Phase 1/2 Trial of GCLAM with Dose-Escalated Mitoxantrone for Newly Diagnosed AML or Other High-Grade Myeloid Neoplasms

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Abstract

Outcomes with "7+3" are often unsatisfactory in acute myeloid leukemia (AML). Trials demonstrating improved outcomes with high-dose cytarabine, addition of cladribine, or escalated anthracycline doses prompted a phase 1/2 study (NCT02044796) of G-CSF, cladribine, high-dose cytarabine, and dose-escalated mitoxantrone (GCLAM) in adults with newly-diagnosed AML or other high-grade myeloid neoplasms. 121 patients, median age 60 (range: 21–81) years, were enrolled. In phase 1, cohorts of 6–12 patients were assigned to 12–18mg/m^2/day of mitoxantrone as part of GCLAM. Because all dose levels were well-tolerated, mitoxantrone at 18mg/m^2 was declared the recommended phase 2 dose (RP2D). 74/94 (79%) patients treated at the RP2D achieved a complete remission (CR; 67/74 without measureable residual disease [MRD]) for an

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overall MRD^{neg} CR rate of 71% (primary phase 2 endpoint). Seven patients achieved a CR with incomplete blood count recovery (CRi; 7%, 5 MRD^{neg}) for a CR/CRi rate of 81/94 (86%). 4-week mortality was 2%. After adjustment, the MRD^{neg} CR and CR/CRi rates compared favorably to 100 matched controls treated with 7+3 at our center and 245 matched patients treated with 7+3 on a cooperative group trial. Our data indicate GCLAM with mitoxantrone at 18mg/m^2/day is safe and induces high-quality remissions in adults with newly-diagnosed AML.

INTRODUCTION

Cytarabine together with an anthracycline – introduced as "7+3" in 1973 – has, at least until recently, remained the mainstay of intensive chemotherapy for adults with newly-diagnosed acute myeloid leukemia (AML).[1–4] While gemtuzumab ozogamicin[5] and midostaurin[6] have now been approved in the U.S. as 7+3 adjuncts, these drugs provide incremental benefit and most patients who achieve a complete remission (CR) will relapse.[2–4] Studies reporting improved outcomes with higher doses of cytarabine during induction[7] or addition of cladribine (but not fludarabine)[8–10] led us to explore the combination of G-CSF, cladribine, cytarabine, and mitoxantrone (GCLAM).[11] Possibly more effective than mitoxantrone/ etoposide/cytarabine (MEC) in relapsed/refractory AML,[12] only very limited data are available in upfront therapy.[13] When developing this trial, escalated anthracycline doses were reported to be associated with greater efficacy.[14,15] Hence, the first goal of this phase 1/2 trial was to establish the maximum tolerated dose (MTD) of mitoxantrone as part of GCLAM in adults with newly-diagnosed AML. We then determined response and duration of remission at the recommended phase 2 dose (RP2D).

PATIENTS AND METHODS

Study population

Adults aged 18 years with untreated AML[16] (acute promyelocytic leukemia excepted) or other myeloid neoplasms with 10% blasts in blood and/or marrow were eligible if they had a treatment-related mortality (TRM) score of 6.9. This score (online calculator: https:// cstaging.fhcrc-research.org/TRM/) is composed of weighted information from 8 covariates (age, performance status, white blood cell [WBC] count, peripheral blood blast percentage, type of AML [de novo *vs.* secondary], platelet count, albumin, and creatinine) and corresponds to a 6.9% probability of death within 28 days ("TRM") of receipt of intensive chemotherapy for newly-diagnosed AML.[17] Patients had to have a left ventricular ejection fraction 45%, creatinine 2.0mg/dL, and bilirubin 2.5 times the upper limit of normal, no uncontrolled infection, and an expected survival of >1 year absent AML. Prior low-intensity treatment for low-grade myelodysplastic syndrome (MDS; <10% blasts) was permitted. Disease risk was assessed according to MRC/NCRI criteria,[18] and, when molecular data was available, 2017 European LeukemiaNet (ELN) criteria.[4] Best responses, defined according to standard criteria,[19,20] were measured after 1–2 cycles of therapy. Measurable residual disease (MRD) was assessed by multiparametric flow cytometry (MFC). The sensitivity of the MFC MRD assay varies with the type of phenotypic aberrancy and immunophenotypes of normal cells in the background populations. Therefore, the assay does not have uniform sensitivity across all cases but is able to detect MRD when present in the large majority of

cases down to a level of 0.1% and in progressively smaller subsets of patients as the level of residual disease decreases below that level. When identified, the abnormal population was quantified as a percentage of the total CD45^{+} white cell events. Any level of residual disease was considered MRD[pos.21–23] Relapse after study treatment was defined by standard morphologic criteria.[4,19] The protocol (ClinicalTrials.gov: NCT02044796) was approved by the Fred Hutchinson Cancer Research Center (Fred Hutch) Institutional Review Board (IRB), and patients gave written informed consent in accordance with the Declaration of Helsinki.

Treatment plan

The safety of GCLAM using mitoxantrone at $10mg/m^2$/day on days 1–3 in relapsed/refractory AML is well-established.[11] Therefore, in phase 1, patients were assigned to either 12, 14, 16, or $18mg/m^2$/day of intravenous (IV) mitoxantrone on days 1–3. G-CSF was given subcutaneously at 300 or 480μg/day (for weight <76kg *vs.* 76kg; days 0–5), cladribine IV at $5mg/m^2$/day (days 1–5), and cytarabine IV at $2g/m^2$/day (days 1–5).[11] The first 2 doses of G-CSF could be omitted for a WBC count of >20,000/μL. In phase 2, patients received mitoxantrone at the RP2D identified in phase 1. A second identical course of GCLAM was given for patients who did not achieve CR or CR with incomplete blood count recovery (CRi) following cycle 1. Patients in CR/CRi after 1–2 cycles of GCLAM could receive up to 4 cycles of GCLA (mitoxantrone omitted). Patients were taken off study for failure to achieve CR/CRi after 2 cycles of therapy, alternative consolidation including hematopoietic cell transplantation (HCT), excess toxicity including persistent aplasia without evidence of leukemia after day 45 of treatment, or relapse. Toxicities were evaluated based on the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.03 (http://ctep.cancer.gov).

Comparison of GCLAM with 7+3

Data were obtained from patients treated with 7+3 at our institution or on a cooperative group trial (SWOG S0106).[24] As an institutional control group, we identified 100 patients aged 22–78 years with TRM scores 6.9 who received 7+3 (cytarabine $100mg/m^2$/day with either daunorubicin $60–90mg/m^2$/day or idarubicin $10–13mg/m^2$/day) without additional agents between 2006 and 2017. From S0106, we included the 245 adults aged 18–60 years randomly assigned between 2004 and 2009 to receive chemotherapy with 7+3 (cytarabine $100mg/m^2$/day, daunorubicin $60mg/m^2$/day).[24] Covariates collected were age, sex, pre-treatment cytogenetic risk, performance status, TRM score (not available from S0106), WBC count, platelet count, peripheral blood blast percentage, *FLT3* and *NPM1* mutational status, and, for institutional patients, *de novo vs.* secondary disease; all S0106 patients had *de novo* AML.[24] MRD after induction on S0106 was assessed prospectively and centrally at the University of Washington,[25] i.e. the same laboratory that assesses MRD for our institutional patients. This retrospective analysis was approved by the Fred Hutch IRB.

Statistical considerations

Phase 1—Cohorts of 6 patients were assigned to increasing doses of mitoxantrone. Dose-limiting toxicity (DLT) was defined as: 1) any grade 3 non-hematologic toxicity, other than

febrile neutropenia or infection, lasting >48hours that resulted in a >7-day delay of subsequent treatment; 2) any grade 4 non-hematologic toxicity, other than febrile neutropenia or infection or constitutional symptoms if recovery to grade 2 within 14 days. Cumulative toxicities were assessed after every treatment cycle. The MTD was defined as the highest dose studied in which the incidence of DLTs was <33%. If 2/6 (33%) on one dose level had toxicity, 6 additional patients could be enrolled for further evaluation of that level.

Phase 2—We considered GCLAM at the RP2D of no further interest if the true MRD^{neg} CR rate was 60% (null hypothesis) while an MRD^{neg} CR rate 75% would spur further investigation (alternative hypothesis). This null hypothesis was derived from historical control data at our institution showing 60% of 146 patients <65 years who received high-dose cytarabine-based induction chemotherapy achieved an MRD^{neg} CR (E.H. Estey: unpublished observation). A Simon Optimal 2-stage design[26] was used, with 80% power and a 1-sided alpha of 7%, thus calling for enrollment of 21 and 41 patients in the first and second stage, with the null hypothesis accepted if MRD^{neg} CR was seen in <14/21 or <42/62 patients. Because of a high response rate among the first 62 patients, we expanded the trial to gain further information about efficacy at the RP2D. A multivariate logistic regression model was used to compare outcomes to 7+3. Data cut-off date for analysis was February 16, 2018.

RESULTS

Study cohort and treatment

Between June 2014 and March 2017, 121 eligible patients (median age: 60 [range: 21–81] years; median TRM score: 2.9 [range: 0.1–6.9]) were enrolled (Table 1). Eighty-four (69%) had AML, 14 (12%) had MDS with excess blasts-2 (MDS-EB-2), 3 (2%) had blastic plasmacytoid dendritic cell neoplasm (BPDCN), and 20 (17%) had a treatment-related myeloid neoplasm (t-AML in 15, t-MDS in 5). Cytogenetic risk was favorable in 9 (7%), intermediate in 94 (78%), and adverse in 17 (14%) patients; karyotyping failed in one participant. All patients completed at least one course of therapy: 52 received 1, 65 received 2, 3 received 3, and 1 received 4 courses of study therapy.

Phase 1—Thirty-three patients were enrolled in phase 1 and received a median of 2 (range: 1–3) cycles of study therapy (Supplemental Table 1). One DLT occurred at each of dose levels 3 (16mg/m^2) and 4 (18mg/m^2) (respiratory failure in both; Table 2). One of the 33 patients died within 28 days of treatment initiation due to intracranial hemorrhage (TRM rate 3% [95% exact confidence interval: 0–16%]). Supplemental Table 2 summarizes adverse events observed in phase 1. In this cohort, 22 achieved an MRD^{neg} CR (67% [48–82%]) and 3 had an MRD^{pos} CR (9% [19–24%]). There were also 4 MRD^{neg} CRi and 1 MRD^{pos} CRi, for a CR/CRi rate of 91% (76–98%). One patient had resistant disease, 1 died in aplasia, and 1 patient with fully-recovered blood counts refused marrow assessment. Although only 1 DLT occurred at the highest dose of mitoxantrone (18mg/m^2/day) examined, this dose was defined as the RP2D in GCLAM.

Phase 2 and expansion cohort—Ninety-four patients received GCLAM at the RP2D. Best responses after 1–2 cycles of induction chemotherapy for the entire study population as well as those treated at the RP2D are summarized in Table 3. Six of the 94 RP2D patients had received prior therapy with azacitidine for an antecedent hematologic disorder. Because of emerging data indicating that failure of DNA methyltransferase inhibitors is an independent adverse prognostic factor,[27,28] we analyzed patients without or with prior azacitidine exposure separately. For the whole 94-patient R2PD cohort, 67 patients achieved an MRD[neg] CR (71% [95% CI: 61–80%]). Seven patients achieved an MRD[pos] CR, and 7 additional patients a CRi (5 MRD[neg] and 2 MRD[pos]) for a CR/CRi rate of 86% (78–92%). Seventy-two of the 81 responders were negative for MRD, for an overall MRD[neg] CR/CRi rate of 76% (67–85%). One patient with myeloid sarcoma had a partial remission (MRD[neg] marrow, visceral disease unable to be assessed), 5 patients obtained a morphologic leukemia free state ([MLFS]; 4 MRD[neg]), 4 had resistant disease, and 2 died from indeterminate cause while in aplasia. Of the 88 azanucleoside-naïve patients treated at the RP2D, 66 (75% [65–84%]) achieved an MRD[neg] CR). Six (7% [3–14%]) achieved an MRD[pos] CR, and 6 achieved a CRi (7% [3–14%]; 5 MRD[neg] and 1 MRD[pos]) for a CR/CRi rate of 89% (80–94%). Seventy-one of these 78 responders were negative for MRD, for an MRD[neg] CR/CRi rate of 80% (71–88%). One patient with myeloid sarcoma had a partial remission (MRD[neg] marrow), 5 patients obtained a morphologic leukemia free state ([MLFS]; 4 MRD[neg]), 3 had resistant disease, and 1 died from indeterminate cause while in aplasia. Thus, the 6 patients with prior azanucleoside treatment fared worse than the other patients treated at the RP2D, with their best responses being MRD[neg] CR (n=1), MRD[pos] CR (n=1), MRD[pos] CRi (n=1), resistant disease (n=1), and death from indeterminate cause (n=2). Two of the 94 patients treated at the RP2D died within 28 days of treatment initiation (sepsis and multisystem organ failure), for a TRM of 2% (0–8%). Eight-week mortality was 5%. Besides cytopenias, infections and neutropenic fever were the most common grade 3–5 toxicities. Other common grade 3–4 toxicities included maculopapular rash, nausea and hypoxia, with the latter occurring primarily in the setting of infection (Table 4).

Twenty-nine of the 81 responders were taken off protocol specifically to undergo HCT, and 39 received alternative consolidation chemotherapies (many prior to transplant) including high-dose cytarabine alone, DNA methyltransferase inhibitors, or investigational agent(s). Forty-three of the 81 responders (53%) have received HCT to date, including 62% of those age 60. Relapses have occurred in 27 patients, after a median CR duration of 227 (range: 76–850) days, while 8 patients died in remission after CR durations of 9, 81, 132, 168, 266, 360, 478, and 600 days. For the entire phase 1/2 study population, overall survival (OS) and relapse-free survival (RFS) are depicted in Figure 1A and Figure 1B, respectively. With a median follow-up among censored patients of 1.92 years, the median OS (Figure 1C) for the RP2D group was 33.3 months and the median RFS (Figure 1D) was 26 months (33.3 months and 26.1 months for the 88 azanucleoside-naïve patients). The one-year OS and RFS were 69% and 65%.

Duration of cytopenias—Data on duration of neutropenia and thrombocytopenia may be least confounded by residual leukemia in patients who achieved CR. For the 74 RP2D patients who achieved a CR after induction therapy, the median times to neutrophil recovery

to 500/μL and platelet recovery to 50,000/μL were 26 (25th–75th percentile: 23–30) days and 23 (25th–75th percentile: 20–28) days. Forty-eight of these patients subsequently received post-remission therapy with GCLA. Among these, 3 did not recover their neutrophils and 10 did not recovery their platelets prior to the subsequent line of therapy. For the others, the median times to neutrophil and platelet recovery were 30 (25th–75th percentile: 26–36) and 34 (25th-75th percentile: 28–42) days.

Treatment outcomes in adults 65 years of age or older—Since potential risks of intensive regimens can outweigh potential benefits in older patients, we compared response rates and tolerability of GCLAM in adults   65 years of age with those <65 years (Table 5). Consistent with expectations, older patients had slightly higher baseline TRM scores, more likely presented with MDS or secondary disease, and more likely had adverse-risk cytogenetics than younger patients. CR rates in those <65 years vs.   65 years were 85% vs. 70%, MRDneg CR rates were 78% vs. 62%, and CR/CRi rates were 91% vs. 80%. 56% of younger patients subsequently underwent allogeneic HCT, compared to 40% of the older subgroup. TRM rates were low in younger and older patients (2% in both groups). Median OS was 33.3 months for the younger subgroup and 13 months for the older group (Figure 1E); 1-year OS was 81% and 51%. Median RFS was not reached for those <65 and was 13 months for those   65 (1-year RFS of 73% and 52%; Figure 1F). In addition to assessing the impact of age on outcomes in a dichotomized fashion, we also evaluated age as a continuous variable. In multivariable analyses including age, gender baseline laboratory values, secondary disease status, cytogenetic risk, and mutation status, age was not independently associated with CR (odds ratio [OR]=0.99, p=0.24), OS (hazard ratio [HR]=1.03, p=0.09), or RFS (HR=1.02, p=0.12).

Treatment outcomes in various patient subgroups—Cytogenetic risk and *de novo* vs. secondary disease were the factors most strongly associated with response. The MRDneg CR rate was 45% in those with adverse-risk disease vs. 88% in favorable- and 86% in intermediate-risk disease (Supplemental Table 3). In multivariable analysis, the OR for CR in the adverse-risk group compared to favorable/intermediate risk was 0.31 (0.01–0.48; p=.01), whereas the HR for death was 2.04 (0.85–4.91; p=0.11) for the adverse risk group. Likewise, the response rate for those with monosomal karyotype was 6/10 (60%; 5 CR and 1 CRi). Overall response rates for AML and MDS-EB-2 were similar (85% for both cohorts) as were MRDneg response rates (74% for AML and 67% for MDS-EB-2 patients).

Comparison to 7+3—Phase 2 trials are inherently comparative. We therefore used two groups as controls for the 94 patients given GCLAM at the R2PD. First were 100 patients treated at Fred Hutch with 7+3 at daunorubicin doses   60mg/m^2 or idarubicin doses   10mg/m^2 who had TRM scores of   6.9. GCLAM patients were older than the 7+3 patients (median age 62 vs. 56 years) but otherwise the two cohorts were well balanced in terms of median TRM scores, cytogenetic risk, mutational status, and rate of secondary disease (Table 6). MRDneg CR, our primary early efficacy endpoint, was obtained in 71% of GCLAM patients vs. 53% of 7+3 patients (p=0.01). The CR/CRi rate was 86% for GCLAM compared to 70% for 7+3 (p<0.01). In multivariable analysis, the ORs for CR and CR/CRi rates for GCLAM compared to 7+3 were 10 (3.57–25.0, p<0.01) and 11.11 (3.70–33.3,

p<0.01), whereas the OR for MRD^{neg} CR was 8.33 for GCLAM (3.22–20.0, p<0.01; Supplemental Table 4). Survival estimates were not statistically significantly different (HR=0.79 for GCLAM vs. 7+3, 95% CI: 0.49–1.27, p=0.32; Figure 2). Two-year cumulative incidence of relapse was 33% in the GCLAM cohort and 28% in the institutional 7+3 cohort (p=0.31). Rates of 4- and 8-week mortality between the two regimens were similar (5% and 3% 8-week mortality for each). The second comparison involved 245 matched patients treated with 7+3 on SWOG S0106 (Supplemental Table 5) with the subset of the GCLAM cohort (n=34) that matched the inclusion criteria for the SWOG study (e.g. age <60, de novo disease only). After multivariable adjustment, the ORs for CR and CR/CRi rate for GCLAM compared to SWOG 7+3 were 3.5 (1.0–12.2, p=0.05) and 4.59 (1.03–20.4, p=0.04), respectively, whereas the OR for MRD^{neg} CR was 2.69 for GCLAM (0.5–14.16, p=0.25). Survival was similar in the 2 arms (HR for GCLAM: 1.03 [0.54–1.98; p=0.92]). Two-year cumulative incidence of relapse was 14% in this GCLAM cohort and 42% in the SWOG 7+3 cohort (p<0.01). Cumulative incidence rates of transplant in first CR at 2-years (with death and relapse analyzed as competing events) for the GCLAM and institutional 7+3 cohort were 41% and 32% respectively (p=0.074); transplant rates for the SWOG 7+3 cohort are not available. Other variables independently associated with response rates and survival in multivariable analysis included secondary disease status, cytogenetic risk group, and mutational status (Supplemental Table 4).

DISCUSSION

The data presented above suggest that GCLAM at a daily mitoxantrone dose of 18mg/m^2 produces similar TRM rates but higher response rates, particularly MRD^{neg} CR, than 7+3. After adjusting for other covariates, age was not associated with response; GCLAM could therefore be useful for older as well as younger patients provided they are fit. This possibility reflects improvements in anti-microbial prophylaxis and therapy leading to gradually declining TRM rates in both older and younger individuals over the past 20 years. [29–31] Toxicity rates and duration of cytopenias appeared similar with GCLAM and 7+3,[6,32–34] and 53% of responding patients, including 62% of those age 60, underwent HCT.

Although TRM and toxicity are of obvious concern, therapeutic resistance manifested as failure to enter CR or relapse are the principal reasons for failure to cure AML. The main goal of induction therapy is to produce a response least likely to be associated with subsequent relapse. It is now well-established that residual disease in patients who achieve a morphologic CR with induction chemotherapy identifies people at particularly high risk of relapse, even if subsequent therapy includes HCT.[35] Hence, we chose MRD^{neg} CR as our principal endpoint with the expectation that higher rates of MRD^{neg} CR would translate into longer remissions and longer survival. We had previously observed MRD^{neg} CR rates of 60% at our center after administration of other high-dose cytarabine-containing regimens. We thus chose MRD^{neg} CR as our primary outcome, and achieved a rate of 71% among all our RP2D patients and 75% among those who had not failed prior azanucleoside therapy.

Today, 7+3 remains the most commonly used "intensive" induction regimen. To overcome the limitation of our single-arm study, our principal control groups were therefore patients

who received 7+3 either at our center or on the S0106 trial. The former group was perhaps of more interest because they received therapy at the same center as the GCLAM patients, with the implication that supportive care practices were thus more likely to be similar. Multivariate analysis indicated GCLAM was significantly more likely to produce CR without MRD than was 7+3 at our institution. An obvious question is why this higher MRD[neg] CR rate did not translate into a proportionate improvement in survival (Figure 2). Differences in follow-up time and transplantation patterns may provide some explanation, as well as a lack of homogeneity in the 7+3 and GCLAM groups in therapy received once relapse had occurred, noting such therapy can substantially affect survival (indeed, many of these patients initially treated with 7+3 were treated with GCLAM upon relapse). This has led to recent emphasis on event-free rather than overall survival as an indicator of a regimen's efficacy. Attempts to compare relapse-free survival with 7+3 and GCLAM are limited by the different criteria used to initiate "salvage" therapy even at our center, with some physicians initiating new therapy immediately upon detection of MRD rather than waiting for frank morphologic relapse. The seeming superiority of GCLAM over 7+3 was observed with CR with MRD and CRi with/without MRD, as well as with CR without MRD. Given the limitations in generalizing from historically-controlled studies, we acknowledge the need for a randomized comparison of GCLAM with 7+3 as was done with FLAG-Ida and 7+3.[7] A 3-arm randomized trial might compare 7+3, FLAG-Ida, and GCLAM to decide which is best for future use as intensive induction chemotherapy regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Kaplan-Meier estimates of **(a)** overall survival and **(b)** relapse-free survival of the 121 patients who received GCLAM on this phase 1/2 study. Kaplan-Meier estimates of **(c)** overall survival and **(d)** relapse-free survival of the 94 patients who received GCLAM at the RP2D. Comparative Kaplan-Meier estimates of **(e)** overall survival and **(f)** relapse-free survival of the 54 patients age <65 years and the 40 patents age   65 years who received GCLAM at the RP2D.
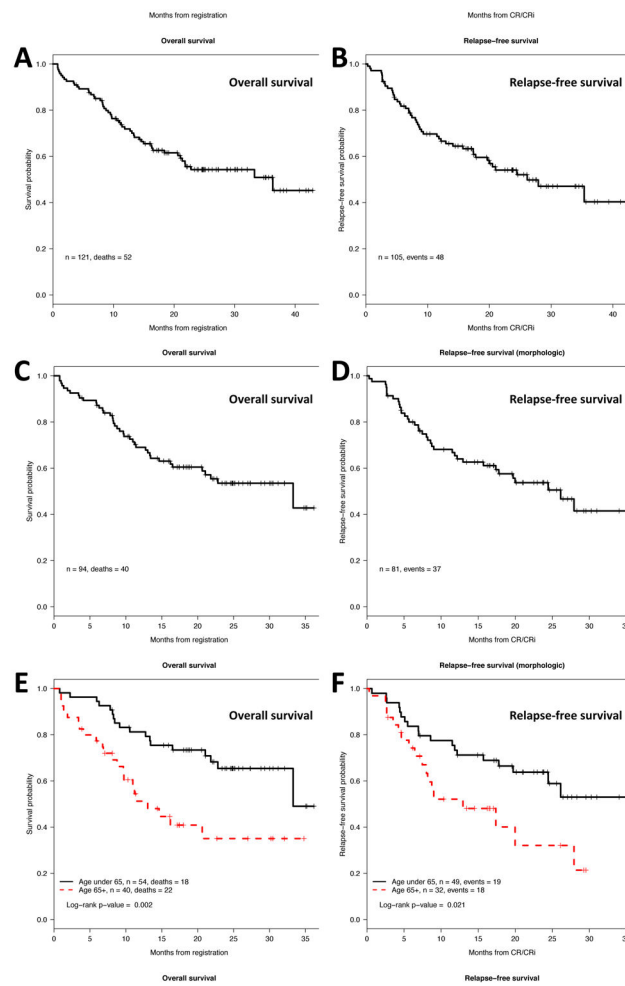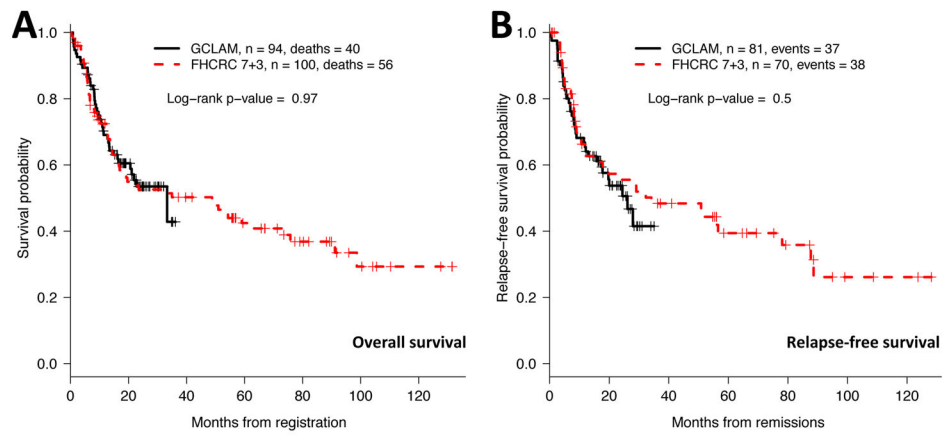
Figure 2.
Kaplan-Meier estimates of **(a)** overall survival and **(b)** relapse-free survival of the 94 patients who received GCLAM at the RP2D and 100 matched patients who received "7+3" at our institution.

TABLE 1

Characteristics of the study cohort

Parameter	n=121
Age, median (range), years	60 (21–81)
Male gender, n (%)	70 (58%)
Disease	
AML	84 (69%)
With recurrent genetic abnormalities	10
With mutated NPM1	20
With bi-allelic mutated CEBPA	1
With mutated RUNX1	3
With myelodysplasia-related changes	21
Myeloid sarcoma	2
AML, not otherwise specified	27
BPDCN	3 (2%)
MDS-EB2	14 (12%)
Treatment-related myeloid neoplasms	20 (17%)
Secondary disease [*]	37 (31%)
Median TRM score (range)	2.9 (0.1–6.9)
Performance status, n (%)	
0	21 (17%)
1	99 (82%)
2	1 (1%)
Cytogenetic risk, n (%) [**]	
Favorable	9 (7%)
Intermediate	94 (78%)
Adverse	18 (15%)
Mutational status, n (%)	
FLT3-ITD	
Wild-type	79 (65%)
Mutated	14 (12%)
Unknown	28 (23%)
NPM1	
Wild-type	66 (55%)
Mutated	22 (18%)
Unknown	33 (27%)
Laboratory findings at baseline, median (range)	

Parameter	n=121
WBC ($\times 10^9$L)	3.4 (0.4–117.9)
Absolute neutrophil count ($\times 10^9$L)	0.7 (0–60)
Peripheral blood blasts (%)	8 (0–96%)
Hemoglobin (g/dL)	9.2 (5.1–19.6)
Platelets ($\times 10^9$L)	58 (10–710)

[*] Defined either as AML transformed from antecedent hematologic disorder, or AML/MDS in a patient who had previously received cytotoxic therapy

[**] Karyotyping failed in 1 patient

TABLE 2

Dose escalation scheme, best responses, and dose-limiting toxicities during phase 1, n=33

Dose Level	G-CSF (D0 to D5)	Cladribine (D1 to D5)	Cytarabine (D1 to D5)	Mitoxantrone (D1 to D3)	Patients (n)	Best Response	Dose-Limiting toxicities
1	300 or 480 μg	5 mg/m^2	2 g/m^2	12 mg/m^2	9	4 CR MRD^{neg} 1 CR MRD^{pos} 3 CRi MRD^{neg} 1 RD	None
2	300 or 480 μg	5 mg/m^2	2 g/m^2	14 mg/m^2	9	8 CR MRD^{neg} 1 DI	None
3	300 or 480 μg	5 mg/m^2	2 g/m^2	16 mg/m^2	9	5 CR MRD^{neg} 2 CR MRD^{pos} 1 CRi MRD^{neg} 1 Unknown *	n=1 (respiratory failure **)
4	300 or 480 μg	5 mg/m^2	2 g/m^2	18 mg/m^2	6	5 CR MRD^{neg} 1 CRi MRD^{pos}	n=1 (respiratory failure ***)

* The response for 1 patient treated at dose level 3 was unable to be assessed due to refusal of bone marrow exam.

** Occurrence during first dose of cytarabine, thought to be due to SIRS response to tumor lysis, cytarabine, or TRALI.

*** Severe ARDS with refractory hypoxemia 2 weeks after the initiation of therapy, thought to be due to concurrent streptococcal and viral pneumonia.

Abbreviations: CR, complete remission; MRD, measurable residual disease; CRi, complete remission with incomplete blood count recovery; DI: death from indeterminate cause; RD, resistant disease

TABLE 3

Best response after 1–2 cycles of study therapy

Response, n (%)	All patients (n=120)[*]	All patients treated at the RP2D (n=94)	Patients treated at RP2D, no prior HMA (n=88)[**]
CR			
MRD^{neg}	84 (70%)	67 (71%)	66 (75%)
MRD^{pos}	10 (8%)	7 (7%)	6 (7%)
CRi			
MRD^{neg}	9 (8%)	5 (5%)	5 (6%)
MRD^{pos}	2 (2%)	2 (2%)	1 (1%)
Overall response	105 (88%)	81 (86%)	78 (89%)
Partial response[+]	1 (1%)	1 (1%)	1 (1%)
MLFS			
MRD^{neg}	4 (3%)	4 (4%)	4 (4%)
MRD^{pos}	2 (2%)	1 (1%)	1 (1%)
Resistant disease	4 (3%)	4 (4%)	3 (%)
Death from indeterminate cause	4 (3%)	3 (3%)	1 (3%)
Early death[^]	3 (2%)	2 (2%)	1 (1%)

[*] The response for 1 patient treated at dose level 3 was unable to be assessed due to refusal of bone marrow exam; she had normalized her blood counts without peripheral blasts but is excluded from this analysis

[**] This excludes the 6 patients who had received prior DNA methyltransferase inhibitor therapy

[+] Partial response occurred in patient with myeloid sarcoma who obtained an MRD^{neg} marrow but visceral disease was unable to be assessed

[^] Death within 28 days of initiation of study therapy

Abbreviations: RP2D, recommended phase 2 dose; HMA, "hypomethylating" agents (i.e. azanucleosides); CR, complete remission; MRD, measurable residual disease; CRi, complete remission with incomplete blood count recovery; MLFS, morphologic leukemia free state; DI, death from indeterminate cause; RD, resistant disease

TABLE 4

Safety and tolerability of GCLAM at the RP2D

Parameter, n=94	Grade 3–4, n (% of cycles)	Grade 5, n (% of cycles)
Fever, infection		
Bloodstream infection	42 (28%)	-
Catheter-related infection	3 (2%)	-
Dental infection	2 (1%)	-
Lung Infection	36 (24%)	-
Neutropenic fever	118 (78%)	-
Sepsis	10 (7%)	1 (0.7%)
Sinusitis	1 (0.7%)	-
Soft-tissue infection	14 (9%)	-
Upper respiratory tract infection	1 (0.7%)	-
Urinary tract infection	4 (3%)	-
Cardiac		-
Atrial tachycardia	7 (5%)	-
Cardiac Arrest	-	1 (0.7%)
Cardiomyopathy	5 (3%)	-
Edema	3 (2%)	-
Hypertension	1 (0.7%)	-
Hypotension	4 (3%)	-
Myo/pericarditis	1 (0.7%)	-
Pericardial effusion	1 (0.7%)	-
Gastrointestinal		
Abdominal pain	1 (0.7%)	-
Colitis	3 (2%)	-
Cholecystitis	1 (0.7%)	-
Diarrhea	5 (3%)	-
Diverticulitis	1 (0.7%)	-
Dysphagia	1 (0.7%)	-
Esophagitis	3 (2%)	-
Ileus/obstruction	3 (2%)	-
Mucositis	8 (5%)	-
Nausea	4 (3%)	-
Ulcer	1 (0.7%)	-
Vomiting	3 (2%)	-
General		
Flu-like symptoms	1 (0.7%)	-
Multi-system organ failure	2 (1%)	2 (1%)
Myalgia	1 (0.7%)	-

Parameter, n=94	Grade 3–4, n (% of cycles)	Grade 5, n (% of cycles)
Immune System		
Anaphylaxis	1 (0.7%)	-
Fever	4 (3%)	-
Infusion reaction	2 (1%)	-
Investigations		
Acute kidney injury	7 (5%)	-
Alanine aminotransferase increase	1 (0.7%)	-
Alkaline phosphatase increase	2 (1%)	-
Aspartate aminotransferase increase	1 (0.7%)	-
Bilirubin increase	5 (3%)	-
Cardiac troponin	2 (1%)	-
INR increased	1 (0.7%)	-
Metabolism and Nutritional		
Hyperglycemia	7 (5%)	-
Hyperkalemia	1 (0.7%)	-
Hypokalemia	7 (5%)	-
Hyponatremia	9 (6%)	-
Hypophosphatemia	2 (1%)	-
Tumor lysis	9 (6%)	-
Nervous System Disorders		
Ataxia	1 (0.7%)	-
Dysarthria	1 (0.7%)	-
Delirium/encephalopathy	3 (2%)	-
Headache	1 (0.7%)	-
Intracranial hemorrhage	1 (0.7%)	-
Stroke	2 (1%)	-
Syncope	4 (3%)	-
Vertigo	1 (0.7%)	-
Respiratory		
Hypoxia	28 (19%)	-
Pleuritic pain	1 (0.7%)	-
Pulmonary edema/effusion	7 (5%)	-
Pulmonary hemorrhage/hemoptysis	2 (1%)	-
Respiratory Failure	2 (1%)	3 (2%)
Other		
Bladder spasm	1 (0.7%)	-
Bone pain	5 (3%)	-
Deep vein thrombosis	3 (2%)	-
Depression	1 (0.7%)	-

Parameter, n=94	Grade 3–4, n (% of cycles)	Grade 5, n (% of cycles)
Fall	2 (1%)	-
Joint swelling	1 (0.7%)	-
Keratitis	2 (1%)	-
Pulmonary embolism	1 (0.7%)	-
Rash	26 (21%)	-
Retinal hemorrhage	1 (0.7%)	-
Urinary retention	1 (0.7%)	

Table describing grade 3–5 non-hematologic effects considered definitively, probably, or possibly related to study treatment by the investigator that were experienced by the 94 patients treated at the RP2D over 151 treatment cycles.

TABLE 5

Comparison of characteristics and treatment outcomes of younger vs. older study participants treated at the RP2D, n=94

Patient Characteristic	Age<65 (n= 54)	Age 65 (n=40)
Median age, years (range)	53 (21–64)	70 (65–81)
Disease		
AML/BPDCN	46 (85%)	33 (82%)
MDS-EB-2	8 (15%)	7 (18%)
Secondary disease [*]	14 (26%)	15 (38%)
Median TRM score (range)	2.32 (0.08–5.85)	4.18 (0.06–6.88)
Performance status, n (%)		
0	8 (15%)	6 (15%)
1	46 (85%)	33 (82%)
2	0	1 (3%)
Cytogenetic risk, n (%)		
Favorable	6 (11%)	2 (5%)
Intermediate	36 (67%)	24 (60%)
Adverse	12 (22%)	14 (35%)
Mutational status, n (%)		
FLT3-ITD		
Wild-type	37 (69%)	25 (63%)
Mutated	5 (9%)	2 (5%)
Unknown	12 (22%)	13 (32%)
NPM1		
Wild-type	30 (56%)	17 (43%)
Mutated	10 (19%)	5 (12%)
Unknown	14 (26%)	18 (45%)
Response		
CR, n (%)	46 (85%)	28 (70%)
MRD[neg] CR, n (%)	42 (78%)	25 (62%)
CRi, n (%)	3 (6%)	4 (10%)
CR/CRi, n (%)	49 (91%)	32 (80%)
PR/MLFS	4 (7%)	2 (5%)
Resistant disease	0	4 (10%)
Death from indeterminate cause	1 (2%)	2 (5%)
Subsequent allogeneic HCT	30 (56%)	16 (40%)
1-year overall survival	81%	51%

Patient Characteristic	Age<65 (n= 54)	Age ≥65 (n=40)
1-year relapse-free survival	73%	52%
TRM	1 (2%)	1 (2%)

*Defined either as AML transformed from antecedent hematologic disorder, or AML/MDS in a patient who had previously received cytotoxic therapy

Abbreviations: RP2D, recommended phase 2 dose; CR, complete remission; MRD, measurable residual disease; CRi, complete remission with incomplete blood count recovery; PR, partial remission; MLFS, morphologic leukemia free state; TRM, death within 28 days of therapy initiation

TABLE 6

Comparison of baseline study characteristics across induction regimens

Regimen	GCLAM RP2D (n=94)	7+3 (n=100)	P-value
Median age, years (range)	62 (21–81)	56 (22–76)	<0.01
Male gender, n (%)	53 (56%)	54 (54%)	0.77
TRM score, median (range)	2.89 (0.06–6.88)	3.09 (0.15–6.9)	0.25
Performance status, n (%)			0.12
0–1	93(99%)	94 (94%)	
2–3	1 (1%)	6 (6%)	
Cytogenetic risk, n (%) *			0.10
Favorable	8 (8%)	19 (19%)	
Intermediate	61 (65%)	61 (61%)	
Adverse	25 (27%)	17 (17%)	
Mutational status, n (%)			
FLT3-ITD			0.61
Wild-type	61 (65%)	55 (55%)	
Mutated	8 (8%)	10 (10%)	
Unknown	25 (27%)	35 (35%)	0.84
NPM1			
Wild-type	47 (50%)	49 (49%)	
Mutated	16 (17%)	14 (14%)	
Unknown	31 (33%)	37 (37%)	
Secondary disease **	29 (31%)	21 (21%)	0.14
Laboratory findings at baseline, median (range)			
WBC ($\times 10^9$L)	3 (1–117)	6 (1–119)	0.06
Peripheral blood blasts (%)	9 (0–96%)	23 (0–90%)	0.09
Platelets ($\times 10^3$)	58 (11–710)	60 (5–547)	0.74
Overall response, n **			
CR	74 (79%)	60 (60%)	<0.01
MRD^{neg} CR	67 (71%)	53 (53%)	0.01
CR + CRi	81 (86%)	70 (70%)	<0.01
2-year cumulative transplant rate	39 (41%)	32 32%)	0.07
1-year overall survival	69%	72%	0.97
Treatment-related mortality			
4-week	2 (2%)	2 (2%)	
8-week	5 (5%)	3 (3%)	

*
Cytogenetic data were missing or karyotyping failed in 3 patients in the 7+3 cohort

**
Defined either as AML transformed from antecedent hematologic disorder, or AML/MDS in a patient who had previously received cytotoxic therapy

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