Retrospective comparison of clofarabine *versus* fludarabine in combination with high-dose cytarabine with or without granulocyte colony-stimulating factor as salvage therapies for acute myeloid leukemia

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ABSTRACT

We recently reported that clofarabine, high-dose cytarabine, and granulocyte colony-stimulating factor (GCLAC) produced a 46% complete remission rate in relapsed/refractory acute myeloid leukemia. GCLAC differs from FLAG by substitution of clofarabine for fludarabine, raising the question of the relative efficacy of these two regimens. We compared GCLAC given at the University of Washington Medical Center/Fred Hutchinson Cancer Research Center to fludarabine and cytarabine (FA) and FLAG given at MD Anderson Cancer Center. Independent multivariate analyses conducted at both institutions showed that after accounting for duration of first complete remission, salvage number, age, and cytogenetics, GCLAC was associated with a higher complete remission rate (odds ratio 9.57, $P<0.0001$) and longer survival (mortality hazard ratio 0.43, $P=0.0002$). Despite the retrospective nature of the analyses, GCLAC may be superior to FA/FLAG, particularly in patients with short duration of first complete remission or unfavorable cytogenetics.
The study was registered as NCT00602225 on ClinicalTrials.gov.

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Introduction

We recently reported that clofarabine and high-dose cytarabine preceded by G-CSF priming (*GCLAC*) produced a 46% complete remission rate in patients with relapsed or refractory acute myeloid leukemia (AML), although the relapsed patients had a median CR duration of only six months, with 36% of patients not having had a prior CR.[1] GCLAC was similar to the FLAG regimen (fludarabine, cytarabine (ara-C) and G-CSF priming),[2-9] with clofarabine substituted for fludarabine. Metabolism of both compounds to their triphosphates may increase synthesis of the triphosphate of ara-C (ara-CTP).[10,11] However, clofarabine can also inhibit ribonucleotide reductase,[12,13] resulting in fewer normally occurring deoxynucleotide triphosphates available to compete with ara-CTP for incorporation into DNA. Because the relative efficacies of GCLAC and fludarabine + ara-C + /- G-CSF remain uncertain, we performed a multivariate analysis comparing these salvage regimens.

Design and Methods

Patient selection

The GCLAC phase I/II protocol was approved by the Fred Hutchinson/University of Washington Cancer Consortium Institutional Review Board; all patients gave informed consent. Full details of the eligibility and patient characteristics are provided in the publication by Becker and colleagues.[1]

Treatment with GCLAC

Patients began G-CSF, 5 mcg/kg daily by subcutaneous injection one day prior to chemotherapy, and received G-CSF daily until ANC rose to at least 2.0 for two consecutive days. Clofarabine was administered intravenously over one hour, daily for five days at 15, 20, or 25 mg/m^2. Ara-C 2g/m^2 was given over 2 hours for five days, beginning 4 hours after the start of the clofarabine infusion.

Treatment with FA or FLAG

FA and FLAG used fludarabine at 30 mg/m^2 daily for 5 days with ara-C 2 g/m^2 daily for 5 days with (FLAG) or without (FA) G-CSF. We initially regarded these 2 regimens as equivalent based on previous results.[2] All FA or FLAG patients were treated at MD Anderson Cancer Center (MDACC), 98% before 2008 while all GCLAC patients were treated at Fred Hutchinson Cancer Research Center (FHCRC) in 2008-2010. Table 1 lists other pre-treatment characteristics of the GCLAC, FA, and FLAG cohorts. Forty percent of the GCLAC patients were refractory rather than relapsed, as compared to 25% of the FA patients, and 15% of the FLAG patients (p value not significant).

Statistical analysis

Comparison with fludarabine with (FLAG) or without (FA) G-CSF

Analyses of CR and survival were done independently at FHCRC and MDACC, with both centers using data from all patients given

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GCLAC or FA/FLAG. There were slight differences in the approaches, which we believe strengthen their independent conclusions. Criteria for CR and CRp were as typically specified.[14] Survival was calculated from the start of salvage therapy to death from any cause or to time of last follow up. Survival probabilities were estimated using the method of Kaplan and Meier and compared using the log rank test. Differences in binary variables, including CR or CRp, were assessed with Fisher's exact test and differences in continuous variables with the Kruskal-Wallis test. Multivariate analyses were used to inquire whether after accounting for relevant prognostic covariates treatment (GCLAC rather than FA/FLAG) affected CR or survival. Logistical regression was used for CR analyses and Cox's regression for analyses of survival. The proportional hazard assumption was verified by the Grambsch-Therneau test.[15] Non-treatment covariates examined were those found to be associated, at $P<0.05$, with CR or survival on univariate analyses. The covariates evaluated in the latter were: age, cytogenetics (with cytogenetic risk defined by Southwest Oncology Group criteria),[16] duration of first CR, number of prior treatments for active AML, and at MDACC prior hematopoietic cell transplant (SCT) and year treated (1990-1994, 1995-1999, 2000-2004, 2005-2007, 2008-2010). Terms were retained in the multivariate model if their P value was less than 0.05, and the same applied for terms describing interactions between treatment and covariates.

Results and Discussion

To compare GCLAC with a similar regimen but with fludarabine replacing clofarabine, we used the MD Anderson Cancer Center salvage database. Because the database contained only 20 FLAG salvage patients, we also included the 81 patients who received FA salvage (FLAG without G-CSF); previous MD Anderson data indicated that there was no difference between FA and FLAG in untreated patients.[2] CR rates were 22 of 81 with FA (27%; 95% CI: 18-38%) and 4 of 20 with FLAG (20%, 6-44%) salvage (Table 2). Survival probabilities are shown in Figure 1. The higher CR rate with GCLAC (46%) largely reflected results in patients with no initial CR (*primary refractory*, 2 of 20 FA, 0 of 3 FLAG, 12 of 18 GCLAC) or with short (< 6 months) initial CR (2 of 19 FA, 0 of 5 FLAG, 4 of 12 GCLAC, Table 2). Response rates with GCLAC were also superior in patients with prognostically unfavorable cytogenetics (Table 2, P=0.04 for comparison with FA/FLAG), probably reflecting the association between adverse cytogenetics and early relapse or failure to obtain an initial CR with FA/FLAG. The 30 day mortality for FA was 18 of 81, for FLAG 4 of 20, and for GCLAC 0 of 50.

FHCRC and MDACC each independently performed univariate and then multivariate analyses considering all treated patients (GCLAC, FA, FLAG). Although each center considered somewhat different covariates and selected somewhat different cut-off points, each analysis arrived at similar conclusions (Table 3). In particular, after accounting for the covariates illustrated in Table 3 each found that treatment with GCLAC was independently associated with a higher CR rate and longer survival. Multivariate

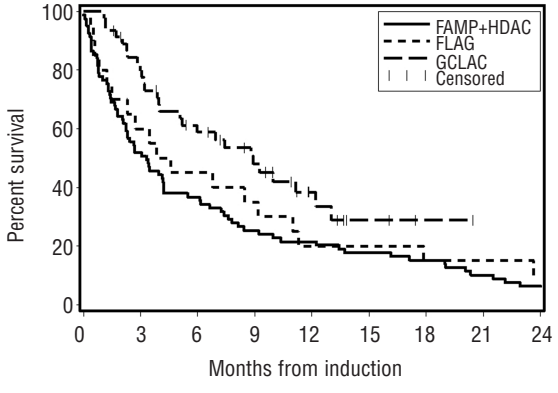

Figure 1. Overall survival of patients treated on GCLAC (long dash) *versus* FLAG (short dash) *versus* FA (FAMP+HDAC) (line). The survival was better for GCLAC as compared to FA, P=0.005, no significant difference in survival between FLAG and FA (P=0.37). GCLAC: clofarabine in combination with high-dose cytarabine and G-CSF priming; FLAG: fludarabine, cytarabine, and G-CSF; FA: fludarabine and high-dose cytarabine.

Table 1. Characteristics of patients enrolled on study.

	GCLAC patients N=50		FA patients N=81		FLAG patients N=20	
	Number	%	Number	%		
Sex						
Female	14	28%	35	43%	11	55%
Male	36	72%	46	57%	9	45%
AML onset						
De novo	32	64%	NA	NA	NA	NA
Secondary	18	36%	NA	NA	NA	NA
Relapsed (median first CR duration)	32 (26 wk)	64%	61 (47 wk)	75%	16 (33 wk)	42%
First salvage	32	64%	41	51%	12	60%
Second or greater salvage	18	36%	40	49%	8	40%
Refractory	18	36%	20	25%	3	15%
Cytogenetics (at initial diagnosis)						
Favorable	3	6%	0	0%	0	0%
Intermediate	27	54%	56	70%	13	68%
Unfavorable	20	40%	24	30%	6	32%
	Median	Range	Median	Range	Median	Range
Age (years)	53	19-69	56	18-82	57	22-87

Table 2. Response rates by regimen, duration first CR, salvage number, and cytogenetics risk category.

	CR with GCLAC 21/46 (46%)	CR with FA 22/81 (27%)	CR with FLAG 4/20 (20%)
Duration CR1 (months)			
0	12/18 (67%)	2/20 (10%)	0/3 (0%)
1-6	4/12 (33%)	2/19 (11%)	0/5 (0%)
>6-12	2/11 (18%)	3/17 (18%)	1/7 (14%)
> 12	3/5 (60%)	15/25 (60%)	3/5 (60%)
Salvage number			
1	16/33 (48%)	18/41 (44%)	2/12 (17%)
2	5/8 (63%)	4/24 (17%)	2/6 (33%)
≥3	0/5 (0%)	0/16 (0%)	0/2 (0%)
Cytogenetics risk			
Favorable	2/3 (67%)	--	--
Intermediate	10/25 (40%)	20/64 (31%)	2/13 (15%)
Unfavorable	9/18 (50%)	2/16 (13%)	2/7 (29%)

Table 3. Univariate and multivariate analyses.

Characteristic	CR rate	Median survival (months)	MD Anderson analysis Univariate *P* CR	Multivariate *P* Survival	CR	Survival
Age						
<56	23/74(31%)	4.3				
56-60	9/27 (33%)	4.6				
61-65	4/19 (21%)	3.9				
> 65	8/23 (35%)	4.2	NS	NS	NS	NS
Cytogenetics						
Normal	23/66 (35%)	7.4				
Other/intermediate risk	7/26 (27%)	4			Unfavorable *vs.* other HR1.67	
Unfavorable risk	14/50 (28%)	2.6	NS	0.03	NS	0.01
Prior SCT						
Yes	1/7 (14%)	1.1				
No	43/136 (32%)	4.3	NS	0.01	NS	NS
Duration of first CR (wks)						
0	13/41(32%)	5.2				
1-26	6/37 (16%)	2.4				
≥52 wk *vs.* <52 wk						
27-52	5/31 (16%)	3.5		< *vs.* ≥ 52 wk	O.R.9.35	
>52	20/34 (59%)	12.2	<0.001	<0.001	<0.001	<0.0001
Salvage number						
1	34/83 (41%)	7.9				
2	10/38 (26%)	4.2			>1 *vs.*1 O.R. 0.31	2 *vs.* 1 0.02 HR1.66
3	0/22 (0%)	2.0	<0.001	<0.001	0.009	≥3 *vs.* 1 <0.0001 HR 3.45
Year treated						
1990-1994	15/45 (33%)	4.2				
1995-1999	6/36 (17%)	2.6				
2000-2004	1/10 (10%)	2.5				
2005-2007	2/7 (29%)	2.1				
2008-2010	20/45 (44%)	8.8	0.05	0.06	NS	NS
Regimen						
FA	22/81 (27%)	3.4				<0.0001 HR 0.39 (GCLAC *vs.* FA)
FLAG	3/19 (16%)	3.8				
GCLAC	19/43 (44%)	8.8	0.05	0.04	0.003 O.R. 4.26 (GCLAC *vs.* FLAG/FA)	0.09 HR 0.58 (GCLAC *vs.* FLAG)

	CR Odds ratio (95% CI)		Hazard proportional assumption met FHCRC Analysis Mortality Hazard ratio (95%CI)		Univariate *P*		Multivariate *P*	
Characteristic	Univariate	Multivariate	Univariate	Multivariate	CR	Mortality	CR	Mortality
Age								
<60	1.0	1.0	1.0	1.0				
≥ 60	1.09 (0.5-2.2)	1.23 (0.5-2.9)	1.20 (0.8-1.7)	1.17 (0.8-1.7)	NS	NS	NS	NS
Cytogenetics								
Favorable/Intermed	1.0	1.0	1.0	1.0				
Unfavorable	1.15 (0.5-2.4)	1.13 (0.4-3.0)	1.31 (0.9-2.0)	1.46 (1.0-2.2)	NS	NS	NS	0.04
Duration of first CR								
≥ 38 wks	1.0	1.0	1.0	1.0				
1-37 *vs.* ≥ 38 wks	0.2 (0.1-0.5)	0.09 (0-0.3)	2.57 (1.7-3.9)	2.66 (1.7-4.1)	<0.0001	0.0004	<0.0001	<0.0001
0	0.51 (0.2-1.2)	0.16 (0-0.5)	1.37 (0.9-2.2)	1.52 (0.9-2.5)	NS	NS	0.002	NS
Salvage number								
1st	1.0	1.0	1.0	1.0				
2nd	0.4 (0.2-0.9)	0.35 (0.1-0.9)	1.75 (1.2-2.6)	1.65 (1.1-2.5)	0.03	0.006	0.03	0.02
>2nd	0.05 (0-0.4)	0.03 (0-0.3)	4.15 (2.5-6.9)	4.04 (2.4-6.9)	0.003	<0.0001	0.003	<0.0001

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Regimen								
FA/FLAG	1.0	1.0	1.0	1.0				
GCLAC (*vs.* FA/FLAG)	4.49 (2.1-9.4)	9.57 (3.4-27)	0.46 (0.3-0.7)	0.43 (0.3-0.7)	<0.0001	0.0002	<0.0001	0.0002

analysis of CR rate demonstrated superior CR rate for GCLAC *vs*. FA/FLAG with odds ratio 4.26, p=0.003 (MDACC) or odds ratio 9.57, *P*<0.0001 (FHCRC). For overall survival, multivariate analysis at MDACC showed superiority for GCLAC (median 8.8 months) *vs*. FA (median 3.4 months, hazard ratio 0.39, *P*<0.0001) or *vs*. FLAG (median 3.8 months, hazard ratio 0.58, *P*=0.09). The multivariate analysis at FHCRC also showed longer survival for GCLAC *vs*. FA/FLAG (hazard ratio 0.43, *P*=0.0002. Consistent with the observation that the principal benefit of GCLAC was in patients with no or a brief CR1 (Table 2), multivariate analysis also indicated that there was an interaction between CR1 duration and treatment with GCLAC (*P*=0.02)

The activity of clofarabine + ara-C as salvage therapy for AML has been previously described,[17] but questions regarding its efficacy relative to other regimens have arisen. Here we use multivariate analyses, done independently at MDACC and FHCRC, to demonstrate that GCLAC is at least as good and plausibly superior to regimens using the same dose and schedule of ara-C but employing fludarabine rather than clofarabine. Furthermore the analyses found that response to GCLAC was less dependent on first CR duration than was the case with FA or FLAG.

Several potentially confounding factors deserve mention. First, a higher proportion of patients who were "primary refractory" prior to receiving GCLAC as opposed to FA or FLAG had not received an initial induction regimen containing ara-C at a dose of ≥1.5g/m^2 daily X 4 (15/18 *vs*. 6/23). Second, a smaller proportion of primary refractory GCLAC patients had received 2, rather than one course of prior induction therapy (4 of 18 *vs.* 10 of 23). Restricting attention to patients who were primary refractory to 2 prior induction courses CR rates were 1 of 10 with FA/FLAG and 2 of 4 with GCLAC. Moreover, 5 patients who had previously not responded to fludarabine-containing therapy (FA 1, FLAG-AMSA 1, FLAG-Ida 2, FLAG 1), 4 entered CR after receiving GCLAC, again suggesting that leukemia that is refractory to fludarabine may be susceptible to clofarabine, although it is conceivable they may have responded equally well to a second course of fludarabine. Lastly, there is the issue of retrospective comparison of treatment administered 2008-2010 (GCLAC) *versus* treatment administered 1990-2007 (FA/FLAG). However, it is not clear that supportive care has changed dramatically in this time frame, and this multivariate analysis would represent the first step in trying to compare efficacy of GCLAC to other similar salvage regimens.

The utilization of salvage regimens as a bridge to transplant may lead to higher survival rates for more effective regimens. For the GCLAC study, half of the patients were able to proceed to allogeneic stem cell transplant. In summary, a multivariate analysis shows that the GCLAC regimen exhibited higher overall efficacy than fludarabine/cytarabine combinations, and a randomized comparison between GCLAC and other salvage regimens is warranted.

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Authorship and Disclosures

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