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ORIGINAL ARTICLE

Fractionated gemtuzumab ozogamicin combined with intermediate-dose cytarabine and daunorubicin as salvage therapy in very high-risk AML patients: a bridge to reduced intensity conditioning transplant?

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Abstract Outcome of patients with primary refractory/relapsed (R/R) acute myeloid leukemia (AML) remains dismal. Herein, we present a retrospective monocentric study of 24 very high-risk AML patients who received a combination of fractionated gemtuzumab ozogamicin (GO) with intermediate-dose cytarabine and daunorubicin as salvage therapy. Median age was 55.3 years. Diagnostic was secondary AML for 33% of them. Seven patients had favorable risk, 8 had intermediate-1 or intermediate-2, and 6 had unfavorable risk of AML according to the European LeukemiaNet prognostic index. Complete remission was achieved in 50% of cases (46% in refractory and 55% in relapsed AML) without excessive toxicity. Thirteen patients could be referred for transplant. Only allogeneic hematopoietic stem cell transplantation provided a benefit in this patient cohort with a 1-year overall survival of 50.7 versus 18.1% in the absence of transplantation. Patients treated with reduced intensity conditioning (RIC) showed a longer survival as compared to those undergoing myeloablative conditioning regimen mainly because of decreased toxicity.

Our data suggest that salvage therapy with fractionated GO combined with intermediate-dose cytarabine and daunorubicin in very high-risk patients may serve as a potential bridge therapy to RIC transplant.

Keywords Acute myeloid leukemia · Relapse · Refractory · Salvage · Gemtuzumab ozogamicin · Bridge to allogeneic stem cell transplantation

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Introduction

Despite the recent improvements in the treatment of adult acute myeloid leukemia (AML), many patients still fail to achieve complete remission (CR) or relapse early after response to initial induction chemotherapy [1]. The prognosis of primary refractory/early relapsed (R/R) AML patients remains poor. Nevertheless, most of these patients undergo salvage chemotherapy [2, 3]. The likelihood of response to salvage therapy ranges from 25 to 50%, and only 11% are still alive at 5 years [4, 5]. Prolonged survival was only observed in patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) [6]. The best outcome after allo-HSCT is achieved when transplantation is performed in CR or in partial remission (PR) after salvage therapy [7]. Response to salvage therapy therefore appears essential to improve overall survival (OS) in R/R AML patients, and new studies should focus on better remission induction and consolidation regimens containing innovative agents.

Gemtuzumab ozogamicin (GO) (Mylotarg®, Pfizer, New York, NY, and Ben Venue Laboratories, Bedford, OH, USA) is an antibody-targeted chemotherapy agent consisting of a

humanized murine anti-CD33 monoclonal antibody (clone P67.6) linked to calicheamicinϒ1, a potent anti-tumor antibiotic, derivative via a hydrolysable bifunctional linker [8]. Binding of GO induces cell death in CD33-positive cells by internalization of the calicheamicin drug and toxin release intracellulary leading then to cleavage of double-stranded DNA [9].

In relapsed AML, GO used alone with an unfractionated dose of 9 mg/m^2 on days 1 and 14 resulted in 13% of CR and a median OS of 5 months. Combined with intermediate-dose cytarabine and mitoxantrone, unfractionated dose of GO provided 50% of CR rates in patients with R/R AML, and a 2-year OS of 41%. However, early toxic deaths were reported related to sinusoidal obstruction syndrome (SOS) [10]. These results were confirmed by subsequent studies evaluating unfractionated GO combined with various agents in patients with R/R AML [11–17]. CR rates ranged from 12 to 63% and median OS from 2.0 to 9.5 months but with a significant hepatotoxicity and absence of full platelet recovery in roughly half of responders. These results led to regulatory approval of the drug in the US for the use in older patients in the first relapse. However, GO was voluntarily withdrawn from the market in 2010 on the basis of results from a phase 3 Southwest Oncology Group (SWOG) randomized study comparing a single infusion of GO at 6 mg/m2 combined with standard induction chemotherapy versus standard induction chemotherapy alone [18]. Since then, randomized studies combining GO with intensive chemotherapy in newly diagnosed AML have been reported. However, a recent meta-analysis showed that addition of GO does not increase the rate of CR but only significantly improves OS [19]. To reduce toxicity while conserving efficacy, fractionated dosing using the lower dose of 3 mg/m^2 GO was proposed. In a phase 2 study of patients with relapsed AML fractionated doses of GO demonstrated efficacy with a CR rate of 26% and no grade 3–4 liver toxicity [20]. This was confirmed by the French ALFA group using a GO schedule of 3 mg/m^2/day on days 1, 4, and 7 during induction chemotherapy followed by a single dose in each of two consolidation courses in older patients with untreated AML that showed a CR rate of 81% and event-free survival of 40.8% compared to 17.1% in the control group [21]. Further studies evaluating regimens combining fractionated GO with various chemotherapies in the first R/R AML showed CR rates ranging from 22 to 75% [22–24].

The main objective of the present retrospective study was to evaluate the efficacy of the combination of fractionated GO with intermediate-dose cytarabine and daunorubicin in very high-risk patients with AML, and its potential use as bridge to transplant in this patient population.

Patients and methods

Study cohort

A retrospective chart review of 24 successive R/R AML patients treated with GO associated to cytarabine (1000 mg/m^2 per 12 h, infused over 2 h for 3 days) and daunorubicin (60 mg/m^2 per day for 2 days) in our institution was performed. Results for cytogenetic analysis were classified according to standard International System for Human Cytogenetic Nomenclature criteria [25] as favorable, intermediate, or unfavorable subgroups. Karyotype abnormalities that involved t(16;16), inv(16), or t(8;21) with or without additional cytogenetic abnormalities were considered favorable cytogenetics. Monosomies and deletions of chromosomes 5 and 7 [-5, -7, del(5)q-, del(7)q-]; abnormalities of the long arm of chromosome 3; t(6;9), or t(9;22); abnormalities involving the long arm of chromosome 11 (abn 11q23) [except t(9;11)]; or complex cytogenetic abnormalities (defined as at least three unrelated cytogenetic clones) were considered unfavorable risk factors. Other cytogenetics were designated intermediate risk factors including normal karyotypes and other cytogenetic abnormalities. Screening for mutations in the nucleophosmin gene (NPM1), FMS-like tyrosine kinase 3 gene (FLT3) internal tandem duplication (ITD) was done centrally; favorable genotypes were defined as normal karyotype and NPM1 mutation without FLT3-ITD, in accordance with international recommendations [25].

Treatment

R/R AML patients were given intravenous GO (3 mg/m^2 [maximum dose 5 mg] infused over 2 h on days 1, 4, and 7) with a premedication consisting of intravenous dexchlorpheniramine and corticosteroids associated to a 3+7 induction course of intravenous daunorubicin (60 mg/m^2 per day for 3 days and intravenous) and cytarabine (200 mg/m^2 as continuous infusion for 7 days). Patients with an identified potential donor achieving CR were then proposed allo-HSCT.

As the first line, 11 patients received a time-sequential induction including the first sequence combining daunorubicin (80 mg/m^2 per day intravenously on days 1–3) and cytarabine (500 mg/m^2 per day continuous intravenous infusion over the same period). The second sequence, administered after a 4-day free interval, consisted of mitoxantrone (12 mg/m^2 per day, intravenously on days 8 and 9) and cytarabine (500 mg/m^2 per 12-h bolus intravenous infusion on days 8–10). Thirteen patients received a "3+7" induction schema: 9 had idarubicin (12 mg/m^2 per day for 3 days) and 4 daunorubicine (90 mg/m^2 per day for 3 days) associated with cytarabine (200 mg/m^2 per day for 7 days). Fifty-four percent of patients had the first salvage treatment relying on high-dose

cytarabine. Tandem salvage was defined by conducting salvage treatment, then bone marrow aspiration at day 15 and in the absence of more than 30% marrow blasts initiating bone marrow transplantation conditioning. Among 13 patients achieving the first CR, 8 patients had consolidation courses with intermediate dose of cytarabine for 3 of them or high dose for 5 of them.

Ethics statement

Written informed consent was obtained from all patients and the procedures followed were in accordance to the Helsinki declaration as revised in 2008. The review board protocol of the Hospices Civils de Lyon approved this study. All data were analyzed anonymously. Each patient was identified with a personal number. Patients were aware that their data were stored in a specific database, but were not informed that these data were used for research purposes. This procedure has been disclosed to the Ethics Committee that, in accordance with national legislation, approved it.

Response criteria and evaluation

Response to salvage therapy was evaluated by bone marrow (BM) studies scheduled after peripheral blood cell recovery. BM studies were performed earlier in the case of suspected disease progression. A CR was defined as <5% blasts in a BM of adequate cellularity and no Auer rods, absence of leukemic blasts in the peripheral blood, an absolute neutrophil count (ANC) of greater than or equal to 1 G/L, and platelets of greater than or equal to 100 G/L with transfusion independence [26]. Hematological relapse or refractory disease was considered when more than 5% blasts were seen in two BM aspirates obtained at a 15-day interval. Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.

Immunostaining and flow cytometry analysis

Analysis of leukemia was performed with FACS Canto II (BD Biosciences) using Diva software (BD Biosciences). Standardized calibration particles (SPHERO 8-peak Rainbow; BD Biosciences) were used daily to adjust instrument settings, set fluorescence compensation, and check instrument sensitivity. Daily instrument setup was performed using FACSComp software (version 4.0; BD Biosciences) according to the manufacturer's recommendations. Due to different optical properties of leukocytes and calibration beads, peripheral blood leukocytes from healthy donors and StatusFlow PRO flow cytometry control (R&D Systems) were also used for instrument setting optimization before acquisition and analysis. StatusFlow PRO reagent allowed determination of daily percent and absolute numbers of leukocyte subsets and comparison of these values with the target values defined by the manufacturer. Target values for CD33 marker were provided with this reagent. Cells were stained with the appropriate monoclonal antibody (mAb) or isotype-matched control mAb. Expression of cell surface molecules was analyzed without fixation on leukemic cells at diagnosis by identifying such cells on their low CD45 expression [27] after whole blood lysis.

Statistical analysis

All patients were analyzed. Descriptive statistics were used to characterize patients and their disease. OS was the main endpoint for this analysis. OS was defined as the time from the initiation of GO therapy to death from any cause and was censored at the date of last information. Survival was estimated by the method of Kaplan-Meier and was compared by the use of the log-rank test. Hazard ratio (HR) with 95% confidence interval (CI) was calculated by the use of Cox models. The binary data were compared by using the Fisher test and continuous data were compared using the Mann-Whitney test. Multivariate analysis was performed using Cox proportional hazard model. Cutoff of CD33 expression was determined with cutoff finder [28]. All analyses were two-sided, with a P value <0.05 considered statistically significant. All analyses were performed using STATA® (version 12) and GraphPad Prism® (version 5).

Results

Patient characteristics

From March 2013 to November 2015, 24 patients with R/R (12 relapses and 12 primary refractory to chemotherapy) AML were treated by fractionated dose of GO combined with intermediate-dose cytarabine and daunorubicin. Median follow-up was of 6 months (range, 0.6–30.9 months). The main clinical and biological characteristics are summarized in Table 1. The median age was 55.3 years (range, 22.2–70.1). The sex ratio (male/female) was 1.7. De novo AML represented 67% of cases, while 37% had secondary AML following myelodysplastic syndrome (6 patients), or a previous hematologic chronic myeloproliferative disorder (1 patient), or therapy-related (1 patient). At the time of salvage therapy, 3 patients (12.5%) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) > 2. Cytogenetic features were available in 22 patients. Two patients had favorable-risk, 13 had intermediate-risk, and 6 had unfavorable-risk cytogenetics. Ten patients (42%) displayed a

Table 1 Characteristics of patients at the time of salvage therapy

Patients (*n*)	24
Sex ratio (M/F), *n*	(15/9), 1.7
Median age, years (range)	55.3 (22.2–70.1)
>60 years, *n* (%)	9 (38)
Cytogenetics, *n* (%)	
Favorable	2 (8)
Intermediate	13 (54)
Unfavorable	6 (25)
Not available	3 (13)
NPM1 status, *n* (%)	
Mutated	7 (29)
Wild type	15 (63)
Not available	2 (8)
FLT3-ITD status, *n* (%)	
ITD	4 (17)
Wild type	18 (75)
Not available	2 (8)
Prognosis index, *n* (%)	
Favorable	7 (29)
Intermediate	1 + 2 8 (33)
Unfavorable	6 (25)
Not available	3 (13)
AML type, *n* (%)	
De novo AML	16 (67)
Secondary AML	8 (33)
Induction, *n* (%)	
3+7	13 (34)
Sequential induction	11 (46)
1st induction CR achievement, *n* (%)	12 (50)

M male, *F* female, *CR* complete remission, *NR* not reached, *OS* overall survival

normal karyotype. By adding NPM1 and FLT3 status, 7 had favorable, 8 had intermediate-1 or intermediate-2, and 6 had unfavorable prognostic index according to the European LeukemiaNet [25]. Bone marrow median blast count at the time of salvage treatment was 40% (range 10–85).

Survival and efficiency

CR rate was 50% (95% CI 28 to 72%): 46% in refractory and 55% in early relapsing AML (p = ns). Median OS was 6.7 months (95% CI, 3.3 to 19.2 months) (Fig. 1a). One-year OS was 35% (95% CI 17–54%). Median OS was similar among the different European LeukemiaNet (ELN) classification groups (Fig. 1b). Patients in ELN favorable group had a median OS of 11.8 months (95% CI 2.2—not reached), intermediate 17.6 months (95% CI 3.3—not reached), intermediate 25.4 months (95% CI 3.2—not reached), and unfavorable 5.2 (95% CI 0.9—not reached). There were no significant differences in terms of OS between relapsed (median OS 6.7, 95% CI 3.2—not reached) and refractory (median OS 7.7, 95% CI 2.2—not reached, p = 0.91) groups (Fig. 1c), between patients younger (median OS 11.4, 95% CI 2.2—not reached) and older than 60 (median OS 11.4, 95% CI 2.6—not reached) (p = 0.68) (Fig. 1d). There were also no significant differences in terms of OS regardless FLT3 status (median OS rates for FLT3 wild type and ITD were 6.7 (95% CI 4.3–19.2) and 3.3 (95% CI 3.2—not reached), respectively (p = 0.92) (Fig. 1e), and between patients who achieved CR (median OS 3.3 with 95% CI 0.9–16.4) after GO and those who did not (median OS 7.7 (4.8—not reached)) (p = 0.10) (Fig. 1f).

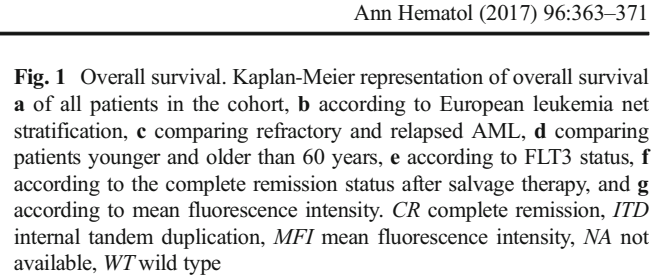

Fig. 1 Overall survival. Kaplan-Meier representation of overall survival **a** of all patients in the cohort, **b** according to European leukemia net stratification, **c** comparing refractory and relapsed AML, **d** comparing patients younger and older than 60 years, **e** according to FLT3 status, **f** according to the complete remission status after salvage therapy, and **g** according to mean fluorescence intensity. *CR* complete remission, *ITD* internal tandem duplication, *MFI* mean fluorescence intensity, *NA* not available, *WT* wild type

CD33 expression

Mean leukemic cells expressing CD33 was 84% (95% CI 77–89%) before GO introduction versus 74% (95% CI 65–84%) at diagnosis (p = 0.052). Mean fluorescence intensity (MFI) of CD33 on leukemic cells was 5277 (95% CI 3852–6721) versus 3515 (95% CI 2442–4588) at diagnosis (p = 0.017). The best cutoff was determined using cutoff finder® at a MFI of 6000, p = 0.2 (Fig. 1g).

Safety

The 24 treated patients developed grade 3–4 neutropenia, thrombocytopenia, and anemia. Median duration of neutropenia ($<0.5 \times 10^9$/L) was 26 days and the median duration of thrombocytopenia ($<20 \times 10^9$/L) was 25 days (range 16–34). No SOS was reported after GO therapy.

Allogeneic hematopoietic stem cell transplantation

Eleven patients did not undergo allo-HSCT. Five of them were considered not fit enough, while no donor was identified for the remaining 6 patients.

Thirteen patients underwent allo-HSCT. Four had refractory AML at the time of transplant with BM blast count inferior to 30%. Patient characteristics at the time of allo-HSCT are summarized in Table 2. The median age was 51.2 years. Four patients received myeloablative conditioning (MAC). One patient had fludarabine and busulfan

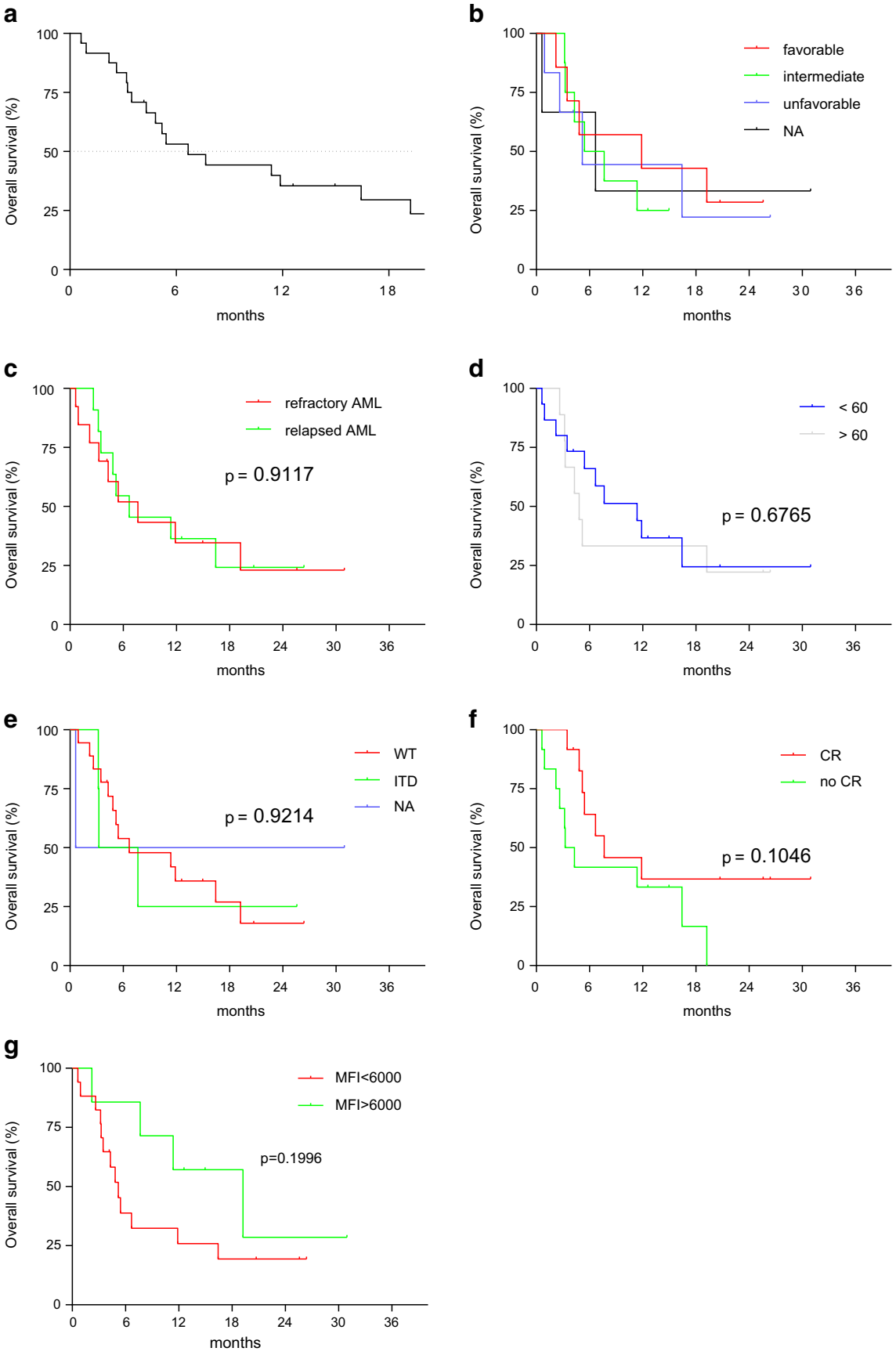
a
Overall survival (%)
months
b
favorable
intermediate
unfavorable
NA
c
refractory AML
relapsed AML
p = 0.9117
d
< 60
> 60
p = 0.6765
e
WT
ITD
NA
p = 0.9214
f
CR
no CR
p = 0.1046
g
MFI<6000
MFI>6000
p=0.1996

Table 2 Characteristics of allografted patients after salvage therapy

Patients (*n*)	13
Sex ratio (M/F), *n*	(6/7), 0.9
Median age, years (range)	51.2 (24.3–63.6)
>60 years, *n* (%)	3 (23)
MAC, *n* (%)	4 (31)
Donor, *n* (%)	
10/10 MRD	5 (38)
10/10 MUD	3 (23)
9/10 MMUD	3 (23)
UCB	2 (15)
Acute GVHD > grade II, *n* (%)	5 (38)
Post ASCT relapse, *n* (%)	6 (46)
TRM, *n* (%)	2 (15)
Death, *n* (%)	8 (62)

ASCT allogeneic stem cell transplantation, *GVHD* graft versus host disease, *MAC* myeloablative conditioning, *MMUD* mismatch unrelated donor, *MRD* matched related donor, *MUD* matched unrelated donor, *TRM* treatment related mortality, *UCB* cord blood units

conditioning and one received 2 Grays total body irradiation with cyclophosphamide and fludarabine. The remaining 11 patients had conditioning using FLAMSA BU2 or BU4 (fludarabine, cytarabine, and amsacrine followed by 2 or 4 days of busulfan) [29] for respectively 7 and 4 of them regarding stem cell sources, 5 patients were transplanted with matched related donors (MRD), 3 with matched unrelated donors (MUD), and 5 with alternative donors [two mismatch unrelated donors (MMUD) and two umbilical cord blood (UCB)].OS was significantly higher in the allo-HSCT group than in the not transplanted patients (P = 0.02) (Fig. 2a). One-year OS after allo-HSCT was 51% (95% CI 21–74) versus 11% (95% CI 1–38) in the absence of allo-HSCT. Five patients had a tandem allo-HSCT following salvage therapy. The median OS was 7.6 months (95% CI 2.2—NR) (Fig. 2b) after tandem salvage allo-HSCT. No SOS was reported after allo-HSCT. Two patients died consecutively to grade IV cutaneous and digestive of graft versus host disease (GVHD) after allo-HSCT. Both of them had cortico-refractory acute GVHD. All patients suffered from febrile neutropenia.

Prophylactic donor lymphocyte infusions (DLIs) were only administered in the 8 patients who had a 10/10 MUD or MRD. All patients with 9/10 MMUD presented acute GVHD contraindicating such a strategy. No differences were noted in terms of survival.

In multivariate analysis in a model including the age older than 60 years, allo-HSCT, refractory AML, and achievement of CR after GO, only the performance of allo-HSCT predicts patients' outcome with a hazard ratio (HR) of 0.25 (95% CI 0.07–0.86), p = 0.02(Fig. 2c).

Discussion

R/R AML remains of poor prognosis. In this setting, there is currently no consensual salvage treatment. The use of GO in AML has been controversial despite several positive clinical trials [21, 30–32]. Indeed, the use of greater than or equal to 6 mg/m^2 doses increases the incidence of SOS [33]. Because of a rapid in vitro and ex vivo turnover of the CD33 antigen on myeloblastic cell surface after exposure to GO [9], fractionated dose regimen was employed as induction therapy and was supposed to reduce liver toxicity [20].

Herein, we report a retrospective monocentric study evaluating GO combined with intermediate-dose cytarabine and daunorubicin in very high-risk AML. With a CR rate of 50% after salvage treatment (46% in refractory and 55% in relapsed AML), our results are consistent with the previous data from the literature [34]. However, most of these studies using fractionated doses of GO as salvage treatment were conducted in patients in the first relapse. Chantepie et al. reported the use of fractioned GO combined with fludarabine, cytarabine, granulocyte colony-stimulating factor (G-CSF), and idarubicin (FLAG-IDA) in R/R AML [23]. The response rate was 38% with 22% of CR, but treatment mortality was relatively high (5/36 patients, 14%). GO combined with intermediate-dose cytarabine and daunorubicin was well tolerated without any death related to salvage therapy. Furthermore, no SOS were reported following salvage treatment, and similarly during and after allo-HSCT, even after conditioning regimen containing busulfan. This was less than expected since a rate of 8.5% moderate/severe SOS was previously reported in this patient population [35].

In our study, we did not identify any prognosis factors except for allogeneic transplant. ELN classification, FLT3 status, and cytogenetic abnormalities seem to lose their prognostic value in front of a relapsed or a refractory AML. However, our study suffers from several limitations such as a small number of patients and the high heterogeneity of patient characteristics. Furthermore, it is a retrospective monocentric study.

Our results are consistent with literature data, and our objective is to identify new prognostic factors in this subgroup of patients with R/R AML. Several scoring systems have been introduced for patients with R/R AML in order to identify patients with an improved outcome [4, 36–38].

Allo-HSCT has a role in the treatment of R/R AML either as salvage therapy or as subsequent therapy following CR2 achieved by salvage chemotherapy. Outcomes for allo-HSCT for AML are superior when the transplant is performed in second CR rather than during the first relapse with a 3-year survival of 25% [39].

In pediatric [40] and adult [41] cohorts, CD33 expression has been shown to be correlated with prognosis in the first-line AML patients treated with GO. In our study, CD33 expression was not correlated with survival or CR achievement. This might be due to a small number of patients, or a high

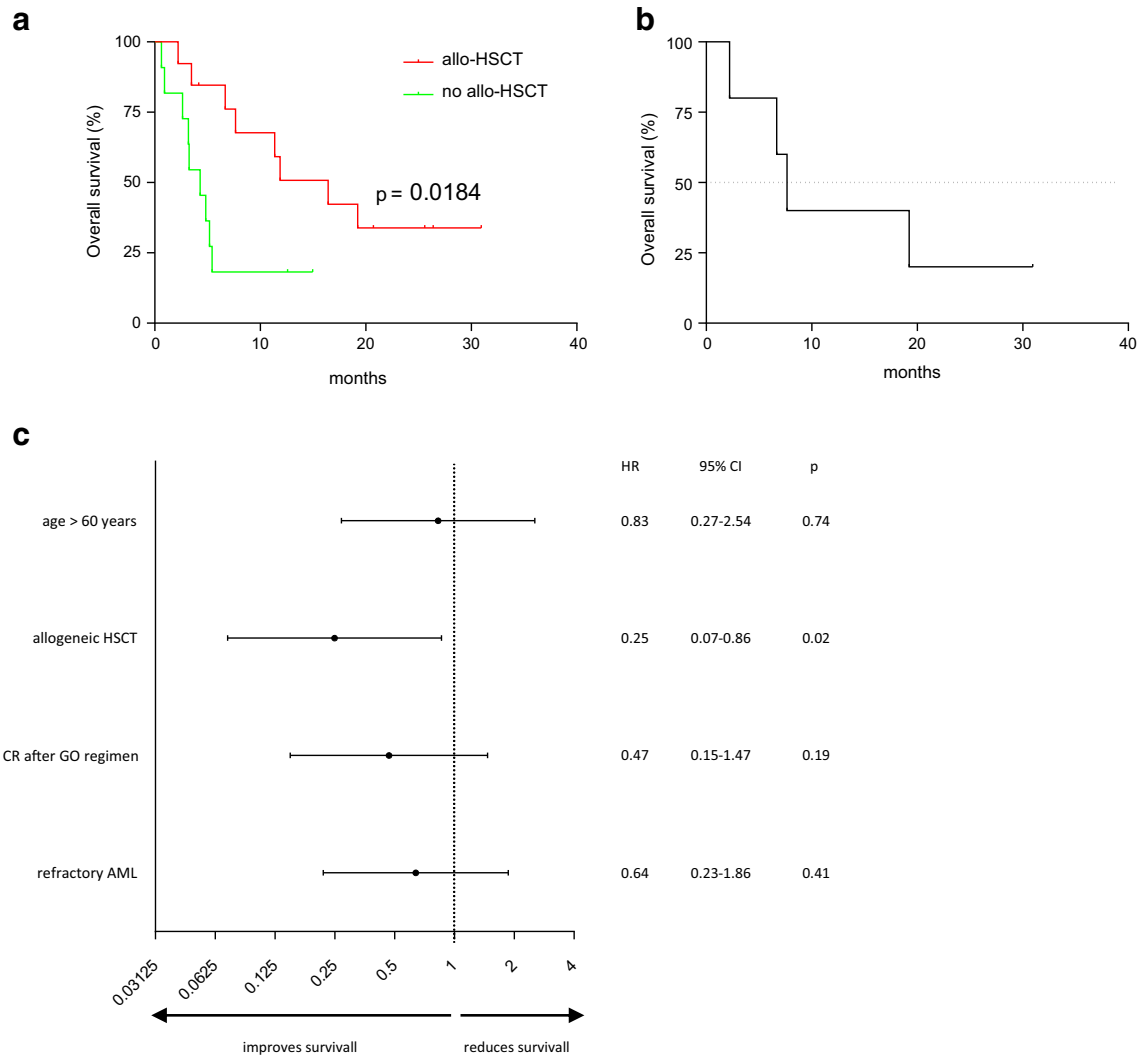

Fig. 2 Overall survival in allografted patients. Kaplan Meier representation of overall survival **a** comparing allografted patients versus non-allografted patients, **b** in patients with tandem salvage and allo-HSCT, **c** comparing RIC versus MAC regimen, and **d** effects of covariate on mortality. *Allo-HSCT* allogeneic hematopoietic stem cell transplantation, *MAC* myeloablative conditioning, *RIC* reduced intensity conditioning

expression significantly superior at relapse than at diagnosis. Multiparametric flow cytometry studies have shown that AML cells displayed an immature phenotype at relapse. In our study, membranous CD33 expression was significantly higher at relapse, meaning that the control of the phenotype at relapse is essential in order not to disregard the possibility of the use of the GO.

No SOS was seen after GO salvage regimen or after allogeneic stem cell transplantation conditioning. In 2010, GO was voluntarily withdrawn from the market on the basis of preliminary results from a phase III SWOG randomized study in more than 600 young adults with untreated AML because of an increased mortality up to 5% due to GO-induced SOS [18]. Later on, different studies have shown that fractioned dose of GO might contribute to keep the effectiveness without having to deplore an excess of toxicity [42]. Our study demonstrates that fractioned dose of GO in heavily treated high-risk AML patients associated to chemotherapies is a strategy with a certain effectiveness with an acceptable toxicity profile.

To our knowledge, there is no comparative study of different strategies in refractory AML patients. In R/R AML, standard chemotherapies are disappointing. Targeted therapies are emerging but need to be validated. GO associated to intermediate-dose cytarabine and daunorubicin as salvage treatment in R/R AML is feasible with low toxicity and might represent in this particularly very high-risk patient population a bridge to allo-HSCT.

In conclusion, data from studies published after withdrawing of GO from the market, of which our present study, suggest that the license status of GO might be reviewed, at least for certain subtypes of patients and certain situations of which R/R AML patients.

Authors' contributions EP included patients, designed the study, acquired, analyzed and interpreted the data, conducted the statistical analysis, and wrote the manuscript; XT included patients, designed the study, analyzed data, and reviewed the manuscript; AP was responsible for coordinating immunophenotyping data; SDL, EW, HLW, FEN, and FB included patients; GS reviewed the manuscript. All authors gave their final approval on the definitive version of the manuscript.

Conflict of interest XT received consultancy fees from Pfizer. The other authors reported no potential conflicts of interest.

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