A phase II study of the efficacy and safety of an intensified schedule of azacytidine in intermediate-2 and high-risk patients with myelodysplastic syndromes: a study by the Groupe Francophone des Myelodysplasies (GFM)

Although Azacytidine improves survival over conventional treatments in higher-risk myelodysplastic syndromes (MDS),[1] median overall survival (OS) with Azacytidine is only about two years. Moreover, the marrow response rate remains low [complete remission (CR) + partial remission (PR) rate of about 30%], and further improvements are needed. Gene hypomethylation appears to be a major mechanism of action of hypomethylating agents (HMA) but, with the administration of Azacytidine every 28 days, global DNA methylation is only a transient phenomenon, which peaks 7-10 days after treatment exposure and returns gradually to baseline at the end of each cycle,[2] suggesting that increasing the number of treatment days could improve the results of this therapy. Because Azacytidine nucleosides need first to be incorporated into DNA during S-phase to induce hypomethylation, an intensified schedule of Azacytidine could theoretically be superior to the standard schedule.

A few clinical studies using intensified schedules of HMA have shown promising results in MDS. In particular, decitabine (DAC) given for ten consecutive days was associated with a high probability of response in poor risk acute myeloid leukemia (AML) and MDS with TP53 mutation, even if the duration of response was limited.[3] Another study using a 10-day regimen of Azacytidine (50 mg/m^2)[4] showed a CR+PR+ trilineage response rate of 32%, twice that observed in a previous study using standard dosing by the same group[4] (C9221 study). Finally, Lyons *et al.*[4] in lower-risk MDS, suggested the superiority of a 10-day schedule of Azacytidine compared to shorter schedules, especially in patients with thrombocytopenia.[5] Given the potentially greater myelosuppression with prolonged (10-day) administration of HMA, we chose to evaluate another intensified schedule of Azacytidine using a 5-day schedule repeated every 14 days for a few months, hoping to improve the response rate.

Patient inclusion criteria were: age 18-75 years with Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and no major comorbidities preventing administration of an intensified regimen of Azacytidine, with International Prognostic Scoring System (IPSS) int-2 or high-risk MDS or chronic myelomonocytic leukemia (CMML) with low white blood cell (WBC) count (<13x10^9/L) and marrow blasts above 10%, or AML with 20-30% marrow blasts, and who had received no prior treatment for their MDS/AML except erythropoiesis-stimulating agent could be included. Treatment consisted of Azacytidine 75 mg/m^2/d given for five days every 14 days for 4 cycles (Azacytidine-14, cycles 1-4). Patients achieving CR or PR then received 4 additional cycles of Azacytidine 75 mg/m^2/d for five days every 21 days (Azacytidine-21, cycles 5 to 8) followed by classical cycles of Azacytidine 75 mg/m^2/d for seven days every 28 days, to be continued until progression, relapse or toxicity arose. This schedule corresponded to a 20% increase in the number of days of Azacytidine during the first eight weeks of treatment. If patients did not obtain CR or PR after the initial 4 cycles of Azacytidine-14, 4 additional cycles of Azacytidine-14 were performed (cycles 5 to 8). If patients did not obtain CR, PR or hematologic improvement (HI) after 8 cycles of Azacytidine-14, they were taken off study. The primary end point was response after 4 and 8 cycles according to International Working Group 2006 criteria. The trial was approved by the Comité de Protection des Personnes Paris, Ile de France (the ethical committee whose approval is valid for all French participating institutions). The Groupe Francophone des Myélodysplasies was trial sponsor, and Celgene (Paris, France) provided a scientific grant, but was not involved in trial analysis or writing the manuscript.

Between July 2011 and June 2013, 27 patients were enrolled in the study; one of these was excluded for consent withdrawal (Table 1). The remaining 26 patients

Table 1. Main baseline characteristics of the patients included.

	N	%
Age [IQR]	66 [58.6;70]	
Gender		
Female	7	27%
Male	19	73%
WHO diagnosis		
RARS	1	4%
RCMD	2	%
RAEB-1	3	12%
RAEB-2	13	50%
CMML	2	8%
AML	5	19%
Cytogenetics		
Favorable	11	42%
Intermediate	6	23%
Poor	9	35%
IPSS group		
Int-2	17	65%
High	9	35%

IQR: interquartile range; WHO: World Health Organization; IPSS: International Prognostic Scoring System; RARS: refractory anemia with ring sideroblast; RCMD: refractory cytopenia with multilineage dysplasia; RAEB: refractory anemia with excess of blast; CMML: chronic myelomonocytic leukemia; AML: acute myeloid leukemia.

Table 2. Prognostic factors of overall survival (univariate analysis).

Variable	HR	95%CI	*P*
Age	1.15	(1.05;1.26)	0.002
Gender			
Male	1.00		0.37
Female	0.61	(0.2;1.83)	
IPSS			
Int-II	1.00		0.0012
High	3.47	(1.32;9.12)	
Karyotype			
Favorable	1.00		
Int/poor	1.82	(0.71;4.66)	0.21
WBC	1.63	(1.08;2.45)	0.019
Hemoglobin	2.55	(0.2 ;33.28)	0.48
Platelet	0.96	(0.91 ;1.01)	0.12
Bone marrow blast	1.08	(1.01 ;1.15)	0.03

HR: hazard ratio; CI: confidence interval; IPSS: International Prognostic Scoring System; WBC: white blood cell count.

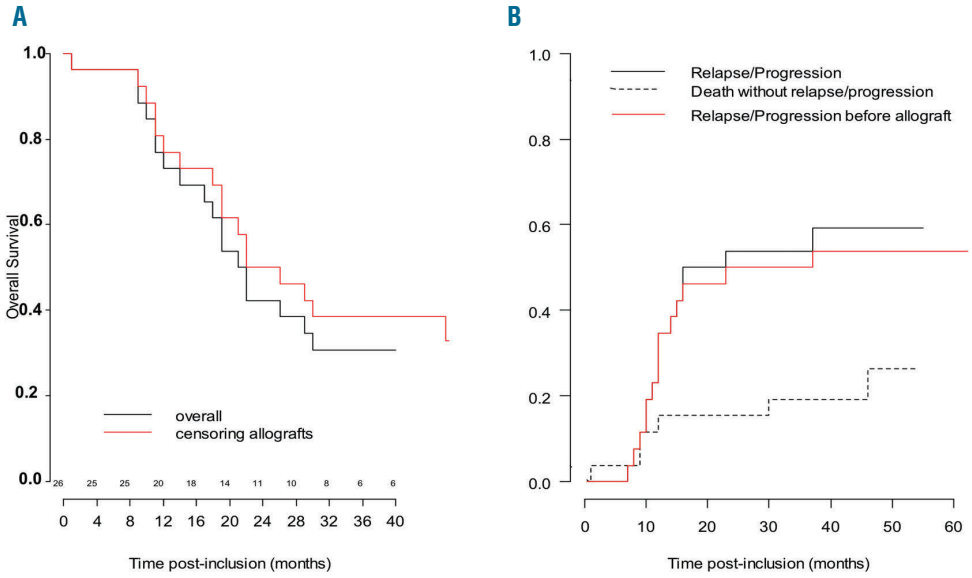

Figure 1. Outcome of patients treated with an intensified schedule of Azacytidine. Overall survival (A) and cumulative incidence of relapse or progression (B).

included 19 males, with a median age of 66 years [interquartile range (IQR): 58.5;70]. At inclusion, according to the World Health Organization 2008 classification, 13 patients had refractory anemia with excess blasts (RAEB)-2, 5 had AML (with 20-30% marrow blasts), 3 RAEB-1, 2 CMML, 2 refractory cytopenia with multilineage dysplasia (RCMD), and 1 refractory anemia with ring sideroblasts (RARS). Median baseline WBC count, platelet count and hemoglobin levels were 2.4 g/dL [1.705;5.125], 72.5 g/dL [43.5;177] and 9.8 g/dL [8.95;10.6], respectively. Median marrow blast was 13.5% [9.65;18]. Cytogenetics were favorable, intermediate and poor in 11 patients, 6 patients and 9 patients, respectively. Thus, according to IPSS classification, 9 patients were IPSS high and 17 were IPSS int-2. The median number of cycles administered was 12 [IQR 10;15], and 24 (92%) patients received at least 6 cycles. Cycle 2 was performed at a median of 14 days [IQR 14;14] after cycle 1 and had to be delayed beyond day (d)15 in only one patient. Cycles 3 and 4, scheduled on day (d)28 and d42, were slightly delayed in many patients and were administered at a median of d35 and d51 from Azacytidine onset. Two patients terminated the study after fewer than 4 cycles (1 and 3, respectively). The safety profile of the intensified schedule of Azacytidine was good, and only 1, 1, 2, and 3 patients required hospitalization during cycles 1, 2, 3, and 4, respectively, suggesting that myelosuppression was not increased compared to what we observed with a standard schedule of Azacytidine. The median number of RBC transfusions was 2 [0;2] and of platelet transfusions 0[0;1] during each of the first 4 cycles of Azacytidine, suggesting no additional hematologic toxicity compared to Azacytidine administration at European Medicines Agency/US Food and Drug Administration approved schedule. No other significant unexpected toxicity was observed in the study.

After 4 cycles, 5 (19%) patients had responded, including one CR and 4 PR. After 8 cycles, 3 had achieved CR and 3 PR, leading to an overall marrow response of 22% (6 out of 27). The median time to first and best response were 30 days (IQR 18.5 ;59.5) and 69 days (IQR 63; 126), respectively. When marrow CR and HI were taken into account, the response rate was 65% ((95%CI: 44 ; 83) after 4 cycles and 62% after 8 cycles.

With a median follow up of 41.6 months, the median duration of CR/PR was 10.5 months and the median duration of all responses was 14 months [95%CI: 10 ; not available (NA)]. Among the 26 patients enrolled, 6 received allogeneic stem cell transplant (3 being alive in CR at the time of the present analysis), and 3 patients received more than 20 cycles of treatment (21+, 38+ and 43+ cycles, respectively). Overall, 19 died during the follow up, including 17 from disease progression and 2 patients died in CR from graft-*versus*-host disease. Median OS was 21.5 months ((95%CI: 17; NA) and a 2-year cumulative incidence of disease progression was 54% (95%CI:34; 74). After censoring, in allografted patients at the time of transplant results were similar, with median OS still at 21.5 months, and 2-year progression rate of 50% (95%CI: 29.4; 67.6) (Figure 1).

By univariate analysis, no pretreatment factor was significantly associated with response to Azacytidine. Seven of the 9 patients with poor cytogenetics achieved a response (77%), including 2 (22%) CR and the 2 patients with chromosome 17p abnormalities who responded. Prognostic factors associated with shorter OS were a higher WBC count (P=0.019), higher marrow blast % (P=0.030), High IPSS score (*vs.* INT-2) (P=0.012) and older age (P=0.002), while cytogenetics had no significant influence (Table 2). In a multivariate Cox model, only age remained of prognostic value for survival.

This study shows the feasibility of an intensified schedule of Azacytidine in selected patients with high-risk MDS. Our response rate of 62% appeared somewhat higher to that observed in our previous studies using conventional dose Azacytidine, even if the number of patients was limited. Recently, prolonged administration of DAC was associated with a high response rate in patients with poor risk cytogenetics and those with *TP53* mutation.[3] The *TP53* mutation status was not assessed in the present study, but both of the 2 patients with

del(17p) (strongly correlated with *TP53* mutation in MDS/AML with complex karyotype) responded.

Our intensified Azacytidine regimen was not associated with increased hematologic toxicity, consistent with a study comparing, in low-risk MDS, a 7-day schedule to a 10-day schedule of Azacytidine.[5] This may be in contrast with more intensive 10-day DAC cycles, frequently associated with severe hematologic toxicity (66% of infection events during the first 2 cycles). Nevertheless, in a recent meta-analysis comparing hematologic toxicity of DAC and Azacytidine, no significant difference was observed, suggesting a similar toxicity profile, at least using the standard schedule of both drugs.[6] Thus, whether the favorable toxicity profile observed in our series is linked to the Azacytidine schedule or patient selection remains to be determined.

In addition, response in the present study (1-2 months) appeared to occur earlier than usually reported with Azacytidine, and even shorter than the three months median time of response reported in the trial evaluating a 10-day schedule of 50 mg/m^2 of Azacytidine. Finally, the median overall survival was 21.5 months, one of the longest reported in high-risk MDS treated with HMA (but similar to the Azacytidine-001 study[1] using a standard dosing schedule), although the patient number was too small to allow any conclusions to be drawn. Despite its limitation given the small number of patients enrolled, the selection of patients able to receive an intensified schedule of Azacytidine, and the absence of randomized comparison with a conventional Azacytidine regimen arm, our results showed a promising and early response rate and an encouraging survival, suggesting that this schedule deserves to be further evaluated in a randomized trial.

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References

1. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3):223-232.
2. Braiteh F, Soriano AO, Garcia-Manero G, et al. Phase I study of epigenetic modulation with 5-azacytidine and valproic acid in patients with advanced cancers. Clin Cancer Res. 2008;14(19):6296-6301.
3. Welch JS, Petti AA, Miller CA, et al. TP53 and Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndromes. N Engl J Med. 2016;375(21):2023-2036.
4. Prebet T, Sun Z, Figueroa ME, et al. Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia Intergroup trial E1905. J Clin Oncol. 2014;32(12):1242-1248.
5. Lyons RM, Cosgriff TM, Modi SS, et al. Hematologic response to three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. J Clin Oncol. 2009;27(11):1850-1856.
6. Gao C, Wang J, Li Y, et al. Incidence and risk of hematologic toxicities with hypomethylating agents in the treatment of myelodysplastic syndromes and acute myeloid leukopenia: A systematic review and meta-analysis. Medicine (Baltimore). 2018;97(34):e11860.