American Journal of Hematology 58:105–109 (1998)

Fludarabine, Cytarabine, and G-CSF (FLAG) for the Treatment of Poor Risk Acute Myeloid Leukemia

Marco Montillo,[1] Salvatore Mirto,[2] Maria Concetta Petti,[3] Roberto Latagliata,[3] Silvana Magrin,[2] Antonio Pinto,[4] Vittorina Zagonel,[4] Giuseppina Mele,[5] Alessandra Tedeschi,[1] and Felicetto Ferrara[5]*

[1]Clinica di Ematologia, Ospedale Torrette, Ancona, Italy
[2]Dipartimento di Ematologia, Ospedale Cervello, Palermo, Italy
[3]Cattedra di Ematologia, Universita' la Sapienza, Roma, Italy
[4]Unita' Leucemie, C.R.O., Aviano, Italy
[5]Divisione di Ematologia, Ospedale Cardarelli, Napoli, Italy

Thirty-eight patients with primary resistant or relapsing acute myeloid leukemia (AML) were treated with fludarabine, cytarabine and granulocyte colony-stimulating factor (FLAG). Median age was 41 (range 11–70). Sixteen patients had AML that was primary resistant to induction treatment, while 22 were relapsed, 11 after autologous bone marrow transplant (AuBMT), 8 less than 6 months from complete remission (CR) achievement, and 3 were second relapse from chemotherapy alone. Overall, 21 of 38 patients (55%) obtained CR. Age, sex, length of CR, and interval between autoBMT and FLAG administration did not significantly influence the CR rate. On the contrary, a normal karyotype at diagnosis was significantly related to a better outcome. There were 4 induction deaths (10%), due to fungal infection in 2 patients and hemorrhagic complications in the remaining two. All patients experienced profound cytopenia. Median time to neutrophil (>500/μl) recovery was 21 days, while a platelet count >20,000/μl was reached after 23 days. The median period of hospitalization was 31 days. The nonhematological toxicity was mild, mainly consisting of mucositis. There were 17 documented infections and 17 episodes of fever of unknown origin. Following CR achievement, 6 patients received autoBMT, 3 alloBMT, 2 high-dose arabinosil-cytosine, and 2 are on a waiting list for transplantation procedure. We conclude that FLAG is an effective and well-tolerated regimen for refractory or recurrent AML, mainly useful for patients to be admitted to bone marrow transplantation. Am. J. Hematol. 58:105–109, 1998.

Key words: acute myeloid leukemia; FLAG; resistant or relapsing patients

INTRODUCTION

Despite the improvements in the treatment of acute myeloid leukemia (AML), overall only 30 to 40% of adults aged less than 60 years are currently cured from their disease [1]. Treatment failures are related to either leukemic cells' resistance to cytotoxic chemotherapy or severe morbidity and mortality associated with induction or post-remission therapy. In particular, early relapsing or primary resistant AML is characterized by unsatisfactory response to current therapeutic approaches and short survival [2–5]. Hence, for these categories of patients, innovative strategies are needed. High or intermediate dose of arabinosil-cytosine (ARA-C), alone or in combination, has been reported as effective in the salvage treatment of AML [6–8]. The clinical efficacy of this therapeutic approach strictly depends on the higher intracellular concentration of the active metabolite ara-C 5′ triphosphate (ARA-CTP). Gandhi et al. demonstrated that the combination of fludarabine (FAMP) plus ARA-C results in a relevant increase of intracellular retention of ARA-CTP [9]. On the other hand, in vitro studies have shown that hematopoietic growth factors (HGF) may render previously dormant leukemic cells more sensitive

*Correspondence to: Dr. Felicetto Ferrara, Via Pigna 76 F/G, 80128 Napoli, Italy. E-mail: ferrara@itb.it

Received for publication 2 May 1997; Accepted 11 February 1998

to cytotoxic drugs by a mechanism of cycling [10–12]. In addition, HGFs significantly enhance (ARA-C) incorporation into DNA unrelated to the number of cells in S-phase [13]. Based on the above observations, Estey and coworkers developed a chemotherapy program called FLAG, that is a combination of FAMP, ARA-C, and G-CSF reported as effective and well tolerated in newly diagnosed as well as in resistant or relapsing AML patients [14,15]. In the present study, we report the clinical characteristics and treatment results of a series of 38 patients suffering from primary resistant or relapsing AML treated with the FLAG program.

MATERIALS AND METHODS

Between March 1994 and March 1996, after obtaining informed consent, 38 eligible patients received the FLAG protocol. Patients were treated with FAMP 30 mg/sqm administered at the same time daily for 5 consecutive days intravenously (iv) over 0.5 hours. Three and a half hours after completing each daily fludarabine infusion, 2 g/sqm of ARA-C were administered iv over 4 h. Recombinant human G-CSF (filgrastim or lenograstim) was administered at a dose of 5 μ/kg from day 0 (24 hr before starting chemotherapy) to polimorphonuclear recovery (more than 0.5×10^9/L). An additional cycle was programmed in the case of partial remission. As compared to the M.D. Anderson schedule, a lower dose of G-CSF (i.e., 5 μg/kg vs. 400 μg/sqm) was employed in the present study.

As shown in Table I, all patients had AML, 32 de novo and 6 secondary to MDS (3 to RAEB, 2 to RAEB-t, 1 to CMML). Diagnosis and classification were carried out according to FAB criteria [16]. Twenty-three were males and 15 females with a median age of 41 (range 11–70). Sixteen patients were considered as primary resistant; 13 had received the induction schedule of the current GIMEMA/EORTC AML10 clinical trial, while 3 (aged over 60 years) had been treated according to the AML13 protocol. Induction of the AML10 protocol consists of a 3+5+10 randomized schedule containing idarubicn (10 mg/sqm on days 1, 3, 5) or daunorubicin (45 mg/sqm on days 1, 3, 5) or mitoxantrone (10 mg/sqm on days 1, 3, 5) plus ARA-C (100 mg/sqm as continuous intravenous infusion from day 1 to 10) plus etoposide (100 mg/sqm from day 1 to 5), while the AML13 induction includes mitoxantrone (6 mg/sqm on days 1, 3, 5), ARA-C (100 mg/sqm as continuous intravenous infusion from day 1 to 7), and etoposide (100 mg/sqm from day 1 to 3). Resistance was defined either as induction therapy failing to induce significant bone marrow hypoplasia in patients surviving 15 days at least, or as leukemic regrowth following marrow aplasia [17]. Twenty-two patients were relapsed: 11 after AuBMT (9 in first CR, 2 in second CR), 8 were early relapses (less than 6 months from CR achievement) from the AML10 protocol while waiting for transplantation procedure, and finally 3 patients were in second relapse from chemotherapy alone. The median interval between AuBMT and relapse was of 11 months (range 3–29). In 28 patients (73.6%), karyotype was determined at diagnosis: 12 patients showed a normal karyotype, while 16 had various abnormalities. Of note, in patients with aberrant karyotype neither t(8;21) nor inv(16) were detected. Performance status was assessed according to World Health Organization (WHO) criteria: 2 patients were in PS 0, 21 in PS 1, 13 in PS 2, and 2 in PS 3. In order to be included in the FLAG program, patients had to present with a serum bilirubin level less than 2 mg/dl, creatinine less than 2 mg/dl, no evidence of cardiac dysfunction, and a PS between 0 and 3. CR was defined as less than 5% of blasts in a normocellular or hypercellular bone marrow with normal peripheral and differential count in the absence of extra-medullary leukemia lasting 2 months at least. Side effects were assessed by WHO criteria.

TABLE I. Characteristics of the Patients*

No.	38
Age	41 (11–70)
Sex (M/F)	23/15
Performance status (WHO)	
0	2
1	21
2	13
3	2
De novo AML	31
AML secondary to MDS	7
Disease status	
Primary resistant	16
Early relapse	8
Relapse from AuBMT	11
Second relapse	3
Karyotype[a]	
Normal	12
Aberrant[b]	16

*WHO, World Health Organization; AML, acute myeloid leukemia; BMT, bone marrow transplant.
[a]Successfully done in 28 patients (73.6%).
[b]No patient with t(8;21) or inv(16).

Survival curves were obtained by Kaplan-Meier method [17]. Patients achieving CR received a second identical consolidation treatment followed by an individualized program of post-remission therapy, depending mainly upon clinical status, age, matched donor availability, and previous exposure to cytotoxic drugs.

RESULTS

Of the 38 patients accrued, all received the five days of chemotherapy. The overall CR rate was 55.3% (21 out of

TABLE II. Response to FLAG by Characteristics of the Disease*

	CR (%)
Total	21/38 (55.3)
Primary resistant	7/16 (43.7)
Relapsing	14/22 (63.6)
Relapsing after AuBMT	6/11 (54.5)
Relapsing after chemotherapy[a]	8/11 (72.7)
Normal karyotype	9/12 (75)
Aberrant karyotype	4/16 (25)

*CR, complete remission; BMT, bone marrow transplant.
[a]Less than 6 months from CR achievement or second relapse.

TABLE III. Nonhematological Toxicity (WHO > 3)*

	No. (%)
Documented infections	17 (44)
Bacterial	13 (34)
Fungal	4 (10)
Fever of unknown origin	17 (44)
Mucositis	4 (10)
Diarrhea	3 (8)
Hepatic	3 (8)
Neurologic (lethargy)	1 (2)

*WHO, World Health Organization.

38). Details of response to FLAG according to status of disease are shown in Table II. Age, sex, length of CR, and interval between AuBMT and FLAG administration did not significantly influence the CR rate. On the contrary, a normal karyotype was signifcantly related to CR achievement (9 out of 12 or 75% for patients with normal karyotype vs. 4 out of 16 or 25% for patients with various karyotypic abnormalities, $P = .02$) as indicated in Table II. As far as the response rate to FLAG according to disease status, CR was obtained in 7 out of 16 primary resistant patients (43.7%), and in 14 out of 22 (63.6%) in relapsing patients. The difference was not statistically significant ($P = .37$ as evaluated by chi-square test). It is worthy of note that 6 out of 11 (54.5%) patients relapsing from AuBMT achieved CR, while the CR rate was of 72.7% (8 out of 11) in patients relapsing from chemotherapy alone; once again, the difference was not significant ($P = .65$). Among no responders, 4 patients died early in induction (10.5%), while 13 (29%) were resistant to FLAG. Two patients (both refractory to platelet transfusion) died from hemorrhagic complications (one from cerebral and one from gastrointestinal bleeding) and two from pulmonary aspergillosis. As concerns resistant patients, 11 had a phase of bone marrow hypocellularity followed by leukemic regrowth, while in two chemotherapy failed to induce bone marrow aplasia. All patients experienced profound granulocytopenia (less than 200/μl); median time to reach PMN > 500/μl and > 1,000μl was 21 and 23 days, respectively. Twenty-six and 28 days was the median time to achieve platelet levels >20,000/μl and 100,000/μl, respectively. In remitters, median time of G-CSF administration was 21 days (range 12–55). The median period of hospitalization was 31 days (range 17–61). Supportive treatment consisted of a median of 9 packed red cell units (range 0–23) and 10 platelet units (0–24). Thirty-four episodes of fever were documented during the period of neutropenia secondary to chemotherapy exposure and in 17 cases remained of unknown origin. Documented infections were 17, 13 bacterial and 4 fungal as detailed in Table III. Median number of days with febrile neutropenia was 6; 4 patients had no fever at all. The nonhematological toxicity was mild. The most common side effects were mucositis and diarrhea (Table III). One patient experienced severe neurologic toxicity (lethargy) that resolved spontaneously. Toxicity of the consolidation cycle was negligible in most cases; in particular there were no WHO >2 episodes of nonhematological toxicity.

Following CR achievement and consolidation, 6 patients received AuBMT, 3 alloBMT, 2 HD-ARA-C, 2 are waiting for a match unrelated donor, and 2 were treated with interleukin-2. Two patients relapsed 2 and 4 months after CR achievement and were refractory to regimens including high-dose ARA-C. Only 3 of 21 patients who obtained CR were considered unable to receive any further treatment, while one refused any type of post-consolidation therapy. Median survival of the whole patient's population was 9 months (Fig. 1) while the disease-free survival was 13 months (Fig. 2). Overall, 10 patients are at present in continuous CR after a median follow-up of 11 months.

DISCUSSION

Among AML patients there is a subset with a very low chance of obtaining long survival or cure [18]. In particular, patients refractory to or early relapsing from induction treatment, those relapsing from bone marrow transplantation or in second or subsequent relapse have a poor clinical outcome [1–5]. Previous studies have shown that the combination of FAMP plus ARA-C with G-CSF is highly effective and well tolerated in poor-risk AML patients as compared to other salvage schedules including high- or intermediate-dose-ARA-C [14,15]. We treated with the FLAG combination a cohort of 38 patients with poor-risk AML observed at 5 different institutions in Italy. Overall, 55% of patients achieved CR, confirming that the FLAG is an effective regimen for refractory or relapsed AML. FLAG showed to be highly effective in relapsing AML with a CR rate >60%. Favourable results were obtained both in patients relapsing early from chemotherapy and in those relapsing from AuBMT. These results are quite remarkable given the poor clinical outcome of patients in both these settings. A

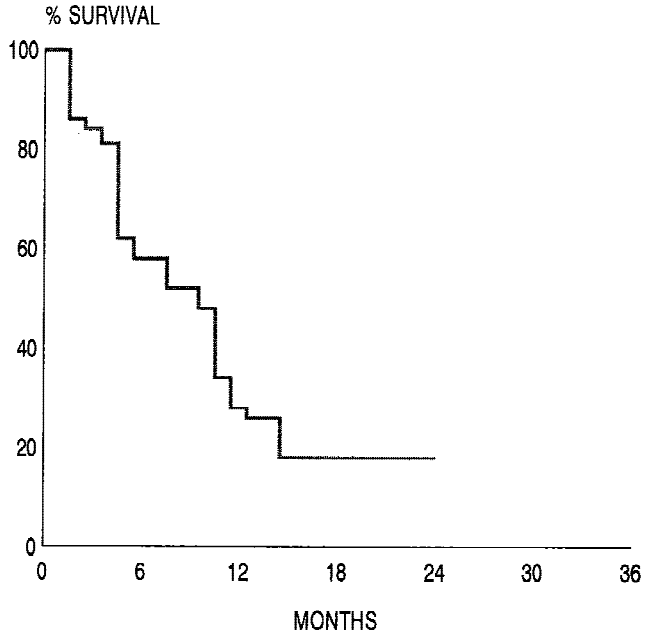

Fig. 1. Kaplan-Meier estimate of the overall survival of the whole patient population.

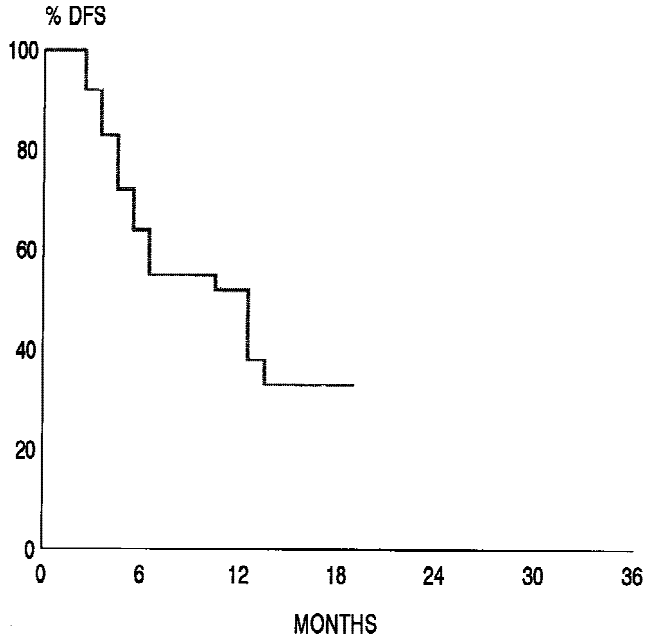

Fig. 2. Kaplan-Meier estimate of the disease-free survival.

lower CR rate was observed in patients primary resistant to induction. However, it should be considered that most of these patients had received the current AML 10 EORTC/GIMEMA protocol, which is an aggressive combination for AML. Poor results were also obtained in patients with karyotypic abnormalities at diagnosis, confirming the pivotal role of cytogenetics in predicting the clinical outcome in AML [19]. For patients with primary resistant AML as well as for those with adverse cytogenetic features at diagnosis, it may be reasonable to potentiate the FLAG with additional drugs such as idarubicin or mitoxantrone [20–22].

The toxicity of the regimen was mild considering that most patients had been heavily pretreated. The treatment-related mortality was 10% and only 2 patients (4%) died from infection (both fungal), while two deceased from hemorrhagic complications. In a recent study dealing with resistant or relapsing AML patients treated with FLAG, none of the 22 patients died of infectious complications [23]. However, in order to be included in the trial, patients had to be afebrile and free of infections; furthermore, there were no patients relapsing from AuBMT. In the study by Visani and coworkers (including 5 patients relapsing from AuBMT), only one patient out of 22 died of infection, confirming that the FLAG is associated with an extremely low rate of life threatening infections [24]. Similarly to Visani et al.'s study, we adopted a lower dose of G-CSF as compared to the M.D. Anderson group, obtaining comparable results in terms of CR rate and tolerability. Whether the prolonged administration of G-CSF has a role in protecting patients from infections remains unclear. In a not randomized study [25], Estey et al. compared two cohorts of AML/MDS patients treated with either FLAG or FA. There were no differences as to CR or infection rates; however most patients received treatment in laminar air flow rooms until granulocyte count reached 500 or 1,000 and this may have substantially accounted for these results; in addition, all patients had newly diagnosed AML. In our study, the situation was quite different because heavily pretreated patients were managed in single or double bedrooms; thus we cannot exclude that G-CSF may have played a substantial role in reducing fatal infections. Nonetheless, the potential benefit of growth factors in AML remains unclear because conflicting results have emerged from different trials [26–29].

Nonhematological toxicity was low, mainly consisting in mucositis and diarrhea. There was only one episode of lethargy, which resolved spontaneously. The low toxicity of the regimen allowed the administration of an aggressive post-remission treatment to 13 out of 20 patients (6 AuBMT, 3 allo-BMT, 2 allo-BMT from matched unrelated donor, and 2 high-dose ARA-C) and 2 patients early relapsing from FLAG were treated with high-dose ARA-C regimes. In conclusion, our results confirm that FLAG is a highly effective and well-tolerated regimen for poor-risk AML patients. Given the relatively low toxicity, in particular the low rate of fatal infections, the FLAG may be useful mainly for patients admitted for transplantation procedures.

REFERENCES

1. Stone RM, Mayer RJ: Acute myeloid leukemia in adults. In: Abeloff MD, Armitage JO, Lichter AS, Nirderhuber JE (eds): ''Clinical Oncology.'' Churchill-Livingstone, 1950–1976, 1995.
2. Keating MJ, Kantarjian H, Smith T, Estey E, Walters R, Andersson B, Beian M, McCredie K, Freireich E: Response to salvage therapy and survival after relapse in acute myelogenous leukemia. J Clin Oncol 7:1071–1080, 1989.
3. Davis CL, Rohatiner AZ, Lim J, Whelan JS, Oza AM, Amess J, Love S, Stead E, Lister TA: The management of recurrent acute myelogenous leukaemia at a single center over a fifteen-year period. Br J Haematol 83:404–411, 1993.
4. Hiddemann W, Martin WR, Sauerland CM: Definition of refractoriness against conventional chemotherapy in acute myeloid leukemia: a proposal based on the results of retreatment by thioguanine, cytosine arabinoside, and daunomycin (TAD 9) in 150 patients with relapse after standardized first line therapy. Leukemia 4:184–188, 1990.
5. Liso V, Iacopino P, Avvisati G, Petti MC, Broccia G, Carotenuto M, Falda M, Fazi P, Lazzarino M, Leoni P, Mirto S, Pucci G, Nobile F, Nosari AM, Specchia G, Stasi R, Tabilio A, Mandelli F: Outcome of patients with acute myeloid leukemia who failed to respond to a single course of first-line induction therapy: A GIMEMA study of 218 unselected consecutive patients. Leukemia 10:1443–1152.
6. Herzig RH, Lazarus HM, Wolff SN, Philips GL, Herzig GP: High-dose cytosine arabinoside therapy with and without anthracyclin antibiotics for remission reinduction of acute nonlymphoblastic leukemia. J Clin Oncol 3:992–997, 1985.
7. Hiddemann W, Kreutzmann H, Straif K, Ludwig WD, Mertelsmann R, Donhuijsen-Ant R, Lengfelder E, Arlin Z, Buchner T: High-dose cytosine arabinoside and mitoxantrone: A highly effective regimen in refractory acute myelogenous leukemia. Blood 69:744–749, 1987.
8. Amadori S, Arcese W, Isacchi G, Meloni G, Petti MC, Monarca B, Testi AM, Mandelli F: Mitoxantrone, etoposide, and intermediate dose-cytarabine: An effective and tolerable regimen for the treatment of refractory acute myeloid leukemia. J Clin Oncol 9:1210–1214, 1991.
9. Gandhi V, Estey E, Keating MJ, Plunkett W: Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 11:116–124, 1993.
10. Tafuri A, Andreef M: Kinetic rationale for cytokine induced recruitment of myeloblastic leukemia followed by cycle-specific chemotherapy in vitro. Leukemia 4:826–834, 1990.
11. Tafuri A, Lemoli RM, Chen R, Gulati SC, Clarkson BC, Andreef M: Combination of hematopoietic growth factors containing IL-3 induce acute myeloid leukemia cell sensitization to cycle specific and cycle non-specific drugs. Leukemia 8:749–757, 1994.
12. Van der Lely, De Witte T, Wessels J, Raymakers R, Muus P, Preijers F: In vitro response of blasts to IL-3, GM-CSF, and G-CSF is different for individual AML patients: Factors that stimulate leukemic clonogenic cells also enhance ARA-C sensitivity. Ann Hematol 68:225–232, 1994.
13. To Boekhorst PAW, Lowemberg B, Sonneveld P: Hematopoietic growth factor stimulation and cytarabine toxicity in vitro: Effects in untreated and relapsed or primary refractory acute myeloid leukemia cells. Leukemia 8:1480–1486, 1994.
14. Estey E, Plunkett W, Gandhi V, Rios MB, Kantarjian H, Keating MJ: Fludarabine and arabinosylcytosine therapy of refractory and relapsed acute myelogenous leukemia. Leuk Lymph 9:343–350, 1993.
15. Nokes TJC, Johnson S, Harvey, Goldstone AH: FLAG is a useful regimen for poor prognosis adult myeloid leukemias and myelodysplastic syndromes. Leuk Lymph 27:93–101, 1997.
16. Bennet JM, Catovski D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposed revised criteria for the classification of acute myeloid leukemia. Ann Int Med 103:620–625, 1985.
17. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481.
18. Estey E: Treatment of refractory leukemia. Leukemia 10 (Suppl 2):39–43, 1996.
19. Mròzek K, Heinonen K, de la Chapelle A, Bloomfield C: Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol 24:17–31, 1997.
20. Steinmetz HT, Staib A, Glasmacher A, Neufang I, Katay I, Diehl V, Wickramanayake PD: Phase II study of Idarubicin, Fludarabine, ARA-C, and G-CSF (IDA-FLAG) for treatment of refractory, relapsed or secondary acute myeloid leukemia. Br J Haematol 93(Suppl 2):219a, 1996.
21. Parker JE, Cullis JO, Mijovic A, Pagliuca A, Mufti GJ: Idarubicin, Fludarabine, cytarabine and G-CSF for the treatment of high risk myeloid malignancies. Blood 88(Suppl 1):454a, 1996.
22. Clavio M, Carrara P, Miglino M, Pierri I, Canepa L, Balleari E, Gatti AM, Cerri R, Celesti L, Vallebella E, Sessarago M, Patrone F, Ghio R, Gobbi M: High efficacy of fludarabine-containing therapy (FLAG-FLANG) in poor risk acute myeloid leukemia. Haematologica 81:512–519, 1996.
23. Huhmann IM, Watzke HH, Geissler K, Gisslinger H, Jager U, Knobl P, Pabinger I, Korninger L, Mannhalter C, Mitterbauer G, Schwarzinger I, Kahls P, Haas OA, Lechner K: FLAG (fludarabine, cytosine arabinoside, G-CSF) for refractory and relapsed acute myeloid leukemia. Ann Hematol 73:265–271, 1996.
24. Visani G, Tosi P, Zinzani PL, Manfroi S, Ottaviani E, Testoni N, Clavio M, Cenacchi A, Gamberi B, Carrara B, Gobbi M, Tura S: FLAG (fludarabine + high-dose cytarabine + G-CSF): An effective and tolerable protocol for the treatment of ''poor risk'' acute myeloid leukemias. Leukemia 8:1842–1846, 1994.
25. Estey E, Thall P, Andreeff M, Beran M, Kantarjian H, O'Brien S, Escudier S, Robertson LE, Koller C, Kornblau S, Pierce S, Freireich E, Deisseroth A, Keating M: Use of granulocyte colony stimulating factor before, during and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: Comparison with fludarabine plus cytarabine without granulocyte colony stimulating factor. J Clin Oncol 12:671–678, 1994.
26. Dombret H, Chastang C, Fenaux P, Reiffers J, Bordessoule D, Bouabdallah R, Mandelli F, Ferrant A, Auzanneau G, et al.: A controlled study of recombinant human granulocyte colony stimulating factor in elderly patients after treatment for acute myelogenous leukemia. New Engl J Med 332:1678–1683, 1995.
27. Rowe JM, Andersen JW, Mazza JJ, Bennett JM, Paietta E, Hayes FA, Oette D, Cassileth PA, Stadtmauer EA, Wiernik PH: A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (>55 to 70 years of age) with acute myelogenous leukemia: A study of the Eastern Cooperative Oncology Group. Blood 86:457–462, 1995.
28. Heil G, Hoelzer D, Sanz MA, Lechner K, Liu Y, Papa G, Noens L, Ho J, O'Brien C, Matcham J, Barge A: Results of a randomised, double-blind-placebo-controlled phase III study of filgratim in remission induction and early consolidation therapy for adults with de-novo acute myeloid leukemia. Blood 86:267a, 1995.
29. Lowemberg B, Boogaerts MA, Daenen SM, Verhoef GE, Hagebbeek A, Vellenga E, Ossenkoppele GJ, Huijgens PC, Verdonck LF, van der Lelie J, Wielenga JJ, Schouten HC, Gmur J, Gratwohl A, Hess U, Fey MF, van Putten WL: Value of different modalities of granulocyte-macrophage colony-stimulating factor applied during or after induction therapy of acute myeloid leukemia. J Clin Oncol 15:3496–3506, 1997.