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FLAG (Fludarabine, High-Dose Cytarabine, and G-CSF) for Refractory and High-Risk Relapsed Acute Leukemia in Children

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Background. The treatment of relapsed and refractory leukemia in children remains a challenge. The morbidity of further chemotherapy is considerable, as most patients have already been exposed to intensive multiagent chemotherapy. The FLAG (fludarabine, high-dose cytarabine, and G-CSF) regimen is as intensive but less cardiotoxic because of the avoidance of anthracyclines. ***Procedure.*** Nineteen children were treated in two U.K. centers with the FLAG regimen for relapsed and refractory acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). There were 13 males and 6 females, with an age range of 1.9 to 14.2 years. AML was the diagnosis in 12 children, ALL in 4, biphenotypic leukemia in 3. Eight patients had refractory disease, 11 were in relapse (5 in first relapse, 4 in second, and 2 in third). ***Results.*** Complete remission was obtained in 13 patients, partial remission was obtained in 4, and 2 patients were considered nonresponders. There were seven patients alive at 12 months (mean) posttherapy; one of these is awaiting bone marrow transplantation (BMT). All patients experienced grade 4 hematological toxicity; no patient died of infection. Thirteen patients received BMT as consolidation (seven unrelated donor; six sibling allografts). Six of these have died, four due to pneumonitis. ***Conclusions.*** FLAG can be regarded as an effective protocol for inducing remission in a group of heavily pretreated children. Its toxicity is acceptable due to the avoidance of anthracyclines. Med. Pediatr. Oncol. 32:411–415, 1999. © 1999 Wiley-Liss, Inc.

Key words: fludarabine; cytarabine; G-CSF; childhood leukemia; refractory

INTRODUCTION

The prognosis for childhood leukemia has improved considerably over the past 15 to 20 years. The event-free survival at 5 years is 65% for acute lymphoblastic leukemia (ALL) [1] and approximately 55% for acute myeloid leukemia (AML) [2]. The successful treatment of both refractory or relapsed disease remains the major dilemma. At relapse, the majority of patients have been exposed to numerous chemotherapeutic agents, in particular anthracyclines. As further intensive therapy will be required to induce remission, the cumulative dose of anthracyclines contributes significantly to the subsequent morbidity and mortality. With the use of an effective nonanthracycline-based regimen, the induction remission rate could be maintained while the overall morbidity reduced.

Fludarabine is a fluorinated purine analog with established antineoplastic activity in lymphoproliferative malignancies [3]. The combination of fludarabine with cytarabine and granulocyte colony stimulating factor (G-CSF) known as FLAG appears to have a synergistic effect. This regimen has been used successfully in adults for the treatment of refractory and relapsed AML and ALL [4–7] with minimal toxicity. We assessed this protocol in children with either ALL or AML refractory to primary therapy or had repeatedly relapsed following standard chemotherapy. Nineteen therapy were treated with this regimen at two U.K. centers.

METHODS

Patient Selection

Between May 1995 and November 1996, 19 children with refractory or relapsed ALL and AML attending the pediatric oncology/hematology units at the Royal Hospital for Sick Children, Bristol, and Great Ormond Street Hospital for Children, London, were treated with the FLAG regimen. There were 13 male and 6 female patients. The median age was 6.4 years (1.9–14.2 years). Twelve patients had a primary diagnosis of AML; four patients had ALL and three patients had biphenotypic leukemia. Six patients with AML and two with biphenotypic leukemia had refractory disease, with persistent blasts present after initial remission induction chemotherapy. Eleven patients (six AML, four ALL, and one biphenotypic) had relapsed disease. Five patients were in

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TABLE I. Patient Characteristic, Times of Relapse, and Treatment Received Prior to FLAG*

Patient number	Age (years)	Diagnosis	Treatment 1	Age at treatment 1	Treatment 2	Age at treatment 2	Treatment 3
1	6.9	AML	AML X	7.7	FLAG × 2		
2	8.05	AML	AML X	8.3	FLAG × 2	8.6	CLASP
3	6.4	ALL	SW good-risk female	9.4	UKALL X D + cranial R/T	12.1	R1 + UD BMT (failed engraftment)
4	7.4	ALL/AML	Steroids, ADE, MACE	7.6	FLAG × 2		
5	2.4	ALL	UKALL XI	5.2	UKALL R2	5.3	FLAG × 2
6	14.2	AML	AML X	14.6	FLA (no GCSF)		
7	11.6	AML	AML X	12.5	FLAG × 2		
8	14	AML	AML 12	14.1	FLAG		
9	13.5	AML	AML 12	13.8	FLAG × 2		
10	10.4	ALL/AML	UKALL XI	10.5	FLAG × 2		
11	3.1	ALL/AML	UKALL XI	6.5	UKALL R1	7.1	FLAG × 2
12	5.9	AML	mod. BFM-83/AML X	7.4	FLAG + ATRA		
13	9.2	AML	AML X	9.6	FLAG		
14	4	ALL	UKALL XB + cranial R/T	6.3	R1/test/R/T + HD MTX	10.7	R1; FLAG
15	6.5	AML	AML XII	6.7	FLAG × 2		
16	6	ALL	UKALL XID + cranial R/T	8.6	UKALL R1	10.7	Asp, V, pred, + FLAG (consolidation)
17	3	AML	AMl X	7.5	MACE, CLASP, auto BMT	8.6	FLAG × 3
18	3.1	AML	AML X + ATRA	4.3	FLAG + sib allo BMT	5.3	FLAG + T-Cell infusion
19	1.9	AML	AML XII + ATRA	2.5	FLAG × 2		

*R/T, radiotherapy; R1, first relapse; R2, second relapse; UDBMT, unrelated donor BMT; ATRA, all transretinoic acid; SW good risk female, local protocol used to treat low-risk disease; UKALL R1, relapse protocol for ALL; UKALL R2, relapse protocol for ALL; Asp, asparaginase; Pred, prednisolone; V, vincristine; ADE, cytosine arabinoside, daunorubicin, etoposide; MACE, amsacrine, cytosine arabinoside, etoposide.

first relapse, four in second, and two in third at the time they received FLAG. The median elapsed time between initial therapy and receiving FLAG therapy was 34 months (range, 8–177 months). All eight patients with refractory disease received FLAG as their second-line chemotherapy within 12 months of their initial diagnosis. Three patients with relapsed disease received FLAG as second-line therapy within 12 months of diagnosis; all three had AML. Patient details, including all the treatment received prior to FLAG, can be found in Table I.

Study Design and Treatment Protocol

The criteria for receiving FLAG were as follows. First, bone marrow relapse in a child with AML after having fully completed standard AML therapy. Second, bone marrow relapse in a child with ALL who had already received standard second-line chemotherapy. Third, persistence of blasts in either ALL or AML following standard induction remission chemotherapy. Fourth, aged 18 years and below. Standard therapy was defined as the current Medical Research Council (MRC) trial for that disease. All the patients were recruited from these trials. The diagnosis and classification of both ALL and AML were made according to the French-American-British (FAB) criteria. Exclusion criteria included patients with severe left ventricular failure, identified by ejection fractions less than 50%, and patients with moderate to severe renal failure (creatinine clearance <30 ml/min).

The FLAG protocol has been previously described in other studies [5], but briefly it consists of G-CSF (Lenograstim, Chugai) 0.5 mi.u/kg intravenously from day 1 to remission (i.e., stop on the first day of neutrophils >1.0 × 10^9/L) 24 hr following the first dose of G-CSF; fludarabine 25 mg/m^2 IV by 30-min infusion (days 2–6), followed 4 hr later by cytarabine 2 gm/m^2 by a 4-hr infusion (days 2–6).

Prophylactic steroid eyedrops were administered to all patients while receiving the cytarabine. Cotrimoxazole was administered to all patients as prophylaxis against pneumocystis carinii (PCP) infection. All administered blood products were irradiated and CMV-negative in patients known to be CMV-negative. Antibiotics were given in accordance with the febrile neutropenia protocol of the respective unit.

Bone marrow aspirates were performed after each course of FLAG upon hematological recovery (neutrophils >0.5 × 10^9/L; platelets >100 × 10^9/L) to assess response.

Complete remission (CR) was defined as less than 5% blasts in a normocellular marrow. Partial remission (PR) was defined as a normocellular marrow containing between 5% and 25% of leukemic blast cells. Nonresponders (NR) were defined as a bone marrow containing

more than 25% leukemic blast cells. Relapsed disease was defined as greater than 5% blasts in a bone marrow that had previously achieved complete remission. Patients in CR went on to bone marrow transplantation (BMT), provided an HLA-matched donor was available. The toxicity of the FLAG regime was assessed using the Common Toxicity Criteria (WHO).

RESULTS

Response

Thirteen patients (70%) achieved complete remission (CR) following one course of FLAG chemotherapy. A partial remission was achieved in four patients (20%) and two patients (10%) were considered to be nonresponders.

Toxicity

Overall the regimen was well tolerated by all the patients. Grade 4 hematological toxicity and at least one episode of severe sepsis during the period of neutropenia was experienced by all the patients. No patients died because of infection. The mean duration of time before the neutrophils recovered to a level $>1.0 \times 10^9$/L was 27 days (range, 7–32 days). The mean duration of time for platelet to recovery to a level of $>50 \times 10^9$/L was 22 days (range, 16–33 days). The mean lymphocyte count at 28 days was 0.47×10^9/L. Eleven patients received a second consolidation course of FLAG. Overall, this second course was well tolerated by all patients. Again, profound myelosuppression was encountered by all but no life-threatening septic episodes was documented.

Most patients developed grade 2 gastrointestinal (GI) toxicity, with stomatitis and severe diarrhea being most commonly reported. One patient developed pancreatitis, which resolved with conservative treatment. This grade of GI toxicity was encountered following each course of FLAG.

One patient had a seizure following FLAG, which was initially ascribed to fludarabine. This patient was subsequently found to have CNS leukemia. Conjunctivitis was experienced in four patients despite steroid eyedrop prophylaxis. All resolved with prolonged topical steroid treatment. There were no new cardiac problems encountered as a result of this treatment.

BMT

To date, 13 patients have received bone marrow transplant as consolidation therapy. A median of 65 days (range, 10–126 days) passed between receiving the first course of FLAG and undergoing bone marrow transplant. Seven of the BMTs were from unrelated donors, of which 4 have died. Of these four, three were in CR at the time of death and one in relapse. This patient had only achieved a PR prior to BMT. Death in all cases was due to complications of BMT.

Six patients received sibling allogeneic transplants. Three have subsequently died. Only one of these three had achieved a CR (using FLAG) prior to BMT. He unfortunately relapsed post-BMT and failed to respond to a further course of FLAG. The other two patients had only achieved a PR with FLAG prior to BMT. Both of these patients died with pneumonitis, in which CMB was isolated in one case.

Six patients did not receive BMT. Two of these (patients 4 and 16) were the nonresponding patients, in whom a remission status was never documented. Patient 2, who had achieved a partial remission with two courses of FLAG, relapsed and died of disease progression. Patient 9 achieved a good CR with FLAG and commenced conditioning for an allogeneic sibling BMT. However, during this time period, his sibling donor developed infectious mononucleosis. The transplant was subsequently deferred and he died with fungal infection, albeit in CR. Patient 19, in whom a CR had been achieved, subsequently relapsed and died during a prolonged and unsuccessful search for a compatible donor. The last patient (patient 17) achieved CR and remains in CR at the time of writing while the search for a potential donor is taking place.

Survival

Seven patients are alive at 11.7 months (range, 1–18 months) post-FLAG therapy. One is awaiting BMT. Eleven patients have died from either progressive disease or transplant-related causes. Both of the nonresponding patients have died.

DISCUSSION

Children with relapsed or refractory acute leukemia are an exceptionally difficult group of patients to cure. The use of intensified regimens has made it possible to induce second remissions in more than 90% of patients with ALL [8,9] who relapse after treatment with modern protocols, but less than a third of these may be cured with chemotherapy alone [10]. The two most important prognostic variables are the duration of the first remission and the site of relapse. The prospects of long-term survival remains bleak in patients who have a bone marrow relapse during treatment or within the first 6 months after cessation of treatment [11–13]. In the MRC UKALL X study, only 3 of 106 children with marrow or combined relapse, on or within 2 years of completing therapy, were in second CR at the time of follow-up [10].

In AML, success is limited in treating relapsed or refractory disease. In this situation, however, outcome depends not only on the time of relapse, but also on the initial therapy. Therapeutic advances in the last decade have led to cure rates for AML of greater than 50% using first-line therapy [2]. Several relapse regimens do exist

for AML, the majority being based on the use of anthracyclines and cytarabine [14,15].

Second-line chemotherapeutic regimens, because of the aggressive nature of the underlying disease, need to be intensive. Toxic agents, such as the anthracyclines, are often incorporated into these protocols in an attempt to regain remission. These agents induce a considerable morbidity in patients who have already been heavily treated with multiagent chemotherapy. The subsequent myeloblative therapy as part of BMT conditioning adds further to this morbidity.

The experience with the use of fludarabine has increased worldwide since initial phase 1 and 2 trials in the early 1980s [16–19]. The combination of fludarabine with cytarabine (Ara-C) has a synergistic effect because of the ability of fludarabine to potentiate Ara-CTP (the active metabolite of Ara-C) accumulation [20]. This combination allows lower and hence more tolerable doses of fludarabine to be used effectively. The addition of granulocyte colony-stimulating factor (G-CSF) has further improved the efficacy of the combination by shortening the duration of neutropenia, particularly in the treatment of AML and myelodysplastic syndrome (MDS) [4,5]. The administration of G-CSF before, during, and after the completion of chemotherapy should increase the sensitivity of the leukemic blasts by pushing more cells into the G1 phase of the cell cycle. Theoretically, this should improve overall cell kill, but definitive proof of this phenomenon has yet to be established by clinical trials. The successful use of FLAG has prompted its use as initial therapy for AML in adults, with some encouraging results [21].

High-dose cytarabine has been used in the treatment of acute leukemia, more so in AML than in ALL [15,22]. There has been published reports of its use in ALL [23], with overall remission induction rates of 34%. Harris et al. [24] recently reported their experience of high-dose cytarabine in refractory acute lymphoblastic leukemia in 52 children. They encountered quite considerable toxicity with this therapy, particularly from sepsis, with 3 fatal bacterial infections, 12 fatal fungal infections, 1 fatal adenoviral pneumonia, and 1 fatal pneumocystis carinii pneumonia. We did not encounter such toxicity, possibly due to the adjuvant use of G-CSF.

There is less experience with the use of FLAG in children [22]. We have used it in a heterogeneous group of children, all of whom had been heavily pretreated with intensive multiagent chemotherapy because of either multiple relapses or primary resistant disease. A CR rate of 70% with a PR rate of 20% was extremely encouraging. The toxicity of the regimen was acceptable. Although all patients suffered a prolonged neutropenic episode following FLAG, there were not any deaths directly due to bacterial infection. A number of patients, however, died because of pneumonitis posttransplant. In two, an attributable cause was found (CMV and RSV), but in two other cases, no etiological agent was identified. Case reports of interstitial pneumonitis consistent with drug-induced pulmonary injury have been published in patients treated with fludarabine [25,26]. All of these patients responded to corticosteroid therapy. It is impossible to say with certainty that fludarabine was the causative agent in our cases of pneumonitis, as other factors may also have played a part, but we do believe that intensive monitoring for reversible causes of pneumonitis is warranted in these patients.

FLAG was used in a very-high-risk group of children with relapsed or refractory ALL and AML with a remission rate of 70%. It is well tolerated, particularly in those previously treated with chemotherapy. This regimen is particularly immunosuppresive and therefore particular care must be taken to avoid transfusion-associated graft vs. host disease and PCP. Similarly, intensive cytomegalovirus monitoring and management is needed because of the probable increase risk of infection during subsequent BMT. An MRC study using FLAG for remission induction in children with relapsed AML is now open.

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