original paper
Haematologica 1996; 81:513-520

HIGH EFFICACY OF FLUDARABINE-CONTAINING THERAPY (FLAG-FLANG) IN POOR RISK ACUTE MYELOID LEUKEMIA

Marino Clavio, Paola Carrara, Maurizio Miglino, Ivana Pierri, Letizia Canepa, Enrico Balleari,* Anna Maria Gatti,* Raffaella Cerri,° Lidia Celesti, Emanuela Vallebella, Mario Sessarego,# Franco Patrone,# Riccardo Ghio,* Eugenio Damasio,° Marco Gobbi

*Cattedre di Ematologia, *Patologia Medica 1, #Semeiotica Medica 2 DIMI e °Divisione di Ematologia 1, Azienda Ospedale S. Martino e Cliniche Universitarie convenzionate, Genoa, Italy*

ABSTRACT

Background. **Elderly patients with acute myeloid leukemia (AML), those refractory to induction chemotherapy and those with so-called secondary leukemia have unfavorable prognoses and require innovative therapeutic approaches. Fludarabine allows an increased accumulation of Ara-CTP in leukemic cells and inhibits DNA repair mechanisms; therefore its association with Ara-C and mitoxantrone results in a synergistic effect.**

Materials and Methods. **From May 1993 to February 1996, fludarabine-containing regimens (FLAG and FLANG) were employed as induction therapy in 51 high-risk AML patients. Diagnosis of AML in 22 patients was preceded by a myelodysplastic syndrome lasting more than six months; 8 of the 29 *de novo* AML cases (28%) were refractory to previous chemotherapy, 9 (31%) were treated for early relapse, 12 (41%) presented poor prognostic factors at diagnosis. The median age was 64 (range 33-76) years and the FAB subtypes were the following: M0 3, M1 5, M2 28, M4 7, M5 8. Forty-eight per cent of patients showed poor prognosis chromosomal abnormalities. FLAG (24 patients) consisted of both fludarabine 30 mg/sqm over 30 minutes followed 4 hours later by Ara-C 2 g/sqm over 4 hours (for 5 days) and G-CSF 300 µg/day administered 12 hours before fludarabine, for a total of 5 doses. FLANG (27 patients) had a shorter duration (3 days), reduced Ara-C dosage (1 g/sqm) and administration of mitoxantrone (10 mg/sqm) at the end of Ara-C infusion.**

Results. **Recovery of both neutrophils (PMN > 0.5×10^9/L) and platelets (Plt > 20×10^9/L) required a median of 16 days from the end of therapy. Overall, 30 patients (59%) achieved CR, 6 (11%) PR and 10 (20%) were refractory; 5 (10%) experienced early death (cerebral hemorrhage or infection). The length of complete response ranged from 2 to 26 months with a median follow-up of 8 months. *De novo* and secondary AML registered 62 and 54% CR rates, respectively. Eight out of 10 patients refractory to conventional schemes achieved CR (80%) but only 3 out of 10 treated for relapse obtained CR (30%).**

Conclusions. **FLAG and FLANG showed similar activity and toxicity while proving to be highly effective and relatively well-tolerated treatments for high-risk *de novo* AML. Secondary leukemias seemed to be responsive as well, but the presence of an unfavorable karyotype alteration lowered the response rate.**

Key words: fludarabine, cytosine arabinoside, mitoxantrone, secondary acute myeloid leukemia, cytogenetics

The results of AML treatment have improved in recent years thanks to the introduction of myeloablative therapy followed by autologous or allogeneic stem cell transplantation.[1] Moreover, even patients treated with intensive multidrug chemotherapy alone show long-term survival approaching 30% in selected series,[2,3] whereas the prognosis of poor

Correspondence: Prof. Marco Gobbi, Chair of Hematology DIMI, University of Genoa, viale Benedetto XV 6, 16132 Genoa, Italy. Tel. & Fax. international +39.10.3538953.

Acknowledgements: work supported in part by CNR 94.02713CT04, 95.02441.CT04 Roma and AIRC Milano. We wish to thank Mr. Paolo Canepa for his editorial assistance.
Received June 21, 1996; accepted August 30, 1996.

risk patients remains unfavorable. This latter group includes elderly patients (> 60 years), patients refractory to conventional induction chemotherapy, patients with poor prognosis chromosomal abnormalities and those with so-called secondary leukemias (i.e. AML evolving from myelodysplasia or after chemo-radiotherapy).[4] The generally accepted policy for most of these patients is supportive treatment alone. More intensive approaches are being explored in selected patients in the hope of producing long-lasting, complete remission.[5-8] Cytosine arabinoside (Ara-C) remains one of the most effective drugs in AML therapy.[2] Previous studies revealed a direct correlation between the outcome of AML patients treated with intermediate- or high-dose Ara-C and leukemic cell retention of Ara-CTP, which is the biologically active Ara-C metabolite.[9] Fludarabine (FLU), an adenosine nucleoside analogue used mainly for the treatment of chronic lymphoproliferative diseases[10] Recent studies have shown that this drug can induce apoptosis of freshly isolated chronic myeloid leukemia cells.[11] It is able to enhance Ara-CTP accumulation in leukemic blasts by inhibiting the enzyme ribonucleotide reductase and the subsequent increase of dCK activity.[12,13] Pharmacokinetic studies demonstrated a median twofold increment in Ara-CTP in almost every patient evaluated after a fludarabine infusion at a conventional dosage four hours before Ara-C administration.[13] Moreover, the inhibition by FLU and Ara-C of DNA repair mechanisms is a good reason for combining this therapy with DNA-damaging agents (i.e. mitoxantrone). The sequential administration of FLU, Ara-C and mitoxantrone proved to be effective in advanced chronic and acute myelogenous leukemias and, in addition, was found to increase the formation of topoisomerase II-DNA complexes induced by mitoxantrone in leukemic cells.[14] The combination FLU+Ara-C was initially explored in relapsed and refractory AML patients and then as first-line therapy in poor prognosis AML with unexpected results.[15] The complete remission rate (CR 41%) was particularly impressive in the latter group, which included patients with adverse karyotype with or without AML evolving from a myelodysplastic syndrome. In the latest published series, which extended this combination therapy to good prognosis patients, granulocyte colony-stimulating factor (G-CSF) was added to the combination of FLU, Ara-C and mitoxantrone beginning one day prior to therapy.[16] Thanks to its ability to recruit quiescent cells to S phase, G-CSF could make leukemic blasts more sensitive to cycle-specific drugs such as Ara-C.[17] Furthermore, recent studies regarding the quantitative evaluation of programmed cell death showed that FLU+Ara-C+G-CSF induce apoptosis to a greater extent than FLU+Ara-C.[18] On the basis of these encouraging results we evaluated the efficacy of combining fludarabine+Ara-C+G-CSF with or without mitoxantrone (FLANG, FLAG) in a group of AML patients that included secondary AML and poor risk primary AML.

Patients and Methods

Between May 1993 and February 1996, 51 patients with high-risk acute myeloid leukemia (AML) were treated with FLAG or FLANG schemes.

A myelodysplastic syndrome lasting more than 6 months preceded the diagnosis of AML in 22 patients, whereas 29 others had *de novo* disease. All secondary AML but three (one case treated in relapse and two resistant to previous therapy) received FLAG or FLANG as first-line therapy. Among the 29 primary AML patients, 8 were refractory to conventional protocols, 9 presented an early relapse (within 12 months from diagnosis) or a second relapse, while the remaining 12, who had received FLAG or FLANG at diagnosis, presented poor prognostic factors at diagnosis (advanced age and high leukocyte count). Diagnosis and classification of AML were carried out according to the FAB criteria.[19] Clinical and hematological features are summarized in Table 1.

Cytogenetic analysis was performed on 24h cultured BM cells according to standard procedures. At least 10 Q-banded metaphases were examined for each sample. Cytogenetic analysis was carried out at diagnosis in 46 patients and 22 (48%) abnormal karyotypes were found (Tables 1 and 3). Patients in complete or partial

Table 1. Clinical features of AML patients treated with FLAG-FLANG.

	All	*De novo*	*Secondary*
Treated patients	51	29 (57%)	22 (43%)
Median age (range)	64 (33 -76)	59 (33-76)	66 (41-75)
Male / female	26 / 25	14 / 15	12 / 10
WHO performance status			
0	5	2	3
1	23	15	8
2	17	6	11
3	6	6	–
FAB subtype			
M0	3	3	–
M1	5	4	1
M2	28	11	17
M4	7	4	3
M5	8	7	1
Disease status			
non pretreated	31 (61%)	12 (41%)	19 (86%)
relapsed	10 (20%)	9 (31%)	1 (4%)
refractory	10 (19%)	8 (28%)	2 (10%)
Karyotype			
abnormal	22 (48%)	11 (39%)	11 (55%)
normal	26 (52%)	17 (61%)	9 (45%)
Hematological parameters before therapy			
median WBC x 10^9/L	7.6 (0.9-310)	8.4 (0.9-310)	6.8 (1.4-120)
median Hb g/dL	9.4 (5.4-13.2)	10 (6.7-13.2)	9 (5.4-11)
median Plt x 10^9/L	30 (6-288)	49 (6-288)	25 (6-156)
Blasts (median)			
peripheral blood	32%	32%	40%
bone marrow	80%	80%	80%

remission also received cytogenetic follow-up.

Treatment

The FLAG regimen consisted of five days of treatment with a 30-minute infusion of fludarabine 30 mg/sqm/day followed four hours later by a 4-hour infusion of Ara-C 2 g/sqm/day. G-CSF 300 μg/day s.c. was administered 12 hours before starting fludarabine, continued for five days and then given again one week after the end of therapy until complete neutrophil recovery.

Patients treated with the FLANG regimen received three days of fludarabine 30 mg/sqm/day as above, followed four hours later by a 2-hour infusion of Ara-C 1 g/sqm/day and a 30-minute infusion of mitoxantrone 10 mg/sqm/day. Twelve hours prior to the fludarabine infusion G-CSF 300 μg/day s.c. was administered. This was continued for three days and then started again as in the FLAG regimen.

Twenty-four patients received the FLAG regimen until August 1994; thereafter the FLANG protocol was applied in 27 patients in order to reduce the treatment period. Patients with creatinine levels above 3 mg/dL and very severe impairment of heart and liver function were excluded .

We considered complete remission (CR) to be morphologically normal marrow with less than 5% blasts concomitant with normal peripheral and differential counts, including a neutrophil count greater than 1×10^9/L and a platelet count greater than 100×10^9/L, normalization of karyotype and normal physical findings for more than 2 months. Partial response (PR) was defined as an improvement in clinical status and hematological parameters lasting at least 3 months, with the persistence of marrow blasts (5-30%) or myelodysplastic features (i.e pseudo-Pelger, erythroblastic nuclear fragmentation, etc.). Patients who did not respond to induction therapy were considered treatment failures. Patients in PR after FLAG or FLANG received a second identical course of treatment and those in CR (after 1 or 2 courses) received a further identical consolidation cycle.

Statistical analysis was performed using the t-test and Kaplan-Meier survival curves were produced. We considered a two-tailed p value of less than 0.05 to be an indication of statistical significance.

Results

Forty-six out of 51 patients were evaluated for response. Five patients (10%) died during induction therapy of cerebral hemorrhage (3 cases), left ventricular failure (1 case) or infection (1 case). Overall, complete remission was achieved in 30 patients (59%) and partial response in 6 (11%). Ten patients showed resistant disease (20%). Table 2 summarizes the overall outcome, Table 3 the outcome of patients with altered karyotype, and Table 4 analytically reports the response rate in primary

Table 2. Treatment and outcome of AML patients treated with FLAG-FLANG.

	All	*De novo*	*Secondary*
Therapy:			
FLAG	24	9	15
FLANG	27	20	7
Hematological recovery (median)			
PMN>0.5 x 10^9/L	16 (10-39)	17 (11-21)	15 (10-39)
PMN>1 x 10^9/L	18 (14-60)	18 (13-26)	17 (14-60)
Plt >20 x 10^9/L	16 (8-56)	17 (8-43)	16 (9-56)
Plt >50 x 10^9/L	19 (14-58)	19 (12-56)	20 (14-56)
Transfusional need:			
RBC transfusion	8 (2-20)	8 (2-17)	11 (3-20)
PLT transfusion	8 (1-16)	7 (1 - 15)	9 (6 -12)
Days with T > 38°C	6 (0-18)	7 (0 - 18)	6 (1 - 18)
Complications:			
FUO	16	11	5
sepsis	10	2	8
bronchopneumonia	7	5	2
aspergillosis	4	4	–
cardiac	4	3	1
neurologic	3	2	1
acute renal failure	1	1	–
DIC	1	1	–
Responses:			
CR	30 (59%)	18 (62%)	12 (54%)
PR	6 (11%)	2 (7%)	4 (18%)
NR	10 (20%)	6 (21%)	4 (18%)
NE	5 (10%)	3 (10%)	2 (10%)
Relapsed	17	9	8
Alive	18	11	7
Dead	33	18	15
Causes of death:			
disease	27	14	13
hemorrh. stroke	3	2	1
acute left ventr fail.	1	1	–
lung cancer	1	1	–
infection	1	–	1
DFS (months)	8 (2-26)	9 (2-13)	9 (3-26)
Overall survival (months)	9 (2-27)	9 (2-19)	10 (2-27)

and secondary AML according to patient and disease features. *De novo* and secondary AML patients demonstrated 62 and 54% CR rates, respectively. Eight out of 10 patients refractory to conventional schemes achieved CR (80%). On the other hand, this result was achieved by only 3 out of 10 patients treated for relapse (30%). As already shown, almost all patients (17/20) treated for refractory or relapsed AML belonged to the *de novo* AML group.

Table 3. Outcome of AML patients with altered karyotype treated with FLAG-FLANG.

Disease	*Disease status*	*Karyotype*	*Response*
de novo AML	non pretreated	+13	NE
de novo AML	refractory	complex	NE
de novo AML	non pretreated	–7,+m1,+m2	CR
de novo AML	refractory	+8	CR
sec AML	non pretreated	+4, D.M.	CR
sec AML	non pretreated	6 p–	CR
de novo AML	non pretreated	+8	CR
sec AML	non pretreated	+8, +21	PR
de novo AML	non pretreated	20 q–	CR
sec AML	non pretreated	–5	PR
de novo AML	relapsed	+8	CR
sec AML	non pretreated	+9	PR
sec AML	non pretreated	–21	REF
de novo AML	non pretreated	8q–	REF
de novo AML	non pretreated	complex	CR
sec AML	non pretreated	5q–	CR
de novo AML	refractory	complex, D.M.	CR
sec AML	non pretreated	+8	CR
de novo AML	refractory	complex	CR
sec AML	non pretreated	complex	REF
sec AML	non pretreated	–7, +21	REF
sec AML	refractory	complex	CR

NE = not evaluable; REF = refractory; PR = partial response; CR = complete response; D.M.= double minutes; m1,m2 = markers.

The FAB subtype did not influence the outcome of *de novo* AML. Abnormal karyotype had a negative impact on the CR rate in secondary AML (45%) compared to the CR rate of patients with normal cytogenetic analysis (78%). By contrast, *de novo* AML with altered karyotype showed a better outcome (75% vs 53%). Considering the absence of favorable prognosis karyotypic alterations, the lower CR rate of the *de novo* AML with normal karyotype is most likely related to the fact that 9 out of 17 patients belonged to the unresponsive group of relapsed AML. The outcome of AML patients with abnormal karyotype is reported in Table 3. In secondary AML, patients over 60 years of age had a better outcome than the younger ones (64% and 38%, respectively); however, the difference is not statistically significant and is due to the clinical and biological features of the individuals < 60 years of age (one received FLAG in

Table 4. Characteristics and outcome of AML patients treated with FLAG-FLANG.

		Primary ANLL CR	(%)	p	*Secondary ANLL* CR	(%)	p
Age	< 60	8/13	(61)		3/8	(38)	
	> 60	10/16	(62)	n.s.	9/14	(64)	0.2
FAB	M0-M2	11/18	(61)		10/18	(55)	
	M4-M5	7/11	(64)	n.s.	2/4	(50)	n.s.
Disease status							
	first line	9/12	(75)		10/19	(53)	
	relapsed	3/9	(33)		0/1	–	
	refractory	6/8	(75)	n.s.	2/2	(100)	n.s.
Karyotype							
	abnormal	8/11	(73)		5/11	(45)	(+3 PR)
	normal	9/17	(53)	0.2	7/9	(78)	0.1
Therapy							
	FLAG	5/9	(55)		7/15	(47)	(+4 PR)
	FLANG	13/20	(65)	n.s.	5/7	(71)	n.s.

relapse after autologous stem cell transplantation and 7 showed unfavorable chromosomal abnormalities). In the *de novo* group, age did not influence the CR rate (62% and 61%, respectively).

Among patients with abnormal cytogenetic analysis at diagnosis, all complete responders showed a normalization of the karyotype, whereas secondary chromosomal changes disappeared in 4 out of 6 partial responders.

FLAG and FLANG seem to be equally effective in both primary and secondary AML.

At the time of analysis 13 patients were disease free with a median follow-up of 8 months (range 2-26 months) and 17 had relapsed (median CR length of 6 months). Median overall survival is 9 months (range 2-27). Thirty-three patients died and the cause of death in 27 of them was the recurrence of AML.

Figure 1 shows the survival of *de novo* and secondary AML patients who received FLAG and FLANG as first-line treatment.

The median time of granulocyte recovery (> 1 $\times 10^9$/L) was 16 days (range 10-39), and 50×10^9/L platelets were reached at a median of 19 days (range 14-58). No differences in hematological recovery were observed between the *de novo* and secondary AML groups. Further details are reported in Table 2.

During the neutropenic phase, 10 episodes of documented sepsis (*Klebsiella p., Staphylococcus a., Serratia m., Enterobacter c., Haemophilus i., Escherichia c., Corynebacterium, Pseudomonas a.*), 4 of pulmonary aspergillosis and 7 of pneumonitis of unknown etiology, together with 16 cases of fever of unknown origin were observed. Only one case of bronchopneumonia contributed to causing the death of a patient.

Minor signs and symptoms of cardiovascular (3 episodes of supraventricular arrhythmia) and central nervous system impairment were observed, but their relation to therapy is uncertain.

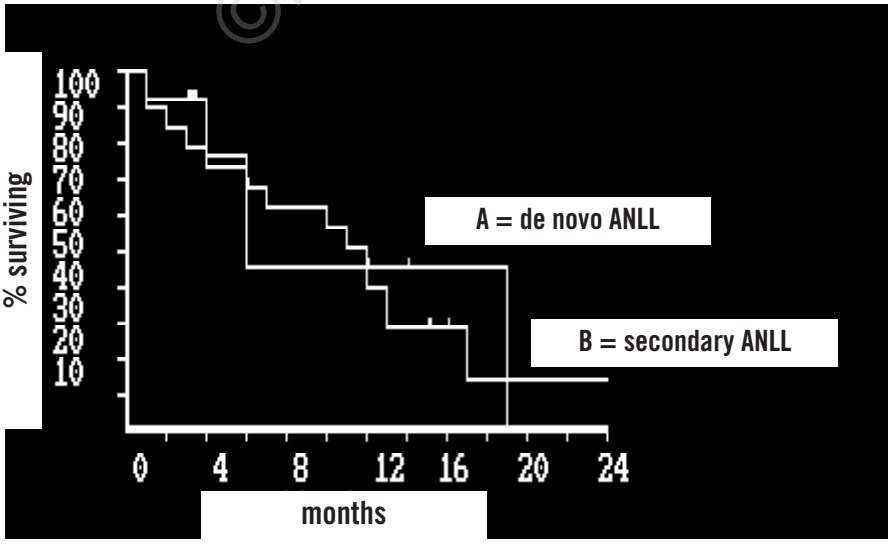

Figure 1. First-line treatment with FLAG-FLANG of *de novo* and secondary ANLL.

Discussion

On the basis of the encouraging results of recently published preliminary trials,[16,20-22] we tested the activity of FLU, intermediate-dose Ara-C and G-CSF with or without mitoxantrone in a cohort of secondary and high-risk *de novo* acute myeloid leukemias. The poor prognosis of our series is emphasized by the high median age (59% of patients were over 60 years of age)[23] and the high incidence of unfavorable karyotypic alterations (Tables 1 and 3). Our results confirm that a substantial percentage of patients in this group can achieve remission. Some issues of this study need to be discussed.

First, the overall complete remission rate (59%) is comparable to CR rates already reported. In particular, it is identical to that reported by Estey *et al.*,[16] who treated 197 patients affected by AML and MDS with fludarabine and Ara-C (FA) or the FLAG scheme. It is worth noting that in their study 112 patients were previously untreated, whereas in our study 40% of *de novo* AML were refractory to or had relapsed after conventional antileukemic treatment and patients with as yet unevolved myelodysplastic syndromes were excluded. The overall results compare favorably with other salvage protocols for refractory or relapsing AML, including Ara-C alone or combined with other drugs and with conventional chemotherapy for secondary AML.[24-26]

Second, patients with secondary AML achieved a CR rate which was comparable to that of high-risk *de novo* AML (54% and 62%, respectively; the difference is not statistically significant). This result appears to be worse than that reported by Visani *et al.*,[21] who obtained 89% in a small group of ten sAML patients. Noteworthy is the fact that in complete responders karyotypic alterations disappeared and marrow was morphologically normal. Moreover, the CR rate of this subgroup is identical to that recently reported by De Witte *et al.*,[27] who collected 50 patients under 60 years of age from 15 different institutions treated with 7-day continuous i.v. infusion of Ara-C and 3 doses of idarubicin. Therefore it appears that this association is at least as effective as the most widely used conventional treatment, even in poor prognosis leukemia.

Third, an unexpectedly high CR rate (75%) was observed in patients refractory to conventional therapy. This is in agreement with Visani *et al.*,[21] who reported 80% CR in patients unresponsive to one or two conventional cycles. However, as already observed,[21] poor results were seen in relapsed patients. It may be that the increased concentration of Ara-CTP and the impaired DNA repair caused by FLU are able to induce remission early in treatment, whereas they are not enough to overcome well-established resistance mechanisms in relapsed leukemic populations. This consideration could also apply to secondary AML with unfavorable cytogenetic aberrations.

Fourth, both FLAG and FLANG displayed low toxicity and only one death due to infection was observed. Moreover, hematological recovery time was in line with that reported in other trials using conventional therapy. Although ours was not a randomized trial, the two schemes appeared equally effective and tolerable, in both *de novo* and secondary AML. The infection rate, the transfusional need and the number of days of antibiotic infusion were also equivalent. It may be that the potential increase in efficacy due to the introduction of mitoxantrone is balanced by the reduction of the Ara-C dose. The inclusion of a third cytotoxic drug did not increase toxicity.

This approach to the treatment of poor prognosis AML is highly effective and well-tolerated, and allows the general cost of managing these patients to be contained by reducing the period of cytotoxic therapy administration.

The duration of remission goes beyond the aim of the study, which was designed to assess the possibility of increasing the efficacy of Ara-C in poor risk AML. However, bearing in mind that many of the patients we treated would have never been offered cytotoxic drugs, the results obtained are of some value.

Long-term survival remains an unresolved problem in the majority of AML patients, especially those with secondary leukemia and structural chromosomal abnormalities. Some patients in this subgroup reached partial response, clini-

cal and hematological parameters improved, and they were followed as outpatients without transfusional support for several months. In this group, therapy may have killed the most malignant clone of the overt leukemic phase but it was not able to eliminate cells belonging to the preexisting myelodysplastic clone. A typical example is that of a patient with a 5q- abnormality who, at progression to overt AML, showed a complex karyotype interpreted as 45,xy, –3, del(5)(q13q31),–7, del (7)(p15), add (17q), +marker. After FLANG therapy karyotype analysis revealed a return to the 5q– abnormality in all the metaphases examined.

It is not clear whether further cycles of chemotherapy might improve DFS and overall survival. Autologous or allogeneic stem cell transplantation[28] probably represents the best consolidation program for selected cases. Accordingly, an allogeneic bone marrow transplant was performed in three patients for whom an HLA-matched sibling was available. Despite the encouraging results published by Estey *et al.*,[16] the role of FLAG-FLANG in the treatment of MDS before the emergence of an overt leukemic phase must be better defined by randomized trials. Further studies must also assess the biology of remission. Some reports have suggested the persistence of clonal hemopoiesis after the induction of remission in secondary myeloid leukemias.[29] If this is true, FLAG-FLANG can probably cure the subclonal evolution of MDS and re-establish apparently normal hemopoiesis sustained by a myelodysplastic stem cell. If, on the other hand, polyclonal hemopoiesis is re-established then early treatment of MDS could have a greater possibility of eradicating the disease.

References

1. Zittoun RA, Mandelli F, Willemze R, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. N Engl J Med 1995; 332:217-23.
2. Mayer R, Davis R, Schiffer C, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med 1994; 331:896-903.
3. Mitus J, Miller K, Schenkein D, et al. Improved survival for patients with acute myelogenous leukemia. J Clin Oncol 1995; 13:560-9.
4. Rigolin GM, Fagioli F, Spanedda R, et al. Study of prognosis in acute myeloid leukemias (AML) by cluster analysis. Haematologica 1994; 79:233-40.
5. Gajewski JL, Ho WG, Nimer SD, et al. Efficacy of intensive chemotherapy for acute myelogenous leukemia associated with a preleukemic syndrome. J Clin Oncol 1989; 7:1637-45.
6. Anderson JE, Appelbaum FR, Fisher LD, et al. Allogeneic bone marrow transplantation for 93 patients with myelodysplastic syndrome. Blood 1993; 82:677-81.
7. De Witte T, Gratwohl A. Bone marrow transplantation for myelodysplastic syndrome and secondary leukaemias. Annotation. Br J Haematol 1993; 84:361-4.
8. Bassan R, Barbui T. Remission induction therapy for adults with acute myelogenous leukemia: towards the ICE age? Haematologica 1995; 80:82-90.
9. Plunkett W, Liliemark JO, Estey E, Keating MJ. Saturation of Ara-CTP accumulation during high-dose Ara-c therapy: pharmacologic rationale for intermediate-dose Ara-C. Semin Oncol 1987; 14(Suppl.1):159-66.
10. Spriano M, Clavio M, Carrara P, et al. Fludarabine in untreated and pretreated B CLL patients: a report on efficacy and toxicity: Haematologica 1994; 79:218-24.
11. Zinzani PL, Buzzi M, Farabegoli P, et al. Apoptosis induction with fludarabine on freshly isolated chronic myeloid leukemia cells. Haematologica 1994; 79:127-31.
12. Gandhi V, Plunkett W. Modulation of arabinosyl nucleoside metabolism by arabinosyl nucleotides in human leukemia cells. Cancer Res 1988; 48:329-34.
13. Gandhi V, Estey E, Keating MJ, et al. Fludarabine potentiates metabolism of arabynosylcytosine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 1993; 11:116-24.
14. Feldman E, Gandhi V, Plunkett W, et al. Sequential administration of fludarabine, ara-C and mitoxantrone enhances topoisomerase II-DNA complex formation and has efficacy in acute leukemia. Blood 1992; 80 (Suppl 1):208a.
15. Estey E, Plunkett W, Gandhi V, Rios MB, Kantarjian H, Keating MJ. Fludarabine and arabinosylcytosine therapy of refractory and relapsed acute myelogenous leukemia. Leuk Lymphoma 1993; 9:343-50.
16. Estey E, Thall P, Andreeff M, et al. 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 1994; 12:671-8.
17. Tafuri A, Andreef M. Kinetic rationale for cytokine induced recruitment of myeloblastic leukemia followed by cycle specific chemotherapy in vitro. Leukemia 1990; 4:826-34.
18. Tosi P, Visani G, Ottaviani E, Manfroi S, Zinzani PL, Tura S. Fludarabine+ARA-C+G-CSF: cytotoxic effect and induction of apoptosis on fresh acute myeloid leukemia cells. Leukemia 1994; 8:2076-82.
19. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of the acute leukemias: French-American-British (FAB) Cooperative Group. Br J Haematol 1976; 33:451-8.
20. Suki S, Kantarjian H, Gandhi V, et al. Fludarabine and cytosine arabinoside in the treatment of refractory or relapsed acute lymphocytic leukemia. Cancer 1993; 72:2155-60.
21. Visani G, Tosi P, Zinzani PL, et al. FLAG (Fludarabine+ high-dose cytarabine+G-CSF) an effective and tolerable protocol for the treatment of poor risk acute myeloid leukemias. Leukemia 1994; 8:1842-6.
22. Visani G, Tosi P, Zinzani PL, et al. FLAG (Fludarabine + cytosine arabinoside+G-CSF) induces complete remission in acute-phase chronic myeloid leukemia: a case report. Br J Haematol 1994; 86:394-6.

23. Resegotti L. The therapy of haematological malignancies in the elderly. Haematologica 1993; 78:141-4.
24. Tricot G, Boogaert A. The role of aggressive chemotherapy in the treatment of the myelodysplastic syndromes. Br J Haematol 1986; 63:477-83.
25. Preisler HD, Raza A, Barcos M, et al. High-dose cytosine arabinoside in the treatment of preleukemic disorders: a leukemia intergroup study. Am J Hematol 1986; 23:131-4.
26. De Witte T, Muus P, De Pauw B, Haanen C. Intensive antileukemia treatment of patients younger than 65 years with myelodysplastic syndromes and secondary acute myelogenous leukemia. Cancer 1990; 66:831-7.
27. De Witte T, Suciu S, Peetrmans M, et al. Intensive chemotherapy for poor prognosis myelodysplasia (MDS) and secondary acute myeloid leukemia (sAML) following MDS of more than 6 months duration. A pilot study by the *Leukemia Cooperative Group of the European Organization for Research and Treatment in Cancer (EORTC-LCG)*. Leukemia 1995; 9:1805-11.
28. Aglietta M, De Vincentiis A, Lanata L, et al. Peripheral blood stem cells in acute myeloid leukemia: biology and clinical applications. Haematologica 1996; 81:77-92.
29. Jowitt SN, Liu Yin JA, Saunders MJ, et al. Clonal remissions in acute myeloid leukemia are commonly associated with features of trilineage myelodysplasia during remission. Br J Hematol 1993; 85:698-705.