5-Azacytidine in the Treatment of Intermediate-2 and High-risk Myelodysplastic Syndromes and Acute Myeloid Leukemia. A Five-year Experience with 44 Consecutive Patients.

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Abstract. *Background/Aim: The hypomethylating agent 5-azacytidine has been the standard-of-care for patients with higher-risk myelodysplastic syndrome (MDS) during the past few years. Its efficacy has been proven in large clinical trials, and its safety has been shown to be superior to that of conventional treatments. Patients and Methods: We conducted a retrospective study on the efficacy and safety of 5-azacytidine in 44 consecutive patients with MDS and acute myeloid leukemia treated with 5-azacytidine during a 63-month period. We recorded the clinical and laboratory characteristics of the patients and we analyzed the response to treatment, overall survival and adverse events during treatment. Results: The median overall survival was 13 months, while serious adverse events consisted mostly of neutropenic infections. Conclusion: We reached two possibly valuable conclusions: Younger patients (<73 years), as well as patients receiving treatment at longer than 28-day intervals had a significantly higher overall survival.*

The management of patients with myelodysplastic syndrome (MDS) is problematic and usually ineffective. There exist several therapeutic approaches available, ranging from watchful waiting and supportive care to aggressive chemotherapy and allogeneic stem cell transplantation (SCT). Supportive treatment is the mainstay in the management of patients with lower-risk MDS. 5-Azacytidine has emerged as a promising option in the treatment of patients with higher-risk MDS and acute myeloid leukemia (AML) with 20-30% marrow blasts. The terms 'lower' and 'higher' refer collectively to low- and intermediate 1 risk groups and to intermediate 2 and high-risk groups, respectively, according to the International Prognostic Scoring System (IPSS). (1) These terms have been widely used during the last few years for classification and grouping purposes.

5-Azacytidine is a chemical analog of cytidine and acts as a hypomethylating agent. DNA methylation is a process utilized *in vivo* to silence and regulate gene expression without changing the actual original DNA sequence. This epigenetic modification has been linked to cancer development since it has been shown that methylation of tumor-suppressor genes promotes tumorigenesis. (2) By hypomethylation, 5-azacytidine deactivates DNA-methyl transferase thus re-activating previously silenced genes. 5-Azacytidine and its deoxy derivative decitabine have been approved as frontline therapy for patients with higher-risk MDS, including AML with 20-30% marrow blasts, as defined by the WHO, (3) who are not eligible or cannot proceed immediately to allogeneic SCT. This agent has already been used in clinical trials for the management of patients with lower-risk MDS with mixed results (4-5) while more trials are underway, and the need for combination treatments cannot be over-emphasized.

The results of the AZA-001 trial (6-7) and of Cancer and Leukemia Group B (CALGB) studies (8-9) established 5-azacytidine as a reference first-line treatment for higher risk MDS. 5-Azacytidine treatment was associated, in the AZA-

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Key Words: 5-azacytidine, myelodysplastic syndrome, acute myeloid leukemia.

Table I. *Epidemiological, clinical and hematological characteristics of the patients.*

Characteristic	Result
Male to female ratio	30:14 (2.1)
Median age (range), years	73 (54-81)
WHO classification of MDS/AML, N (%)	
RAEB-I	9 (20.5)
RAEB-II	18 (40.9)
RCMD-RS	2 (4.5)
RCMD	3 (6.7)
RARS	1 (2.3)
CMML	4 (9.1)
AML	7 (15.9)
IPSS risk classification, N (%)	
Low	0 (0)
Intermediate-1	3 (6.8)
Intermediate-2	29 (65.9)
High	5 (11.4)
Not applicable (AML)	7 (NA)
Classification according to karyotype risk (37 patients)*, N (%)	
Good	24 (54.5)
Intermediate	4 (9.1)
Poor	9 (20.5)
Complete blood count parameters	
Median hemoglobin (range), g/dl	8.55 (4.5-12.5)
Median absolute neutrophil count (range), ×109/l),	1.08 (0.0-16.3)
Median platelet count (range), ×109/l	80.0 (2-820)
Transfusion dependence, N (%)	39 (88.6)
Median transfusions per month (packed RBC units) (range)	3 (0-7)
Previous treatments, N (%)	3/44 (6.8)

*The remaining 7 patients had AML (IPSS classification, not applicable). WHO, World Health Organization; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; RS, ring sideroblasts; RARS, refractory anemia with ring sideroblasts; CMML, chronic myelomonocytic leukemia; AML, acute myeloid leukemia; IPSS, International Prognostic Scoring System; RBC, red blood cell.

Table II. *Results of efficacy data.*

Characteristic	Result
5-azacytidine cycles, Median (Range)	5 (1-22)
Actual 5-azacytidine dose (mg/m^2/cycle), Mean (Range)	75 (59-75)
Actual cycle duration (days), Median (Range)	30 (28-40)
Dose reductions, N (%)	6 (13.6)
Reason for dose reduction	
Sustained neutropenia	6/6 (100)
Temporary 5-azacytidine interruption, N (%)	26 (59.1)
Reason for 5-azacytidine interruption, N (%)	
Sustained cytopenia	10/26 (38.5)
Neutropenic infection	15/26 (57.7)
Hemorrhagic complication	1/26 (3.8)
Permanent 5-azacytidine discontinuation, N (%)	23/44 (52.3)
AML transformation	17/23 (73.9)
Recurrent or severe infection	4/23 (17.4)
Pyoderma gangrenosum	1/23 (4.3)
Allogeneic bone marrow transplantation	1/23 (4.3)
Combination therapy (5-azacytidine+X*), N (%)	3/44 (6.8)
5-azacytidine cycles till response (according to the IWG criteria), Median (Range)	4 (1-7)
Response (according to the IWG criteria), N (%)	
Complete response	7 (15.9)
Partial response	8 (18.2)
Stable disease	29 (65.9)
Failure	0 (0)
Overall survival (months), median (range)	13 (1-101)
Survival since initiation of 5-azacytidine (months), Median (Range)	10 (1-47)
Post treatment transfusion dependence, N (%)	34 (77.3)
Transfusions per month (post-treatment), Median (Range)	1 (0-5)
Progression to AML, N (%)	21 (56.8)
Death rate, N (%)	29/44 (65.9)
Cause of death, N (%)	
Infection	24/29 (82.8)
Hemorrhage	3/29 (10.3)
Cardiac dysrhythmia	2/29 (6.9)

AML, Acute myeloid leukemia; X*, methotrexate, cytarabine, hydroxyurea; IWG, International Working Group.

001 trial, with fewer grade 3-4 cytopenias and shorter hospitalization time than low_dose cytarabine in higher-risk patients with MDS. (10) The AZA-001 trial showed that 5-azacytidine significantly prolongs overall survival in patients with AML with low bone marrow blasts, and significantly improves several patient morbidity measures, in comparison to conventional care regimens. (11)

The efficacy of 5-azacytidine is an intriguing study subject, even more so as 5-azacytidine combination regimens are currently under study in an effort to discover the most effective combination therapy for higher risk MDS patients.

We conducted a retrospective study to evaluate the efficacy and safety of 5-azacytidine in patients with higher risk MDS and AML with low (20-30%) marrow blast counts who had been treated at two centers during a period of 63 months.

Patients and Methods

In the present retrospective study, we recorded the epidemiological, clinical and hematological characteristics of 44 consecutive patients with higher risk MDS or AML with 20-30% bone marrow blasts treated at two Centers with 5-azacytidine in a 63-month period (January 2009 to May 2014). All patients were to receive 5-azacytidine at a dose of 75 mg/m^2 subcutaneously or intravenously,

Table III. *Survival correlations*.

	Median survival (months), (range)	*p*-Value
WHO classification of MDS/AML		0.37
RAEB-I	13.8 (2-42)	
RAEB-II	13.5 (1-93)	
RCMD-RS	17.5 (15-20)	
RCMD	13.0 (10-35)	
RARS	59 (59)	
CMML	11.5 (4-17)	
AML	30.0 (6-101)	
IPSS risk classification		0.94
Low	NA	
Intermediate-1	13 (5-59)	
Intermediate-2	13 (1-51)	
High	10 (3-93)	
Karyotype risk classification		0.94
Good	13 (1-59)	
Intermediate	13 (4-17)	
Poor	11 (3-93)	
Karyotype		0.048
-Y	51.0	
Normal	11.4 (1-36)	
8	12.0 (4-17)	
-7	28.7 (11-40)	
Complex (>3 abnormalities)	21.4 (3-93)	
Age		0.007
<73 years (n=23)	25.6 (2-101)	
≥73 years (n=21)	14.5 (1-59)	
Cycle duration		0.04
28 days (n=18)	12.4	
>28 days (n=26)	25.8	

WHO, World Health Organization; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; RS, ring sideroblasts; RARS, refractory anemia with ring sideroblasts; CMML, chronic myelomonocytic leukemia; AML, acute myeloid leukemia; IPSS, International Prognostic Scoring System.

Table IV. *Results and Safety data (adverse events and supportive treatment)*.

Adverse event description	Result
Clinical adverse event, N (%)	29/44 (65.9)
One or more neutropenic infections, N (%)	26/29 (89.7)
Bloodstream Infection, N (%)	9/26 (34.6)
Lower respiratory infection, N (%)	10/26 (38.5)
Neutropenic fever, N (%)	8/26 (30.1)
Septic shock, N (%)	2/26 (7.7)
Hemorrhagic events, N (%)	2/29 (6.7)
Cerebral hemorrhage (grade 5), N (%)	1/2 (50.0)
Epistaxis (grade 3), N (%)	1/2 (50.0)
Other (pyoderma gangrenosum), N (%)	1/29 (3.4)
Laboratory incidents, N (%)	44/44 (100)
Neutropenia (all grades[1]), N (%)	36/44 (81.8)
Neutropenia (grades 3/4), N (%)	34/44 (77.3)
Neutropenia duration (days/cycle), Median (range)	17.5 (5-30)
Anemia (all grades), N (%)	44/44 (100)
Anemia (grades 3/4), N (%)	24/44 (54.5)
Anemia requiring transfusion, N (%)	
Anemia duration (days/cycle), Median (range)	20 (6-30)
Thrombocytopenia (all grades), N (%)	31/44 (70.5)
Thrombocytopenia (grades 3/4), N (%)	21/44 (47.7)
Thrombocytopenia duration (days/cycle), Median (range)	15 (5-30)
Supportive treatment (during AZA administration)	
G-CSF administration, N (%)	16/44 (36.4)
G-CSF dose (mcg/cycle), Median (range)	3000 (1500-30000)
Erythropoietin administration, N (%)	7/44 (15.9)
Erythropoietin (darbepoetin) dose (μg/cycle), Median (range)	600 (150-600)
Red blood cell transfusions, N (%)	39/44 (88.6)
Red blood cell transfusions (units/cycle), Median (range)	3 (0-7)
Pooled random donor platelet transfusions, N (%)	17 (38.6)
Pooled random donor platelet transfusions (units/cycle), Median (range)	10 (3-30)

[1]Grading of all adverse events is based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. G-CSF, Granulocyte-colony stimulating factor.

daily for seven days, in cycles repeated every 28 days. The patients were classified according to the WHO classification of MDS, the IPSS and the karyotype risk. (3) Complete blood count parameters were recorded for all patients, before, during and after treatment with 5-azacytidine, as well as their transfusion dependence, and the administration of granulocyte colony-stimulating factor (G-CSF), erythropoietin and platelet transfusions.

We evaluated treatment efficacy in terms of response according to the modified IWG criteria, (12) the time to response and its duration, the overall survival and progression to AML, as well as the correlation of survival and progression to AML with the patients' baseline characteristics. We also recorded the adverse events reported by the patients and by the treating physicians during the follow-up period. Clinical adverse events and laboratory incidents and their grading according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0 (13) were recorded and analyzed.

IBM SPSS statistics, version 19.0 (IBM Corporation, NY, NY, USA) was used for the statistical analysis of the results. All the correlations of survival to categorical variables were performed using the independent samples Kruskal–Wallis test.

Results

Forty-four consecutive patients were included in the study; 37 (84.1%) patients with higher-risk MDS and 7 (15.9%) patients with AML (with 20-30% marrow blasts) were treated with 5-

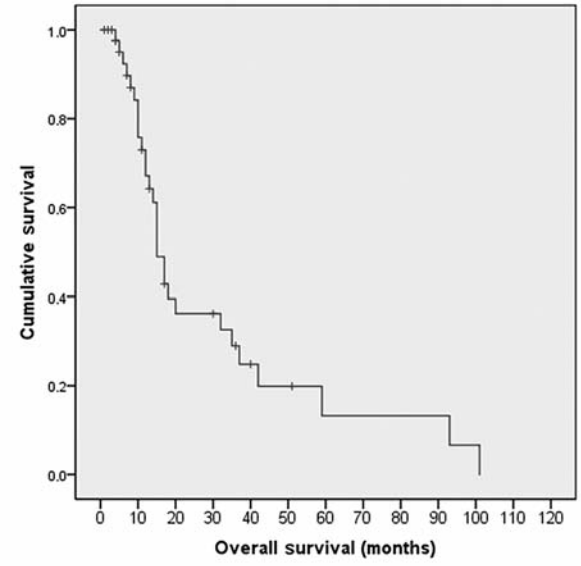

Figure 1. *Kaplan–Meier survival curve (all patients).*

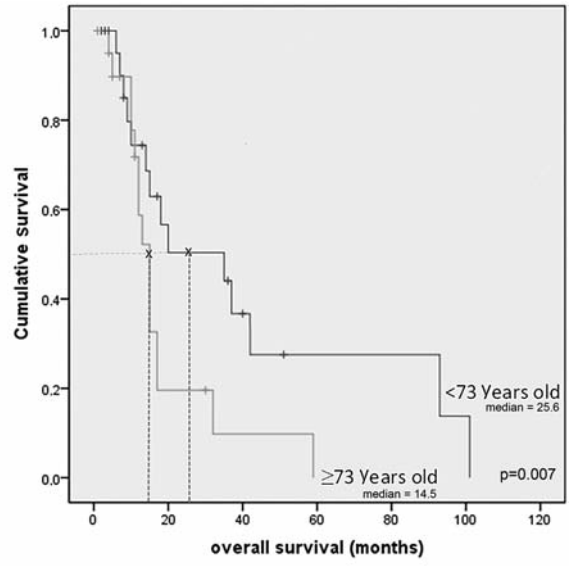

Figure 2. *Kaplan–Meier survival curve (younger vs. older patients).*

azacytidine between January 2009 and May 2014. Their epidemiological, clinical and hematological characteristics are shown in Table I. Among the MDS patients, 29 (78.4%) had an intermediate-2 (1.5-2.0) and 5 (13.5%) had a high (≥2.5) IPSS score. Moreover, 24/37 (54.5%) patients had a favorable cytogenetic profile, 4/37 (9.1%) an intermediate karyotype, and 9/37 (20.5%) had a poor karyotype (seven patients with a complex karyotype with ≥3 abnormalities, and three patients with chromosome 7 deletion).

The patients received a median of five cycles (range=1-22) of 5-azacytidine until the end of the follow-up period. The mean actual dose of 5-azacytidine administered to the patients was 75 (range=65-75) mg/m^2/cycle, and the median cycle duration was 28 (range=28-40) days. Six (13.6%) patients required a dose reduction during treatment due to sustained neutropenia and 26/44 (59.1%) had one or more temporary interruptions of their treatment due to sustained neutropenia and/or thrombocytopenia (n=10/26, 38.5%), neutropenic fever or other neutropenic infections (mostly pneumonia) (n=15/26, 57.5%) and hemorrhagic complications (n=1/26, 3.8%). The data are presented in detail in Table II. Thirty (68.2%) patients started with 5-azacytidine in an out-patient context, but 76.7% of them were hospitalized during treatment, especially during the first three treatment cycles, due to adverse events. The rest of the patients (31.8%) were hospitalized in order to receive treatment, mainly due to comorbidities and complications of MDS.

Response to treatment. Best response to treatment according to the modified IWG criteria was achieved after a median number of four cycles (1-7). Interestingly, 5/44 (11.4%) patients achieved an initial response after the sixth cycle and 1/44 (2.3%) after the seventh. Seven (15.9%) patients achieved a complete response (CR), 8 (18.2%) a partial response (PR) and 29 (65.9%) stable disease (SD). No primary treatment failures were noted.

Overall survival. The median overall survival of the patients (Table II) was 13 (range=1-101) months, while, 12 (27.3%) patients had a survival that exceeded 18 months. It should be noted that even patients that achieved a CR (7/44, 15.9%) had a median survival of 15 (5-51) months, comparable to that of patients achieving a PR or even those with SD (p=0.96). The cause of death was an infection in 24/29 (82.8%), hemorrhage in 3/29 (10.3%) and cardiac dysrhythmia in 2/29 (6.9%). The Kaplan–Meier survival curve is presented in Figure 1.

Interestingly, survival of younger patients was much higher than that of older patients as outlined in Table III. Patients younger than 73 years old had a mean overall survival of 25.6 months, while those aged 73 years old or more had a mean overall survival of 14.5 months (two-sided test, p=0.007). The comparative Kaplan–Meier survival curves are presented in Figure 2. The same applies for the survival of the patients since the onset of treatment with 5-azacytidine (14 months *vs.* 9.5 months, two-sided test, p=0.007). Even so, advanced age was not correlated to higher rate of progression to AML (independent Mann–Whitney *U*-test, two-sided test, p=0.43). The survival rates for each group of patients according to the WHO classification, the IPSS, the karyotype and the karyotype risk groups are detailed in Table III. No correlation

was noted between the survival and the baseline blood count parameters (hemoglobin, white blood cell and platelet count). Among patients achieving a CR or a PR, quick-responders (response noted on cycle 3 or earlier) did not have a longer survival compared to slow-responders (20.6 *vs*. 23.6, two-sided test, p=0.86). Moreover, pre-treatment transfusion dependence did not affect overall survival, nor did the use of G-CSF and erythropoietin administration during or before treatment, or dose reductions of 5-azacytidine during treatment. On the contrary, the duration of the cycles was correlated with survival in an intriguing way. Patients that received treatment at longer intervals than those recommended (*i.e*. >28 days), due to adverse events (mainly severe infections), had a longer overall survival than patients that faithfully followed the program (25.8 *vs*. 12.4 months, two-sided test, p=0.04). To our knowledge, such correlation has never been reported in clinical trials or smaller cohorts of patients treated with 5-azacytidine.

Renal or liver function impairment did not seem to affect survival, although the number of patients with these conditions was too small to allow further correlations.

Transfusion dependence. The transfusion dependence of this cohort of patients was significantly reduced after 5-azacytidine treatment. There was a 11.3% reduction in the number of the transfusion-dependent patients after 5-azacytidine treatment, but, most importantly, fewer transfusions were required for the management of these patients after 5-azacytidine treatment [median=1 transfusion per month (range=0-5) *vs*. 3 (0-7), paired samples two-sided t-test, p=0.0001). It should be noted, however, that the transfusion needs were significant during the first cycles of treatment. The reduction of the transfusion dependence of the patients was correlated to their response to treatment (independent samples Mann–Whitney *U*-test, two-sided test, p=0.048), as was expected.

Progression to AML. Progression to AML was noted in 21/37 (56.8%) patients and was not correlated to the presence of neutropenia (Pearson Chi-square two-sided test, p=0.312) or its grade (Pearson Chi-square two-sided test, p=0.555). Moreover, progression to AML was not correlated to G-CSF administration (Pearson Chi-square, two-sided test, p=0.69). There was a trend (p=0.14) for higher rate and faster progression to AML in high-risk patients compared to patients with intermediate 2 risk, as expected.

Safety assessment. Clinical adverse events were recorded in 29/44 (65.9%) and laboratory incidents in 44/44 (100%) patients (Table IV). Neutropenia during treatment occurred in 36/44 (81.8%) patients, but was attributed to the treatment (as evidenced by a decrease from baseline neutrophil count) in 31/44 (70.5%), and was grade 3/4 (according to the CTCAE, V. 4.0) in 34 (77.3%). Neutropenia was strongly correlated to clinical adverse events (Pearson Chi-square, two-sided test, p=0.002). Thrombocytopenia was experienced by 31/44 (70.5%) patients and grade 3/4 thrombocytopenia (according to the CTCAE) occurred in 21 of them (47.7%). Renal impairment was noted in six (13.6%) patients and led to a dose reduction in two of them, while liver function impairment was noted in three patients and was fully reversible. Metabolic or other commonly tested laboratory parameters were not significantly affected during treatment with 5-azacytidine, and only two serious non-hematological or infectious clinical adverse events were noted during the follow-up period (cerebral hemorrhage and pyoderma gangrenosum). It should be noted that although survival was adversely correlated to the age of the patients, there was no statistically significant difference in the clinical adverse events or laboratory incidence experienced by younger and older patients.

Anemia was the most common laboratory finding (44/44, 100%). A decrease from the baseline hemoglobin level was reported in 33/44 (75.0%) patients, and in 24/44 (54.5%) it was grade 3/4 according to the CTCAE.

Neutropenic infections were the most common and severe adverse events noted during the follow-up period. Twenty-six (59.1%) patients experienced a neutropenic infection (mostly bloodstream infection and pneumonia), and in 17 (38.6%) patients, this was a grade 5 adverse event. In fact, neutropenic infections were the cause of death in 24/29 (82.7%) patients, but this number refers both to patients actively treated and patients that had progressed to AML.

Supportive treatment was needed in the majority of patients. G-CSF was administered in 16/44 (36.4%) patients. The vast majority of patients (39/44, 88.6%) had to be supported with red blood cell transfusions during treatment. Platelet transfusions were needed in 17/44 (38.6%) patients during treatment.

Discussion

The management of patients with higher-risk MDS is problematic due mainly to the advanced age of the patients, frequent comorbidities, lack of an effective and safe treatment, and the high frequency of adverse events. The establishment of hypomethylating agents as first-line treatment for patients with higher risk MDS, and AML with 20-30% blasts, for which allogeneic SCT is not a feasible option, has enriched our choices in the treatment of this difficult-to-treat patient group. Initial clinical trials (2-4) have proven a survival benefit and an improved safety profile, with toxicities manageable even in an outpatient context with the use of 5-azacytidine.

In our cohort of patients, the overall survival did not differ from that reported in large patient series treated with conventional treatments, (9) although it should be noted that almost a third (15/44, 34.1%) of the patients were still alive

and under 5-azacytidine treatment during the last follow up visit, a fact that may underestimate the median overall survival of the group.

A significant finding in this cohort of patients is that no primary treatment failures were noted. All patients had at least SD, but only 15.9% of them achieved a CR. There are two points that should be further analyzed. Firstly, younger patients did much better when treated with 5-azacytidine than did older patients. This observation is expected, given the frailty and comorbidities of older patients. Nevertheless, there was no difference in the rate and grading of anemia, thrombocytopenia and neutropenia, nor in the rate of clinical adverse events between younger and older patients. It is highly probable that older patients are unable to handle these adverse incidents as efficiently as younger patients do.

Another interesting point that should be analyzed is the fact that patients that actually adhere to the program had a shorter OS. Indeed, according to our data, patients that did not have any interruptions in their scheduled doses of 5-azacytidine had a shorter OS than those that had one or more interruption in their administration schedule. This is an observation that is very hard to interpret due to the multiple factors that may contribute to this outcome. Nevertheless, one could guess that a delay in the scheduled dose might provide enough time for the bone marrow to recover from the toxicity of 5-azacytidine, or that a patient with an infection that was considered as treated, may have relapsed after another incident of neutropenia, due to scheduled rechallenge with 5-azacytidine. These assumptions cannot be further investigated due to a lack of more detailed data about the severity and the duration of infectious complications in our study group. Nevertheless, it should be taken into consideration in future clinical trials involving patients with MDS and AML.

Our experience suggests that treatment with 5-azacytidine is as effective as previously used treatments, (9) with generally predictable toxicities, although hospitalization is frequently inevitable. The most severe adverse events were neutropenic infections, primarily of the lower respiratory tract and bloodstream, that lead to significant morbidity and prolongation of inpatient treatment, and were the cause of death in most of the patients. Patients treated with 5-azacytidine, in most cases, have to be supported with red blood cell transfusions, and a considerable percentage of them has to be supported with platelet transfusions and G-CSF during treatment until hematological response is achieved. Nonetheless, after response is achieved, the need for red blood cell transfusions is remarkably decreased in transfusion-dependent patients ($p<0.0001$), and there is no need for G-CSF administration or platelet transfusion.

The administration of 5-azacytidine in an out-patient context, its manageable toxicities and the fact that it can be used safely enough in older patients, have contributed to the popularity of the drug. Although these features are very appealing, according to our experience, the administration of 5-azacytidine in an out-patient context is often not feasible due to the hematological toxicities of the treatment, which can be serious. Despite the attractive features of treatment with 5-azacytidine, the results of monotherapy need to be improved. Drug combinations with 5-azacytidine are already under study in order to improve treatment efficacy. The co-administration of hypomethylating agents with other chemotherapeutic or immunotherapeutic agents such as sorafenib, (14) cytarabine (15) and standard chemotherapeutic regimens (16) in AML, lenalidomide (17-18) in AML and 5q-syndrome, and valproic acid (19) in MDS, shows promising results for the development of more potent regimens.

Conflicts of Interest

Nothing to declare.

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