytidine treatment of 9.6 months (HR 0.58, $p = 0.0045$), as well as between Aza and LDAC with an OS benefit of 9.2 months (HR 0.36, $p = 0.0006$) [7]. No statistically significant differences in OS were seen when Aza was compared with IC; OS was prolonged by 9.4 months with a hazard ratio of 0.76, but the p value was not significant at 0.51 [7]. This apparent discrepancy was likely due to the low numbers in this subgroup ($n = 42$); 17 patients in this group were randomized to Aza and 25 to intensive chemotherapy.

No differences in response to Aza were seen across the IPSS risk groups enrolled (although most patients were int-2 or high risk $n = 313$ (87%)), nor within the cyto-genetic risk groups identified by the IPSS (good, intermediate, poor). Patients with del-7 or del(7q), a group recognized to have particularly poor prognosis, had an OS of 13.1 months vs. 4.6 months in the CCR group [7, 29].

Responses on this trial were similar to those observed in CALGB 9221. Overall, 29% of those assigned to Aza achieved either CR (17%) or PR (12%) compared with 12% (8% CR, 4% PR) assigned to CCR ($p = 0.0001$) [7]. Any hematological improvement (HI) was observed in 49% of those treated with Aza vs. 29% of those treated with CCR ($p = 0.0001$) [7]. In addition, for those treated with Aza, major erythroid responses were seen in 40% of patients, major platelet responses in 33% and major neutrophil responses in 19%. By contrast, for those receiving CCR major erythroid responses were seen in 11% ($p < 0.0001$), major platelet responses were seen in 14% ($p < 0.0003$) and major neutrophil responses were seen in 18% ($p = 0.58$, not statistically significant) [7]. Patients treated with Aza experienced

a statistically significant reduction in the need for intravenous antibiotics (33% relative risk reduction vs. CCR; RR 0.66 95% CI:0.49–0.87 $p = 0.0032$). Furthermore of the 111 patients with red cell transfusion dependence at the time of study enrollment, 50 (45%) became transfusion independent vs. 13 (11.4%) of the 114 patients randomized to receive CCR (p value significant at 0.0032) [7].

Secondary endpoints in this trial included time to AML transformation and hematological response according to the IWG 2000 criteria for MDS [11]. Treatment with Aza in the entire group was associated with delayed leukemic transformation; the median time to transformation was 17.8 months in the Aza treated group vs. 11.5 months in the CCR group ($p < 0.0001$) [7].

Among the most notable findings on this trial was that achievement of CR or PR was not necessary in order to achieve an improvement in OS; any patient who achieved a hematological response showed a survival benefit.

13.2.1.3 AZA in AML—Changes in the diagnostic criteria for AML based upon the WHO guidelines published in 2008 resulted in the reclassification of patients enrolled on both the CALGB and AZA-001 from the previous FAB classification of Refractory Anemia with Excess Blasts in Transformation (RAEB-T; 20–30% bone marrow blasts) to a new diagnosis of AML [1, 30, 31]. The WHO now defines any patients with 20% blasts as having AML [30].

A pooled analysis of previously published CALGB studies including 9221, 8921, and 8421, in which enrolled patients treated with Aza would now be re-assigned as AML was published in 2006 [28]. This reported the response to Aza given either intravenously or subcutaneously at a dose of 75 mg/m²/day for 7 days of a 28 day cycle in 103 patients who would now be classified as having AML, 91 of whom received Aza [28]. Of these patients 33 (36%) developed a response (8 CRs, 2 PRs, 23 HIs), with a median duration of response of 7.3 months (range 2.2–25.9 months) [28]. Formal comparison with supportive care alone across the three studies was not possible, but 27 patients enrolled in 9221 were randomized to upfront Aza and a further 13 crossed over to receive Aza before the 6 month analysis. Of these, 7% in the Aza group achieved CR or PR compared with 0% in the observation-only group [28]. Median survival time for the 27 patients assigned upfront to Aza was 19.3 months compared with 12.9 months for the 25 AML patients randomly assigned to observation. Of 13 patients with WHO AML at the time of study entry who crossed over to receive Aza, one achieved a PR, and one HI.

Of the 358 patients originally enrolled on AZA-001, a third would now be identified as having AML. A second analysis of these patients was undertaken in order to assess outcome in this group of older adults treated with either Aza or CCR [7, 32]. Of the 113 patients now designated as AML, 63 were assigned to BSC, 34 to LDAC and 16 to IC [32]. The median age in all groups was 70 years with a range of 58–80. Patients were evenly distributed with respect to age, cytogenetic risk group, and ECOG scores. Bone marrow blast percentages were similar in both groups at 23% with a range of 20–34%. In all, 55 patients were randomized to the Aza arm and 53 to CCR. After a median follow-up of 20.1 months, OS

was significantly ($p=0.005$) longer in those patients treated with Aza (24.5 months) than in those receiving CCR (16 months). The 2 year survival was also superior in the Aza group at 50% compared with 16% in the CCR group ($p=0.001$) [32]. Adverse events in this group of patients were primarily grade 3 and 4 cytopenias, which remain difficult to distinguish from the underlying disease. Four patients in the Aza group and three patients in the CCR group discontinued treatment as a result of adverse events.

Several prospective studies of Aza given on the conventional schedule of 75 mg/ m²/day for 7 days in patients identified as AML at diagnosis have been reported. One such study enrolled 82 patients with AML (27 (33%) with secondary disease) and a median age of 72 years (range, 29–87 years) [33]. Thirty-five patients (43%) received Aza as their first treatment, and 47 patients (57%) had previously received 1 or more lines of chemotherapy. The overall response rate in this group was 32% (26/82 patients) with 16 patients (20%) achieving a CR or a CR with incomplete count recovery, and 10 patients (12%) achieving a PR [33]. Untreated patients responded more often than those previously treated with 31% of untreated patients achieving either a CR or a CR with incomplete count recovery compared with only 9 (19%) such responses in the previously treated group ($p = 0.006$). The response duration in untreated patients who achieved a response was 13 months with 1 and 2 year survivals of 58 and 24% respectively [33]. Another study from Germany evaluated medically unfit ($n = 20$) or relapsed/refractory ($n = 20$) patients with AML and a median bone marrow blasts count of 42% [34]. This study showed similar statistically significant differences in response between untreated patients, who demonstrated overall responses (CR + PR + HI) of 50%, and patients with relapsed or refractory disease, who had an overall response rate of only 10% ($p=0.008$) [34]. These response rates are striking and compare favorably with responses seen with induction therapy although additional data are necessary in order to determine whether Aza or Dac will end up the therapeutic agent of choice in this context [6, 35].

Results from the CALGB trials were sufficient in the United States and the AZA-001 trial satisfied the European regulators for the approval of Aza as standard therapy for patients with MDS and low blast count AML. In the United States, approval was granted for all IPSS defined MDS subtypes, while in Europe approval is confined to patients with Int-2 and high risk IPSS scores not eligible for bone marrow transplantation, those with CMML-2 and those with WHO defined AML with 20–30% blasts or multilineage dysplasia.

Both large phase III trials demonstrated this drugs activity in MDS and AML, and further showed that unlike previous therapies, DNMTi require prolonged exposure to elicit a clinical benefit. In the CALGB trials most responses were seen by cycle 4 (75%), with a median number of cycles to any response (CR, PR, HI) of three cycles [27]. The range for this response was 1–17 cycles, however and although 90% of patients achieved a response by cycle 6, some patients got their response as late as cycle 17 [27]. In the AZA-001 trial where the goal was to provide at least six cycles of therapy and there was no predefined stopping point, the investigators demonstrated that continuing the Aza dosing as long as possible can result in improvements in the observed responses, and these results were reiterated by additional analysis of the studies conducted by the CALGB [28, 32]. The secondary analysis of CALGB studies demonstrated a response in 91 of 179 patients, and

responders received a median of 14 cycles of therapy (range 2–30) [28]. The median time to first response in this study was slightly shorter than that seen in 9221, at 2 cycles (but with a range of 1–16) and although most responses (91%) were achieved by the sixth cycle, continuation of Aza was able to improve the quality of the first response in 48% of those treated, and this best response was seen in most patients (92%) by the 12th cycle [28]. Overall 30 patients achieved a best response of CR 3.5 cycles beyond the first response (with a 95% CI of 3.0–6.0 cycles), and in 21 patients whose best response was PR, this was seen as a median of 3.0 cycles after the first response (95% CI was 1.0–3.0) [28].

13.2.1.4 Other Considerations of Dose and Schedule—Additional questions which remain about the use of single agent Aza therapy are related to administration schedule (to weekend or not to weekend, are 7 days enough) and optimal drug delivery (subcutaneous vs. intravenous vs. oral).

In community practice there is often difficulty in giving this drug on the FDA approved schedule due to inadequate availability of personnel to administer the drug on weekends. This practical consideration resulted in a trial of several schedules of Aza administered in a community setting during weekdays only [36]. In this trial, 151 patients, for the most part with lower risk MDS (low, int-1 in 63% of patients), were randomized to receive Aza on one of the three schedules: 75 mg/m² daily for 5 days, off 2 days and then on 2 days (5-2-2), 50 mg/m² daily for 5 days, off 2 days and then on for 5 further days (5-2-5), and lastly 75 mg/m² daily for 5 days alone (5-0-0) [36]. These schedules seemed to result in similar hema-tological improvement rates (44%, 45%, 56%, respectively), but this study was not designed to produce statistically significant results, nor have these schedules been directly compared with the approved 7 day schedule. Thus it is difficult to condone alteration of the schedule at this time, based upon the lack of survival data in these schedules and the demonstrated survival benefit with administration of these drugs on the approved schedule. One additional schedule question has been raised by the preliminary data from the Eastern Cooperative Oncology Group trial 1905, which was a randomized phase II trial comparing Aza 50 mg/m²/day subcutaneously for 10 days to the same Aza schedule given in combination with the Histone deacetylase (HDAC) inhibitor entinostat (4 mg/m²/day PO days 3 and day 10) [37]. This abstract reported only on patients with baseline cytogenetic abnormalities (*n* = 40 evaluable) but demonstrated complete cytogenetic responses of 13% and a partial cytogenetic responses of 23% for an overall response in this subgroup of 51% (21/40) [37]. No differences in response were seen between the two treatment groups. Notably the responses observed were significantly higher than those reported with conventional Aza dosing raising the question of whether a lower dose, longer administration schedule may be of some benefit. At present these data are insufficient to change practice, however as additional groups publish the results of ongoing clinical trials of different dosing schedules, practice changes may be in order.

With respect to optimal drug delivery there is only a single study which directly compares the pharmacokinetics of intravenous to subcutaneous dosing within individual patients. In this study the pharmacokinetic profile of intravenous administration was almost identical to that seen with subcutaneous dosing, although the peak drug concentration was higher in patients receiving intravenous drug [38]. Despite these data, published clinical trials using

20 min IV infusion schedules are limited to two studies, one which gave Aza for 5 days and the other for 7 [39, 40]. Both of these studies demonstrated response rates which were similar to those seen with subcutaneous dosing (27% in the 5 day and 56% for the 7 day schedule), but neither of them was powered to detect a survival benefit [39, 40]. Despite the dearth of published response data, it seems reasonable to switch to intravenous administration in patients who suffer significant injection site reactions with subcutaneous dosing, and the FDA approved a New Drug Application for intravenous Aza in January 2007, supporting this practice [41].

Initial studies with oral Aza were limited by rapid catabolism of the compound in aqueous environments but the development of a film-coated formulation improved stability [42, 43]. Since that time the first phase I study of oral Aza has been published, demonstrating activity for the oral drug in patients with both MDS and CMML, with promising response rates [44]. Six of 17 (35%) previously treated patients had a response (CR + PR + HI) and 11 of 15 (73%) untreated patients responded (CR + PR + HI). This study demonstrated no overall response in the 8 patients with AML, however two patients had stable disease for 14 and 15 cycles [44]. Overall these results suggest that oral Aza may be a real possibility for the future and clinical trials of this drug are ongoing.

13.2.2 Dac

5-Aza-2'-deoxycytidine (Dac) is a deoxynucleoside analog of cytidine in which the carbon 5 position of the pyrimidine ring has been substituted with nitrogen (Fig. 13.2b) [15]. It is imported into cells by the action of nucleotide transporters, where it is activated by deoxycytidine kinase and then phosphorylated (Fig. 13.3) [15]. After its phosphorylation to the triphosphate form, 100% of the drug is incorporated into DNA, where it interrupts the action of DNA methyltransferases as described above for Aza. Similar to Aza, Dac has been demonstrated to cause both DNA hypomethylation and DNA damage, albeit at lower concentrations [45]. The identification of DNA hypomethylation as a functional consequence of exposure to both Aza and Dac, in conjunction with the recognition of DNA methylation changes as a frequent abnormality in cancer, spurred significant clinical interest in the development of these drugs for clinical use [20, 45].

Although effects upon DNA methylation were recognized and noted early in its development, initial clinical trials focused on conventional dosing strategies aimed at developing a maximum tolerated dose schedules [46–48]. These studies demonstrated considerable activity but with toxicity not significantly superior to cytarabine, with several studies performed investigating combinations with other chemotherapeutics in the salvage setting [49, 50].

Several early studies showed promising results with “low dose” Dac regimens, however these studies provided the drug at doses of 40–50 mg/m²/day, and toxicity remained a serious problem [51–53]. The first study to investigate the “optimal” lower dose Dac schedule for maximal demethylation was published in *Blood* in 2002 by Jean-Pierre Issa and colleagues [54]. This trial enrolled 48 patients at doses ranging from 5 to 20 mg/m²/day for 10–20 days of a 6 week schedule depending upon count recovery. Most interestingly in this study, responses appeared to be superior for the lower dose schedules studied, prompting the

authors to suggest further investigations of the drug be undertaken at truly lower dose schedules [54].

Based upon extensive phase I/II data at moderate to higher doses, the first large scale trial of Dac enrolled 170 patients with MDS between 2001 and 2004 and randomized them to either Dac (89 patients), given at 15 mg/m² iv every 8 h (45 mg/m²/day) for 5 days, or BSC (81 patients) [10]. Patients were removed from the study for disease progression, transformation to AML, failure to achieve a PR after six cycles of therapy, or failure to achieve a CR after eight cycles of therapy. Additionally, patients who did achieve a CR were removed from therapy after two cycles of sustained CR. The groups were well matched for all important variables with a median age of 70 years (range, 30–85 years). A majority of the patients (71%) had int-2 or high risk disease by IPSS criteria. The primary study endpoints were overall response rate and time to AML transformation or death. Overall 30% ($n = 27$) of patients experienced improvement on the study (CR + PR + HI) compared with 7% ($n=6$) patients randomized to BSC, and this difference was statistically significant $p=0.001$ [10]. In a retrospective central review of pathology nine patients enrolled on Dac and three patients on the supportive care arm were designated as having AML (by FAB criteria, >30% bone marrow blasts). Response rates in these nine patients were 56% (5/9), while none of the patients enrolled on the supportive care arm developed a response [10]. It is important to note that in this randomized controlled non-crossover trial there was no survival benefit for the use of Dac, although one might argue that the dose used (45 mg/m²/day × 5 days) was not low enough to maximize hypomethylation over cytotoxicity and the median number of cycles administered was low (3).

Following the results of this trial (which were disappointing from a survival perspective, but represented the first active agent for patients with high grade myelodysplasia), in 2006 the FDA approved Dac for all MDS subtypes. Based upon the results of earlier studies suggesting that lower dose Dac dosing might be superior, two pivotal phase II studies were performed aimed at identifying the “optimal” hypomethylating dose for Dac [55, 56]. The first of these was published in 2007 and enrolled 95 patients, again with a majority (66%) of patients having int-2 or high risk disease [55]. All patients were randomized to receive one of the three different Dac schedules, 10 mg/m² intravenously over 1 h daily for 10 days, 20 mg/m² intravenously over 1 h daily for 5 days, or 20 mg/m² subcutaneously daily for 5 days. Patients received a median of seven cycles of treatment and the CR rate overall was significantly better than anticipated at 37%, and an overall improvement (including CR + PR + HI) was observed in a staggering 73% of patients [55]. The 5 day schedule was deemed superior with 25/64 patients on this arm achieving CR and this schedule was selected for further investigation in subsequent trials [55]. The second analogous trial published in 2009 by Steensma and colleagues enrolled 99 patients in a single arm trial of Dac 20 mg/m² over 1 h daily for 5 days [56]. A lower percentage of patients on this trial were high grade (46%), and the median number of administered courses were slightly lower (5) than in the prior investigation. These authors observed a 15% CR rate and an overall response rate of 43% (CR + PR + HI) [56]. Both trials demonstrated that the lower dose schedule of Dac 20 mg/m²/day for 5 days had at least equivalent efficacy when compared with the FDA

approved schedule, and furthermore that maintaining 4 week dosing intervals and repeated cycles of therapy were important in order to maximize response.

One additional phase III study of Dac has been published [13]. It is important to note that this study did not employ the 5 day, 20 mg/m²/day schedule described above. This trial was designed to demonstrate a survival benefit for the use of Dac in patients with MDS, comparable to that observed with Aza. Two-hundred and thirty-three patients with a median age of 70 years (range 60–90) were enrolled; 53% had poor-risk cytogenetics and 33% fulfilled WHO AML diagnostic criteria (> 20% blasts) [13]. The primary end point for this trial was OS. Patients were stratified by IPSS risk group, cytogenetics and enrollment site, and were randomly assigned to receive either Dac or BSC. This study design specifically prohibited patient crossover to the experimental arm in an effort to eliminate crossover bias. The Dac was given intravenously at a dose of 15 mg/m² every 8 h for 3 days. Cycles were scheduled to repeat every 6 weeks, but the interval could be extended up to 10 weeks for failure of count recovery, eight cycles of treatment were planned. In total 119 patients were randomized to receive decitabine and 114 patients were randomized to the control arm; only 21% of patients received the planned eight cycles of treatment. At the planned analysis point of 2 years, OS in the Dac treatment cohort was 10.1 months vs. 8.5 months in the supportive care arm, this difference was not statistically significant ($p = 0.38$, HR, 0.88; 95% CI, 0.66–1.17) [13]. Sixteen patients on the Dac arm (13%) achieved a CR and 25 patients (21%) improved (PR + HI), for an overall response rate of 34%. The median time to best response was 3.8 months (range, 1.4–11.8 months) for all responders, with a median of 5.8, 2.9, and 3.8 months to reach CR, PR, and HI, respectively. Two patients (2%) in the supportive care arm had a HI, there were no CRs or PRs in this group. Dac did not have a statistically significant impact upon time to AML transformation; patients on Dac transformed to AML after 8.8 months vs. 6.1 months in the supportive care arm (HR 0.85; 95% CI 0.64–1.12; $p = 0.24$) [13].

Disappointing results, in terms of survival benefit, from two large phase III trials of Dac in MDS have resulted in a significant shift in terms of practice away from Dac in this population [10, 13]. Despite these results, some clinicians continue to use Dac in the first line treatment of MDS patients, and it is certainly notable that none of the three phase III studies of Dac used the most common low dose schedule of Dac at 20 mg/m²/day for 5 days, a dose schedule which is pharmacologically more consistent with the 75 mg/m² Aza dose demonstrated to prolong survival. Additionally, the European phase III trial delayed subsequent Dac cycles based upon cytopenias, a strategy which is increasingly recognized as inferior. As a result of these caveats it is likely that Dac has similar efficacy to Aza, although at present the data have not definitively demonstrated this equivalence.

13.2.2.1 DAC in AML—Despite disappointing results in patients with MDS, many clinicians favor Dac in patients presenting with AML, particularly in those with very proliferative disease, as a result of its relative cytotoxicity when compared with Aza. A dosing strategy employing 20 mg/m² for 10 days has been studied by investigators at the Ohio State James Cancer Center [35, 57]. This dose schedule was initially developed in a phase I trial designed to assess combination therapy with valproic acid, however a single agent response of 73% in a group of very elderly (median age 70) patients with high risk

AML prompted phase II investigation (see below) [57]. The Phase II trial enrolled 53 patients of median age 74 years (range 60–85) with AML (16 complex karyotype, 19 with an antecedent hematological diagnosis) and produced a response rate of 64% (34/53) composed of 25 CRs and 9 CRs without count recovery [35]. Patients enrolled on study had a median survival of more than a year, suggesting that this strategy is similarly effective to conventional chemotherapeutics in this patient population [6, 8, 9]. These very promising results have produced an ongoing cooperative trial using this dose schedule in older patients with AML and may yet demonstrate statistically significant improvements in survival for this particular subgroup of elderly AML patients.

13.3 Azanucleotides and CMML

Dac remains the most studied drug in patients with CMML, a distinct entity within the WHO diagnostic criteria form MDS. Several studies have examined the activity of Dac both prospectively and retrospectively in this group. One recently published phase II study enrolled 39 patients of median age of 71 years with advanced CMML to receive Dac on the 20 mg/m²/day intravenous schedule for 5 days of a 28 day cycle [58]. Enrolled patients received a median of ten cycles of drug (range, 1–24) and the overall response rate was 38%, composed of 4 (10%) CRs, 8 (21%) marrow responses, and 3(8%) His [58]. With a median on trial follow-up of 23 months the OS was 48%. Another study examined the response to Dac in 31 patients diagnosed with CMML who were treated on two phase II and one phase III clinical trials [59]. Patients included in the analysis had similar demographics and disease characteristics across the three studies. The median age was 70 and patients were predominantly male (71%). The overall response rate in this group was 36% (14% CR + 11% PR + 11% HI) [59]. Although Aza has also been shown to have activity in this disease, the number of published reports in this group are limited, and thus most experts would likely favor the use of Dac for patients with CMML outside the context of a clinical trial [60]. An ongoing clinical trial designed to prospectively enroll patients with CMML is ongoing in order to address the efficacy of Aza in this disease.

13.4 Outcomes Following Azanucleotide Failure

As we develop our experience with azanucleotides it has become clear that patients who lose their response to azanucleotides have a dismal prognosis [14]. As a result of these poor outcomes, current standard practice is to maintain patients on therapy with hypomethylating drugs on a monthly schedule indefinitely and to stop only in the context of overt progression. Unfortunately, analysis of patients enrolled on early studies of Aza who develop disease progression have now been published, showing that in patients who fail azanucleotides, survival is remarkably short with a median life expectancy of 5.6 months and a 2-year survival probability of 15% [61]. Similar results have been reported in patients who fail Dac [14, 62]. Outcomes in these reports suggest that enrollment on clinical trials and bone marrow transplantation may result in superior outcome in these patients, however in the absence of successful bone marrow transplantation the OS reported at 1 year remains a mere 28% [14, 61, 62].

13.5 Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) are a novel class of drugs whose putative mechanism of action depends upon the ability to alter gene expression. Intracellularly, DNA is stored in the form of “beads on a string” in which the DNA duplex winds around a nucleosome composed of eight histones (two each of H2A, H2B, H3 and H4) [63]. The DNA/histone unit (the nucleosome) is condensed to form higher order chromatin structures such as heterochromatin, which has densely packed nucleosomes and euchromatin, which has loosely packed nucleosomes [63]. Modifications, including ubiquitination, methylation, phosphorylation, poly(ADP) ribosylation, and acetylation, of specific amino acid residues within each histone make up the “histone code” which determines the state of the regional chromatin at specific genes and thus their transcriptional activity [63]. DNA methylation events are thought to induce changes within the local “histone code” which promote gene silencing, although whether methylation events or histone marks are primary remains a matter of some controversy. Perhaps the most studied histone modification is acetylation of lysine N-terminal tails which are common to most histones. Acetylation of lysine results in an open chromatin conformation and promotes gene transcription while deacetylation of lysine residues promotes gene silencing [63].

HDACs are enzymes that remove acetyl groups from a variety of different protein targets including histones. Increased HDAC activity has been described in cancer cells, and aberrant HDAC activity is characteristic of a number of well recognized recurrent genetic anomalies characteristic of leukemia including the core binding factor gene fusions (t(8;21) (q22;22) and inv(16)), and the sine qua non of acute promyelocytic leukemia t(15;17) (q24;21) [64–66]. The gene products of such fusions result in aberrant recruitment of HDACs to genes important for myeloid differentiation. Recognition of HDACi as a potential novel therapy in myeloid malignancy resulted from the observation that drugs known to induce differentiation in vitro induced histone hyperacetylation, potentially leading to re-expression of epigenetically silenced genes [67]. Many different diverse chemical compounds can inhibit HDACs, including short chain fatty acids (e.g., phenylbutyrate), hydroxamic acid derivatives (e.g., vorinostat), non-hydroxamate small molecules (e.g., entinostat), and cyclic peptides (e.g., romidepsin) [68].

Most of the published clinical trials of HDACi in MDS and AML are phase I. As single agents the response rates observed have been relatively low, usually between 10 and 20% [68]. Toxicities with these agents demonstrate a common pattern and include fatigue, nausea, vomiting, and diarrhea. Although most of these studies evaluated the correlative endpoint of histone acetylation, no associations between hyperacetylation of histones and response to therapy have been demonstrated. For a more complete review of HDACi in cancer please see Chap. 3, Sect. 3.5 of this book.

13.6 Azanucleotides and HDACis

There has been significant enthusiasm for a combination strategy which includes azanucleotides in conjunction with HDACis. This stems from the observation in vitro that sequential exposure to Dac or Aza followed by HDACi result in synergistic re-expression of

DNA methylation silenced genes [69]. Several studies evaluating such combinations have been published to date and the results remain mixed. Although some studies suggest a higher response rate than for single agent azanucleotides, most data are in the phase I or II setting, at a single center, and employ alternative dosing strategies for the azanucleotide making it difficult to distinguish whether these responses are truly superior. In those studies where a single agent arm was also enrolled response rates do not appear to be consistently superior [37, 57]. Although early correlative endpoints did demonstrate evidence to support a connection between reversal of methylation events and response to therapy, subsequent studies (even at the same institution by the same investigators) have failed to substantiate a correlation between gene specific reversal of methylation and response [70, 71].

The first two studies published reports on a combination of Aza at doses between 25 and 75 mg/m²/day subcutaneously for 5–10 days [70, 72]. These studies enrolled a total of 42 patients with MDS (16) and AML (26), of median age 66. These studies reported that the combination was well tolerated and resulted in response rates of 34 (11/32, 5 CRs) and 50% (5/10, no CRs) respectively (CR + PR + stable disease) [70, 72]. The second study reported correlative epigenetic data in three responders and three non-responders, with those patients who developed a response showing robust demethylation of the tumor suppressor gene *p15^{INK4B}* while those who did not retained methylation at this locus, suggesting that changes in methylation were indeed a marker for responsiveness [70].

Two phase I/II studies have evaluated the combination of Dac with valproic acid. The first employed Dac 15 mg/m²/day for 10 days with a dose escalation of valproic acid from 20 to 50 mg/kg/day for 10 days in patients with high grade MDS or AML [73]. Fifty four patients of median age 60 (range 5–80 years) were enrolled, 48 patients had AML and 6 had MDS, 11 patients were previously untreated. Twelve patients responded to therapy; 10 developed a CR and 2 a CR with incomplete platelet recovery. Median responses were seen after 2 months (range 29–130 days) and responders survived a median of 15.3 (range 4.6–20.2+) months vs. 4.9 (0.6–17.8+) months in non-responders [73]. Responders were more likely to have been randomized to a higher dose of valproic acid. Although changes in methylation (both gene specific events, including *p15^{INK4B}*, and genome wide methylation, by LINE-1 pyrosequencing) and gene expression were analyzed in the patients on this study no correlations with response were observed [73]. All patients experienced a decrease in genome wide methylation which correlated with Dac exposure. In a second study, this one employing Dac 20 mg/m²/day for 10 days intravenously, responses were also encouraging with an overall response rate of 44% in 11 of 25 enrolled patients [57]. This trial enrolled 25 AML patients, in whom the median age was 70 years; 12 patients were untreated and 13 had relapsed disease. In this group of slightly older patients, encephalopathy was the principal toxicity and this was dose limiting at 20–25 mg/kg/day. In an intent-to-treat analysis, the response rate was 52% (13). CR was observed in 8 patients and PR in 4. Responses appeared similar for patients who received Dac alone and for those who received valproic acid in addition. In this study, re-expression of estrogen receptor was statistically significantly associated with clinical response ($p = 0.05$), however although the investigators also demonstrated ER promoter demethylation, global DNA hypomethylation, depletion of

DNA methyltransferase enzyme, and histone hyperacetylation, these markers did not correlate with response [57].

The combination of Aza with vorinostat (SAHA) has also been explored. In one phase I trial in patients with MDS and AML this combination produced an impressive overall response rate of 64% [74]. A second phase II trial of this combination in patients with MDS and AML has also been reported [75]. This trial enrolled 17 untreated patients and demonstrated an overall response rate of 41% ($n = 7$) [75]. Similar outcomes (overall response of 37%) were observed in patients receiving a combination of Aza with the compound MGCD0103, an oral isotype-selective HDACi [76]. Although these responses appear to be encouraging, a majority of these combination studies have been published to date only in abstract form and larger studies are necessary in order to verify their superiority.

Data from one of the first randomized phase II studies to enroll patients either on single or double agent therapy was presented at the 2010 ASH meeting and reviewed in detail earlier in this manuscript (see Aza section under Sect. 5.2.1.4), this study, at least, suggests that combination therapy may not be superior [37]. In this trial patients with either MDS or AML with MDS related changes were randomized to receive either Aza at 50 mg/m² for 10 days subcutaneously alone or Aza in combination with entinostat 4 mg/m² orally on days 3 and 10. Although the final results of this trial have not yet been published, it is important to note that the response rates for patients enrolled to receive Aza alone were indistinguishable from those who got the combination.

These results and others with a variety of HDACis may underestimate the value of combined therapy. It is important to note that among the many mechanisms postulated to be responsible for the efficacy of HDACis are induction of apoptosis and cell cycle arrest [77]. Since azanucleotides require DNA replication in order to produce DNA demethylation, it may be that administration of HDACi simultaneously or even in advance of the azanucleotide may result in diminished incorporation and limit responsiveness. Presently, a multi-institution phase II sequence study designed to address this question is open for enrollment [78].

13.7 Azanucleotides and Conventional Chemotherapy

One study has been published which explores the possible role of azanucleotide in “priming” leukemia cells for death [79]. This open label, phase I study was designed to address the safety and feasibility of Dac at a dose of 20 mg/m² either as a continuous infusion or a short infusion for 3, 5, or 7 days followed by standard dose 7 + 3 IC (cytarabine 100 mg/m²/day continuous intravenous infusion for 7 days + dauno-rubicin 60 mg/m²/day for 3 days). The study enrolled 30 patients of median age 55 (range 23–60) with newly diagnosed AML and a less than favorable karyotype (*inv*(16), *t*(8;21) and APL patients were excluded). Thirteen patients had complex, 11q23 or chromosome 7 abnormality associated leukemias and 8 had an antecedent hematological diagnosis. Toxicity was not dissimilar to that seen with 7 + 3 alone, although there appeared to be slightly more gastrointestinal toxicity in the group treated with 7 days of Dac priming, and there were no deaths. All subjects received consolidation, 20 patients went on to receive allogeneic bone

marrow transplantation. Overall 27 (90%) of patients responded to one course of induction therapy, 17 patients achieved a CR and 10 a PR, patients scored as a PR all achieved hemato-logical remission, but went on to receive a second course of induction resulting in a CR in 8/10 patients [79]. The overall CR rate following 1 or 2 cycles of induction therapy was therefore 83%. With a median follow-up of 32 months, 53% of patients (16/30) remained alive and in CR, 14 subjects died, 3 of complications related to allogeneic bone marrow transplant and the remainder died of relapsed or refractory AML [79]. The correlative DNA methylation analysis of this study revealed universal demethylation at both gene specific and genome wide loci with all schedules of Dac. The most potent hypomethylation was observed in patients treated with bolus, rather than continuous infusion schedules of Dac.

Although preliminary, this phase I trial demonstrated a remarkably good CR rate and a randomized phase II study designed to assess the two most potent demethylation schedules of Dac priming identified by this study should begin accrual in 2012.

13.8 Azanucleotides and Bone Marrow Transplantation

Allogeneic bone marrow transplant (allo-transplant) is the only curative strategy currently available for patients with MDS and high risk AML. Presently the role of hypomethylating agents both prior to and following transplant is under investigation.

Several small retrospective studies of azanucleotide induction prior to allo-transplant have been reported, two using Dac and two using Aza. The first of these reported outcomes in 17 patients with MDS of median age 55.5 (range 36–66) years undergoing allo-transplant (12 sibling donor, 5 unrelated donor) after prior therapy with Dac (various dosing regimens) [80]. These patients received predominantly reduced intensity conditioning and peripheral blood stem cells (13/17). With median follow-up of 12 (range 3–35) months, 8 patients remained in CR [80]. A second prospective study performed in Europe reported similar results in 15 patients of median age 69 (range 60–75) years with either MDS ($n = 10$) or AML ($n=5$) [81]. All patients were treated with upfront Dac followed by reduced intensity allo-transplant (4 sibling donor, 11 unrelated donor). Fourteen patients achieved a CR (93%), with a median duration of 5 (range 1–51) months [81]. The relapse rate in this group was similar (4/15) to that reported retrospectively. The third study examined outcomes in 54 patients with MDS or CMML who either received (30) or did not receive (24) prior therapy with Aza [82]. Patients treated with Aza received a median of 4 (range 1–7) courses prior to transplant. The overall, relapse free and cumulative relapse 1 year following transplant were 47, 41, and 20%, for those patients treated with Aza and 60, 51, and 32% for untreated patients and these results were not statistically significantly different [82]. The final trial using Aza was a retrospective review of 68 patients undergoing allo-transplant for MDS or AML arising from MDS [83]. Thirty five patients received Aza followed by either myeloablative (40%) or reduced intensity (60%) conditioning. Thirty three patients received IC followed by allo-transplant. In these two, albeit somewhat different groups, the OS at 1 year was 57% in those treated with Aza and 36% in the IC group [83]. Overall these data suggest that Dac and Aza are a reasonable pre-transplantation strategy that does not adversely affect outcome when compared with high dose induction or supportive care. A

phase II clinical trial of Dac prior to allo-transplant is ongoing in Singapore using the currently favored schedule of 20 mg/m²/day for 5 days intravenously.

Post-transplant relapse remains a significant problem in MDS and high risk AML patients. Traditionally relapses in this population have been managed with donor lymphocyte infusions (DLI) (in those who do not demonstrate graft vs. host disease) or re-induction with traditional chemotherapeutic agents. Although limited prospective data exist on the use of azanucleotides for salvage of patients relapsing following allogeneic transplant, or as a preventive strategy following transplant, several small studies have been published, suggesting that these agents may have a significant role to play.

The first of these examined the efficacy of Aza at a flat dose of 100 mg subcutaneously days 1–3 followed by planned DLI on day 10 [84]. Cycles were repeated every 22 days for a median of 2 (range 1–10) courses to 26 patients with relapsed AML ($n = 24$) or CMML ($n = 2$) following allo-transplant. Toxicity with this combination was as expected and consisted of infections and GVHD. Four patients (15%) were salvaged with a complete and lasting CR following this combination [84].

A second study, this one retrospective, described the results of salvage with Aza 100 mg/m² for 5 days in 22 patients of median age of 50 (range 28–69) years, with either AML (17) or MDS (5) relapsed following allo-transplant [85]. A majority (20/23) of these patients had received a myeloablative conditioning regimen and half (10/23) had a sibling donor. On average two cycles of Aza were administered (range 1–8). Most patients also received DLI (18/23). In this group, 5 patients (23%) achieved a CR lasting a median of 433 days (range 114–769) with a 2-year survival rate of 23% [85].

A third single institution study, retrospectively reviewed Aza 75 mg/m² for 5 or 7 days as salvage in 10 patients with MDS (9) or AML (1) of median age 55 (range 25–67) years [86]. Seven patients achieved CR or stable disease with this regimen, 3 of whom progressed after a median of 6 cycles. The median OS (OS) for the group was 422.5 days (range 127–1,411).

Taken together these results are encouraging and a variety of studies are ongoing to determine prospectively the role of azanucleotides both before and after allo-transplant [87].

13.9 Molecular Determinants of DNMTi Response in MDS and AML

Early on in the development of azanucleosides for the treatment of myeloid disease there was considerable enthusiasm for the identification of molecular markers of disease response. Initially several authors examined gene specific methylation reversal, including *p15^{INK4B}* and ER as discussed earlier in this manuscript [10, 55, 57, 70, 71]. Disappointingly, although reversal of methylation at many loci has been documented following azanucleotide exposure, it has not been demonstrated to correlate with or predict response to treatment, but rather seems to reflect duration of exposure to hypomethylating agents [88]. Another marker of response which has been studied is p53-inducible-ribonucleotide-reductase (p53R2), a gene identified in cell line screens to be induced following decitabine exposure [89, 90]. Link and colleagues demonstrated a statistically significant concordance between response to therapy and induction of p53R2 both at the mRNA and protein levels [90]. Although

these results are thought provoking, they require sampling after many cycles of therapy and it is difficult to determine how useful a biomarker of response this would be clinically.

The identification of mutations in the genes encoding *TET2* (ten–eleven translocation2) and *DNMT3A* in patients with MDS and AML have raised questions about whether response to therapy may depend upon genetic characteristics of the underlying myeloid neoplasm. Recently a number of authors have demonstrated that up to 26% of patients with MDS demonstrate mutations in *TET2*, and further that MDS patients with *TET2* mutations appear to have a superior prognosis (although this is not as clear in patients with AML) [91, 92]. Since *TET2* encodes a dioxygenase which functions to convert 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA demethylation at selective loci, defects in *TET2* function would be expected to result in hypermethylation. One recent study suggests that patients bearing *TET2* mutations have a superior response (CR + PR + HI) to Aza treatment 82% vs. 45% ($p = 0.007$), although OS was not different in the two groups and these results have yet to be validated [93]. By contrast with mutations involving *TET2*, mutations in *DMNT3A* have been demonstrated to predict adverse outcome in both MDS and AML, although as yet no evaluation has been made of the impact of such mutations on response to epigenetic therapies [94–96].

13.10 Conclusions

Azanucleotides have changed the landscape of treatment for patients with MDS and AML with MDS related changes. Ongoing work with these agents in patients with a variety of myeloid diseases is likely to result in advances over the next few years. Despite the considerable efficacy of these drugs, patients with underlying myelo-dysplasia continue to have a remarkably poor outcome and novel strategies in these diseases remain essential. As we continue to develop insight into the mechanism(s) which underlie the activity of these drugs, perhaps we will be able to understand why they work so well for some patients and what strategies will maximize the longevity of these responses. Certainly it has become clear that single agent azanu-cleotides given on a conventional schedule are not a panacea. Whether responses can be optimized with continuous dosing strategies, combination with other drugs, or allogeneic bone marrow transplantation remains a question yet to be answered by well designed clinical trials.

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Prognostic Variable	Score value				
	0	0.5	1.0	1.5	2.0
Bone Marrow Blast %	<5	5-10		11-20	21-30
Karyotype	Good= normal,-y, del(5q), del(20q)	Intermediate= all others	Poor= ≥3 or any 7 abnormality		
Cytopenias	0/1	2/3			

IPSS risk group	Score	Median Survival
Low	0	5.7 years
Int-1	0.5-1	3.5 years
Int-2	1.5-2.0	1.2 years;
High	≥2.5	0.4 year

Fig. 13.1. Clinical criteria for and IPSS risk group classification of patients with myelodysplasia, from ref. [2]

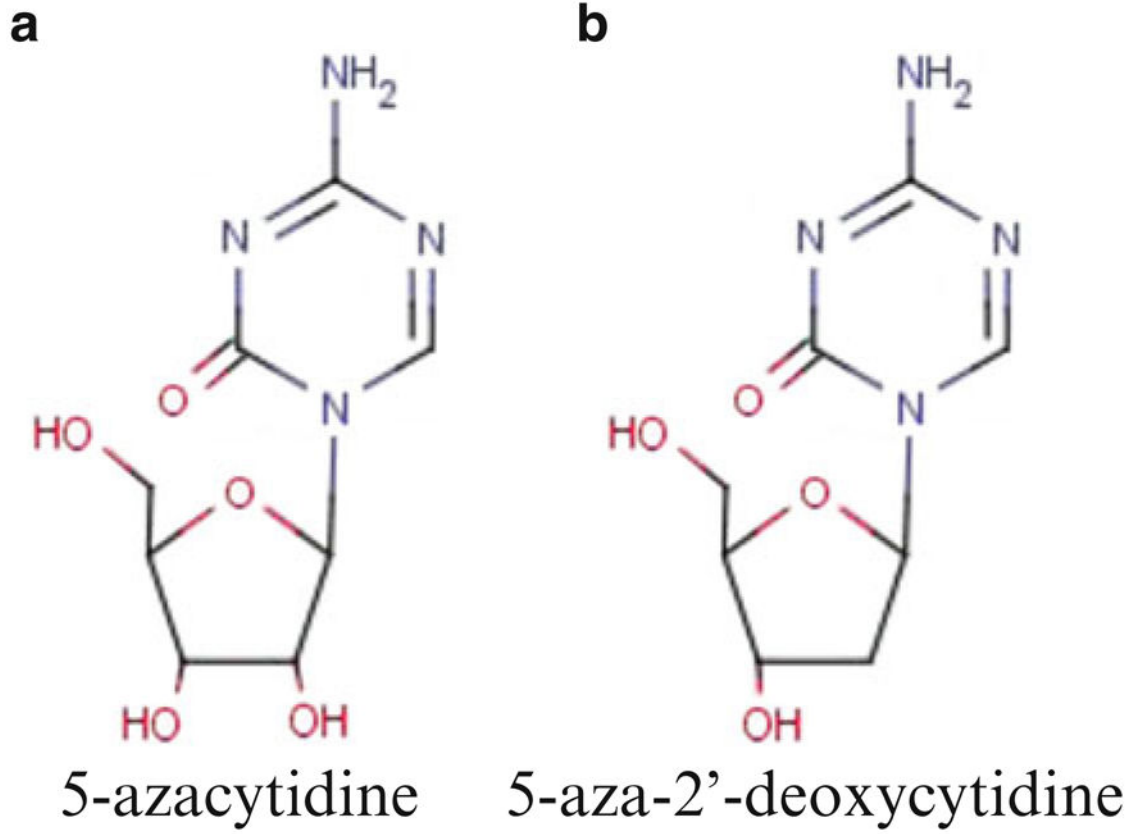


Fig. 13.2.
Molecular structure of Aza (**a**) and Dac (**b**)

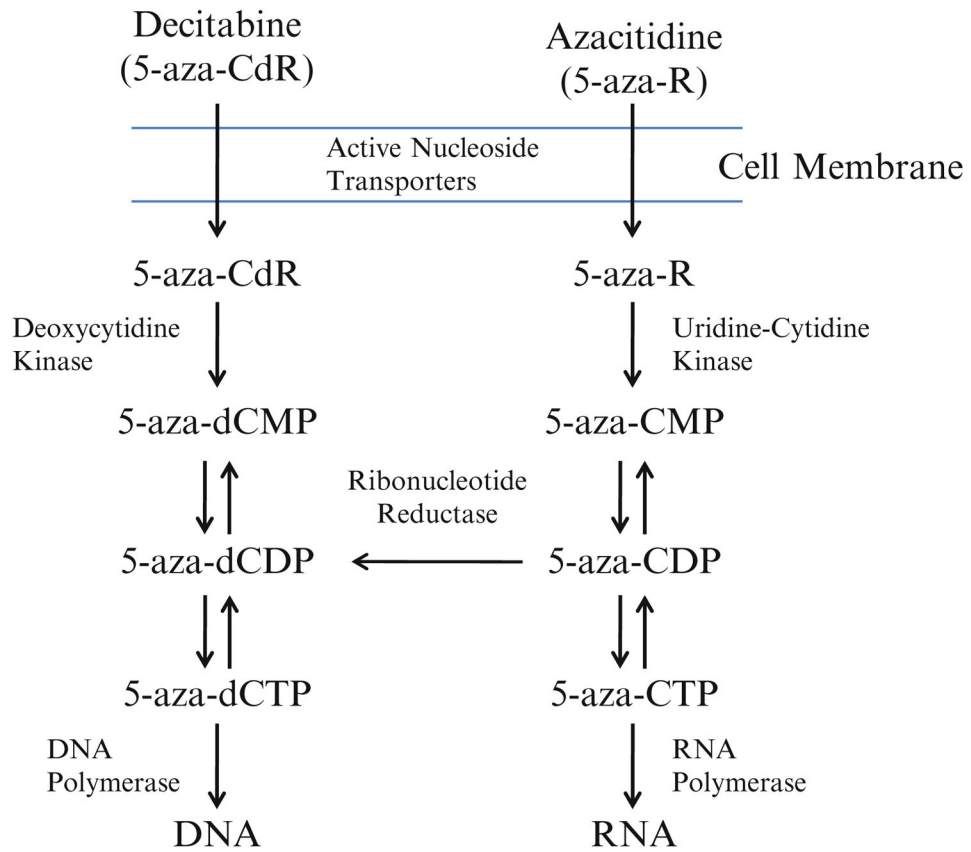


Fig. 13.3.
Uptake and serial steps for the incorporation of Aza and Dac into RNA and DNA

Table 13.1

Selected clinical trials with azacitidine (aza) or decitabine (dac) in MDS

Trial	CALGB 9221	D-0007	ICD03-180	AZA-001	US Oncology	ADOPT	EORTC 06011
Author (publication year)	Silverman (2002) [27]	Kantarjian (2006) [10]	Kantarjian (2007) [55]	Fenaux (2009) [7]	Lyons (2009) [36]	Steensma (2009) [56]	Lubbert (2011) [13]
Number enrolled	191	170	95	358	151	99	233
Number treated with study drug	150 (99 upfront, 51 crossovers)	89	95	179	151	99	119
Phase	III	III	II	III	II	II	III
Study regimen	Aza SQ 75 mg/m ² × 7 days	Dac IV 15 mg/m ² q8h × 3 days	Dac IV 10 mg/m ² × 10 days IV 20 mg/m ² × 5 days SQ 20 mg/m ² × 5 days	Aza SQ 75 mg/m ² × 7 days	Aza SQ 75 mg/m ² × 5 days-2 days off-2 days × 5 days-2 days off-5 days × 5 days	Dac IV 20 mg/m ² × 5 days	Dac IV 15 mg/m ² q8h × 3 days
Int-2 or high IPSS (%)	46	70	66	87	Not reported	46	93
Median cycles administered	4	3	7	9	6	5	4
CR % (by IWG 2000)	9	9	37	17	Not reported	15	13
CR + PR + HI% (by IWG 2006)	48	30	73	49	48	43	34

Table 13.2

Selected clinical trials with azacitidine (aza) or decitabine (dac) in MDS

Trial	CALGB 9221	D-0007	ICD03-180	AZA-001	US Oncology	ADOPT	EORTC 06011
Author (publication year)	Silverman (2002) [27]	Kantarjian (2006) [10]	Kantarjian (2007) [55]	Fenaux (2009) [7]	Lyons (2009) [36]	Steensma (2009) [56]	Lubbert (2011) [13]
Number enrolled	191	170	95	358	151	99	233
Number treated with study drug	150 (99 upfront, 51 crossovers)	89	95	179	151	99	119
Phase	III	III	II	III	II	II	III
Study regimen	Aza SQ 75 mg/m ² × 7 days	Dac IV 15 mg/m ² q8h × 3 days	Dac IV 10 mg/m ² × 10 days IV 20 mg/m ² × 5 days SQ 20 mg/m ² × 5 days	Aza SQ 75 mg/m ² × 7 days	Aza SQ 75 mg/m ² × 5d-2 days off-2 days × 5 days-2 days off-5 days × 5 days	Dac IV 20 mg/m ² / days × 5 days	Dac IV 15 mg/m ² q8h × 3 days
Int-2 or high IPSS (%)	46	70	66	87	Not reported	46	93
Median cycles administered	4	3	7	9	6	5	4
CR % (by IWG 2000)	9	9	37	17	Not reported	15	13
CR + PR + HI% (by IWG 2000)	48	30	73	49	48	43	34