Randomized multicenter phase II study of flavopiridol (alvocidib), cytarabine, and mitoxantrone (FLAM) versus cytarabine/daunorubicin (7+3) in newly diagnosed acute myeloid leukemia

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ABSTRACT

Serial studies have demonstrated that induction therapy with FLAM [flavopiridol (alvocidib) 50 mg/m^2 days 1-3, cytarabine 667 mg/m^2/day continuous infusion days 6-8, and mitoxantrone (FLAM) 40 mg/m^2 day 9] yields complete remission rates of nearly 70% in newly diagnosed poor-risk acute myeloid leukemia. Between May 2011-July 2013, 165 newly diagnosed acute myeloid leukemia patients (age 18-70 years) with intermediate/adverse-risk cytogenetics were randomized 2:1 to receive FLAM or 7+3 (cytarabine 100 mg/m^2/day continuous infusion days 1-7 and daunorubicin 90 mg/m^2 days 1-3), across 10 institutions. Some patients on 7+3 with residual leukemia on day 14 received 5+2 (cytarabine 100 mg/m^2/day continuous infusion days 1-5 and daunorubicin 45 mg/m^2 days 1-2), whereas patients on FLAM were not re-treated based on day 14 bone marrow findings. The primary objective was to compare complete remission rates between one cycle of FLAM and one cycle of 7+3. Secondary end points included safety, overall survival and event-free survival. FLAM led to higher complete remission rates than 7+3 alone (70% vs. 46%; *P*=0.003) without an increase in toxicity, and this improvement persisted after 7+3+/-5+2 (70% *vs.* 57%; *P*=0.08). There were no significant differences in overall survival and event-free survival in both arms but post-induction strategies were not standardized. These results substantiate the efficacy of FLAM induction in newly diagnosed AML. A phase III study is currently in development. This study is registered with *clinicaltrials.gov identifier: 01349972.*

Introduction

Adults with acute myeloid leukemia (AML) have a poor prognosis with conventional chemotherapy agents. There are approximately 18,000 new cases of AML with close to 11,000 deaths yearly in the United States.[1] Unfortunately, treatment of AML has changed little over the last four decades. "7+3" [7 days of continuous infusion (CI) cytarabine and 3 days of anthracycline] remains the standard induction chemotherapy regimen for newly diagnosed non-acute promyelocytic leukemia (APL) AML patients who are fit for intensive therapy.[2] Despite many attempts to improve the 7+3 regimen with the addition and/or substitution of mechanistically diverse agents, no regimen has proven to be consistently superior to 7+3.[3]

Timed sequential therapy (TST) has been shown to improve outcomes in some newly diagnosed adults and children with AML.[4-8] TST relies on the opportune timing of cell-cycle specific cytotoxic agents in order to exert maximal effect on leukemic cell death. *In vitro* studies of flavopiridol, a pan cyclin-dependent, multi-serine-threonine kinase inhibitor, followed by cytarabine in a TST manner demonstrated increased cytotoxicity compared to either agent alone.[9] FLAM (flavopiridol followed by cytarabine and mitoxantrone) was evaluated in 138 newly diagnosed poor-risk AML patients in serial phase II trials, with overall complete remission (CR) rates of 67%-80% and reproducibly low rates of morbidity and mortality.[10-12] These data suggest that FLAM might improve outcomes relative to 7+3 induction therapy in newly diagnosed AML patients. Therefore, we sought to compare FLAM to 7+3 in a multicenter randomized phase II trial in newly diagnosed adult AML patients with intermediate- and adverse-risk cytogenetics.

Methods

Patient eligibility

Between May 2011 and July 2013, newly diagnosed AML patients aged 18-70 years with pathological confirmation of bone marrow (BM) blasts 20% or more were enrolled in a multi-institutional study. Eligibility criteria were similar to those from previous studies.[10-12] FISH

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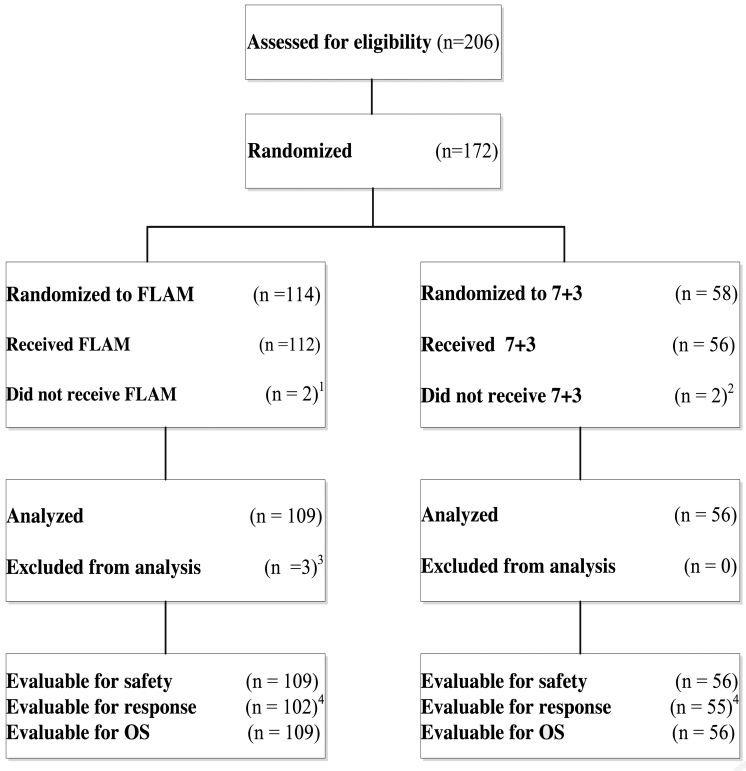

Figure 1. Consort Diagram. 172 patients were randomized between FLAM (n = 114) and 7+3 (n = 58), 109 patients on FLAM and 56 patients on 7+3 were analyzed for response and overall survival (OS). [1]2 patients did not receive FLAM after randomization: progressive deterioration of performance status (n=1), exceeded maximum prior anthracycline dose (n=1) [2]2 patients did not receive 7+3 after randomization: withdrawal of consent (n=1), death before intervention (n=1). [3]3 patients were excluded from the analysis after initiating treatment with FLAM due to ineligibility: T-cell acute lymphoblastic leukemia (n=1), Favorable-risk cytogenetics: t(8;21) (n=1), Prior treatment for AML (n=1). [4]7 patients treated with FLAM and one patient treated with 7+3 were non-evaluable (NE) for response due to early death.

for core-binding factor (CBF) AML (t(8;21); inv(16); (t(16;16)) was performed at each institution prior to enrollment, and patients were excluded if CBF positive. The study was conducted in accordance with the Declaration of Helsinki after approval by the ethics committee of each participating center.

Treatment

Patients were randomized by centralized computer-generated allocation procedure (REDCap[13]) 2:1 to receive FLAM (arm A): flavopiridol 50 mg/m^2 IV days 1-3, cytarabine 2 gm/m^2 CI IV days 6-8 (667 mg/m^2/day), and mitoxantrone 40 mg/m^2 IV day 9 or 7+3 (arm B): cytarabine 100 mg/m^2/day CI IV days 1-7, and daunorubicin 90 mg/m^2 IV days 1-3 (idarubicin 12 mg/m^2 IV days 1-3 was substituted as needed for lack of daunorubicin availability). Patients were stratified according to the following risk factors: 1) age 50 years or over; 2) secondary AML (defined as treatment-related AML or AML from antecedent hematologic disorder) and/or known adverse cytogenetics;[14] and 3) hyperleukocytosis [white blood cell (WBC) count >50x10^9/L].

All patients received a BM biopsy on day 14 unless medically contraindicated. Residual leukemia on day 14 was defined as BM blasts 5% or more morphologically with overall cellularity 10% or more. Arm B patients were eligible to receive an additional cycle of induction therapy, 5+2 (cytarabine 100 mg/m^2/day CI IV days 1-5, daunorubicin 45 mg/m^2 IV days 1-2) in the setting of residual leukemia on day 14. Post-induction treatment was performed according to physician preference.

Response and toxicity

Bone marrow (BM) aspirates and biopsies were performed before treatment, on day 14 of treatment, and at hematologic recovery or when leukemia regrowth was suspected. Response criteria were defined according to standard definitions.[14] Adverse events were graded by NCI Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0.

Statistical analysis

The study was designed to compare CR rates between FLAM and one cycle of 7+3, using a Bayesian approach for interim monitoring for futility. The primary analysis would conclude a significant benefit for FLAM if the one-sided *P* value from a Fisher's exact test less than 0.10. A sample size of 165 patients, randomized 2:1 to FLAM or 7+3, respectively, yielded 85% power to detect an increase in the probability of CR from 55% with 7+3[15-17] to 75% with FLAM. In addition to the planned primary end point analysis, CR rates between FLAM and 7+3+/-5+2 were analyzed by Fisher's exact test with a one-sided *P* value analogous to the primary end point analysis.

Secondary end points included toxicity comparisons, overall survival (OS), and event-free survival (EFS). OS was defined from date of randomization to death or last known follow up. EFS was defined as date of randomization to the first occurrence of persistent AML after one cycle of induction, relapse or death. Patients were censored for EFS if they had received non-protocol therapy or an allogeneic stem cell transplant (SCT). All significance tests for secondary end points were two-sided with $P<0.05$ considered significant. To explore heterogeneity of treatment effects between various subgroups, logistic regression models with terms for treatment, the patient subgroup, and their interaction were fit. *P* values were derived from likelihood ratio tests and are considered exploratory. OS, EFS, and time to hematologic recovery probabilities were calculated using the Kaplan-Meier method and tested for differences between treatment arms with the log rank test.

Further details of Methods are provided in the *Online Supplementary Appendix*.

Table 1. Patients' characteristics.

	FLAM (n=109) N (%)	7+3 (n=56) N (%)
Age- median (range)	59 (19-70)	60 (22-69)
Age ≥50	82 (75%)	42 (75%)
Age ≥60	48 (44%)	31 (55%)
Male	61 (56%)	31 (55%)
Adverse genetics[2]	58 (58%)	27 (55%)
FLT3-ITD mutation	13 (14%)	6 (15%)
Adverse cytogenetics	48 (44%)	21 (38%)
Complex cytogenetics	30 (28%)	16 (29%)
Monosomal cytogenetics	24 (22%)	12 (21%)
NPM1 mutation	26 (29%)	10 (25%)
ELN risk group		
Favorable	17 (16%)	8 (14%)
Intermediate-1	32 (29%)	22 (39%)
Intermediate-2	12 (11%)	5 (9%)
Adverse	48 (44%)	21 (38%)
Secondary AML	52 (48%)	26 (46%)
Treatment-related	15 (14%)	4 (7%)
Prior hematologic disorder	37 (34%)	22 (39%)
WBC >50x10^9/L	13 (12%)	5 (9%)
No poor-risk features[3]	25 (23%)	18 (32%)
≥1 poor-risk feature	84 (77%)	38 (68%)
≥2 poor-risk features	34 (31%)	20 (36%)
Site		
Johns Hopkins	46 (42%)	22 (39%)
University of North Carolina	15 (14%)	8 (14%)
Mayo Clinic- MN	15 (14%)	5 (9%)
BMT of GA	11 (10%)	7 (12%)
Vanderbilt	8 (7%)	3 (5%)
Moffitt	3 (3%)	4 (7%)
VCU	3 (3%)	3 (5%)
Baylor- Dallas, TX	3 (3%)	3 (5%)
Mayo Clinic- AZ	4 (4%)	1 (2%)
University of MD	1 (1%)	0 (0%)

There were no significant differences between both arms for all of the patients' characteristics; [2]adverse genetics included patients with adverse cytogenetics according to ELN risk criteria[14] and/or FLT3-ITD mutations; [3]poor-risk features defined by: 1) WBC >50x10^9/L) Adverse genetics (adverse cytogenetics and/or FLT3-ITD mutation), 3) Secondary AML (treatment-related or AML from antecedent hematologic disorder).

Table 2. Toxicity comparisons.

Grade ≥3 toxicity	FLAM (n=109)	7+3 (n=56)	*P*
Tumor lysis syndrome	9 (8%)[1]	4 (7%)[2]	>0.99
Myocardial dysfunction	8 (7%)	3 (5%)	0.75
GI toxicity	12 (11%)[3]	5 (9%)	0.79
Hepatic dysfunction	23 (21%)	13 (23%)	0.84
Infection	38 (35%)[4]	21 (38%)	0.74
Pulmonary toxicity	8 (7%)	4 (7%)	>0.99
Renal toxicity	3 (3%)	1 (2%)	>0.99
Thromboembolic events	3 (3%)	1 (2%)	>0.99
Febrile neutropenia events	52 (48%)	25 (45%)	0.74
Day 30 mortality	5 (5%)	1 (2%)	0.67
Day 60 mortality	11 (10%)	2 (4%)	0.22

TLS: tumor lysis syndrome; GI: gastrointestinal; [1]2 early deaths due to TLS; [2]1 early death due to TLS; [3]1 early death due to GI bleed; [4]3 early deaths due to sepsis.

Results

Patients' characteristics

One hundred and sixty-five patients (FLAM: n=109, 7+3: n=56) from 10 institutions were randomized, treated, and included in the analysis, as shown in Figure 1. Clinical demographics and disease biological features of all patients are presented in Table 1. Adverse-risk according to ELN criteria[14] was seen in 44% and 38% of patients on FLAM and 7+3, respectively. In addition, 47% of patients (FLAM: 48%, 7+3: 46%) had secondary AML. The majority of patients (FLAM: 77% *vs.* 7+3: 68%) had one or more poor-risk features, defined as: 1) WBC >50x10^9/L; 2) adverse genetics (ie. adverse cytogenetics and/or FLT3-ITD mutation); and/or 3) secondary AML.

Toxicity

Grade 3 or more toxicities were similar in both arms (Table 2). Although the rates of tumor lysis syndrome (TLS) were similar between FLAM and 7+3 (FLAM: 8% *vs.* 7+3: 7%), there were 2 early deaths on the FLAM arm due to TLS (compared with 1 early death on 7+3), and 3 grade 4 TLS toxicities on FLAM: acute kidney injury requiring dialysis (n=2), and cytokine release syndrome (n=1). While there was no significant difference in treatment-related mortality between both arms (day 60 mortality: FLAM: 10%, 95%CI: 5%-17% *vs.* 7+3: 4%, 95%CI: 0-12%; *P*=0.22), the majority (8 of 11) of early deaths on FLAM were in patients aged 60 years or over. Causes of early mortality (day 60 mortality) on FLAM included refractory leukemia (n=4), infection/sepsis (n=3), TLS (n=2), multi-organ failure (n=1), and gastrointestinal (GI) bleed (n=1). Causes of early mortality on 7+3 included refractory leukemia (n=1) and TLS (n=1).

Time to full hematologic recovery (ANC >1x10^9/L and platelet count >100x10^9/L) for all patients who achieved CR was similar for FLAM (37 days, 95%CI: 34-40 days) and 7+3 (34 days, 95%CI: 32-37 days) (*P*=0.30). Patients who received 5+2 (n=13) had a median time from start of initial therapy to full hematologic recovery of 49 days.

Clinical outcomes

FLAM led to a 70% CR rate (71 CR + 5 CRi; 95%CI: 60%-78%), while 7+3 led to a 46% CR rate (25 CR + 1 CRi; 95%CI: 33%-60%); odds ratio = 2.64, 95%CI: 1.29-5.45, one-sided *P*=0.003 (Table 3). The treatment effect was of similar magnitude and significance after controlling for the randomization stratification factors (odds ratio = 2.94, 95%CI: 1.44-5.99, one-sided *P*=0.001). Thirteen of 56 (23%) patients on the 7+3 arm received 5+2 for evidence of residual leukemia on day 14 (median % cellularity: 10%, range: 5%-50%; median % blasts: 33%, range: 12%-90%). Six of 13 (46%) patients achieved CR [including one complete remission with incomplete hematologic recovery (CRi)] after receiving 5+2. A comparison of CR rates between FLAM and 7+3+/-5+2 was 70% (95%CI: 60%-78%) *versus* 57% (95%CI: 43%-70%), respectively (one-sided *P*=0.08).

A day 14 BM biopsy was performed on 102 of 109 FLAM patients and 55 of 56 7+3 patients (Table 4). Residual leukemia on day 14 was significantly less with FLAM compared with 7+3 (25%, 95%CI: 17-35% *vs.* 44%, 95%CI: 30%-58%, respectively; *P*=0.03). Patients in CR received diverse post-induction treatment strategies

Table 3. Response comparisons.

Response	FLAM (n=109)	7+3 (n=56)	P*	7+3 +/- 5+2 (n=56)	P*
CR/CRi- n. (%)	76 (70%)	26 (46%)	0.003	32 (57%)	0.08
Failure to achieve CR- n. (%)	26 (24%)	29 (52%)		23 (41%)	
Early death- n. (%)	7 (6%)	1 (2%)		1 (2%)	

**P values were derived from one-sided Fisher's exact test.CR: complete remission; CRi: complete remission with incomplete hematologic recovery.*

based on physician/institution preference. Of the 76 FLAM patients who achieved CR, 35 (46%) underwent FLAM consolidation, 21 (28%) high-dose cytarabine (HiDAC) consolidation, 13 (17%) early allogeneic SCT without consolidation, 5 (7%) had poor performance status preventing further therapy, one had early relapse, and one underwent clofarabine treatment for persistent cytogenetic abnormalities. Of the 32 CR patients on 7+3 (with or without 5+2), 26 (81%) underwent HiDAC consolidation, 3 (9%) early allogeneic SCT without consolidation, 2 (6%) had poor performance status preventing further therapy, and one patient refused further therapy. A total of 82 (50%) patients underwent allogeneic SCT (FLAM: 55 of 109=51%; 7+3: 27 of 56=48%), and one patient underwent an autologous SCT in CR on the 7+3 arm. Of the 83 patients who underwent a SCT (autologous + allogeneic), myeloablative conditioning was performed on 44% and 64% of FLAM and 7+3 patients, respectively, based on institution preference. Donors on the FLAM arm included matched related (n=17), matched unrelated (n=19), haploidentical (n=16), and cord blood (n=3) donors. Donors on the 7+3 arm included matched related (n=7), matched unrelated (n=14), haploidentical (n=5), and cord blood (n=1) donors. Relapses were similar in patients who underwent SCT in CR1 (FLAM: 32% *vs.* 7+3: 38%).

Subset analyses

Although not powered to demonstrate significance, FLAM increased CR rates across different risk groups, as seen in exploratory *ad hoc* analyses (Figure 2). When testing for heterogeneity of treatment effect, patients under 50 years of age derived greater benefit from FLAM *versus* one cycle of 7+3 (CR rates: FLAM: 96% vs. 7+3: 57%; odds ratio = 19.5, 95%CI: 2.03-187.0) compared to those aged 50 years or over (FLAM: 61% *vs.* 7+3: 43%; odds ratio = 2.08, 95%CI: 0.98-4.43; *P* for interaction = 0.04). This difference also persisted for FLAM *versus* 7+3+/-5+2 (*P* for interaction = 0.007). Those with no poor-risk features also saw greater benefit from FLAM (CR rates: FLAM: 100% *vs.* 7+3: 72%; odds ratio = 20.8, 95%CI: 1.07-405.0) when compared with one or more poor-risk features (FLAM: 61% *vs.* 7+3: 34%; odds ratio = 2.9, 95%CI: 1.32-6.0; *P* for interaction = 0.04). Notably, patients with secondary AML (CR rates: FLAM: 60% *vs.* 7+3: 35%) derived similar benefit from FLAM as *de novo* AML (FLAM: 79% vs. 7+3: 57%).

Overall survival and event-free survival outcomes

Median follow up was 553 days (range: 1-1217 days). To date, relapses were similar for FLAM and 7+3 (43% *vs.* 50%, respectively). There was no significant OS difference between FLAM and 7+3: median OS = 17.5 months, 95%CI: 12.7-25.4 months, on FLAM *versus* 22.2 months, 95%CI: 16.2-40.0 months, on 7+3 (*P*=0.39) (Figure 3A). Two-year OS on FLAM was 50% *versus* 59% on 7+3. Although not significantly different, there was an apparent clinical improvement with FLAM with a median EFS = 9.7 months (95%CI: 5.0-11.7 months) on FLAM *versus* 3.4 months (95%CI: 1.3-13.3 months) on 7+3 (*P*=0.15) (Figure 3B).

Table 4. Day 14 bone marrow biopsy comparisons.

	FLAM (n=109)	7+3 (n=56)	P[1]
Day 14 BM biopsy results	102 (94%)[2]	55/56 (98%)[3]	
Residual leukemia on day 14	26 (25%)	24 (44%)	0.03
Achievement of CR	10 (38%)	10 (42%)[4]	
5+2 given	N/A	13 (54%)	
No residual leukemia on day 14	76 (75%)	31 (56%)	0.03
Achievement of CR	63 (83%)	22 (71%)	

[1]P value derived from a two-sided Fisher's exact test and included for descriptive purposes only; [2]7 of 109 patients on FLAM arm did not receive a day 14 BM biopsy due to: Early death (n=4); logistical concerns and/or patient refusal (n=2); patient in Intensive Care Unit (n=1); [3]1 of 56 patients on 7+3 arm did not receive a day 14 BM biopsy due to: early death; [4]of the 24 patients on the 7+3 arm with residual leukemia on day 14: 10 (42%) achieved a CR (6/13 who received 5+2, 4/11 who did not receive 5+2).

Overall, 65 (60%) patients died on the FLAM arm compared with 32 (57%) deaths on the 7+3 arm. Causes of death on the FLAM arm included refractory leukemia (n=43), sepsis/infection (n=8), allogeneic SCT complications (n=6), hemorrhage/coagulopathy (n=3), TLS (n=2), stroke (n=1), multi-organ failure (n=1), and unknown causes (n=1), whereas causes of death on 7+3 included refractory leukemia (n=26), allogeneic SCT complications (n=4), TLS (n=1), and sepsis/infection (n=1). In addition, 13 (17%) patients on FLAM died while in CR compared with 3 (10%) on 7+3. Causes of death in CR on FLAM included allogeneic SCT complications (n=6), infections (n=3; including one patient who died of *E. coli* sepsis during HiDAC consolidation), progressive bone marrow failure (n=1), presumed central nervous system (CNS) leukemia (n=1), intracerebral hemorrhage (n=1), and unknown causes (n=1). Causes of death in CR on 7+3 included allogeneic SCT complications (n=2), and sepsis/infection (n=1).

Discussion

The main study objective of this phase II trial was to assess the relative activity of FLAM compared with 7+3 in order to determine whether there was justification for further development of FLAM based on CR rate. The study findings support the hypothesis that FLAM induction leads to superior CR rates compared with 7+3 therapy

(70% *vs.* 46%; *P*=0.003) in patients with intermediate and adverse-risk cytogenetics. The cycle 1 CR rate was chosen as a more specific indicator of relative activity between the 2 regimens because of variations in the approach following one cycle of induction therapy in practice. For instance, many leukemia clinicians utilize a re-induction strategy (ie. 5+2) in some patients with persistent disease on day 14 after 7+3 induction. In contrast, FLAM is not intensified on the basis of early BM biopsy findings. We considered it to be important to compare the FLAM regi-

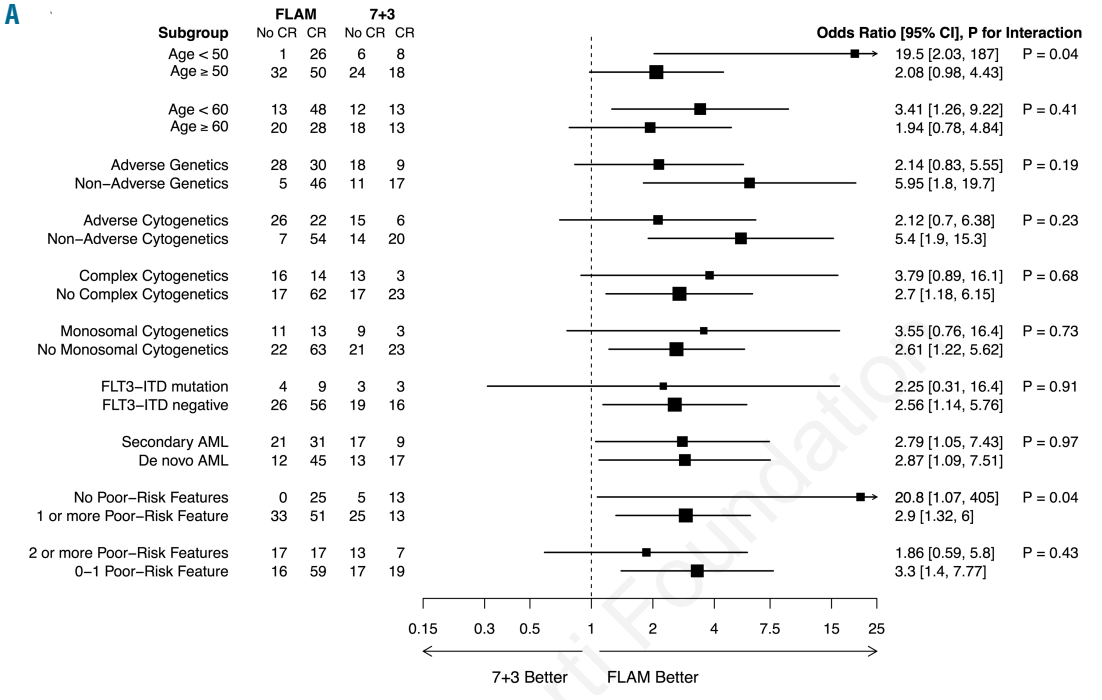

Figure 2. Subset analyses. Forest plot of odds ratios for complete remission in patient subgroups, plotted on a log scale. *P* values were derived from likelihood ratio tests of logistic regression models for interaction between treatment: (A) FLAM *vs.* 7+3 and (A) FLAM *vs.* 7+3+/-5+2, and patient subgroup on complete remission. To provide information about patients in the 'no poor-risk features' subgroup, 0.5 was added to all cell counts in this analysis for calculating odds ratios and 95% confidence intervals.

men to an appropriate contemporary standard control regimen of 7+3; thus, we allowed a re-induction strategy (ie. 5+2) in those patients with residual leukemia on day 14, as recommended by the National Comprehensive Cancer Network Guidelines in AML.[18] There are a number of confounders when determining whether to give a second cycle of induction, such as patient age, performance status, toxicities, and day 14 BM findings.[19] Therefore, a second cycle of induction was recommended, but not mandated, in those patients with residual leukemia on day 14. The comparison of FLAM to 7+3+/-5+2 (CR rates: 70% *vs.* 57%; *P*=0.08) also substantiates the improved efficacy with FLAM and further supports the development of this regimen.

The CR rates seen on this study were lower than other recently reported phase III studies in AML due to differences in the patient populations studied.[16,20,21] Patients with favorable-risk cytogenetic features (ie. CBF AML) were excluded on this study. In addition, newly diagnosed elderly AML patients were included on this study up to 70 years of age, whereas other trials only included patients up to 60 years of age.[16,20,21] Forty-seven percent of patients on this study had secondary AML, a subgroup with poor outcomes.[22] A larger proportion of patients had adverse-risk cytogenetics (41% total) when compared with other contemporary phase III studies in AML.[16,20,21] The relatively low CR rates seen on the 7+3 arm of this study were consistent with other recent reports in similar non-favorable risk patient populations.[23,24]

Importantly, there was no difference in overall toxicity between FLAM and 7+3. TLS was a major concern given the high rates of TLS with flavopiridol in chronic lymphocytic leukemia[25-27] and consistent 7%-10% incidence of grade 3 or more TLS in our prior phase II studies.[10-12] Three patients treated with FLAM on this study had grade 4 TLS, and 2 patients died due to complications of TLS. Patients with WBC more than 20x10^9/L, monocytic phenotypes (ie. M4/M5, or arising from pre-existing MPN), and baseline renal dysfunction appear to predispose to grade 4 or more TLS with FLAM. Though all patients received allopurinol and sevelamer prophylaxis, it may be necessary to use rasburicase and other supportive care measures to blunt cytokine release syndrome (eg. steroids) in order to decrease the severity of TLS in future trials with FLAM.

In addition, older age (>60 years) appears to predispose to FLAM toxicity. Although overall treatment-related mortality rates were similar between FLAM and 7+3, 8 of 11 early deaths (<60 days) on FLAM occurred in patients aged 60 years or over. On subset analyses, younger patients had higher CR rates with FLAM compared with 7+3, and this difference remained statistically significant after 7+3+/-5+2.

A major question in the management of AML patients is whether or not to give more chemotherapy for a day 14 BM biopsy revealing residual leukemia. There is, unfortunately, a lack of a consistent approach in managing these patients. Moreover, some patients with residual leukemia on a day 14 BM biopsy after 7+3 induction will ultimately achieve CR and may not need additional induction therapy.[28-30] There is considerable investigator bias regarding whether or not to give a second cycle of induction therapy for patients with residual leukemia on day 14.[19] In our study, 24 patients on the 7+3 arm had residual leukemia on day 14, but only 13 (54%) received 5+2, and 6 of 13 (46%) achieved CR (compared with 4 of 11 without 5+2). The proportion of patients achieving a CR with a second cycle of induction was similar to other reports.[16,30] Although Rowe *et al.*[31] reported that adults who achieve a CR after one or two courses of induction therapy have similar overall outcomes, this analysis was retrospective, and the patients included on this analysis received an identical course of induction therapy (ie. 7+3), as opposed to 5+2 for day 14 residual leukemia. In contrast, the GOELAMS study group[32] performed a multicenter prospective study in which a second induction course was given to patients with 5% or more blasts on a day 15 BM biopsy, and showed that those with residual leukemia on day 15 had significantly worse outcomes, even after a second induction course. In the present study, patients on the FLAM arm did not receive more intensive therapy in the presence of residual leukemia on day 14, and a proportion of those (38%) still achieved CR.

Secondary AML represents a subgroup of patients with an extremely poor outcome who may benefit from FLAM induction. Secondary AML can be confirmed based on his-

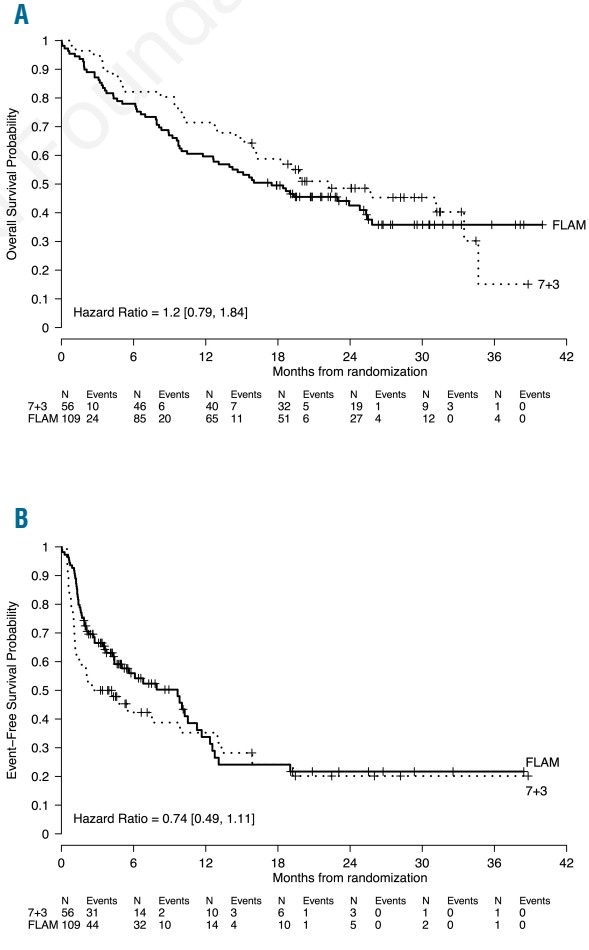

Figure 3. Kaplan-Meier estimates of overall survival (OS) and event-free survival (EFS). (A). OS for the entire cohort and treatment arm measured by Kaplan-Meier methodology. OS is defined as the date of randomization to date of last follow up or death. (B). EFS for the entire cohort and treatment arm measured by Kaplan-Meier methodology. EFS is defined as time from randomization to persistent AML after one cycle of induction therapy, relapse, death, or date of last follow up (censored).

tory alone in most cases. While 60% of patients with secondary AML in this current study achieved CR, we have now treated 158 newly diagnosed secondary AML patients with FLAM across all 4 phase II studies with an overall CR rate of 66% (105 of 158).[10-12] The similar effect with FLAM in secondary *versus de novo* AML highlights FLAM activity in secondary AML, despite the overall decreased response rates and poor outcomes seen in this subpopulation. Moreover, although small numbers, all 6 patients with secondary AML and aged under 50 years treated with FLAM achieved a CR on this study (compared to 1 of 3 patients with secondary AML and under 50 years of age achieving a CR on 7+3). Thus, these data substantiate the benefit of FLAM in secondary AML, particularly in younger patients, and compare favorably to the promising findings seen with CPX-351.[23]

Despite the significant improvement in response rates seen with FLAM, our study did not show a difference in OS. These findings must be interpreted cautiously as the study was not powered to detect an OS difference. Other recently performed randomized studies in AML have also reported improved CR rates without OS advantage.[33] Moreover, lack of an OS advantage from a randomized phase II study aimed primarily to assess activity does not diminish the pertinence of these findings. It also reinforces the importance of a larger randomized phase III study specifically powered to detect an OS advantage, as evidenced by the finding that the OS of the 56 patients on the 7+3 arm of this poor-risk patient population was significantly better than the results of recently reported phase III studies.[16,20,21,34] An additional limitation of the OS/EFS analyses on this study was the lack of standardized post-induction treatment strategies in both arms. FLAM was initially developed as two cycles (induction and consolidation therapy) in patients who achieve CR but post-induction treatment for both arms in this study was not defined. For example, only 46% of CR patients on FLAM received FLAM consolidation, while 28% received HiDAC, and 17% received early allogeneic SCT without consolidation therapy. In addition, post-remission transplantation conditioning therapies and donor selections were also variable. This lack of a consistent treatment approach for patients who achieve CR is a major challenge in drug development in AML. It will be essential for these post-induction treatment strategies to be standardized for phase III studies in order to truly verify whether FLAM induction can improve overall outcomes compared with 7+3. Given the inherent variability and unclear utility of a second cycle of induction with 7+3, a phase III study comparing FLAM to one cycle of 7+3 (without early re-induction therapy) would eliminate any potential biases of re-treatment.

In conclusion, FLAM represents a promising induction regimen for AML patients with intermediate- and adverse-risk cytogenetic features. Our findings reveal that FLAM induction leads to superior CR rates when compared with 7+3. On subset analyses, younger patients (<50 years), and those with no poor-risk features, appeared to benefit more from FLAM than their 7+3 counterparts. Moreover, FLAM demonstrated promising activity in secondary AML. While the data clearly demonstrate that FLAM induction leads to superior CR rates compared with 7+3 alone, rigorous clinical trial designs are required to determine OS differences, particularly among select subgroups such as secondary AML.

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