Fludarabine, cytosine arabinoside, granulocyte-colony stimulating factor with or without idarubicin in the treatment of high risk acute leukaemia or myelodysplastic syndromes

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Summary

The combination of fludarabine (FDR), high dose cytarabine and granulocyte colony stimulating factor (FLAG) with or without idarubicin (Ida) was used in the treatment of poor risk acute leukaemia or myelodysplastic syndrome (MDS) in a single centre experience. A total of 105 patients were treated over a 4-year period with 59% achieving a complete remission (CR); no statistical difference observed between FLAG and FLAG-Ida. For patients responding to FLAG ± Ida, the median event-free survival (EFS) was 11 months and 23% at 5 years. Such patients proceeded either to further chemotherapy or a haematopoietic stem cell transplant (HSCT). The median EFS (13 months vs. 8 months) and projected 5-year survival (37% vs. 13%) of patients undergoing HSCT was significantly better than those who did not ($P = 0.021$). In all, 14 of 72 patients remain alive in continuing CR (median duration 43 months) with 10 of 31 having had a HSCT vs. four of 41 that did not ($P = 0{\cdot}033$). Both regimens were well tolerated, with the majority of patients experiencing grade 1 or less non-haematological toxicity (mainly nausea and vomiting). The median time to neutrophil and platelet recovery was 28 and 31 d, respectively. No significant differences were seen with the addition of ida. There was a 17% incidence of treatment-related deaths, of which 39% was caused by invasive aspergillus infection. The results show that FLAG ± Ida is an effective and well-tolerated remission induction regimen for poor risk leukaemia and MDS.

Keywords: leukaemia, myelodysplastic syndrome, fludarabine, idarubicin, haematopoietic stem cell transplant.

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Intensive chemotherapy has significantly improved the prognosis of patients with newly diagnosed acute leukaemia. Nonetheless, a proportion of patients are refractory to first line therapy while others relapse, especially those with unfavourable cytogenetic karyotypes at diagnosis. In addition, other high risk haematological malignancies, such as advanced myelodysplastic syndromes (MDS) and chronic myeloid leukaemia (CML) in blastic transformation, similarly present difficult treatment problems. Improvements to current therapeutic strategies for such cases are needed.

The FLAG and FLAG-idarubicin (Ida) regimens, which combine fludarabine (FDR), high dose cytosine arabinoside (ara-C) and granulocyte colony stimulating factor (G-CSF) with or without Ida, have recently been used with encouraging results in poor risk acute myeloid leukaemia (AML), MDS and refractory or relapsed acute lymphoblastic leukaemia (ALL) (Estey *et al*, 1994; Visani *et al*, 1994; Clavio *et al*, 1996; Nokes *et al*, 1997; Parker *et al*, 1997; Deane *et al*, 1998; Fleischhack *et al*, 1998; Montillo *et al*, 1998; Ferrara *et al*, 1999; Steinmetz *et al*, 1999; Jackson *et al*, 2001). The toxicity of this combination regimen was also found to be low. The rationale for these regimens is the synergistic action between the various agents. FDR triphosphate, the active metabolite of FDR, inhibits ribonucleotide reductase with subsequent accumulation of intracellular ara-CTP (Gandhi and Plunkett, 1988; Gandhi *et al*, 1993). A positive correlation has been found

between intracellular ara-CTP levels and remission rates (Estey *et al*, 1990). G-CSF prior to FDR increases the fraction of cells in cycle when they are most vulnerable to ara-C and enhances the incorporation of ara-C into DNA (Tafuri and Andreeff, 1990; Tosi *et al*, 1994). Idarubicin is used because it was found to be less susceptible to multidrug resistance compared with other anthracyclines in human leukaemia cell lines (Ross *et al*, 1995).

We report here on a single centre experience in treating patients with poor risk acute leukaemia and MDS with the FLAG ± Ida regimen. This was a non-randomized retrospective study.

Patients and methods

Patient characteristics

Between April 1995 and April 1999, 105 consecutive patients with high risk acute leukaemia [refractory or relapsed AML, *de novo* AML with preceding MDS (MDS/AML), secondary MDS or AML, Philadelphia positive (Ph′+ve) ALL, blast crisis of CML] or high risk MDS (*de novo* or relapsed refractory anaemia with excess blasts in transformation (RAEB-t), high risk refractory anaemia with excess blasts (RAEB) – International Prognostic Scoring System (IPSS) ≥1·5 (Greenberg *et al*, 1997) were treated at a single centre with FLAG ± Ida. The median age was 35 years (range 5–81 years) with a male:female ratio of 1·7:1.

One-third of the patients with myeloid diseases had poor risk cytogenetics: defined as −7, 7q−, −5, 5q− with additional abnormalities, 11q23 abnormalities and complex karyotypes (≥4 abnormalities). A smaller proportion (14%) of AML had an immunophenotype indicative of a stem cell disorder with aberrant CD7 expression or biphenotypic with B-lineage markers. In addition, of the 61 patients with relapsed disease, 19 had early relapses (<6 months off treatment), 21 had multiple relapses (median 2, range 2–3); and 13 were refractory to a median of one course (range 1–3) of chemotherapy (various regimens). Ten patients had relapsed following haematopoietic stem cell transplant (HSCT).

Consent

Consent for treatment with the FLAG ± Ida regimen was obtained from all patients, verbally prior to 1997 and subsequently with written consent according to institutional guidelines.

Treatment received

FLAG treatment consisted of FDR 30 mg/m^2 intravenous (i.v.) infusion for 30 min on days 1–5, ara-C 2 g/m^2 i.v. infusion (started 4 h after the start of the FDR) for 2 h on days 1–5 and G-CSF (filgrastim) 300 μg s.c. injection or i.v. infusion for 30 min starting on the day prior to chemotherapy and continuing during postchemotherapy, to shorten the neutropenic period, until a neutrophil count of $>1 \times 10^9$/l was achieved. FLAG ± Ida treatment consisted of FLAG plus idarubicin 8 mg/m^2 i.v. infusion for 30 min on days 1–3.

Whether a patient received idarubicin was a clinical decision, based on patient age and disease type, and was decided by the senior clinician responsible for the patient, although the age limit for receiving idarubicin was ≤65 years. Therefore those treated with FLAG-Ida were predominantly younger with a median age of 28 years (range 5–65 years) in contrast to 57 years (range 8–81 years) ($P < 0{\cdot}001$), with primary refractory/early first relapsed acute leukaemia ($P < 0{\cdot}001$) and CML blast crisis. Those treated with FLAG had more *de novo* ($P = 0{\cdot}002$) or relapsed ($P = 0{\cdot}003$) high risk MDS or MDS/AML.

Patients received one to two courses of FLAG ± Ida as remission induction chemotherapy. Thirty-nine patients received a second course of FLAG ± Ida as either further remission induction or consolidation chemotherapy. Nineteen and 13 patients received various other standard chemotherapy regimens for their second and third courses, respectively, as consolidation chemotherapy. One patient with multiply relapsed ALL went on to maintenance-type therapy. Wherever possible and depending on donor availability, suitable age and performance status, responding patients proceeded to HSCT. Of the 72 patients who responded [complete remission (CR) or partial remission (PR)] to FLAG ± Ida, 31 proceeded to HSCT (in five of whom this was a second allogeneic HSCT). Of these, 15 proceeded to HSCT after one course of FLAG ± Ida, 14 after two courses and two patients after one course followed by mitozantrone and ara-C.

Transplant details

The donors were 14 matched sibling, three one-antigen mismatched related, five matched unrelated, six mismatched unrelated (five one-antigen and one two-antigen) and three autologous. The stem cell source was from 18 bone marrow and 13 peripheral blood stem cells. Conditioning and graft-versus-host disease prophylaxis was chosen according to local policy with regard to donor and recipient characteristics.

Supportive treatment

All patients were nursed in reverse barrier isolation with high efficiency particulate air (HEPA) filtration and received oral antimicrobial prophylaxis as follows: chlohexidine mouthwash 10 ml q.d.s.; ciprofloxacin 500 mg b.d. and colistin 1·5 MU b.d. (Prentice *et al*, 2001); fluconazole 100 mg o.d. and amphotericin suspension 5 ml q.d.s. or, from 1998 to 1999, itraconazole 200 mg b.d. and amphotericin suspension (Paterson *et al*, 2001); cotrimoxazole 960 mg b.i.d. three times a week and aciclovir 800 mg q.d.s.

Outcome analysis

CR was defined as a blast count <5% in a regenerating marrow of normal morphology, with normal cytogenetics (if abnormal

at diagnosis). In the case of CML blast crisis, CR was defined as a blast count <5% and the loss of any newly acquired cytogenetic abnormality (however the patient could remain Ph′+ve on cytogenetic analysis or *BCR/ABL* positive on molecular analysis). PR was defined as a blast count of 5–15% and/or the persistence of a cytogenetic abnormality. Refractory disease was defined as a blast count >15%. Bone marrow examination was performed for disease assessment on neutrophil recovery ($\geq 0{\cdot}5 \times 10^9$/l) unsupported by G-CSF or between 4 and 6 weeks post-treatment in the event of failure to recover counts. Events in event-free survival (EFS) were defined as relapse, disease progression in the case of PR or death in CR. Toxicity was graded according to the National Cancer Institute (NCI) common toxicity criteria (NCI, 1988).

Statistical analysis

Overall survival (OS) and EFS are presented in the form of Kaplan–Meier survival curves. Comparison between two survival curves was made using the log-rank test. Median analysis between two unpaired groups was made using the Mann–Whitney *U*-test. Comparison of binomial data from two unpaired groups was made using Fisher's exact test. $P < 0{\cdot}05$ was taken as significant.

Results

Response rates

Sixty-two patients (59%) achieved CR after treatment with FLAG ± Ida, 10 patients (9·5%) achieved PR and 24 patients (23%) had unresponsive disease. Nine patients (8·5%) were not assessable for efficacy because of death prior to disease assessment. CR rates for various diseases and their subgroups in Table I.

Response duration

The OS for the entire cohort of patients is shown in Fig 1, with a median survival of 8 months (range 1–67 months) and a projected 5-year OS of 15%. The EFS of the 72 patients responding (62 CR, 10 PR) to FLAG ± Ida is shown in Fig 2, with a median of 11 months (range 1–67 months) and a projected 5-year EFS of 23%. A comparison of those patients who responded and then either had an HSCT or did not is also shown in Fig 2. Those patients proceeding to an HSCT did significantly better than those who did not ($P = 0{\cdot}021$), with a median EFS of 13 months (range 4–67 months) and a projected 5-year EFS of 37% compared with 8 months (range 1–67 months) and 13%. Furthermore, 10 of 31 patients who had an HSCT remain alive in continuing CR, with a median duration of 41 months (range 22–67 months), compared with only four of 41 who did not have an HSCT, with a median duration of 48 months (range 37–67 months) ($P = 0{\cdot}033$).

Table I. Distribution of diseases, with individual CR rates, amongst the 105 patients treated with FLAG ± Ida over a 5-year period between April 1994 and April 1999.

Disease	Number of patients ($n = 105$)	CR rate
AML		
Primary refractory/early first relapse*	16	8/16 (50)
Relapsed†	14	11/14 (79)
MDS/AML		
Untreated	14	9/14 (64)
Primary refractory/early first relapse*	7	3/7 (43)
Relapsed†	15	9/15 (60)
ALL		
Untreated	1	0/1
Primary refractory/early relapse*	14	6/14 (43)
Relapsed†	12	8/12 (67)
BC CML	6	4/6 (67)
BC CML – primary refractory/first early relapse*	4	3/4 (75)
BC CML – relapsed†	2	1/2

Percentage values are given in parenthesis.
MDS/AML refers to either high risk MDS or AML with preceding MDS.
CR, complete remission; AML, acute myeloid leukaemia; MDS, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; BC CML, blast crisis chronic myeloid leukaemia.
*First relapse <6 months off treatment; †first relapse >6 months off treatment or second or more relapse.

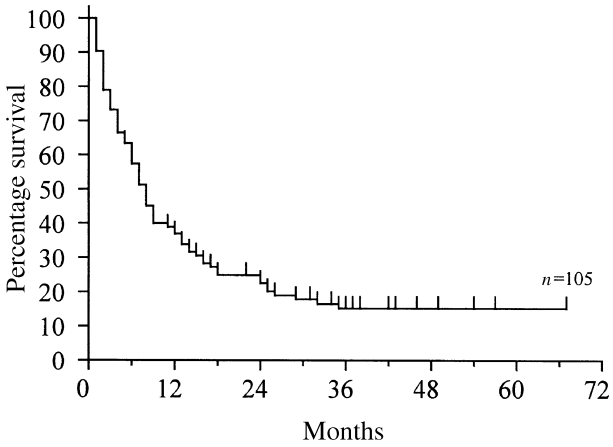

Fig 1. Overall survival of 105 patients with high risk acute leukaemia and myelodysplastic syndromes treated with FLAG ± Ida over a 5-year period between April 1994 and April 1999.

Toxicity

The median time for neutrophil recovery (neutrophil count $\geq 0{\cdot}5 \times 10^9$/l) following FLAG ± Ida was 28 d (range 17–64 d). The median time for platelet recovery (platelet count ≥20 ×

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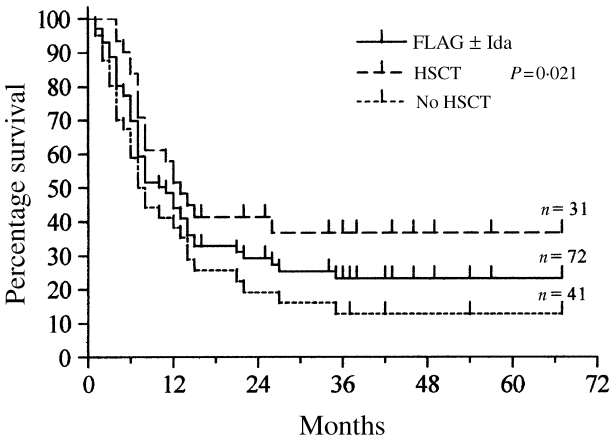

Fig 2. Event free survival of 72 patients with high risk acute leukaemia and myelodysplastic syndromes achieving a response over a 5-year period between April 1994 and April 1999. In addition, a comparison of the EFS of two subgroups of patients responding to FLAG ± Ida depending on whether they received a haemopoeitic stem cell transplant (HSCT) or not is also shown. The outcome of those proceeding to a HSCT was significantly better than those who did not ($P = 0{\cdot}021$).

10^9/l, unsupported) following FLAG ± Ida was 31 d (range 18–70 d).

There were 18 (17%) treatment-related deaths, defined as death resulting from a complication developing prior to recovery from FLAG ± Ida. The majority were related to infectious complications, with seven of 18 deaths (39%) caused by invasive aspergillus infection.

Full NCI common toxicity data was available for 83 patients and 115 of 144 courses of FLAG ± Ida (Table II). Overall FLAG ± Ida was well tolerated with the main toxicity being gastrointestinal, particularly nausea and vomiting. However, the majority of patients experienced minimal (grade 1) or no toxicity. There were only two significant neurological complications: one patient receiving FLAG-Ida had a seizure and one patient receiving FLAG developed vertigo that required medical treatment.

Infections

Infection data was available for 86 patients and 116 of 144 courses of FLAG ± Ida. A total of 105 courses (91%) were associated with one or more febrile episodes (defined as a fever ≥ 38·5°C or ≥ 38°C on two occasions separated by 1 h) with a total of 125 episodes analysed. The distribution of infections seen is given in Table III. Two specific instances should be noted. A high rate of invasive aspergillus infection (22% of patients where data was available) diagnosed by either tissue histology or computerized tomography ± microbiological evidence was seen, with significant mortality (37%). All but two (sinus and brain) were pulmonary aspergillus infections. In addition, cytomegalovirus (CMV) reactivation [polymerase chain reaction (PCR) positive on blood], one of which resulted in CMV disease (colitis), was seen in 5% of patients (where data was available), none of whom had undergone an HSCT.

Table II. Incidence of NCI common toxicity ≥ grade 2 associated with FLAG ± Ida treatment. (Data are from 83 of 105 patients and 115 of 144 courses of chemotherapy.)

Toxicity	Incidence ($n = 115$)
Nausea	32 (28)
Vomiting	15 (13)
Diarrhoea	5 (4)
Mucositis	2 (2)
Skin rash	11 (10)

Percentage values are given in parenthesis.

Table III. Incidence of infections associated with FLAG ± Ida treatment. (Data are from a total of 86 of 105 patients and 116 of 144 courses of chemotherapy, resulting in a total of 125 febrile episodes.)

Infection	Incidence ($n = 125$)
Pyrexia of unknown origin	68 (54)
Bacteraemia	23 (18)
Gram positive bacteraemia	14 (11)
Gram negative bacteraemia	9 (7)
Aspergillus	19 (15)
Candidaemia	3 (2)
Pneumonia	8 (6)
CMV	4 (3)

Percentage values are given in parenthesis.

Discussion

Although significant advances have been made in the treatment of *de novo* acute leukaemia, the treatment of refractory or relapsed acute leukaemia and MDS/AML remains difficult. Since early 1994 we have treated the majority of such patients, and others with high risk haematological malignancies, with FLAG ± Ida. The data presented is a retrospective analysis of 105 consecutive high risk patients treated with FLAG ± Ida at our institution over a 5-year period between April 1994 and April 1999.

The results show that FLAG ± Ida is an effective remission induction regimen for high risk acute leukaemia and MDS. This was a very poor risk group, including 39% with primary refractory/early first relapsed disease and 41% with late relapsed disease (the majority of which were multiple and/or refractory). The CR rate of 59%, median EFS of 12 months and projected 5-year EFS of 25% of those patients achieving CR with FLAG ± Ida compares favourably with those published for other regimens (Table IV). A group worthy of a specific mention are the 10 patients treated with FLAG ± Ida for relapsed disease post-HSCT. The response rate was 90% (seven CR and two PR) with a median EFS of 11 months (five of nine receiving a second HSCT), and three patients remaining in continuing CR at 38, 56 and 57 months.

Table IV. CR rates of primary refractory (Ref) and relapsed (Rel) acute leukaemia (AL) from other published studies.

Disease	Treatment	Numbers	CR rate %	Reference
Ref and first Rel AML	Various regimens	243	33	Keating *et al* (1989)
Ref and Rel AML	ICE	97	43	Carella *et al* (1993)
Ref AML	Ida + ID ara-C	21	52	De Witte *et al* (1996)
First Rel AML	MEC	50	68	Vignetti *et al* (1996)
Ref and first Rel AML	timed sequential MEC	20	60	Martino *et al* (1999a)
Ref and first Rel AML	HD ara-C ± mitoxantrone	162	38	Karanes *et al* (1999)
Ref and first Rel ALL	RELAL-88	45	74	Martino *et al* (1999b)
Ref and first Rel ALL	Various regimens	314	31	Thomas *et al* (1999)
Ref and Rel AL	HD ara-C + HD mitoxantrone	66	53	Raanani *et al* (1999)

HD, high dose; ID, intermediate dose; ICE, idarubicin, ara-C and etoposide; MEC, mitoxantrone, etoposide and ara-C; MAE, mitoxantrone, HD ara-C and etoposide; RELAL-88, vindesine, mitoxantrone, cyclophosphamide, ID Ara-C, prednisolone and methotrexate. AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia.

It is important to note that this was not a study designed to detect differences in outcome related to the use of idarubicin. Indeed, the treatment was selected on the basis of underlying disease and patient characteristics. Nevertheless no differences were seen. Although the addition of idarubicin to FLAG appeared to increase the overall CR rate (62·7% vs. 52·6%) and projected 5-year EFS of those in CR (32% vs. 17%), this did not reach statistical significance (data not shown) and, as stated, the two groups were not comparable.

FLAG ± Ida was well tolerated and the idarubicin did not result in any increased toxicity, particularly haematological recovery or infectious complications. When FLAG and FLAG-Ida are compared (data not shown), the only trends noted were more vomiting with FLAG-Ida ($P = 0{\cdot}096$) and a longer time to platelet recovery with FLAG ($P = 0{\cdot}071$). The difference in time to platelet recovery probably reflects the underlying disease as there were significantly more patients with MDS/AML treated with FLAG ($P < 0{\cdot}001$). The relatively prompt recovery of neutrophils probably reflects the continued use of G-CSF beyond the administration of chemotherapy. There was no significant difference between neutrophil and platelet recovery times after the first or second course (data not shown). It should be noted that the idarubin dose was lower (8 vs. 10 mg/m^2) than that used in other variations of the regimen.

The high treatment-related death rate of 17% was not unexpected in view of the high risk features of the patients treated. The high incidence of invasive aspergillus infection and the four cases of CMV reactivation reflect the immunosuppressive properties of FDR and vigilance for such infections should be high. It is of interest that three of the cases of CMV reactivation were seen in patients with relapsed ALL in whom previous treatment would also have been immunosuppressive.

Studies suggest that early HSCT following re-induction chemotherapy is an effective treatment strategy in such high risk patients (Vignetti *et al*, 1996; Byrne *et al*, 1999). Wherever possible, patients responding to FLAG ± Ida went on to receive an HSCT with a significant improvement in survival. However these results must be treated with caution as the median age of those receiving an HSCT was significantly lower at 28 years in contrast to 51 years in those who did not ($P = 0{\cdot}003$). This may have introduced bias in favour of those receiving an HSCT. Indeed, when the number of patients in continuing CR having received an HSCT (10 of 31) was compared with those who did not but were eligible on the grounds of age ≤60 years (three of 27), the difference was no longer significant ($P = 0{\cdot}066$). In this analysis there was also no longer a difference between the median ages of the two groups (28 years compared with 29 years).

In the light of our experience with FLAG ± Ida, we recommend it for use as remission induction therapy in patients with high risk acute leukaemia, with the possible exception of relapsed T-ALL (data not shown, CR rate 33%). Recently, we have initiated a prospective non-randomized study of FLAG with liposomal daunorubicin (Cortes *et al*, 1999) (FLAG-X), rather than idarubicin, in the treatment of refractory or relapsed acute leukaemia (Potter *et al*, 2001).

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