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Original Article

# Lyon-University Hospital Experience with Gemtuzumab Ozogamicin Therapy in Acute Myeloid Leukemia: a 'Real-Life' Study

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**Abstract. Ninety-four adults with newly diagnosed or relapsed/refractory acute myeloid leukemia (AML) were treated with fractionated doses of gemtuzumab ozogamicin (GO) at one-single French center over ten years. We attempted to define predictive factors for response and survival. The overall response rate was 70% (86% in newly diagnosed and 65% in relapsed/refractory AML). Mortality during induction was 6%. Disease-free survival (DFS) and overall survival at three years after GO treatment was 36% and 31%, respectively. Median DFS in relapsed/refractory patients was eight months with a 3-year DFS at 34%. Among remitters, allogeneic hematopoietic stem cell transplantation (HSCT) can be performed in 28 cases (42%), including two patients in first-line therapy and 26 in further line. In relapsed/refractory patients undergoing allogeneic HSCT after responding to GO therapy, the median DFS was not reached. Incidences of transplant-related mortality, grade ≥ 3 acute graft-versus-host (GvH) disease, and extensive chronic GvH disease were 11%, 14%, and 25%, respectively. No sinusoidal obstruction syndromes were reported among allografted patients as among the other patients in the studied cohort. GO-based chemotherapy is a viable option for the treatment of relapsed/refractory AML patients and is a feasible schedule as a bridge to allogeneic transplant.**

**Keywords:** Acute myeloid leukemia; Gemtuzumab ozogamicin; Prognosis; Treatment.

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**Introduction.** Acute myeloid leukemia (AML) is a life-threatening hematological disorder characterized by uncontrolled proliferation of abnormal blasts in the bone marrow, disturbing normal hematopoiesis. Over the past few years, several promising concepts have been introduced for the treatment of AML, of which one is based on the expression of CD33 on leukemic cells.[1] Gemtuzumab ozogamicin (GO) (mylotarg®) is a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin, a potent DNA-binding cytotoxic antibiotic that causes single and double-strand cuts. The bond between antibody and drug is stable in circulation and then dissolves, once intracellular, to allow the calicheamicin to bind with the DNA. GO spares the presumably normal precursors, so allowing for restoration of normal hematopoiesis.[2] GO monotherapy has shown a 23% response rate in newly diagnosed AML patients not eligible for intensive

chemotherapy.[3] GO was initially approved by FDA, then subsequently withdrawn; it was reapproved combined with cytarabine and daunorubicin given as standard '7+3' for newly diagnosed CD33-positive AML. Approval was obtained after the French ALFA-0701 trial randomizing newly diagnosed patients aged 50-70 years to receive '7+3' ± GO with fractionated doses (3 mg/m$^2$ on days 1, 4, and 7),[4] and was based on prolongation of event-free survival (EFS) with, however, a benefit limited to patients with favorable or intermediate cytogenetics. Hepatotoxicity (including hepatic veno-occlusive disease) has been reported in association with the use of GO, especially in patients with underlying hepatic disease or abnormal liver function. Improved outcomes have also been reported in patients receiving low-dose GO combined with low-dose cytarabine compared with low-dose cytarabine alone (30% of responses versus 17%).[5] In combination with azacitidine, GO produced a 44% CR rate with a median OS of 11 months in patients aged 60-69 years, and 35% CR rate with again a median OS of 11 months in patients aged ≥70 years.[6]

In this 'real-life' study, we report our experience with fractionated GO administration given as front-line therapy in combination with conventional chemotherapy according to the European recommendations, but also and above all given outside the official indications in the relapsed/refractory setting. The study aim was to evaluate its efficacy, especially in high-risk patients and its potential use as a bridge to transplant.

**Patients and Methods.**

*Patients.* A chart review of 94 AML patients receiving GO between January 2009 and January 2019 at the Lyon University Hospital (France) was retrospectively performed. All patients had Eastern Cooperative Oncology Group Performance Status (ECOG PS)<2 at the time of starting GO therapy. Diagnoses were established according to criteria proposed by the French-American-British (FAB) study group. In all patients, leukemic blasts CD33 expression was > 40%, and a majority of them expressed substantial amounts of CD33 (>70% in 60% of cases). All patients were classified according to the European LeukemiaNet (ELN) stratifications.[7] Cytogenetic data were classified according to standard International System for Human Cytogenetic Nomenclature criteria into favorable-, intermediate-, or unfavorable-risk subgroups.

The screening for the following mutations was performed at diagnosis prospectively or retrospectively in 93 of the 94 patients: FMS-like tyrosine kinase 3 (*Flt3*) gene internal tandem mutation (*ITD*) or tyrosine kinase domain (*TKD*), the nucleophosmin gene (*NPM1*), the *MLL partial tandem duplication (PTD)*, the CCAAT/enhancer-binding protein alpha (*CEBPA*) gene, the ectropic virus integration site 1 protein homolog (*EVI1*) gene (*MECOM*), and the isocitrate dehydrogenase (*IDH1/2*) genes.

*Treatment.* Twenty-two patients received GO as front-line therapy (group 1), while 72 patients received GO in second or further line of treatment of whom 13 were previously allografted. As a front-line treatment, GO (3 mg/m$^2$/day on days 1, 4 and 7) was combined with a conventional '7+3' induction chemotherapy, with cytarabine (200 mg/m$^2$/day on days 1 to 7) and daunorubicin (60 mg/m$^2$/day on days 1 to 3).[4] The total dose of GO per infusion was not to exceed one 5 mg vial. Sixty-six patients in the relapsed/refractory setting (group 2) received the same treatment. Patients who achieved composite complete remission (CRc) can receive two courses of consolidation, including daunorubicin and cytarabine with GO (3 mg/m$^2$/day on day 1). They could also be considered for allogeneic hematopoietic stem cell transplant (HSCT) according to age, Eastern Cooperative Oncology Group Performance Status (ECOG PS), genetic-risk profile, presence of a potential donor, and absence of prior transplantation. Six refractory patients (group 3) with an identified HLA compatible donor received GO (3 mg/m$^2$/day on days 1, 4 and 7) combined with cytarabine (200 mg/m$^2$/day on days 1 to 7) and daunorubicin (60 mg/m$^2$/day on days 1 to 3) followed at day 15 by a FLAMSA sequential conditioning combining fludarabine, cytarabine, amsacrine, followed by cyclophosphamide, and/or either total body irradiation or busulfan, and allogeneic HSCT.[8]

*Ethics statement.* All treatments received approval from the institutional review board and were conducted in accordance with the Declaration of Helsinki. This observational and retrospective study did not require any specific, informed consent or ethics committee approval according to French legislation (articles L.1121-1 paragraph 1 and R1121-2, Public Health Code). However, the patients enrolled in a transplant protocol, and in any case of transplantation, signed informed consent. All data were collected and analyzed anonymously.

*Statistical analyses.* Descriptive statistics were used to characterize patients and their disease. Descriptive data were stratified by study cohorts. Associations between pretreatment characteristics and responses to induction were evaluated by the Pearson $\chi^2$ test. All tests were two-sided with statistical significance set at 0.05. CRc includes all patients who achieved CR, CRi (all CR criteria except for residual neutropenia or thrombocytopenia), and CRp (CR with incomplete platelet counts).[9] CRc was defined as less than 5% blasts in bone marrow aspirates with no blasts with Auer rods and no extramedullary disease. Hematological relapse was considered when more than

5% blasts were seen in two bone marrow aspirates obtained at a 15-day interval. Overall survival (OS) was defined as the time from GO therapy to death or last patient contact. Disease-free survival (DFS) was defined from date of CRc following GO therapy to date of relapse or death, or last contact with patient in continuous CRc. DFS and OS distributions were estimated by the method of Kaplan and Meier. All treatment and subgroup comparisons were performed by the log-rank test. Simultaneous effects of multiple covariates were estimated with the maximum-likelihood logistic regression model for response to therapy and with the Cox's proportional hazard model for DFS and OS and tested by the likelihood-ratio test, also used in univariate analyses for continuous variables. Regarding continuous variables the threshold chosen for the analyses was the median value. Estimated hazard ratios (HRs) are reported as relative risks (RR) with 95% confidence intervals (CI). All computations were made using BMDP software (BMDP Statistical Software, Los Angeles, CA).

## Results.

*Population characteristics.* A total of 94 AML patients (53 males and 41 females) were treated with GO at the Lyon University Hospital between January 2009 and January 2019. At the time of analysis, the median follow-up was 3 years (95% CI: 1.8 – 3.3 years). Main characteristics at the time of treatment are summarized in **Table 1**. The median age was 56 years (18 – 75 years). Twenty-seven patients had secondary AML (6 with chronic myelomonocytic leukemia, 5 with prior myelodysplastic syndrome, 4 with prior chronic myeloproliferative syndrome, 4 with non-Hodgkin lymphoma, 2 with breast cancer, 2 with uterus cancer, 1 with mastocytosis, 1 with Hodgkin disease, 1 with colon cancer, and 1 following immunosuppressive treatment for renal transplantation). The patients were grouped into three study cohorts. Twenty-two patients received GO + chemotherapy as front-line therapy (group 1). Seventy-two patients received GO in the relapsed/refractory setting (49 in second line of treatment, 20 in third line, 2 in fourth line, and one in fifth line). Among them, 66 relapsed/refractory patients received GO + chemotherapy (group 2) and 6 very high-risk refractory patients received GO + chemotherapy 2 weeks prior starting conditioning regimen in the setting of allogeneic HSCT (group 3). Overall, 19 patients were classified as 'favorable-risk' according to the ELN classification, 42 as 'intermediate-risk,' and 33 as 'unfavorable-risk.' Molecular profiling of the studied patients included *Flt3-ITD* mutations in 20 patients (21.5%), *Flt3-TKD* in 6 (6.4%), *NPM1* in 23 (24.7%), *EVI1* in 15 (16.1%), *MLL-PTD* in 8 (8.6%), *CEBPA* in 4 (4.3%), *IDH1* in 3 (3.2%), and *IDH2* in 8 (8.6%).

*Response rates.* Overall, the rate of CRc was 70% (66 of the 94 patients). CRc was achieved in 19 patients (86%) in group 1, 42 (63%) in group 2, and 5 (83%) in group 3.

Overall, 28 patients (42% of morphological remitters) underwent allogeneic HSCT after achieving response with GO either in first remission (2 patients) or further remission (18 patients in second line and 8 in third line): 13 transplants from a mismatched

**Table 1.** Patient characteristics. Group 1: patients who received GO as front-line therapy; group 2: patients who received GO after one or further lines of therapy in the relapsed/refractory setting; group 3: very high-risk refractory patients who received GO 2 weeks prior starting conditioning regimen in the setting of allogeneic HSCT.

| Characteristics | Group 1 (22pts) | Group 2 (66pts) | Group 3 (6pts) | All pts (94pts) |
|---|---|---|---|---|
| **M/F ratio** | 2.14 | 1.06 | 2.00 | 1.29 |
| **Age (y)** | 65* (18-75)** | 53 (24-71) | 49 (23-64) | 56 (18-75) |
| **Secondary AML** | 5 (22%) | 20 (30%) | 2 (33%) | 27 (29%) |
| **Prior transplant** | 0 | 13 (20%) | 0 | 13 (14%) |
| **ELN 2017** | | | | |
| **Favorable** | 2 (9%) | 15 (23%) | 2 (33.3%) | 19 (20%) |
| **Intermediate** | 12 (55%) | 28 (42%) | 2 (33.3%) | 42 (45%) |
| **Unfavorable** | 8 (36%) | 23 (35%) | 2 (33.3%) | 33 (35%) |
| **BM blast count (%)** | 40 (25-95) | 30 (20-90) | 20 (8-50) | 35 (8-95) |
| **WBC count (x $10^9$/L)** | 3.81 (1.1-54) | 5.20 (0.3-94) | 1.6 (0.6-69) | 4.42 (0.3-94) |
| **Nb of lines** | | | | |
| **1** | 22 | - | - | 22 |
| **2** | - | 46 | 3 | 49 |
| **>2** | - | 20 | 3 | 23*** |

*median; ** range; *** including 20 patients in third treatment line (17 in Group 2 and 3 in Group 3), 2 patients in fourth line (both in Group 2), and one patients in fifth line (in Group 2).

Abbreviations: BM, bone marrow; ELN, European LeukemiaNet; FAB, French-American-British classification; M/F, male/female; Nb of lines, number of treatment lines; WBC, white blood cells; y, years.

**Table 2.** Multivariate analyses in relapsed/refractory patients (group 2 and group 3).

| Factors | HR | 95% CI | p value |
|---|---|---|---|
| **Associated with CRc** | | | |
| Secondary AML (yes *vs* no) | 6.05 | 2.01 – 17.8 | 0.001 |
| **Associated with DFS** | | | |
| Nb of prior therapeutic lines (> 1 *vs* one) | 2.55 | 1.13 – 3.06 | 0.03 |
| AlloHSCT after GO (no *vs* yes) | 5.88 | 3.89 – 8.84 | < 0.001 |
| **Associated with OS** | | | |
| AlloHSCT after GO (no *vs* yes) | 3.86 | 1.87 – 7.92 | < 0.001 |
| Nb of prior therapeutic lines (> 1 *vs* one) | 1.95 | 1.06 – 3.52 | 0.03 |
| *NPM1* mutation (yes *vs* no) | 0.23 | 0.10 – 0.54 | 0.02 |
| *EVI1* mutation (no *vs* yes) | 0.24 | 0.11 – 0.52 | 0.02 |
| CRc achievement after GO (no *vs* yes) | 3.63 | 1.80 – 7.31 | 0.006 |
| Prior Allo HSCT (yes *vs* no) | 0.29 | 0.13 – 0.65 | 0.004 |

Abbreviations: AlloHSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; CI, confidence interval; CRc, composite complete response; DFS, disease-free survival; GO, gemtuzumab ozogamicin; HR, hazard ratio; Nb, number; OS, overall survival; WBC, white blood cell. A HR < 1 indicated a benefit for one factor over another.

unrelated donor, 6 from an identical unrelated donor, and 9 from an identical sibling. Conditioning regimens included amsacrine/cytarabine/fludarabine/anti-thymoglobulin (ATG)/busulfan or total body irradiation (TBI) (20 patients), fludarabine/busulfan/ATG (4 patients), cyclophosphamide/fludarabine/TBI (2 patients), busulfan/ATG/TBI (one patient), and cyclophosphamide/busulfan (one patient). Graft-versus-host (GvH) prophylaxis used ciclosporin ± methotrexate or mycophenolate mofetil or cyclophosphamide. Sources of cells were peripheral blood (23 patients), bone marrow (2 patients), and cord blood (3 patients). Six out of 20 patients (30%) evaluated for MRD before undergoing allogeneic HSCT were MRD-negative.

The other patients achieving any response received at least one subsequent consolidation therapy following GO treatment. Three patients not achieving CR with GO were allografted after a further line of treatment.

When considering only relapsed/refractory patients (group 2 and group 3), factors influencing response in univariate analyses included AML subtype [78% (*de novo* AML) *vs* 36% (secondary AML); p =0.0006] and ELN classification [100% (favorable-risk) *vs* 60% (intermediate-risk) *vs* 48% (unfavorable); p =0.001]. In a multivariate analysis, only secondary AML [HR: 6.05; 95% CI: 2.01 – 17.8; p =0.001] remained of significant prognostic value (**Table 2**).

*Disease-free Survival.* At the time of analysis, relapse has occurred in 33 of the 66 patients (50%) who responded to GO therapy. The median time from GO therapy to relapse was 5.3 months (1.5 – 53.6 months). Overall, median DFS was 10.5 months (95% CI: 6.0 – 22.6 months) with a 3-year DFS of 34% (**Figure 1A**). Median DFS was 19 months with a 3-year DFS of 36% in patients treated with GO as first-line therapy (group 1), and 7.7 months (3-year DFS: 33%) and 18.6 months (3-year DFS: 40%) in relapsed/refractory patients from group 2 and group 3, respectively (**Figure 1B**). Overall, median DFS in relapsed/refractory patients was 8 months with a 3-year DFS at 34%.

In relapsed/refractory patients (group 2 and group 3), factors predictive for DFS in the univariate analysis included allogeneic HSCT after achieving CRc with GO therapy (median DFS: not reached *vs* 1.5 months; p <0.0001) (**Figure 1C**) and the number of prior therapeutic lines [median DFS: 8.0 months (one prior line) *vs* 10.2 months (2 prior lines) *vs* 3.3 months (3 prior lines]. Adverse ELN stratification AML showed lower DFS than intermediate/favorable-risk AML (**Figure 1D**), as AML with prior history of hemopathy or cancer comparatively to *de novo* AML (**Figure 1E**), but differences were not statistically significant. In a multivariate analysis using a model including age (<55 *vs* ≥55 years), ELN stratification (favorable- and intermediate-risk *vs* unfavorable-risk), antecedents of hemopathy or cancer (secondary AML *vs de novo* AML), the number of prior therapeutic lines (one prior line *vs* > one prior line), *Flt3-ITD, EVI1* and *NPM1* mutation status, pre-treatment percentage of blasts in bone marrow (≤30% *vs* >30%), pre-treatment WBC count (<4 *vs* ≥4 x $10^9$/L), antecedents of allogeneic

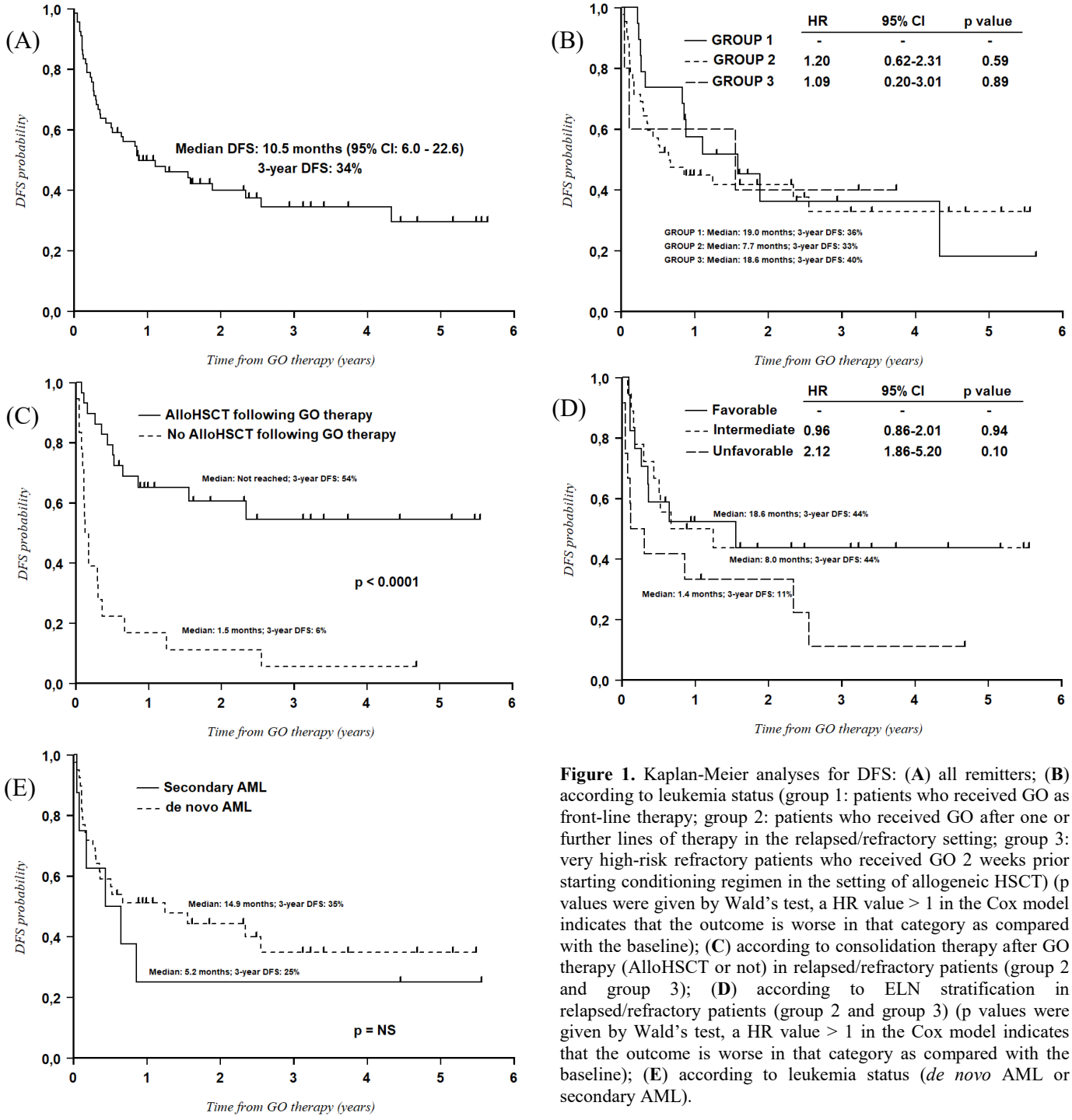


**Figure 1.** Kaplan-Meier analyses for DFS: (**A**) all remitters; (**B**) according to leukemia status (group 1: patients who received GO as front-line therapy; group 2: patients who received GO after one or further lines of therapy in the relapsed/refractory setting; group 3: very high-risk refractory patients who received GO 2 weeks prior starting conditioning regimen in the setting of allogeneic HSCT) (p values were given by Wald's test, a HR value > 1 in the Cox model indicates that the outcome is worse in that category as compared with the baseline); (**C**) according to consolidation therapy after GO therapy (AlloHSCT or not) in relapsed/refractory patients (group 2 and group 3); (**D**) according to ELN stratification in relapsed/refractory patients (group 2 and group 3) (p values were given by Wald's test, a HR value > 1 in the Cox model indicates that the outcome is worse in that category as compared with the baseline); (**E**) according to leukemia status (*de novo* AML or secondary AML).

HSCT, and allogeneic HSCT as consolidation treatment after GO therapy, only the number of prior therapeutic lines [HR: 2.55; 95% CI: 1.13 – 3.06; p =0.03] and allogeneic HSCT after GO therapy [HR: 5.88; 95% CI: 3.89 – 8.84; p <0.001] appeared of significant prognostic value (**Table 2**).

*Overall survival.* Overall median OS after GO therapy was 12.5 months (95% CI: 7.8 – 19.3 months) with a 3-year OS of 31%. Median OS was 25.9 months with a 3-year OS of 31% in group 1, and 8.4 months (3-year OS: 31%) and 2.4 months (3-year OS: 33%) in group 2 and group 3, respectively.

In relapsed/refractory patients (group 2 and group 3), factors predictive for OS in univariate analysis included ELN stratification [median OS: 19.1 months (favorable-risk) *vs* 9.4 (intermediate-risk) *vs* 3.5 months (adverse-risk); p =0.01], allogeneic HSCT prior to GO therapy [3.4 months (yes) *vs* 11.7 months (no); p =0.05), allogeneic HSCT after GO as consolidation therapy [58.8 months (yes) *vs* 4.7 months (no); p <0.0001], achievement of CRc with GP therapy [20.7 months (yes) *vs* 3.4 months (no); p <0.0001], *EVI1* status [11.7 months (not mutated) *vs* 2 months (mutated); p =0.005], and the number of prior therapeutic lines [14.2 months (one prior line) *vs* 5.4 months (2 prior lines) vs 4.0 months (3 prior lines) vs

1.3 (4 prior lines); p =0.01]. In a multivariate Cox proportional hazard analysis including age, ELN stratification, WBC count before GO therapy, the line of treatment when receiving GO, *Flt3-ITD, EVI1* and *NPM1* mutation status, prior history of hemopathy or cancer, prior allogeneic HSCT, blast percentage in bone marrow before GO therapy, achievement of CRc with GO therapy, and use of allogeneic HSCT after GO therapy, the number of prior therapeutic lines (more than one line *vs* one prior line) [HR, 1.95; 95% CI: 1.06 – 3.52; p =0.03], *NPM1* status [HR, 0.23; 95% CI: 0.10 – 0.54; p =0.02], *EVI1* status [HR, 0.24; 95% CI: 0.11 – 0.52); p =0.02], prior allogeneic HSCT [HR, 0.29; 95% CI: 0.13 – 0.65; p =0.004], CRc achievement [HR, 3.63; 95% CI: 1.8 – 7.31; p =0.006], and allogeneic HSCT after GO therapy [HR, 3.86; 95% CI: 1.87 – 7.92; p <0.001] appeared of significant prognostic value (**Table 2**).

*Toxicity.* Overall, GO therapy was well tolerated. All patients experienced severe myelosuppression. A total of 6 patients (6%) died during the period of induction (all patients in group 2). The causes of death were septic shock (2 patients), fungal infection (1 patient), pneumonia (1 patient), acute respiratory distress syndrome (1 patient), and hemorrhagic stroke (1 patient with progressive disease). In remitters, the median duration of aplasia in patients achieving response was 32.5 days (15 – 55 days) in group 1 and 30.5 days (8 – 93 days) in group 2. Severe prolonged thrombocytopenia (platelet count<50 x $10^9$/L at day 45) was observed in 16% of cases.

Among the 28 responders to GO therapy who underwent allogeneic HSCT, 3 died from transplant-related toxicity: 2 from severe pulmonary infections and one from severe GvH disease. Severe acute GvH disease (grade ≥ 3) was observed in 4 cases (3 gut GvH and one liver GvH). Extensive and limited chronic GvH disease was observed in 7 and 3 cases, respectively. No sinusoidal obstruction syndromes were reported among allografted patients as among the other patients in the studied cohort.

**Discussion.** In initial publications, GO was used with an unfractionated dose of 9 mg/m$^2$ on days 1 and 14 or 6 mg/m$^2$ on day 4. In combination with chemotherapy, observed CR rates in relapsed/refractory patients were around 50% with a 2-year OS at 41%, but with the absence of full platelet recovery in roughly half of the responders and often early toxic deaths related to sinusoidal obstruction syndrome.[10-16] In order to reduce toxicity while keeping efficacy, fractionated dosing was proposed, demonstrating in combination with chemotherapy a CR rate of 81% in older patients with untreated AML and 2-year event-free survival of 40.8% versus only 17.1% in a control group without GO.[17] The benefit of fractionated dosing was then confirmed in first relapsed/refractory patients.[18-20]

In our study, we aimed at giving a realistic picture of patient outcomes during and after fractionated dosing GO therapy in newly diagnosed and relapsed/refractory AML patients. We, therefore, reported all AML cases seen in our department over 10 years with a specific interest for those treated outside the official indication of GO therapy in the relapsed/refractory setting. Despite improvements in the treatment of adult AML, the prognosis of relapsed/refractory AML patients remains particularly dismal.[21] Prolonged survival is classically only observed in patients who underwent allogeneic HSCT.[22] In relapsed/refractory patients, our goal with GO salvage therapy was, therefore, first to achieve a morphological CR, but secondly to go to transplantation with a leukemia cell burden as lowest as possible.

Although suffering from several limitations, including the small number of patients, the high heterogeneity of patient characteristics, and the absence of comparator with results that cannot be entirely attributed to GO, our study has the advantage to describe the use of GO in a single-center 'real-life.' All our patients showed an expression of CD33. GO salvage therapy was assessed in 66 relapsed AML patients and in 6 refractory young adults as 'last chance therapy' in a sequential treatment with conditioning regimen followed by allogeneic HSCT. Results in the relapsed/refractory setting tended to be compared to those obtained over the same period with the same treatment in newly diagnosed AML.

Overall, our study showed encouraging results for fractionated doses of GO therapy combined with a traditional '7+3' induction chemotherapy in relapsed/refractory patients. Although the small size of our cohort underpowered subgroup analysis interpretations, this treatment yielded to achieve a promising

CRc rate of 65% in relapsed/refractory patients and a 3-year DFS of 34%. These results were not significantly different from those obtained with the same treatment in front-line therapy patients. Furthermore, a sizeable proportion of patients were bridged to allogeneic HSCT, and an encouraging OS rate was observed. Our results were in accordance with those recently published showing a viable option for GO-based chemotherapy as salvage therapy, with similar survival rates and a feasible schedule as a bridge to allogeneic HSCT.[23] Main prognostic factors appeared related to the intensity of prior therapy since a history of prior transplantation was of adverse influence and also related to leukemia cell characteristics such as the genetic profile. Overall, allogeneic HSCT performed after CR achievement following GO therapy remained the major prognostic factor for both DFS and OS. Analyses using transplant

as a time-dependent covariate would have been suitable but were not relevant because of the small number of involved patients.

Similarly, the length of first remission, classically recognized as a major prognostic factor, was not taken into consideration because of the various number of prior therapeutic lines received by the patients. Although determined on small effectiveness, *NPM1* mutation and *EVI1* mutation emerged as prognostic factors of favorable and unfavorable impact on survival, respectively. On the contrary, *Flt3-ITD* presence did not appear to influence the prognosis, while it was associated with a high rate of CD33 expression.[24] This unusual finding could be explained by a balance between the recognized unfavorable outcome of patients with Flt3 mutation and the high sensitivity of patients with Flt3 mutation to GO therapy. In these patients, the introduction of Flt3 inhibitors is going to be widely used. However, none of our patients received *Flt3* inhibitors. Numerous studies have suggested the lack of efficacy of GO in case of low CD33 expression both in adults and children.[25,26] Because CD33 expression of leukemic blasts was at least 40% in our series, CD33 expression was not introduced in prognostic models. On the other hand, previous therapy emerged as an important prognostic factor after GO treatment. Prior history of allogeneic HSCT was confirmed of poor outcome.[27] This warrants trials using other novel therapeutic agents and strategies in case of relapse following allogeneic HSCT. Reversely, allogeneic HSCT of any type is regarded as the only therapeutic option with curative potential in high-risk AML, including relapsed/refractory patients.[21] However, it represents the treatment of choice once a CR has been reached. Best results are generally achieved when transplantation is performed on a minimal leukemic burden generally estimated by a negative MRD determined either by molecular biology or by immunophenotyping.[28] Based on this concept, our small series of refractory patients starting conditioning regimen at day 15 of GO plus chemotherapy reinduction showed encouraging results for a very high-risk AML population.

While we always used GO in combination with intensive chemotherapy, GO could be combined with lower intensity treatments, such as hypomethylating agents, in patients considered unfit for standard chemotherapy.[29] Such combinations might provide higher response rates in unfit patients. However, they can also generate higher hematological toxicity and potentially alter OS, which remains (with a sustained quality of life) the most important endpoints in the elderly AML population. In our study, GO combined with standard chemotherapy was well tolerated. Like all treatment using monoclonal antibodies, there is, however, always a significant risk of infusion-related reactions with IV administration of GO, which could be avoided by premedication with acetaminophen and methylprednisolone. In previous reports, a variable proportion of patients have been reported to develop clinically apparent sinusoidal obstruction syndrome.[3-6,30-32] The cause is not really known, but it is likely due to the direct toxicity of the conjugate on Kuppfer cells.[33] Endothelial lesions enhance vascular toxicity due to inflammatory state and high doses of reactive oxygen species into Kupffer cells, which express CD33. Symptoms usually arose within 5 to 20 days of the infusion and could be influenced by prior therapies and hepatic biological status. No sinusoidal obstruction syndromes were reported in our series after GO infusion or after allogeneic HSCT following GO therapy even when using conditioning regimen, including busulfan. The good tolerance observed in our series confirmed results reported by the MyloFrance-1 study[20] and could potentially be explained by the use of GO at fractionated dosing. Toxicity was less than expected since sinusoidal obstruction syndrome was generally considered in 8.5% of cases.[2] Patients receiving GO should nevertheless be carefully monitored before, during, and after each course of treatment.

**Conclusions.** Overall, our study confirmed the efficacy and safety of GO-based chemotherapy in a real-life setting. Interestingly patients who received GO after relapse, assuming they did not previously receive allogeneic HSCT, showed no significant difference in terms of response to therapy and duration of response when compared to those who received GO as front-line therapy. In relapsed/refractory patients, this schedule should be used at the stage of the first relapse as a bridge to allogeneic transplant, which might be performed when possible after MRD negativization. These data need, however, to be confirmed in a larger cohort.

**Author contributions.** ML interpreted the data, drafted the manuscript, reviewed the manuscript, and gave final approval; ME, AL and MR collected the data and provided technical support; SL, MVL, FB, HL, SD and EW included patients; MH and GF included patients and reviewed the manuscript; GS reviewed the manuscript and gave final approval; XT included patients, collected the data, conducted the statistical analysis, interpreted the data, and wrote the manuscript.

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