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Outcomes of Substituting Oral Fludarabine for Intravenous Fludarabine in Combination with Cytarabine and Filgrastim for Treatment of Primary Refractory or Relapsed Acute Leukemias

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ABSTRACT

Background: Treatment of relapsed/refractory acute myeloid or lymphoid leukemia consists of salvage chemotherapy followed by allogeneic hematopoietic stem-cell transplantation. Intravenous fludarabine, cytarabine, and filgrastim is an effective regimen in this setting. In view of the lack of availability of intravenous fludarabine in Mexico from 2009-2013, we substituted an equivalent oral fludarabine dose (40 mg) for the intravenous formulation. **Objective**: This is a retrospective comparison of the toxicity and effectiveness of oral fludarabine, cytarabine, and filgrastim versus intravenous fludarabine, cytarabine and filgrastim. **Results***:* A total of 44 patients with relapsed/refractory acute myeloid leukemia or acute lymphoid leukemia treated in an academic medical center from 2005-2013 with oral fludarabine, cytarabine and filgrastim (21 patients) or intravenous fludarabine, cytarabine and filgrastim (23 patients) were included in the analysis. There was a trend towards a higher complete remission rate and a longer overall survival following intravenous fludarabine, cytarabine, and filgrastim as compared with oral fludarabine, cytarabine, and filgrastim: complete remission rates 39.1 vs. 23.8% (p = 0.342) and overall survival 6.14 vs. 10.78 months (p = 0.363), respectively. A higher incidence of neutropenic fever (100 vs. 76.2%; p = 0.019) and septic shock (34.8 vs. 0%; p = 0.003) and a longer hospitalization (26.8 vs. 19.4 days; p = 0.046) were observed with intravenous fludarabine, cytarabine, and filgrastim. In multivariate analysis, factors associated with a shorter survival were septic shock (HR: 3.93; 95% CI: 1.67-9.25; p = 0.002) and a higher number of previous treatments (HR: 2.5; 95% CI: 1.26-4.99; p = 0.009). Complete remission was associated with better survival (HR: 0.18; 95% CI: 0.08-0.44; p < 0.001). **Conclusions:** Further studies are needed to determine the optimal dose and timing of oral fludarabine when given as part of the fludarabine, cytarabine, and filgrastim regimen for relapsed/refractory acute leukemia. Our data suggest that the dose of oral fludarabine used, 40 mg/m^2 per day for five days, may be a lower bioequivalent dose to the intravenous dose in fludarabine, cytarabine, and filgrastim. (REV INVES CLIN. 2015;67:287-95)

Key words: Oral fludarabine. FLAG. Relapsed AML. Refractory AML. Relapsed ALL. Refractory ALL.

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Primary refractory or relapsed adult acute leukemia (acute myeloid leukemia [AML] and acute lymphoblastic leukemia [ALL]) are associated with poor survival. Approximately 30-40% of young adult patients with AML will be primarily refractory to standard chemotherapy and more than 50% will relapse after initial remission[1,2]. Similarly, 10-30% of adults with ALL will be refractory to induction treatment, while the majority of patients who achieve initial complete remission (CR) will eventually relapse[3,4]. The cure rate for AML and ALL in adults is estimated to be between 20 and 40%[5-8]. Therapeutic approaches for refractory and relapsed patients consist of salvage chemotherapy followed by allogeneic hematopoietic stem-cell transplantation (Allo-HSCT).

Given the sub-optimal outcome of the standard treatment for relapsed or refractory acute leukemia, participation in clinical trials is recommended. The National Comprehensive Cancer Network (NCCN) guidelines list the following salvage regimens for relapsed or refractory AML: FLAG (fludarabine, cytarabine and filgrastim ± idarubicin[9,10]; cladribine + cytarabine + G-CSF ± mitoxantrone[11,12]; high-dose cytarabine (HiDAC) ± anthracycline; etoposide + cytarabine ± mitoxantrone[13] and clofarabine ± cytarabine + G-CSF ± idarubicin[14,15]. These salvage regimens have shown CR rates between 46 and 66%. The NCCN guidelines list the following salvage regimens for ALL: combinations with clofarabine[16,17], cytarabine[18], alkylating agents[19] or nelarabine (T-ALL)[20]; augmented-hyper-CVAD[21], liposomal vincristine[22,23], and tyrosine kinase inhibitors for ph-1 positive subset. The FLAG regimen with or without variations has been studied in both diseases (AML and ALL), with variable CR rates of 7-100% (mean 54.5%) and median overall survival (OS) of 6-16 months in AML[9,24-33], and CR rates of 30-83% with median OS of 4-5 months in ALL[34-37]. The main toxicity of this regimen is myelosuppression with a high rate of infectious complications. The original FLAG regimen included intravenous (IV) fludarabine. There are some bioequivalence studies comparing oral and IV fludarabine, mostly in chronic lymphocytic leukemia/low-grade lymphomas[38-43]. To our knowledge there are no previous reports on the outcomes of oral fludarabine-containing FLAG (oFLAG) regimens in relapsed acute leukemia patients. Between 2009 and 2013, there was no IV fludarabine available in Mexico. Utilizing the data from the bioequivalence studies in chronic lymphocytic leukemia, patients with relapsed or refractory acute leukemia were treated with oFLAG. We report a retrospective study of the toxicity and effectiveness of oFLAG compared with FLAG in this population.

MATERIALS AND METHODS

Patients and treatment regimens

A total of 44 patients with relapsed or refractory acute leukemia treated with oFLAG or FLAG as salvage regimens between 2005 and 2013 were included in this analysis. The regimen consisted of: fludarabine (30 mg/m² daily IV for five days as a 30-minute infusion or 40 mg/m² daily orally for five days), cytarabine (2 g/m² IV daily for five days as a four-hour infusion starting 3.5 hours after fludarabine), and filgrastim (300 mcg SQ daily starting on day 1)[9]. The election of oral or IV fludarabine was based on the availability of the IV drug preparation: patients received oral fludarabine only when the IV formulation was not available in Mexico. Except for the type of fludarabine administered, the rest of the regimen was the same. This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. For this type of study, formal consent is not required. As a regular procedure, informed consent is obtained from patients before treatment in our institution.

Definitions

Refractory and relapsed acute leukemia and CR were defined as previously described[44]. Patients were considered refractory if they did not achieve CR after induction chemotherapy (one cycle for ALL and two cycles for AML). Complete remission was defined as having a bone marrow aspirate with bone spicules and with < 5 % blasts, no peripheral blood blasts, and no extramedullary leukemia infiltrations, in the presence of a peripheral blood absolute neutrophil count ≥ 1×10^9/l and platelet counts ≥ 100×10^9/l[44]. Relapse was defined as the reappearance of leukemic blasts in the peripheral blood or more than 5% blasts in the bone marrow or the presence of extramedullary relapse in those patients who previously had achieved CR. Early relapse was defined as that occurring within 12 months of CR.

Table 1. Characteristics of the patients

	Total (n = 44)	Oral fludarabine (n = 23)	IV fludarabine (n= 21)	p
Age (years)	26.66	25.64	27.39	0.760
Gender				
– Male	56.8%	52.2%	63.6%	
– Female	43.2%	47.8%	36.4%	0.317
ECOG*				
– 0-1	93.2%	91.2%	95.4%	
– ≥ 2	6.8%	8.7%	5.5%	0.196
Comorbidities	25%	21.7%	27.2%	0.189
Diagnosis*				
– Pre B-ALL	63.6%	56.5%	72.2%	
– AML	31.8%	39.1%	22.2%	
– T-ALL	2.3%	0%	5.4%	
– APL	2.3%	4.3%	0%	0.334
High risk	47.7%	34.8%	59.1%	0.067
Status				
– Early relapse	63.6%	63.6%	69.6%	
– Late relapse	15.9%	18.1%	13.0%	
– Refractory	20.5%	18.1%	17.4%	0.624
Number of previous lines of treatment				
– 1	59.1%	61.9%	56.5%	
– 2	38.6%	33.3%	43.5%	
– 3	2.3%	4.8%	0%	0.641
Extramedullary leukemia	27.3%	27.3%	26%	0.597
– CNS*	18.2%	18.2%	17.4%	
– Vertebra	2.3%	0%	4.3%	
– Skin	4.5%	4.5%	4.3%	
– Other	2.3%	4.5%	0%	0.734
Karyotype				
– Normal	27.3%	27.3%	26.0%	
– WM*	45.5%	50%	43.5%	
– Complex	11.4%	18.2%	4.3%	
– t(9;22)	4.5%	4.5%	4.3%	
– t(15;17)	2.3%	0%	4.3%	
– Other	9.0%	0%	17.4%	0.234
Time from relapse (months)	7.0	7.2	4.4	0.149

*ECOG: Eastern Cooperative Oncology Group performance status.
Pre B-ALL: precursor B-cell acute lymphoid leukemia; AML: acute myeloid leukemia; T-ALL: T-cell acute lymphoid leukemia; APL: acute promyelocytic leukemia; CNS: central nervous system; WM: without metaphases.

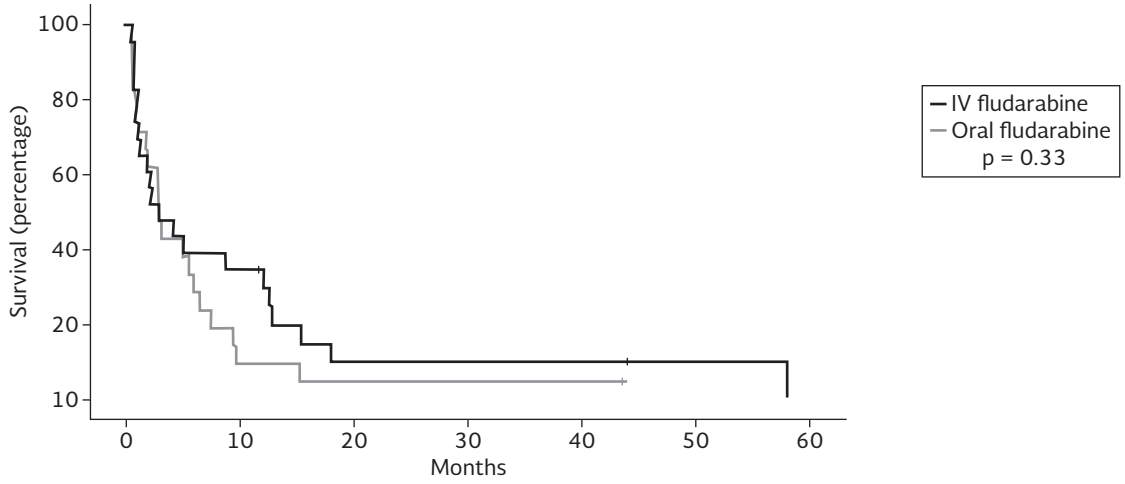
Statistical analysis

Numerical variables were described in terms of means ± standard deviation (SD) or medians and ranges, and categorical variables in frequencies and proportions. The $\chi 2$ test or Fisher exact test and Student's *t* test were used for comparison between groups when appropriate. Also, associations were addressed by odds ratio (OR) with 95% confidence interval (CI). Survival analysis was done using the Kaplan-Meier method and risk factors were determined by Cox logistic regression model. The p-values ≤ 0.05 were considered as statistically significant. Data were analyzed with the support of the statistical software SPSS version 21.

RESULTS

Forty-four patients with relapsed or refractory acute leukemia were treated with FLAG or oFLAG between 2005 and 2013. Twenty-one patients (47.7%) were

Figure. 1. Overall survival according to fludarabine administration.



treated with oral fludarabine and 23 patients (52.3%) with IV fludarabine. Baseline characteristics of the patients are shown in table 1. The diagnoses were: Pre-B ALL (63.3%), AML (31.8%), acute promyelocytic leukemia (APL, 2.3%) and T-ALL (2.3%). There was a trend to a higher frequency of ALL in patients treated with IV fludarabine (72.7 vs. 53.5%; p = 0.11). The patient with APL was a woman with multiple relapses and FLAG was the fourth line of treatment.

Patients had received different first-line treatments: for AML, combinations of cytarabine and anthracycline (31.8%); for ALL, hyper-CVAD (45.5%)[45], pediatric-based protocol from the Princess Margaret Hospital (4.5%)[46], or an institutional protocol HOP-0195 (2.3%)[47]; and others in 15.9%. The majority of cases (63.6%) were early relapses, whereas 20.5 and 15.8% were primary refractory and late relapses, respectively. The median time to initial relapse was 6.8 months. In 59.1% of the cases FLAG was the second line of treatment, and in 40.9% it was the third or fourth line. There was a trend for a higher number of high-risk patients in the IV fludarabine group compared with the oral fludarabine group (59.1 vs. 34.8%, respectively; p = 0.067).

Response and survival

The overall CR rate was 31.8% (33.3% for AML and 31% for ALL; p = 0.56), 23.8% with oral fludarabine, and 39.1% with IV fludarabine (p = 0.342). Median OS was 2.87 months (95% CI: 0.81-4.93) for the entire group, and 2.9 and 2.87 months for the oral and IV groups, respectively (p = 0.363) (Fig. 1).

Median OS was 3.17 months (95% CI: 2.18-4.16) for patients who achieved CR with FLAG or oFLAG, and 2.87 months (95% CI: 0.24-5.50) for patients who did not achieve a CR (p = 0.69) (Fig. 2). Fifty-seven percent of patients who achieved CR received a consolidation course with the same regimen. Only three patients (6.8%) of the total group were consolidated with Allo-HSCT. The median OS was significantly higher in patients who underwent Allo-HSCT: 15.33 vs. 2.83 months (p = 0.028) (Fig. 3).

At the time of analysis, 93.2% of the patients were deceased. The main cause was progression of the disease (81.8%). Treatment-related mortality was 9.1%.

Adverse effects

There was a higher incidence of neutropenic fever with IV fludarabine than with oral fludarabine (100 vs. 76.2%; p = 0.019), as well as a higher incidence of septic shock (34.8 vs. 0%; p = 0.003) and a longer hospitalization (26.8 vs. 19.4 days; p = 0.046). Characteristics of neutropenic fever episodes in both groups are shown in table 2. Only 50% of the patients received prophylactic antibiotics: trimethoprim-sulfamethoxazole 25%, ciprofloxacin 18.2%, acyclovir 11.4%, and itraconazole 20.5%. There was a trend to a higher rate of septic shock in patients without prophylactic antimicrobials: 26 vs. 9% (p = 0.135). We identified the source of infection

Figure. 2. Overall survival according to complete remission.

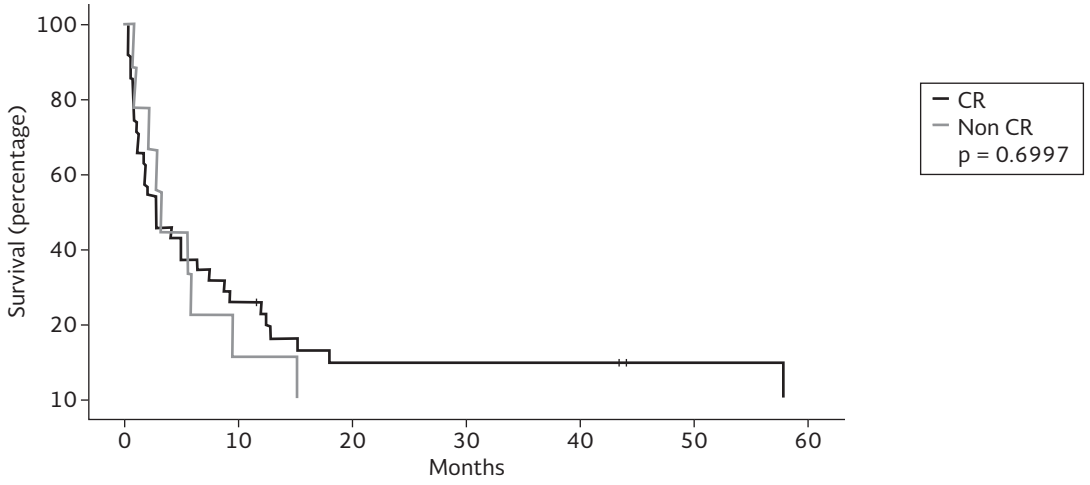
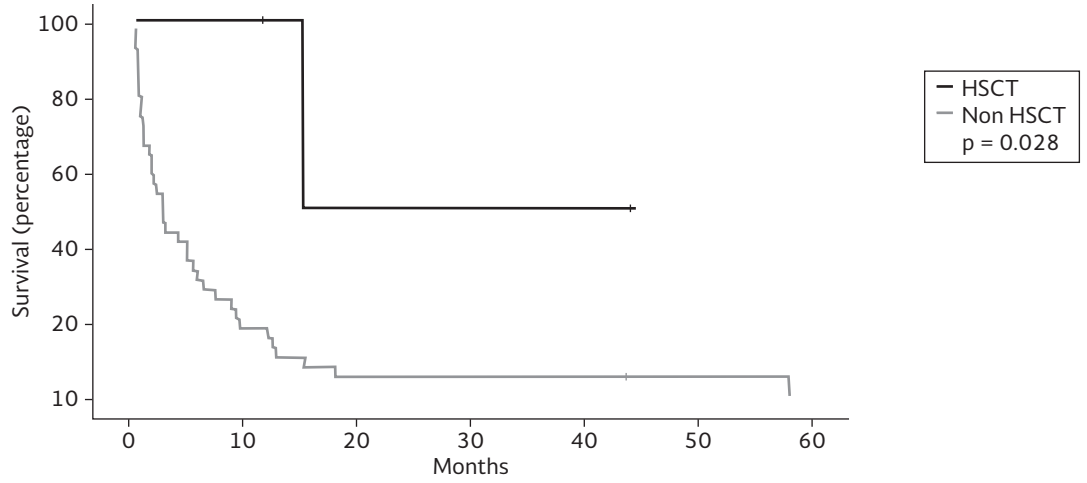


Figure. 3. Overall survival according to allogeneic hematopoietic stem-cell transplantation (Allo-HSCT).



in 65.9% of the cases: pneumonia (29.5%), bacteremia (13.6%), perirectal soft-tissue infection (11.4%), urinary tract infection (4.5%), and other (6.8%). The cause of febrile neutropenia was identified by culture in 52.3% of cases. The most common microorganisms were: gram-negative bacilli (34.1%), *Aspergillus* spp. (6.8%), gram-positive cocci (4.5%), candidemia (4.5%), and combinations with *Aspergillus* spp. (2.3%). The 30-day mortality following FLAG or oFLAG was 9.1%.

Univariate and multivariate analysis

Factors associated with shorter OS by univariate analysis were: number of lines of treatment (more than one prior to FLAG) (HR: 2.03; 95% CI: 1.09-3.78; p = 0.03) and development of septic shock (HR: 2.8; 95% CI: 1.24-6.36; p = 0.023). Factors associated with longer OS were: CR with FLAG or oFLAG (HR: 0.26; 95% CI: 0.12-0.58; p < 0.001) and undergoing HSCT (HR: 0.15; 95% CI: 0.02-1; p = 0.009). By multivariate analysis, factors that remained significant were: number of previous cycles of treatment (HR: 2.5; 95% CI: 1.26-4.99; p = 0.009), septic shock (HR: 3.93; 95% CI: 1.67-9.25; p = 0.002), and CR with FLAG (HR: 0.18; 95% CI: 0.08-0.44; p < 0.001).

DISCUSSION

In this retrospective study comparing the toxicity and effectiveness of oFLAG with FLAG, we found a trend to higher CR rates with IV fludarabine as compared

Table 2. Characteristics of neutropenic fever

	Total	Oral fludarabine	IV fludarabine	p
Neutropenic fever	88.6%	76.2%	100%	0.019
Duration of neutropenia (days)	16.82	17.33	16.35	0.417
Duration of neutropenic fever (days)	6.56	7.19	6.13	0.56
Hospitalization (days)	23.3	19.43	26.83	0.046
Septic shock	18.2%	0%	34.8%	0.003

with the oral presentation, although OS was the same in both groups. There was a significant difference in toxicity with higher rates of neutropenic fever, septic shock, and a longer hospitalization with IV fludarabine. These two facts could indirectly mean that the selected oral dose of fludarabine (40 mg/m^2) was not bioequivalent to the selected IV dose (30 mg/m^2). The bioavailability of the oral drug was estimated to be 55%. Reported bioavailability has ranged from 30 to 80% between patients and this variability may be mostly related to differences in the individual metabolism of fludarabine rather than variations in the oral absorption of the drug[38,39]. Most studies comparing IV fludarabine at a dose of 25 mg/m^2 have shown bioequivalence with 40 mg/m^2 of oral fludarabine[38]. Since the IV fludarabine dose in FLAG is 30 mg/m^2, possibly the most appropriate oral fludarabine dose for the oFLAG regimen should be 48 mg/m^2.

It is also important to mention that data suggest that the time between fludarabine and cytarabine administration may be critical to obtain the desired effect. Current recommendations are that cytarabine should be administered 3.5-4.0 hours after fludarabine. This is because fludarabine enhances the intracellular accumulation of ara-CTP (ara-cytidine-5'-triphosphate), the active metabolite of cytarabine. There is a strong association between the accumulation of ara-CTP in the leukemic blasts and response in AML[26]. The pharmacokinetics of oral fludarabine may therefore be critical to determine the optimal timing for the administration of cytarabine. The time to achieve peak plasma concentrations of fludarabine when administered orally is between 1-2 hours and is slightly extended by the presence of food (2.2 + 1.0 vs. 1.3 + 0.74 hours)[39].

The patients included in this cohort had a particularly poor prognosis since it included a large percentage of early relapse and refractory acute leukemias (84.1%) and 40.9% had received 2-3 previous chemotherapy regimens. This likely explains the lower mean CR in our study compared to previous reports (33.3 vs. 54.5%, respectively) and the shorter OS (2.89 vs. 6-16 months in previous studies). Interestingly, there are very few reports on the use of FLAG regimens in ALL patients, most of them FLAG variants[34-37]. One study reported CR in 2/6 cases (33%) of relapsed/refractory ALL with myeloid markers treated with FLAG[37]. This is similar to the CR rate of 31% observed among ALL patients in our study. Tables 3 and 4 summarize previous reports of FLAG and its variants in AML (Table 3) and ALL (Table 4).

It is important to note the high rate of serious infectious complications. With IV fludarabine, the rate of neutropenia and fever was 100%, and 34.8% of the cases developed septic shock, which is consistent with previous reports. It should be noted that in our series, filgrastim was frequently discontinued after the fifth dose, mainly for financial reasons, whereas this agent was continued until neutrophil recovery in previous reports. Also, only 50% of our patients received prophylactic antibiotics. High-risk patients (expected duration of neutropenia longer than seven days) benefit from prophylactic antibacterial, antifungal, and antiviral agents[48]. We had a slightly higher incidence of fungal infections than previously reported by others (13.6 vs. 7-10%)[9,27,30,33,36]. Only 20.5% of our patients received antifungal prophylaxis. A retrospective analysis of infectious complications of AML patients receiving chemotherapy with combinations of fludarabine showed that 65% developed fever of undetermined origin and 13 and 21% experienced gram-negative or gram-positive bacteremia, respectively. The rate of possible invasive fungal infection after FLAG was 6% in a previous retrospective study in which all patients received prophylactic levofloxacin and itraconazole[49].

Table 3. Fludarabine, cytarabine and filgrastim in acute myeloid leukemia

Reference	Design and characteristics	Setting	CR	Median DFS/OS	NF/TRM/HSCT
Estey, et al. 1993[26]	Prospective non-comparative; FLAG	n = 59 Relapsed or refractory AML	36%	CR duration 39 weeks	–
Clavio, et al. 1996[27]	Retrospective, non-comparative; FLAG and FLANG	n = 51: 28% refractory, 31% early relapse, 41% with poor prognostic factors	Global 59% Relapse: 30% Refractory: 80% High-risk: 61%	CR duration 6 months OS 9 months	NF: 56.5% TRM 10%
Nokes, et al. 1997[28]	Retrospective, non-comparative; FLAG	n = 40: 24 AML (19 relapsed, 4 refractory), 8 MDS and 8 other	Refractory: 100% Relapse: 68%	–	TRM: 7.5%
Montillo, et al. 1998[9]	Prospective, open-labeled, non-comparative; FLAG	n = 38: 16 refractory, 8 early relapse, 11 post-transplant relapse and 3 second relapse	Refractory: 43.7% Relapse: 63.6%	DFS: 13 months OS: 9 months	NF: 88% TRM: 10%, Allo-HSCT: 7.9%
Ferrara, et al. 1999[29]	Prospective, non-comparative; FLAG	n = 26 Relapse post Allo-HSCT	RC: 50%	OS 6 months DFS: 13 months	NF: 89% TRM: 15% Allo-HSCT: 19%
Jackson, et al. 2001[30]	Prospective, non-comparative; FLAG	n = 83 Relapse (early or late) and refractory AML	Late relapse: 81% Early relapse/ refractory: 30%	Late relapse: DFS: 8.2 OS: > 16 months Early relapse/ refractory: DFS: 3.2 OS: 6.3 months	NF: 99% TRM: 18%
Hanel, et al. 2001[31]	Prospective, comparative; FLANG	n = 29 Relapsed and refractory AML	59%	DFS: 3.2 months OS: 6.8 months	NF: 85% TRM: 15%
Pastore, et al. 2003[32]	Prospective, non-comparative; FLAG-Ida	n = 46 Relapsed and refractory AML	Refractory: 50% Relapsed: 53%	DFS: 12 months OS: 11 months	NF: 86.9% TRM: 6.6% Allo-HSCT: 23.9%
Steinmetz, et al. 1999[33]	Prospective, non-comparative; FLAG-Ida	n = 29 Relapsed and refractory AML	Refractory: 7% Relapsed: 80%	–	NF: 98% TRM: 22%
Carella, et al, 2001[25]	Prospective, non-comparative; FLAG	n = 41 Relapsed: 11 Refractory: 19 Secondary AML: 11	Refractory: 45% Relapsed: 83%	DFS: 12 months OS: 11 months	NF: 70.7% TRM: 7%

CR: complete remission; DFS: disease-free survival; OS: overall survival; NF: neutropenic fever; HSCT: hematopoietic stem-cell transplantation; FLAG: fludarabine, cytarabine and filgrastim; AML: acute myeloid leukemia; TRM: therapy related mortality; Allo-HSCT: allogeneic hematopoietic stem-cell transplantation; FLANG: FLAG plus mitoxantrone; FLAG-Ida: FLAG plus idarubicin; MDS: myelodysplastic syndrome.

As previously mentioned, the proportion of patients who underwent Allo-HSCT as consolidation in our group was very small, only three patients. This fact could explain the low OS rate observed in the entire cohort and also why patients who underwent Allo-HSCT had a longer survival in the univariate analysis, but this did not remain significant in the multivariate analysis.

In summary, oral fludarabine-FLAG was as effective as IV fludarabine-FLAG for the salvage treatment of primary refractory or relapsed AML or ALL. It could be an alternative in countries with irregular availability of the IV formulation. The optimal dose and pharmacokinetics of the oral fludarabine preparation in this regimen needs to be determined. This regimen is

Table 4. Fludarabine, cytarabine and filgrastim in acute lymphoid leukemia

Reference	Design and characteristics	Setting	CR	Median DFS /OS	NF/TRM/HSCT
Suki, et al. 1993[34]	Prospective, non-comparative; FLA	n = 30 Refractory or relapsed ALL	30%	CR: duration 22 weeks OS: 12 weeks for responders and 34 weeks for non-responders	NF: 93% TRM: 27%
Montillo, et al. 1997[35]	Prospective, non-comparative; FLA-G-CSF	n = 12 Relapsed/ refractory ALL	83%	–	NF: 50% TRM: 8.3%
Specchia, et al. 2005[36]	Prospective, non-comparative; FLAG-Ida	n = 23 Relapsed/ refractory ALL	39.1%	OS: 4.5 months. For responders: DFS: 6 months and OS: 9 months. DFS for transplanted: 10 months	NF: 56.5% Allo-HSCT: 30%. TRM: 3%
Visani, et al. 1996[37]	Prospective, non-comparative; FLAG	n = 12 Relapsed/ refractory ALL	67% (100% for 6 patients with myeloid antigen expression, 33% without)	CR duration 22.5 weeks	–

CR: complete remission; DFS: disease-free survival; OS: overall survival; NF: neutropenic fever; TRM: therapy related mortality; Allo-HSCT: allogeneic hematopoietic stem-cell transplantation; ALL: acute lymphoid leukemia; FLAG: fludarabine, cytarabine and filgrastim; FLA: FLAG without granulocyte colony stimulating factor; FLA-G-CSF: fludarabine, cytarabine followed by granulocyte colony stimulating factor; FLAG-Ida: FLAG plus idarubicin.

associated with high rates of infectious complications. It would be recommended to treat patients with appropriate prophylactic antimicrobials. The prognosis in this group of patients is still very poor and the responses are only transient in most patients. Salvage chemotherapy with FLAG can lead to prolonged survival if used as a bridge for Allo-HSCT.

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