RESEARCH ARTICLE

G-CSF Priming, clofarabine, and high dose cytarabine (GCLAC) for upfront treatment of acute myeloid leukemia, advanced myelodysplastic syndrome or advanced myeloproliferative neoplasm

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Prior study of the combination of clofarabine and high dose cytarabine with granulocyte colony-stimulating factor (G-CSF) priming (GCLAC) in relapsed or refractory acute myeloid leukemia resulted in a 46% rate of complete remission despite unfavorable risk cytogenetics. A multivariate analysis demonstrated that the remission rate and survival with GCLAC were superior to FLAG (fludarabine, cytarabine, G-CSF) in the relapsed setting. We therefore initiated a study of the GCLAC regimen in the upfront setting in a multicenter trial. The objectives were to evaluate the rates of complete remission (CR), overall and relapse-free survival (OS and RFS), and toxicity of GCLAC. Clofarabine was administered at 30 mg m^{-2} day^{-1} × 5 and cytarabine at 2 g m^{-2} day^{-1} × 5 after G-CSF priming in 50 newly-diagnosed patients ages 18–64 with AML or advanced myelodysplastic syndrome (MDS) or advanced myeloproliferative neoplasm (MPN). Responses were assessed in the different cytogenetic risk groups and in patients with antecedent hematologic disorder. The overall CR rate was 76% (95% confidence interval [CI] 64–88%) and the CR + CRp (CR with incomplete platelet count recovery) was 82% (95% CI 71–93%). The CR rate was 100% for patients with favorable, 84% for those with intermediate, and 62% for those with unfavorable risk cytogenetics. For patients with an antecedent hematologic disorder (AHD), the CR rate was 65%, compared to 85% for those without an AHD. The 60 day mortality was 2%. Thus, front line GCLAC is a well-tolerated, effective induction regimen for AML and advanced myelodysplastic or myeloproliferative disorders.
Am. J. Hematol. 90:295–300, 2015.

Introduction

Clofarabine has been shown to have potent antileukemic activity attributed to its ability to inhibit ribonucleotide reductase and potentiate formation of araCTP. Combinations of clofarabine and cytarabine are active in newly diagnosed acute myeloid leukemia (AML), producing a complete remission (CR) rate of 52% and CR + CRp rate of 60% in patients age ≥50 [1]. Addition of low dose cytarabine to clofarabine resulted in a higher CR rate, as well as longer event free (but not overall) survival than seen with clofarabine in an adaptively randomized trial in newly diagnosed patients aged ≥ 60 [2]. Likewise in a similar trial in relapsed patients age ≥55 clofarabine (40 mg m^{-2} day^{-1} × 5) plus cytarabine (1 g m^{-2} day^{-1} × 5) resulted in a higher response rate, but not improved survival, than cytarabine alone [3]. In relapsed or refractory disease we reported a CR rate of 46% with G-CSF priming, clofarabine, and high dose cytarabine (GCLAC), a regimen derived from the FLAG regimen via substitution of clofarabine for fludarabine; results were similar in patients with "unfavorable" cytogenetics [4]. Furthermore, multivariate analyses suggested that GCLAC as given at the University of Washington/Fred Hutchinson Cancer Research Center (UW/FHCRC) was associated with better CR and survival rates than fludarabine/cytarabine combinations as given at the University of Texas MD Anderson Cancer Center [5]. These results motivated a trial of GCLAC in patients with newly diagnosed AML, or advanced myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN), with the goal of assessing CR rate and survival. The trial was registered at ClinicalTrials.gov as NCT01101880.

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Conflict of interest: PSB discloses research funding from Sanofi. All other authors report nothing to disclose.
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Received for publication: 15 December 2014; **Accepted:** 20 December 2014
Am. J. Hematol. 90:295–300, 2015.
Published online: 24 December 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23927

Methods

Human subjects. This study was conducted with approval of the Fred Hutchinson Cancer Research Center Institutional Review Board and the Institutional Review Boards at Stanford University and City of Hope, and in accordance with an assurance filed with and approved by the US Department of Health and Human Services. Informed consent was obtained from all patients.

Eligibility. Adult patients ages 18 through 64 with a diagnosis of acute myeloid leukemia by World Health Organization (WHO) with the exception of acute promyelocytic leukemia, or advanced myelodysplastic syndrome including RAEB-2 or advanced myeloproliferative neoplasm, including CMML-2 by WHO classification with ≥10% blasts in the bone marrow or peripheral blood. Patients were also required to have Eastern Cooperative Group performance status 0 through 2, and adequate renal and hepatic function as indicated by the following laboratory values: (1) serum creatinine ≤1.0 mg dL^{-1} or if serum creatinine >1.0 mg dL^{-1}, then the estimated glomerular filtration rate (GFR) must be >60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation; (2) serum bilirubin ≤1.5 × upper limit of normal (ULN) unless elevation is thought to be due to Gilbert's syndrome, hemolysis, or hepatic infiltration by the hematologic malignancy, (3) aspartate transaminase (AST)/alanine transaminase (ALT) and alkaline phosphatase ≤2.5 × ULN unless elevation is thought to be due to hepatic infiltration by the hematologic malignancy. Prior therapy with imides or hypomethylating agents for a preceding hematological disorder was permitted. Patients were excluded for serious dysfunction involving the heart, kidney, liver, or other organ system that may place the patient at undue risk to undergo treatment, or significant organ compromise due to systemic fungal, bacterial, viral, or other infection, or prior allogeneic hematopoietic cell transplant.

Treatment. Patients received daily subcutaneous G-CSF priming (filgrastim 5 mcg kg^{-1}), starting 1 day prior to the initiation of the clofarabine and cytarabine induction therapy and continuing until the absolute neutrophil count (ANC) was >2.0 × 10^9/L for 2 consecutive days. Clofarabine (30 mg m^{-2} day^{-1}) was given intravenously over 1 hr on days 1 through 5 with cytarabine (2 g m^{-2} day^{-1}) given intravenously over 2 hr on the same 5 days but beginning 4 hr after the start of clofarabine. A second induction cycle was administered if a patient had >5% marrow blasts 21 days post induction. Post remission therapy was identical except that G-CSF was discontinued once chemotherapy was complete and that doses of clofarabine and cytarabine were reduced: 25 mg m^{-2} day^{-1} of the former and 2 g m^{-2} day^{-1} of the latter were given on days 1–4. Patients could receive up to 3 post remission courses. At any point, after achieving CR or CRi or similar response meeting criteria for allogeneic transplant at the respective institutions, patients could proceed to transplant.

Concomitant medications. Prophylactic administration of steroids (hydrocortisone or dexamethasone or prednisone) was encouraged to minimize clofarabine related toxicities. Antimicrobial prophylaxis was typically done with levofloxacin, fluconazole, and acyclovir, except that fluconazole was not given on days clofarabine was administered.

Data analysis. The revised National Cancer Institute's Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 was used for adverse event reporting.

Response rates were determined by standard criteria [6]. Minimal residual disease (MRD) was defined as any level of cells with the phenotype of the original leukemia by multicolor flow cytometry and this analysis was limited to the patients at UW/FHCRC. Survival (OS) was estimated using the Kaplan–Meier method [7] and measured from the first day of induction therapy to death due to any cause, with patients last known to be alive censored at the date of last contact.

Flow cytometry assessment of minimal residual disease (MRD). MRD was defined for patients with morphologic CR with blast count under 5%, and any level of residual leukemia as assessed by morphology or flow cytometry or cytogenetics/fluorescence in situ hybridization (FISH) or mutation analysis. This analysis was restricted to the patients treated at UW/FHCRC, for whom the bone marrow was assessed for MRD by multiparameter flow cytometry, as described [8].

Results

Patients

Fifty patients were enrolled. Thirty-nine had AML and 11 had MDS or MPN with 10–19% blasts. The median age was 53 years (range of 22–64). Twenty-three of the patients (46%) had a documented abnormality in blood count for >1 month prior to diagnosis of AML or advanced MDS/MDS (AHD) and 2 had therapy-related AML or MDS/MPN. Using SWOG criteria, 13 patients (26%) had unfavorable, 32 patients (64%) intermediate, and 4 patients (8%) favorable cytogenetics with 1 patient in the unknown risk category. Table I notes other pretreatment characteristics for this group.

TABLE I. Patient Characteristics

Characteristic	Number (total = 50)	%
Gender		
Female	23	46%
Male	27	54%
Cytogenetics		
Favorable	4	8%
Intermediate	32	64%
Unfavorable	13	26%
Indeterminate	1	2%
Antecedent hematological disorder (AHD)	23	46%
Flt3 ITD mutation	10 of 42 tested	24%
Median age (range)	53 (22–64)	
Median WBC × 10^9/L	12.0 (0.8–761.3)	
Median peripheral blast (%)	16% (0–100%)	

Definition of cytogenetic risk: Favorable: inv16, *t*(8;21); Intermediate. Trisomy 8, normal, -Y,+6,-12p;Unfavorable; -5/-5q,-7/-7q, abnormal 3q,9q,17p,20q,21q, t(9,22), complex karyotype [9].

Response rate and postremission therapy

Complete remission (CR) was achieved in 38 patients [76% (95% CI 64–88%)], most (33/38) with the first course of induction, and CR or CRp in 41 patients [82% (95% CI 71–93%)]. Six of 11 patients given a 2nd course after not being in CR/CRp after the 1st responded (5CR, 1 CRp). CR rate was higher for patients without an AHD (85% vs. 65%) (Table II). Cytogenetics was also associated with response in the expected pattern: CR rates were 100%, 84%, 62% for patients with "favorable" intermediate, and unfavorable risk and CR+CRp rates of 100%, 84%, and 77%, respectively (Table II). Thirty-five patients of the 38 patients entering CR (92%, 95%) CI received one course of GCLAC postremission therapy, 21 two courses and 13 three courses. Twenty-seven patients eventually received an allogeneic transplant, 23 doing so while in CR1 after treatment on this study (61% of the 38 CRs).

Blood count recovery

In patients whose ANC rose to >500 and/or platelet count to > 100,000 after therapy median times to these counts were 19 days (range 13–35 for ANC, 47 patients) and 24 days (range 16–63 for platelets, 36 patients).

Relapse and survival

Forty-nine of the 50 patients survived 60 days after treatment began, and one patient died on day 44 after induction due to refractory leukemia and infection. Median overall survival was 24.3 months (95% CI 15 months, not reached). At a median follow up of 15 months, 32 patients were alive, of whom 21 were in remission (Fig. 1). Eighteen patients had died: 6 due to refractory AML (at 1.5, 3, 4, 5, 15, and 16 months), 9 relapsed with AML at a median of 7 weeks from CR (range 4–84 weeks) and 3 succumbed to post-transplant complications. MRD was assessed routinely only in UW/FHCRC patients by multicolor flow cytometry in whom it had an effect on survival: median <16 months for patients with MRD, vs. 70% at 30 months for those without MRD (Fig. 2). There were no patients with MRD present after induction who became negative for MRD after consolidation. Patients with AHD exhibited reduced overall survival compared to those without AHD (Fig. 3). Similarly, patients with unfavorable cytogenetics had reduced overall survival compared to those in other risk categories (Fig. 4).

 doi:10.1002/ajh.23927

TABLE II. Response Rates for GCLAC in Patients With or Without AHD, Those With the Flt3 ITD Mutation, and by Cytogenetic Risk Group

	CR	ORR (CR + CRp)
All ($n = 50$)	38 (76%) 95% CI (64–88%)	41 (82%) 95% CI (71–93%)
No AHD ($n = 27$)	23/27 (85%) 95% CI (76–94)	23/27 (85%) 95% CI (76–94)
+ AHD ($n = 23$)	15/23 (65%) 95% CI (51–79)	18/23 (78%) 95% CI (61–95)
Flt3 positive ($n = 10$)	7/10 (70%)	7/10 (70%)
Favorable risk cytogenetics ($n = 4$)	4/4 (100%)	4/4 (100%)
Intermediate risk cytogenetics ($n = 32$)	26/32 (81%)	27/32 (84%)
Unfavorable risk cytogenetics ($n = 13$)	8/13 (62%)	10/13 (77%)

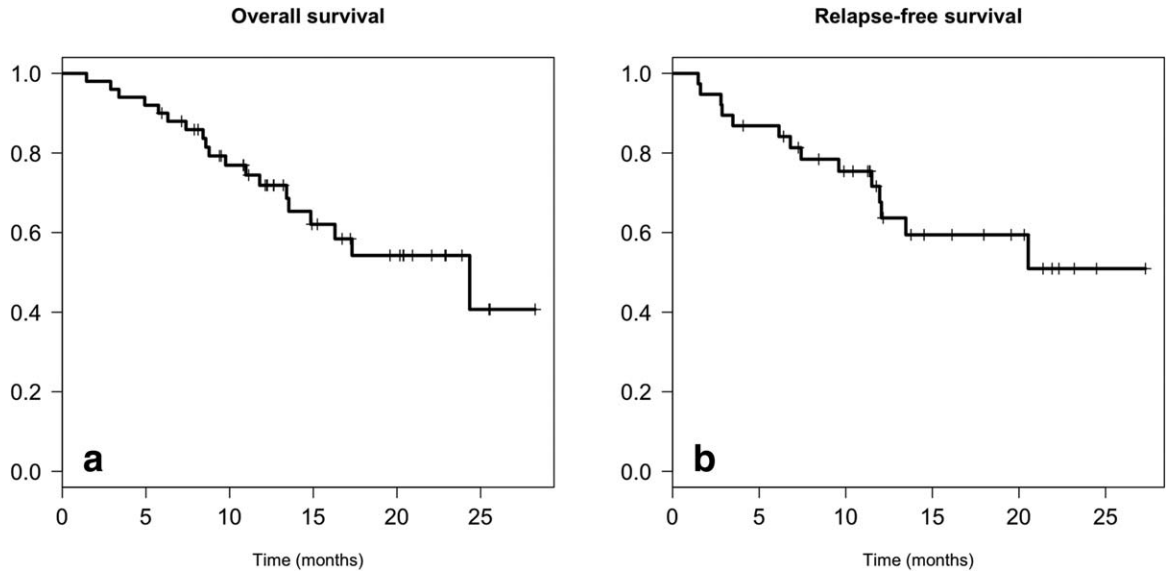

Figure 1. Overall and relapse-free survival (RFS). a. Overall survival. b. Relapse free survival.

Adverse events (AEs)

There were 180 total grade 3 AEs in 38 (76%) of the patients, 116 during induction, of which the majority, 58 (24%) were due to infection, and 64 during consolidation, of which the majority, 35 (15%), were also due to infection. The remainder of the grade 3 events each comprised less than 7% of the events, including pulmonary and hepatic toxicity, for which full details are provided in Table III. There were 13 total grade 4 AEs in 10 (20%) of the patients: 12 during induction including 4 pulmonary (6%), 2 infection (3%), 4 hepatic enzyme elevation (6%), 2 metabolic (3%), and 1 (2%) during consolidation which was a pulmonary event. Included in the grade 4 pulmonary events were 3 occurrences of pulmonary infiltrates, hypoxia, and diffuse alveolar hemorrhage that responded to steroids, and this constellation was rarely seen when patients were premedicated with steroids. The grade 3 and 4 infections for which a pathogen was identified included bacteremias [methicillin resistant *Staphylococcus aureus* (4), *Enterococcus faecalis*, coagulase negative Staphylococcus (4), *Escheresia coli* (3), alpha hemolytic streptococcus (2), micrococcus, *Clostridium innocuum*, *Proteus mirabilis*, *Rothia mucilaginosa*, *Streptococcus viridans*, *Streptococcus mitis* (2), *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, Enterobacter, Salmonella, and vancomycin resistant enterococcus], *Clostridium difficile* colitis (3), viral pneumonias [rhinovirus, parainfluenza, and influenza (2), cytomegalovirus], and *Aspergillus fumigatus* pulmonary infection.

Discussion

The impetus for this trial was our study of GCLAC in relapsed/refractory AML [4]. In this setting multivariate analyses suggested the superiority of GCLAC to fludarabine + ara-C +/G-CSF with respect to CR and survival [5], so we thought we should examine its efficacy in the upfront setting. However, the cooperative group trials have typically excluded patients with AHD, so we sought comparison with other trials that included this group. The CR rate of 65% for patients with AHD in this study compares favorably with the results of the other reported clofarabine plus cytarabine combinations: 33% for clofarabine with 1 g m^{-2} araC dose [1], 49% for clofarabine with low dose araC [2], and 36% for patients who received clofarabine plus low dose araC followed by alternating cycles of this combination and decitabine [10]. Moreover, we observed a CR rate of 70% for patients with the Flt3 ITD mutation after treatment with GCLAC without the inclusion of a Flt3 inhibitor. Patients with unfavorable cytogenetics also fared relatively well, with a CR rate of 62%.

Given the only fair ability of known covariates to predict outcome in AML [11], multivariate analyses cannot substitute for randomized clinical trials. Three randomized trials have compared clofarabine ± - other drugs with more standard therapies. In "fit" older patients with AML the MRC/NCRI noted that clofarabine+daunorubicin produced similar outcomes as ADE; results were similar in various patient subgroups. In less fit older patients clofarabine doubled the CR rate seen with low dose cytarabine but did not affect survival [12]. In the only randomized trial examining clofarabine + higher-dose cytarabine [3], Faderl et al. found that this regimen produced better CR and EFS but not overall survival than the same dose of cytarabine (1 g m^{-2} daily × 5) alone in patients age >55 with relapsed or refractory AML. There were more deaths by day 30 of treatment in the clofarabine + cytarabine group. This led to the suggestion that better pretreatment identification of patients at high risk for treatment-related

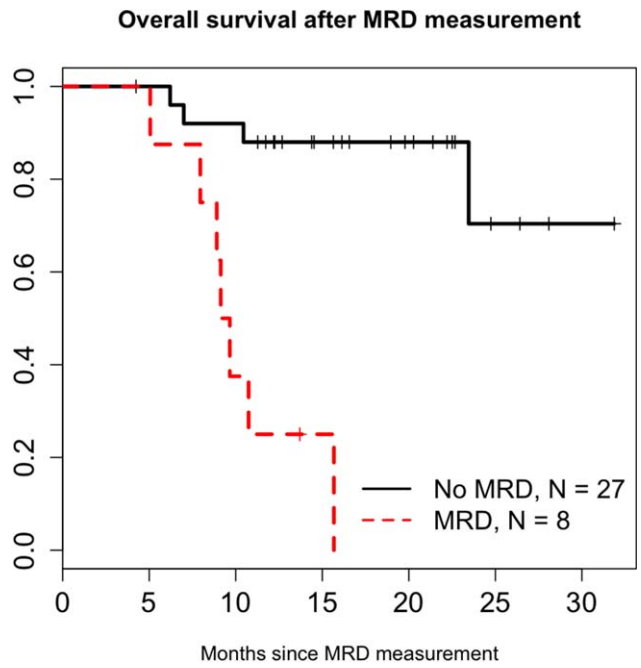

Figure 2. Overall survival by presence or absence of minimal residual disease (MRD). The solid line represents the patients without MRD, the dashed line, the patients with MRD. Note the markedly decreased survival for patients with MRD. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

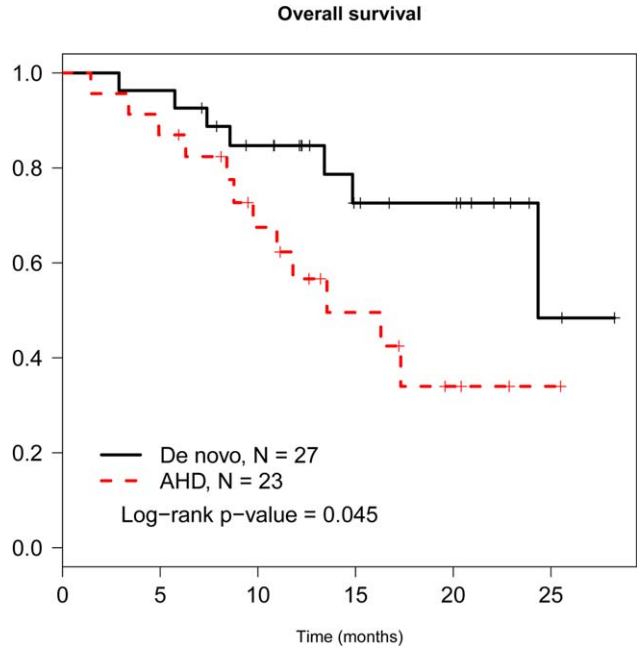

Figure 3. Overall survival after GCLAC for patients with or without antecedent hematologic disorder (AHD). The solid line represents the survival of patients without AHD (de novo) and the dashed line the survival of patients with AHD. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mortality and their exclusion from clofarabine + high-dose cytarabine might have led to improved survival in this arm.

One advantage of the GCLAC regimen for patients with impaired cardiac function is that it does not include an anthracycline yet is intensive with preserved efficacy comparable to the regimens that include anthracyclines. Table IV summarizes the results of the published front-line combination drug regimens of purine analogs with-

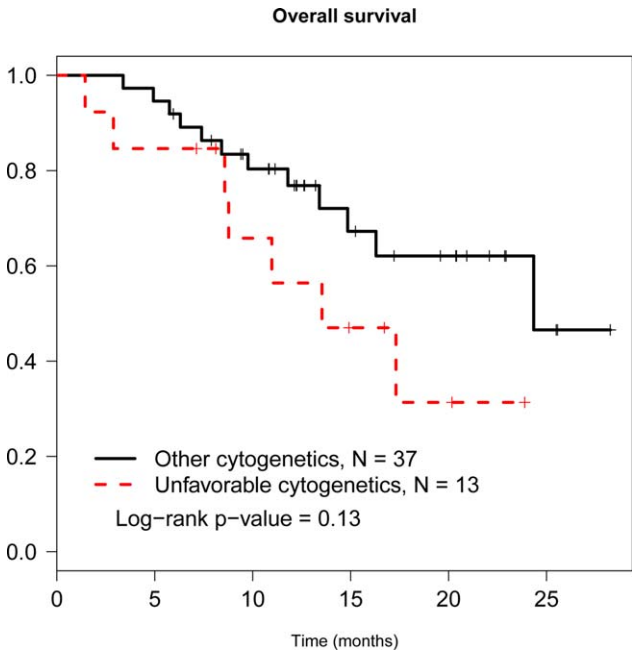

Figure 4. Overall survival after GCLAC by cytogenetic risk group. The dashed line represents the survival of patients with unfavorable cytogenetics, and the solid line represents the patients with other cytogenetic risk categories (favorable, intermediate and unknown). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III. Grade 3 and Grade 4 Adverse Events

Event	Grade 3 number (% of grade 3 events) total $N = 180$ in 38 (76%) of the patients		Grade 4 number (% of grade 4 events) total $N = 13$ in 10 (20%) of the patients	
Cycle type	Induction	Consolidation	Induction	Consolidation
Number in category	116 (64%)	64 (36%)	12 (92%)	1 (8%)
Infection	58 (32%)	35 (19%)	2 (15%)	0
Pulmonary	15 (8%)	11 (6%)	4 (31%)	1 (8%)
Liver enzymes	13 (7%)	3 (2%)	4 (31%)	0
Gastrointestinal	7 (4%)	3 (2%)	0	0
Metabolic	7 (4%)	2 (1%)	1 (8%)	0
Dermatologic	5 (3%)	5 (3%)	0	0
Neurologic	4 (2%)	2 (1%)	0	0
Coagulation	3 (2%)	1 (<1%)	0	0
Cardiovascular	2 (1%)	0	0	0
Ocular	1 (<1%)	0	0	0
Pain	1 (<1%)	0	0	0
Hepatobiliary	0	1 (<1%)	0	0
Urinary	0	1 (<1%)	0	0

out anthracycline. In comparison with the other clofarabine regimens, the dose of araC is increased to 2 g m^{-2} with GCLAC. The CR rate with GCLAC is higher than the regimens with the lower doses of araC [1,2,10], although the ages of the studied populations differ. Fludarabine [13] and cladribine [15] are also effective in combination with cytarabine in upfront AML. Notably, FLAG has exhibited efficacy in core binding factor (CBF) leukemias [16], and the addition of GO to FLAG results in an overall survival of 78%, and RFS 85% at 3 years [14]. The other options for regimens that do not include anthracyclines include hypomethylating agents, imides, histone deacetylase inhibitors and novel investigational drugs.

The presence of MRD has been associated with poor prognosis for patients with AML undergoing allogeneic stem cell transplant [17,18],

 doi:10.1002/ajh.23927

TABLE IV. Front-line Combination Regimens for AML With a Purine Analog Without an Anthracycline

Publication	Regimen	Indication	CR rate	Median RFS/OS (m)	Induction death	Age median (range)
Ferrara et al. [13]	FLAG (fludarabine 30 mg m^{-2}, AraC 2 g m^{-2})	AML with multilineage dysplasia	64%	22/16	9%	61 (31–75)
Borthakur et al. 2014 [14]	FLAG-GO Doses of fludarabine and araC as above, GO 3 mg m^{-2}	CBF-AML	91%	12/Not reached	4%	48 (19–76)
Faderl et al. 2006 [1]	Clo 40 mg m^{-2} AraC 1 g m^{-2}	AML age ≥50	52% (45% in Flt3 ITD, 33% in AHD)	8.1/10.3	7%	61 (50–74)
Faderl et al. 2008 [2]	Clo 20 mg m^{-2} vs. Clo 20 mg m^{-2}-LD-AraC 20 mg m^{-2}	Elderly AML, high risk MDS, CMML	Clo-31%Clo-LD-AraC-63%(14% in Flt3 ITD, 49% in AHD)	Clo not reached/5.8 Clo-LD-AraC-11.4/11.4	Clo 31%Clo-LD-AraC 19%	61 (60–83)
Faderl et al. 2012 [10]	Clo 20 mg m^{-2}-AraC 20 mg m^{-2} then consolidation alternating with decitabine	Elderly AML	58% (86% in Flt3ITD $N = 7$, and 36% in AHD)	14.1/12.7	7% (8 weeks)	70 (60–81)
Rubnitz et al. 2009 [15]	Cladribine 9 m m^{-2}-AraC 500 mg m^{-2} (arm over 2h vs. arm CI)	Pediatric AML	araC over 2 h: 43%araC CI: 65%	ND, other therapy followed	ND, other therapy followed	9 (0.05–21)
Present study	GCLAC (clofarabine 30 mg m^{-2}, AraC 2 g m^{-2})	Adult AML	76% (70% in Flt3 ITD, and 65% in AHD)	Not reached/24.3	2% at 8 weeks	53 (22–64)

LD = low dose, CBF = core binding factor, GO = gemtuzumab ozogamicin, CI = continuous infusion, ND = not determined.

and was also noted to predict poor risk if present after the second cycle of treatment for AML [19]. We observed 0% survival at 15 months for patients who achieved CR with MRD (detectable leukemia by flow cytometry) after GCLAC induction, compared to those without MRD, who exhibited 70% survival at 30 months (Fig. 2).

One plausible future direction for GCLAC is a trial randomizing patients aged <60–65 between it and 3 + 7, the "standard" regimen that results in a CR rates in the range of 60–70% in the cooperative group setting [20–22]. However recognizing that results with neither are optimal, another possibility is addition of an anthracycline to GCLAC, particularly in patients in whom treatment-related mortality is distinctly improbable. Nazha et al. [23] have reported that clofarabine, ara-C (1 g m^{-2} daily × 5) and idarubicin (CIA) is "safe and effective" in newly diagnosed patients aged < 60 and Wierzbowska et al. [24] have reported analogous results in relapsed/refractory patients substituting cladribine for clofarabine (CLAG-M). Randomized comparison of either of these regimens with 3 + 7 as initial therapy might be worthwhile.

In summary, GCLAC is a well-tolerated induction regimen with minimal treatment related mortality, and typical incidence of expected hematological toxicity and infections. There was a high remission rate, and presence of MRD was associated with brief survival.

Acknowledgments

Research support was provided by Sanofi. The authors gratefully acknowledge the contribution of the late Dr. Stephen H. Petersdorf.

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