Fludarabine, Cytarabine, and Granulocyte-Colony Stimulating Factor for the Treatment of High Risk Myelodysplastic Syndromes

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BACKGROUND. The prognosis of patients with high risk myelodysplastic syndromes (MDS) (i.e., refractory anemia with excess of blasts [RAEB] and refractory anemia with excess of blasts in transformation [RAEB-t]) usually is poor. The combination of fludarabine, cytarabine, and granulocyte-colony stimulating factor (G-CSF) (FLAG regimen) has been reported to be effective in patients with these diseases.
METHODS. Forty-two patients (32 with RAEB-t and 10 with RAEB) were treated with the FLAG regimen. The median age was 61 years (range, 27–74 years). Forty patients were diagnosed with primary MDS and 2 patients had treatment-related MDS. Induction therapy was comprised of the FLAG regimen, whereas consolidation therapy included idarubicin and cytarabine. Patients with a compatible donor and who were age $<$ 50 years were scheduled to undergo an allogeneic bone marrow transplantation (BMT), whereas for those patients without a donor and who were age $<$ 60 years autologous BMT with peripheral blood stem cells mobilized by the consolidation regimen plus G-CSF was planned.
RESULTS. Complete remission (CR) was achieved in 31 of 42 patients (74%; 95% confidence interval, 60–87%). Death during induction therapy occurred in 4 patients (9%) whereas 7 patients (17%) were resistant to the FLAG regimen. Toxicity from the consolidation regimen was negligible. All patients age $<$ 50 years and achieving CR were eligible for allogeneic BMT procedures, with early recurrence being the only reason for exclusion. The median overall survival and disease free survival were 13 months and 18 months, respectively. Patients with favorable cytogenetics had a significantly better outcome compared with those patients with an adverse karyotype.
CONCLUSIONS. The FLAG regimen is effective in patients with high risk MDS as well as in patients age $>$ 60 years. The toxicity of the regimen is low and the majority of patients are eligible to undergo allogeneic BMT procedures after induction/consolidation therapy. [See editorial on pages 1893–9, this issue.] ***Cancer* 1999;86: 2006–13.** © *1999 American Cancer Society.*

KEYWORDS: high risk myelodysplastic syndromes, fludarabine, cytarabine, granulocyte-colony stimulating factor (G-CSF), peripheral blood stem cell transplantation.

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterized by one or more cytopenias and dysfunctional blood cells.[1-3] According to the French–American–British (FAB) classification, leukemic MDS such as refractory anemia with excess of blasts (RAEB) and refractory anemia with excess of blasts in transformation (RAEB-t) are distinguishable from acute myeloid leukemia (AML) on the basis of the percent of bone marrow and peripheral blood blast cells.[4,5] Although it is widely accepted that the majority of AML patients require aggressive treatment aimed at

achieving complete remission (CR) and cure, patients with RAEB and RAEB-t still receive AML-like therapy less frequently than do AML patients, despite the fact that the prognosis of patients with high risk MDS usually is poor with a median survival of < 6 months.[6-8] In patients with adverse characteristics at diagnosis, supportive treatment appears to have limited impact on survival and low dose chemotherapy may be of benefit only in rare individuals while inducing a not negligible hematologic toxicity.[9] Therefore, in these patients, a sustained benefit can be achieved only through treatment aimed at eradicating the aberrant clone and restoring polyclonal hematopoiesis.[10] In addition, in MDS patients with no history of cytopenia who are treated intensively at the time of diagnosis, the FAB distinction between AML and RAEB or RAEB-t has emerged as having little therapeutic impact in terms of CR achievement and duration.[11,12]

The combination of fludarabine, cytarabine, and granulocyte-colony stimulating factor (G-CSF) (FLAG) has been reported to be effective in patients with high risk MDS as well as AML with acceptable toxicity.[13-17] The FLAG regimen is based on the synergistic action of fludarabine (FAMP) and cytarabine (ARA-C) by inducing a twofold increase in the intracellular concentration of the active ARA-C metabolite ARA-CTP.[18] Conversely, the addition of G-CSF also may potentiate ARA-C effects through the recruitment of blast cells in S-phase, as well as by enhancing ARA-C incorporation into DNA unrelated to the number of cells in S-phase.[19-21]

Encouraged by promising results obtained in pilot studies of patients with RAEB-t treated by FLAG,[22,23] we decided to evaluate the clinical efficacy and toxicity of the FLAG regimen extensively by accruing all RAEB and RAEB-t patients observed at our respective institutions who were considered eligible for aggressive chemotherapy.

PATIENTS AND METHODS

Between January 1995 and December 1997, 73 consecutive patients were diagnosed at our respective institutions as having RAEB or RAEB-t according to FAB criteria.[4] Forty-four untreated patients (60%) were considered to be eligible for aggressive chemotherapy by meeting the following criteria: age < 75 years, a performance status 0–2, no previous chemotherapy for MDS, serum bilirubin < 3 mg/dL, aspartate aminotransferase/alanine aminotransferase < 3n and serum creatinine < 2 mg/dL, no clinical sign of congestive heart failure, and no severe infectious complications. Twelve patients were excluded from the trial due age ≥ 75 years whereas 17 patients age < 75 years were not accrued because of congestive heart failure (9 patients), liver dysfunction (6 patients), and renal failure (2 patients). It is interesting to note that 27 of the 29 patients not eligible for the trial (93%) were age more than 60 years. Informed consent was obtained from 42 patients who actually were included in the trial. There were 23 males and 19 females with a median age of 61 years (range, 27–74 years). Thirty-two patients had RAEB-t and 10 had RAEB. Two patients had been treated previously with radiotherapy and chemotherapy for breast carcinoma, both with the cyclophosphamide, methotrexate, and 5-fluorouracil combination. The main clinical and hematologic characteristics of the current patient series at the time of diagnosis are summarized in Table 1. All patients underwent cytogenetic analysis; fully valuable banded metaphases were obtained in 35 of 42 patients (83%) and karyotypes were classified according to the International System of Human Cytogenetic Nomenclature.[24] Among those patients with valuable metaphases, 12 (34%) had a normal karyotype whereas 23 patients (66%) had different chromosomal abnormalities as detailed in Table 1. According to the International Prognostic Scoring System (IPSS) for MDS,[8] 36 patients were classified as high risk and 6 were classified as intermediate 2 risk.

The induction schedule was comprised of FAMP,

TABLE 1
Patient Characteristics

No.	42
Diagnosis	
RAEB	10 (24%)
RAEB-t	32 (76%)
Median age (yrs) (range)	61 (27–74)
<60	20 (48%)
≥60	22 (52%)
Gender (M/F)	23/19
Median interval between diagnosis and treatment (mos) (range)	0 (0–3)
de novo MDS	40 (95%)
t-MDS	2 (5%)
IPSS for MDS	
Int-2	6 (14%)
High	36 (86%)
Cytogenetics[a]	
Normal	12 (34%)
−7	3 (8%)
+8	2 (6%)
+5	1 (3%)
6p−	1 (3%)
del20q	1 (3%)
t(8;21)	1 (3%)
complex (≥3 abnormalities)	14 (40%)

RAEB: refractory anemia with excess of blasts; RAEB-t: refractory anemia with excess of blasts in transformation; M: male; F: female; MDS: myelodysplastic syndromes; t-MDS: therapy-related myelodysplasia; IPSS: International Prognostic Scoring System; Int-2: intermediate 2.
[a] Data refer to patients with evaluable metaphases (35 of 42; 83%).

30 mg/m^2, over 30 minutes intravenously (i.v.) at the same time daily from Day 1 to Day 5. Approximately 3.5 hours after completing each day's FAMP infusion, ARA-C, 2 g/m^2, was given i.v. over 4 hours. G-CSF was administered daily at a dose of 300 μg i.v. over 2 hours from Day 0 until a CR was achieved. A second course of FLAG was given in the case of partial remission (PR). Consolidation treatment included 1 course of idarubicin (IDA) at a dose of 10 mg/m^2 and ARA-C, 2 g/m^2, both i.v. on Days 1–2. After consolidation chemotherapy, patients age $<$ 50 years with a compatible sibling were scheduled to receive allogeneic bone marrow transplantation (BMT). For patients lacking a suitable donor and who were age $<$ 60 years, the consolidation regimen (IDA/ARA-C) followed by G-CSF from Day 3 until the day of last apheresis was used for mobilizing peripheral blood stem cells (PBSC) for autologous PBSC transplantation (APBSCT). A minimum of 2×10^6/kg of CD34 positive (CD34+ve) cells was required for APBSCT. No further treatment was given to older patients (i.e., those age $>$ 60 years). Antimicrobial prophylaxis was performed using oral ciprofloxacin. All patients received induction and consolidation chemotherapy in single or double rooms without laminar air flow.

CR was defined as a normocellular bone marrow with $<$ 5% blasts, normal blood count and differential, and the absence of extramedullary leukemia. PR was defined as bone marrow blasts between 5–10% and the absence of blast cells in peripheral blood. Patients who did not achieve at least a PR after induction therapy were taken off study. Resistance was defined either as induction therapy failing to induce significant bone marrow hypoplasia in patients surviving at least 15 days or as leukemic regrowth after the hypocellular phase.[25]

Cytogenetic response was investigated by evaluating the percentage of abnormal metaphases in patients with an abnormal karyotype who achieved a morphologic CR. Survival curves were generated according to the Kaplan–Meier method.[26] Differences between curves were evaluated by the log rank test whereas the influence of different parameters on CR achievement was calculated by the chi-square test. Toxicity was recorded according to the World Health Organization (WHO) criteria.

RESULTS

The great majority of the patients in the current study were treated very early after diagnosis, with the median interval between the time of diagnosis and FLAG delivery being 0 months (range, 0–3 months). Therapeutic results are summarized in Table 2. CR was achieved in 31 of 42 patients (74%; 95% confidence interval [95% CI]), 60–87%); 29 patients obtained a CR after 1 cycle of FLAG, whereas a second course was needed in 2 patients. It is interesting to note that a cytogenetic CR, defined as the complete disappearance of abnormal metaphases, was documented in 10 of 12 patients (83%) with an abnormal karyotype who attained a CR. The remaining 2 patients achieved a cytogenetic PR (i.e., a $>$ 50% reduction of cells bearing the previously documented chromosomal abnormality). Four patients (10%) died early during induction chemotherapy from infection (n = 3) and cerebral hemorrhage (n = 1). Seven patients (17%) were resistant to FLAG. The cytogenetic pattern was not related significantly to CR achievement, whereas age $>$ 60 years did appear to exert a borderline effect. In particular, by subdividing cytogenetic results into 3 groups according to the IPSS criteria for MDS, CR was obtained in 11 of 13 patients in the good prognostic group, 4 of 5 patients in the intermediate prognostic group, and 9 of 17 patients in the adverse karyotype group (P = 0.15). The CR rate was not statistically different even when the patients with good and intermediate cytogenetic patterns were grouped together and compared with the unfavorable subset (15 of 18 vs. 9 of 17; P = 0.11; 95% CI for the odds ratio [OR], 0.05–1.07) (table 2). According to age, a CR was achieved in 18 of 20 patients (90%) age $<$ 60 years and

TABLE 2
Therapeutic Results

CR (total)	31/42 (74%; 95% CI, 60–87%)	
1 cycle	29/31 (94%; 95% CI, 85–99%)	
2 cycles	2/31 (6%; 95% CI, 1–15%)	
CR (pat. with RAEB)	6/10 (60%; 95% CI, 30–90%)	$P = 0.46$[a]
CR (pat. with RAEB-t)	25/32 (78%; 95% CI, 64–92%)	
CR (pat. age <60 yrs):	18/20 (90%; 95% CI, 77–99%)	$P = 0.05$[b]
CR (pat. aged ≥60 yrs)	13/22 (59%; 95% CI, 39–79%)	
CR (good-intermediate cytogenetics[c])	15/18 (83%; 95% CI, 66–99%)	$P = 0.11$[d]
CR (poor cytogenetics[c])	9/17 (53%; 95% CI, 30–70%)	
Deaths in induction	4/42 (10%; 95% CI, 4–14%)	
Resistant:	7/42 (17%; 95% CI, 2–24%)	
Successful collection of CD34+ cells[e]	7/8 (88%)	
AUTO + ALLOBMT (performed/planned)	14/18 (78%)[f]	

CR: complete remission; 95% CI: 95% confidence interval; pat: patients; RAEB: refractory anemia with excess of blasts; RAEB-t: refractory anemia with excess of blasts in transformation; AUTO: autologous peripheral blood stem cell transplantation; ALLOBMT: allogenic peripheral blood stem cell transplantation.

[a] 95% confidence interval for odds ratio, 0.52–10.86.

[b] 95% confidence interval for odds ratio, 0.03–0.87.

[c] Prognostic significance of cytogenetics was assessed according to the International Prognostic Scoring System for myelodysplastic syndromes.[8]

[d] 95% confidence interval for odds ratio, 0.05–1.07.

[e] 2×10^6 kg at least.

[f] Three patients developed a disease recurrence before allogenic peripheral blood stem cell transplantation. At last follow-up one was on a waiting list for a transplantation procedure.

TABLE 3
Hematopoietic Regeneration and Supportive Treatment

Days for neutrophils $> 0.5 \times 10^9$/L (range)	19 (13–27)
Days for platelets $> 20 \times 10^9$/L (range)	20 (16–38)
Packed red blood cell units (range)	7 (0–13)
Platelet units (range)	8 (0–28)
Days of fever > 38° (range)	6 (0–12)
Days of i.v. antibiotics (range)	7 (0–21)
Days of hospitalization (range)	24 (13–39)

i.v.: intravenous.

TABLE 4
Hematopoietic Regeneration and Supportive Treatment According to Age

Age	<60 yrs	≥60 yrs	*P* value
Days for neutrophils $> 0.5 \times 10^9$/L	15	22	0.03
Days for platelets $> 20 \times 10^9$/L	18	24	0.04
Packed red blood cell units	5	11	0.002
Platelet units	6	12	0.009
Days of fever > 38°	5	7	0.19
Days of i.v. antibiotics	7	9	0.15
Days of hospitalization	20	29	0.003

i.v.: intravenous.

in 13 of 22 patients (59%) age ≥ 60 years ($P = 0.05$; 95% CI for OR, 0.03–0.87). Finally, the CR rate did not differ significantly according to the initial diagnosis (60% in RAEB patients vs. 78% in RAEB-t patients; $P = 0.46$; 95% CI for OR, 0.52–10.86) (Table 2).

After the achievement of a CR, all patients received the planned consolidation course. Hematopoietic regeneration and supportive treatment are shown in Table 3. The median time to achieve a sustained neutrophil count $> 0.5 \times 10^9$/L and a platelet count $> 20 \times 10^9$/L was 19 days (range, 13–33 days) and 20 days (range, 16–36 days), respectively. Patients received a median of 7 units of packed red blood cells (range, 0–16 units) and 8 units of platelets (range, 0–28 units). As indicated in Table 4, hematologic recovery was significantly longer in patients age > 60 years, who also required more intensive transfusion support as well as more prolonged hospitalization. However, although the period of neutropenia was shorter for younger patients, the number of days with fever > 38 °C did not differ significantly between the 2 age groups (Table 4). Extrahematologic toxicities that were > WHO Grade 2 (Table 5) were comprised of stomatitis (eight patients), increase in liver enzymes (three patients), increase in liver enzymes plus serum bilirubin (two patients), severe gastrointestinal bleeding (one patient), and diarrhea (four patients). The main toxicity was due to infections; five patients experienced pneumonia (one from pulmonary *aspergillosis*), accounting for the deaths of two patients; an additional patient died from gram- positive sepsis (*staphylococcus aureus*). Fever of unknown origin occurred in 27 patients whereas 9 patients did not experience fever. The median time spent in the hospital for induction chemotherapy was 24 days (range, 13–39 days). The toxicity due to consolidation was negligible apart from chemotherapy-related cytopenia.

TABLE 5
WHO Toxicity of > Grade 2 Due to Induction

Infections	6 (14%)
Bacterial	5
Fungal	1
Stomatitis	8 (19%)
Elevated liver enzymes	3 (7%)
Elevated serum bilirubin plus liver enzymes	2 (5%)
Diarrhea	4 (10%)
Gatrointestinal bleeding	1 (2%)

WHO: World Health Organization.

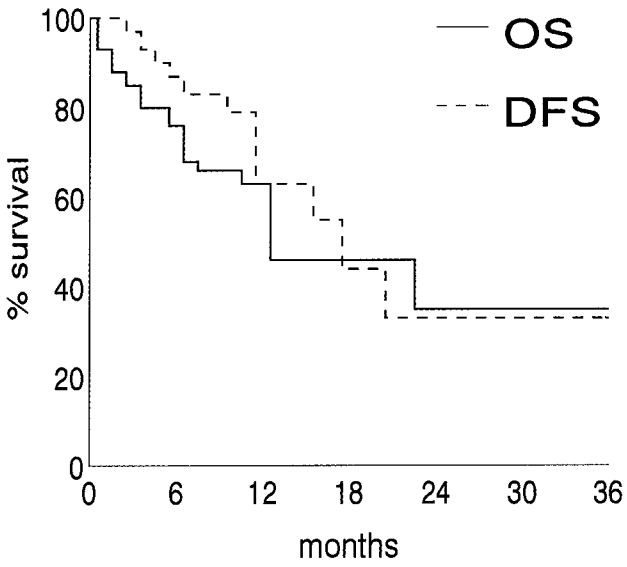

FIGURE 1. Overall survival (OS) and disease free survival (DFS) of the entire patient population (median, 13 months and 18 months, respectively).

After a median follow-up of 17 months, the median disease free survival (DFS) and overall survival (OS) were 18 months and 13 months, respectively (Fig. 1). To evaluate the prognostic impact of karyotype, patients with good and intermediate patterns according to the IPSS were grouped together and compared with those with poor cytogenetics. As shown in Figures 2 and 3, a favorable karyotype was significantly related to a better outcome in terms of both OS and DFS.

Among patients age < 60 years, there were 3 early

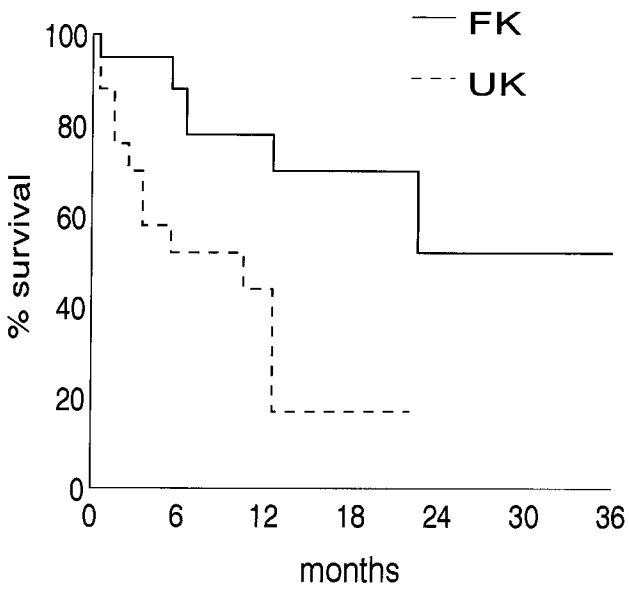

FIGURE 2. Overall survival according to karyotype ($P = 0.006$). FK: favorable karyotype (i.e., good and intermediate patterns according the International Prognostic Scoring System [IPSS] for myelodysplastic syndromes [MDS]); UK: unfavorable karyotype (i.e., poor pattern according the IPSS for MDS).

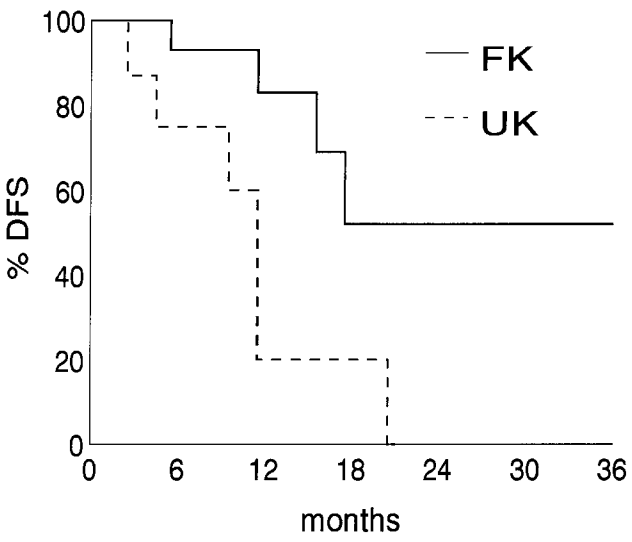

FIGURE 3. Disease free survival (DFS) according to karyotype. P = 0.01. FK: favorable karyotype (i.e., good and intermediate patterns according the International Prognostic Scoring System [IPSS] for myelodysplastic syndromes [MDS]); UK: unfavorable karyotype (i.e., poor pattern according the IPSS for MDS).

recurrences after 2 months, 4 months, and 5 months, respectively, from CR achievement while waiting for transplantation procedures. Two of these patients recurred with RAEB-t and one patient recurred with overt AML. All died within a few months with progressive disease despite the administration of further chemotherapy. Six patients underwent BMT whereas eight patients underwent autografting with PBSC successfully mobilized after consolidation plus G-CSF. The median number of CD34+ve cells collected was 4.1×10^6/kg (range, 2.1–7.8 $\times$ 10^6/kg). One patient who failed to achieve mobilization of CD34+ve cells underwent harvesting from bone marrow. At last follow-up one patient was on a waiting list for transplantation procedures. One patient age 27 years died from acute graft versus host disease after BMT and 2 patients developed disease recurrence after APBSCT at 10 months and 11 months from diagnosis, respectively. Among those patients age > 60 years there were 5 recurrences at 6 months, 8 months, 12 months, 12 months, and 21 months, respectively. Three patients recurred with RAEB-t and two patients recurred with overt AML. None of these patients received further chemotherapy; 3 patients died within 3 months whereas the remaining 2 patients were still alive and receiving supportive care at 6 months and 8 months, respectively, from the time of recurrence. Overall, 19 patients were in continuous CR at the time of last follow-up; it is interesting to note that 6 patients were in their first CR after a range of 12–24 months and 3 patients were in their first CR after a range of 24–36 months.

DISCUSSION

A number of studies have shown that > 50% of patients with high risk MDS may achieve CR after AML-like chemotherapy and/or bone marrow transplantation.[27-32] In addition, two recent surveys have suggested that CR rates and survival are no different between patients with AML and MDS when aggressive treatment is administered.[11,12]

In a pilot study on a small cohort of patients with RAEB-t, we demonstrated that the FLAG regimen induced CR in a high percentage of cases.[22,23] Encouraged by these results, we extended the treatment to all patients with high risk MDS who were considered eligible for aggressive therapy. It is interesting to note that the majority of patients in our series were in the high risk MDS group according to the IPSS for MDS.[8] Nevertheless, in this cohort of patients with adverse prognostic features and a median age of 61 years a CR rate of 74% was obtained, confirming the clinical efficacy of the FLAG regimen in a consistent proportion of patients with leukemic MDS. The synergistic effect of the combination of FAMP plus ARA-C in enhancing ARA-CTP intracellular concentration[18] as well as the potential effect of FAMP plus ARA-C on multiple drug-resistant positive cells[33] may account for the remarkable activity of FLAG in patients with MDS.

Although our CR rate was 90% for patients age < 60 years and 53% for patients age ≥ 60 years, the difference barely reached statistical significance ($P = 0.05$). Although this difference would be expected to become more significant in a larger cohort of patients, it should be taken into account that, as usually occurs with AML in the elderly,[34] there was relevant selection at diagnosis regarding the inclusion of older patients. In particular, the inclusion criteria of the current study did not allow treatment for patients age ≥ 75 years, who accounted for 16% of the current patient series. Therefore, at least for older individuals, it remains to be established whether FLAG, or any other AML-type chemotherapy, truly results in a definite advantage in survival compared with supportive care alone for unselected MDS patients. Nonetheless, the results of the current study confirm previous data from the M. D. Anderson Cancer Center regarding the possibility of achieving CR after the administration of FLAG in a consistent fraction of selected elderly individuals with high risk MDS.[13]

In our series, an adverse karyotype did not appear to influence the CR rate significantly. Accordingly, little impact of cytogenetics, with the exception of chromosome 7 abnormalities on the achievement of CR, has been observed in other larger studies of MDS.[35,36] Conversely, poor cytogenetics appears to exert a relevant influence on DFS and OS.[6-8] Analysis of data regarding survival in the current series of patients remains limited by the relatively short duration of follow-up. In addition, the number of patients enrolled into the trial does not allow a definitive evaluation of parameters significantly related to the duration of DFS and OS. However, patients with a favorable karyotype, as defined by grouping patients with IPSS good and intermediate patterns together, appeared to gain a substantial survival advantage from our treatment schedule whereas a poor outcome clearly was evident for patients with an adverse karyotype. For this patient category, the addition of anthracyclines to induction therapy does not appear to result in a substantial advantage in terms of CR achievement and duration.[14] An alternative approach could be increasing FLAG activity through the addition of investigational drugs with novel mechanisms of action, such as topoisomerase-I inhibitors.[37]

The overall toxicity of the regimen was mild, especially when considering that the median age of our patient population was 61 years. The major cause of early mortality was infection, whereas only one patient died from hemorrhagic complications. Hematologic recovery was fast with a median time to the achievement of sustained neutrophil and platelet counts of 19 days and 20 days, respectively. Obviously, the administration of G-CSF may have played a pivotal role in shortening the period of neutropenia in our patients. Although the role of hematopoietic growth factors remains unclear with regard to their impact on DFS and OS or in reducing infections in patients with AML, the majority of published studies demonstrate a significant advantage in the reduction of the duration of neutropenia and hospitalization.[38,39] It is interesting to note that although in our study the dose of G-CSF was lower than that originally employed in the M. D. Anderson Cancer Center schedule,[13] the time to granulocyte recovery was substantially similar. Finally, in spite of a significantly longer hematologic recovery in older patients, these individuals experienced a period of fever comparable to that of younger patients. This confirms that the FLAG regimen can be administered safely to a consistent proportion of elderly patients with leukemic MDS.

Nonhematologic toxicity was negligible, and was comprised mainly of mucositis and diarrhea occurring in a small minority of patients. Furthermore, toxicity virtually was absent after consolidation therapy. Accordingly, the only reason for which planned transplantations were not performed was early disease recurrence while patients were on the waiting list. The consolidation schedule (IDA/ARA-C) also was remarkably effective as a CD34+ve cells mobilization regimen; it is interesting to note that in the eight patients in whom APSCT was planned, PBSCs were collected successfully in seven patients. In this regard, our experience confirms previous data from Carella et al. indicating that it may be possible to harvest PBSCs successfully from patients with high risk MDS who are treated with aggressive chemotherapy.[40]

The results of the current study demonstrate that the FLAG regimen offers high rates of CR in patients with de novo MDS with acceptable toxicity. Treatment at diagnosis most likely results in a higher response rate and fast hematologic recovery. Finally, the low toxicity after induction and consolidation therapy allows transplantation opportunities in a high percentage of patients. Although our results, along with previously published experience,[13-15] support the clinical activity of the FLAG regimen in patients with MDS, the current study also indicates that a consistent proportion of patients is either excluded from treatments aimed at CR achievement and cure or develops an early disease recurrence. More innovative strategies including the development of new drugs and biologic approaches are warranted to improve what remain to be unsatisfactory results for a relevant fraction of patients with high risk MDS.

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