

**Original**

**Retrospective Analysis of Clinical Factors Relating to the Outcome of Gemtuzumab Ozogamicin Therapy**

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**Abstract:** It is difficult to predict the clinical outcome of gemtuzumab ozogamicin (GO) therapy based solely on the previously identified predictive factors. We retrospectively analyzed the relationship between clinical factors and outcomes in 12 patients with relapsed or refractory acute leukemia who received GO monotherapy. The median patient age at initial GO infusion was 56 years, and the average initial dosage was 8.1 mg/m<sup>2</sup>. Four patients (33%) achieved an overall remission (OR). The time from diagnosis to GO infusion was significantly longer in patients with OR than in patients with no remission (NR) (1747 vs. 501 days, respectively;  $P < 0.01$ ). The number of karyotype abnormalities before GO infusion was significantly greater in NR patients (9.5) than in OR patients (0.5;  $P = 0.03$ ). Monocyte counts in the bone marrow before GO therapy were significantly lower in OR than in NR patients (100/ $\mu$ L vs. 1080/ $\mu$ L, respectively;  $P = 0.048$ ). In a multivariate analysis, monocyte count was significantly associated with overall survival ( $P = 0.005$ ). CD14 expression in OR patients was lower than in NR patients, with the exception of 4 patients whose French-American-British subtypes were M4 or M5 (OR, 0.3%; NR, 2.5%;  $P = 0.04$ ). NR was noted in all 6 patients who underwent allogeneic stem cell transplantation before and/or after GO infusion. Patients showing good sensitivity to conventional chemotherapy with good survival after diagnosis tend to be sensitive to GO as well. A low monocyte count in the bone marrow at infusion of GO might indicate improved efficacy of GO therapy. Further investigation is warranted for establishing appropriate patient selection and for clarifying efficient conditions for GO therapy.

**Key words:** acute myeloid leukemia, gemtuzumab ozogamicin, monocyte, predictive factor

**Introduction**

Gemtuzumab ozogamicin (GO) is a new chemotherapy agent targeting CD33-positive acute myeloid leukemia (AML). GO is composed of recombinant humanized IgG<sub>4</sub> anti-CD33 mouse monoclonal antibody conjugated with the antitumor antibiotic, calicheamicin- $\gamma_1$ <sup>1)</sup>.

In Japan, GO has been approved as a monotherapy treatment for relapsed or refractory CD33-positive AML since 2005. However, GO monotherapy has had limited efficacy<sup>2)</sup>. Similar to non-Japanese studies, the overall remission (OR) rate for GO monotherapy has been reported as 30% in a Japanese phase II study<sup>2, 3)</sup>. The following predictive factors affecting the clinical outcome of GO therapy have been identified in previous studies: P-glycoprotein activity and expression<sup>1, 4)</sup>, multi-drug resistance protein (ATP-binding cassette, sub-family C, member)<sup>4)</sup>, white blood cell (WBC) count at the start of GO therapy<sup>5)</sup>, CD33-antigen loads in peripheral blood (PB)<sup>6)</sup>, duration of initial complete remission<sup>7)</sup>, and cytogenetic risk<sup>7)</sup>. However, it is difficult to predict the outcome of GO therapy based on these factors alone. Additionally, there have been no reports regarding predictive factors that affect clinical outcomes of GO therapy among Japanese patients. In our study, we retrospectively evaluated the use of GO in 12 patients treated at our hospital and analyzed the pretreatment factors that affected their clinical outcome.

## Patients and Methods

### *Patients*

Twelve patients with relapsed/refractory *de novo* acute leukemia who were treated with GO at our hospital between September 2007 and August 2009 were included in this study. Informed consent was obtained from each patient before initiation of GO therapy. Patient follow-ups were updated on December 31, 2009. The subtypes of leukemia were classified as M1 (n = 2), M2 (n = 5), M4 (n = 2), and M5 (n = 2), based on the French-American-British (FAB) classification; the remaining patient was classified as a mixed phenotype acute leukemia, B/myeloid, not otherwise specified (n = 1), based on the WHO classification of tumors of hematopoietic and lymphoid tissues (WHO classification, fourth edition, 2008)<sup>8)</sup>.

Cytogenetic analyses were performed on bone marrow (BM) samples of all patients using the G-banding method at the time of diagnosis of leukemia and before GO infusion. Cytogenetic risk was categorized according to the Southwest Oncology Group (SWOG)<sup>9)</sup> and Medical Research Council (MRC)<sup>10)</sup> classification for cytogenetic risk. Cell surface expression of CD33 and CD14 on the leukemic blasts in the pretreatment BM samples was determined by flow cytometry in all patients.

### *Efficacy measures*

Efficacy measures were categorized into the following 3 groups: complete remission (CR), CR with incomplete platelet recovery (CRp), and no remission (NR)<sup>7)</sup>. CR was defined as: (I) the absence of leukemic blasts in PB; (II) no more than 5% leukemic blasts in the BM, as measured by BM aspiration or biopsy samples; (III) PB counts with hemoglobin concentration  $\geq 9$  g/dL, absolute neutrophil count  $\geq 1500/\mu\text{L}$ , and platelet count  $\geq 100,000/\mu\text{L}$ ; and (IV) red blood cell transfusion independence  $\geq 2$  weeks with platelet transfusion independence  $\geq 1$  week. CRp was similarly defined, but CRp platelet counts were  $<100,000/\mu\text{L}$ . Patients

who did not satisfy the criteria for either CR or CRp were categorized as NR. OR included both CR and CRp. Determination of remission status was evaluated from 15 days to 70 days after the first administration of GO.

Overall survival (OS) was defined as the time from the first GO infusion until death. Event-free survival was defined as the time from the first GO infusion until death or relapse of leukemia. Duration of initial complete remission (CR1) of leukemia was defined as the time from diagnosis of CR1 to the first relapse of leukemia.

#### *Pretreatment factors*

Patients were divided into 2 groups based on the GO efficacy measures, i.e., the OR and NR groups. In a univariate analysis, various factors (mentioned below) were compared between the 2 groups at the time of diagnosis of leukemia, before the first GO infusion, and at the first GO infusion. The following factors were assessed at the time of diagnosis of leukemia: WBC count, percentage of leukemic blasts in PB, lactate dehydrogenase, nucleated cell count in BM, percentage of leukemic cells in BM, percentage of peroxidase-positive leukemic blasts, the Eastern Cooperative Oncology Group (ECOG) performance status, number of karyotype abnormalities, and cytogenetic risk according to the SWOG and MRC classification systems. The factors assessed before the first GO infusion included the following: percentage of leukemic blasts in BM, leukemic blast count in BM, percentage of monocytes in BM, monocyte count in BM calculated as nucleated cell count in BM multiplied by the percentage of monocytes in BM divided by 100, percentage of leukemic cells in BM expressing CD14, percentage of leukemic cells in BM expressing CD33, number of karyotype abnormalities, and cytogenetic risk according to the SWOG and MRC classification systems. The factors assessed at the first GO infusion included patient age, frequency of relapse, ECOG performance status, duration of CR1, time since diagnosis of leukemia, number of chemotherapy regimens administered, WBC count, percentage of leukemic blasts in PB, leukemic blast count in PB, percentage of monocytes in PB, and monocyte count in PB. Multivariate analysis was performed for OS and included significant factors identified in the univariate analysis.

#### *Safety measures*

Adverse events were graded using the Common Terminology Criteria for Adverse Events, version 3.0. WBC counts from day 1 to day 14 after the first GO infusion were compared between the 2 groups using the Wilcoxon test. Unmeasured values of WBC counts were substituted by interpolated values derived from the adjacent values using linear interpolation.

#### *Statistical analyses*

Survival curves were calculated according to the Kaplan-Meier method and compared using the log-rank test. Univariate analysis was performed using the Wilcoxon test and chi-

square analysis, while multivariate analysis was performed using the Cox regression analysis. Statistical analyses were performed using the software program, KyPlot, version 5.0 (Kyen-sLab Co. Ltd., Tokyo, Japan). In all tests,  $P < 0.05$  was considered significant.

## Results

### *Patient characteristics*

We evaluated 12 patients in this study. The median age was 56 years (range, 27–85 years), the male : female ratio was 6 : 6, and the mean observation time was 200 days (range, 16–642 days). The patients' leukemic status and laboratory data are presented in Tables 1 and 2. Patient characteristics are listed according to the categories of GO efficacy in Table 3.

### *Feasibility and treatment outcomes*

All 12 patients received the first dose of GO as a monotherapy, following which 8 patients received 1 course and 1 patient received 3 courses of GO (1 course was defined as 2 doses of GO). For the patients who received 2 or more doses, there was a median gap of 14 days (range, 9–54 days) between the first and second doses. The median initial dose was  $8.1 \text{ mg/m}^2$  (range,  $5.8\text{--}9.1 \text{ mg/m}^2$ ). CR, CRp, OR, and NR were observed in 2 (17%), 2 (17%), 4 (33%), and 8 patients (68%), respectively (Table 1).

### *Pretreatment factors according to efficacy measures*

The pretreatment factors listed above were compared between the OR and NR groups (Table 4). No clinical factors at the time of diagnosis of leukemia were significantly associated with the clinical outcome, however a number of significant differences between the 2 groups were observed for factors before or at GO infusion. The duration of CR1 and the duration between diagnosis of leukemia and the first GO infusion were significantly longer in OR patients. The ECOG performance status was significantly greater in NR patients compared to OR patients ( $P = 0.03$ ). In addition, the number of karyotype abnormalities before GO infusion was significantly greater in NR patients than in OR patients (9.5 vs. 0.5, respectively;  $P = 0.03$ ), and the cytogenetic risk was also significantly different between OR and NR patients, according to both the SWOG ( $P = 0.01$ ) and MRC ( $P = 0.049$ ) classification systems. The percentage of leukemic blasts in the BM showing CD33 expression was not significantly different between groups ( $P = 0.46$ ), but CD14 expression (a monocyte marker) on leukemic blasts in the BM was significantly lower in OR patients than in NR patients (0.35% vs. 2.60%, respectively;  $P = 0.004$ ). The monocyte count in the BM was also significantly lower in OR patients than in NR patients ( $100/\mu\text{L}$  vs.  $1084/\mu\text{L}$ , respectively;  $P = 0.048$ ). In the comparison of 8 patients with the exception of the 4 patients designated as FAB M4 (acute myelomonocytic leukemia) or M5 (acute monocytic leukemia), CD14 expression in 3 OR patients was consistently lower than in 5 NR patients (0.3% vs. 2.5%, respectively;  $P = 0.04$ ). The monocyte count in the PB of OR patients at the initial infu-

Table 1. Patients' status at first GO infusion and efficacy of GO

Case	Age/Sex	Leukemia subtype (FAB/WHO)	Disease state	SCT source and state (GO infusion day from SCT)	Periods of CR1 (day)	Day from diagnosis (day)	ECOG PS at GO infusion	No. of chemotherapy regimens	Efficacy of GO
1	69/M	AML (M2)	3 <sup>rd</sup> relapse	-	1148	1875	1	4	CRp
2	64/F	AML (M1)	3 <sup>rd</sup> relapse	-	397	1113	2	3	CRp
3	74/F	Mixed phenotype	1 <sup>st</sup> relapse	-	1474	1770	1	2	CR
4	85/F	AML (M5b)	2 <sup>nd</sup> relapse	-	1353	2232	2	2	CR
5	44/M	AML (M1)	2 <sup>nd</sup> relapse	post uBMT (107) pre CBT (-18)	317	564	3	8	NR
6	48/M	AML (M4)	Induction failure	post CBT (47)	-	210	4	3	NR
7	48/F	AML (M4)	2 <sup>nd</sup> relapse	post CBT (231)	148	471	3	5	NR
8	38/M	AML (M5a)	2 <sup>nd</sup> relapse	post uBMT (42)	96	201	3	3	NR
9	79/M	AML (M2)	2 <sup>nd</sup> relapse	-	525	803	1	2	NR
10	27/M	AML (M2)	2 <sup>nd</sup> relapse	post CBT (111)	260	562	2	5	NR
11	44/F	AML (M2)	1 <sup>st</sup> relapse	pre rBMT (-23)	349	412	3	3	NR
12	85/F	AML (M2)	1 <sup>st</sup> relapse	-	382	785	3	8	NR

GO, gemtuzumab ozogamicin; FAB, French-American-British classification; WHO, World Health Organization classification 2008; SCT, stem cell transplantation; CR1, initial complete remission; ECOG, Eastern Cooperative Oncology Group; PS, performance status; AML, acute myeloid leukemia; uBMT, unrelated bone marrow transplantation; CBT, cord blood transplantation; rBMT, related bone marrow transplantation; CR, complete remission; CRp, CR with incomplete platelet recovery; NR, no remission.

Table 2. Cytogenetic and hematologic findings at the time of diagnosis and before GO infusion.

Case	Karyotype	Cytogenetic findings at diagnosis of leukemia		Cytogenetic findings before GO infusion		PB at GO infusion		BM before GO infusion					
		SWOG/MRC risk category (No. of abnormalities)	Karyotype	SWOG/MRC risk category (No. of abnormalities)	Karyotype	WBC (/ $\mu$ L)	Blast (%)	Monocyte (%)	NCC ( $\mu$ L)	Blasts (%)	Monocytes (%)	CD14 <sup>+</sup> leukemic blasts (%)	CD33 <sup>+</sup> leukemic blasts (%)
1	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	5400	52	0	27000	26	0.3	0.4	95
2	46,XX,add(15)(q26)[7]/46,XX,del(9)(q2)[2].	Unfavorable / Intermediate (2)	46,XX,add(15)(q26)[7]/46,sl,der(22)t(10;22)(q11.2;p11.2)[13].	Intermediate (2)		7600	75	0	56000	71.5	0.1	0.2	64.3
3	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	9500	78	0	396000	89	0	0.3	172
4	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	2800	1	2	12000	4.2	2.2	0.5	572
5	46,XY,t(11;12)(p15;q13)[20].	Unknown / Intermediate (1)	46,XY,t(11;12)(p15;q13)[10]/46,sl,add(8)(q13),add(17)(q21)[3]/46,XX[6].	Intermediate (1)	46,XY,t(11;12)(p15;q13)[10]/46,XY,t(13)(q42;q21)[5]/46,sl,add(12)(q13)[1]/46,XY,t(3;12)(p21;p13)[1]/46,XY[6].	9100	99	0	42000	28.2	0.5	3.8	94
6	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	24000	51	2	223000	59.4	1.1	1.2	94.1
7	46,XX,t(9;11)(p22;q23)[20].	Unfavorable / Intermediate (1)	45,XX,add(3)(q26),add(6)(p21)-7t(9;11)(p22;q23),del(13)(q?) [2]/44,sl,-X,der(1)add(1)(p34),add(1)(q21),-3,-add(3),der(4;14)(q10q10),-add(6),-del(6)(q?),-del(13),del(16)(q?),add(21)(p11.2),+mar1,+mar2[9]/45,sl,-X,der(1)add(1)(p34),add(1)(q21),+3,-add(3),der(4;14)(q10q10)-add(6),+del(6)(q?),-del(13),del(16)(q?),add(21)(p11.2),+mar1,+mar2,+mar3[4]/46,XX[3].	Intermediate (1)	45,XX,add(3)(q26),add(6)(p21)-7t(9;11)(p22;q23),del(13)(q?) [2]/44,sl,-X,der(1)add(1)(p34),add(1)(q21),+3,-add(3),der(4;14)(q10q10),-add(6),-del(6)(q?),-del(13),del(16)(q?),add(21)(p11.2),+mar1,+mar2,+mar3[4]/46,XX[3].	8700	32	60	172000	68.2	0.2	1.6	98.5
8	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	500	14	2	160000	62.4	2	5.1	977
9	45,X,-Y[4].	Intermediate / Intermediate (1)	Normal karyotype	Intermediate (1)	Normal karyotype	4400	1	8	60000	3.7	2.1	2.9	19.2
10	46,XY,t(8;21)(q22;q22)[20].	Favorable / Favorable (1)	46,XY,t(8;21)(q22;q22)[20].	Favorable (1)	46,XY,t(8;21)(q22;q22)[20].	20500	76	0	263000	57.6	0.3	1.4	68.4
11	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	500	21	9	69000	78.6	0.6	1.7	12.3
12	Normal karyotype	Intermediate / Intermediate (0)	Normal karyotype	Intermediate (0)	Normal karyotype	21300	80	1	137000	75.4	0	2.8	73.9

GO, gemtuzumab ozogamicin ; SWOG, Southwest Oncology Group ; MRC, Medical Research Council ; PB, peripheral blood ; BM, bone marrow ; WBC, white blood cell ; NCC, nucleated cell counts.

Table 3. Characteristics of the patients according to the gemtuzumab ozogamicin efficacy measures

	OR (4)		NR (8)	Total (12)
	CR (2)	CRp (2)		
Sex				
Male	0	1	5	6
Female	2	1	3	6
Age, median (range)	71.5 (64-85)		46 (27-85)	56 (27-85)
State at 1 <sup>st</sup> GO infusion				
Induction failure	0	0	1	1
1 <sup>st</sup> relapse	1	0	2	3
2 <sup>nd</sup> relapse	1	0	5	6
3 <sup>rd</sup> relapse	0	2	0	2
Subtype of leukemia				
AML M1 (FAB)	0	1	1	2
AML M2 (FAB)	0	1	4	5
AML M4 (FAB)	0	0	2	2
AML M5a (FAB)	0	0	1	1
AML M5b (FAB)	1	0	0	1
Mixed phenotype (WHO2008)	1	0	0	1
GO infusion after SCT*	0	0	5	5
uBMT	0	0	2	2
CBT	0	0	3	3
Days from SCT to 1 <sup>st</sup> GO , median (range)	none in OR		107 (42-231)	
GO infusion before SCT*	0	0	2	2
CBT	0	0	1	1
rBMT	0	0	1	1
Days from 1 <sup>st</sup> GO to SCT, median (range)	none in OR		20.5 (18-23)	

GO, gemtuzumab ozogamicin; OR, overall response; CR, complete remission; CRp, CR with incomplete platelet recovery; NR, no remission; AML, acute myeloid leukemia; FAB, French-American-British classification; SWOG, Southwest Oncology Group; MRC, Medical Research Council; SCT, stem cell transplantation; uBMT, unrelated bone marrow transplantation; CBT, cord blood transplantation; rBMT, related bone marrow transplantation; \*, One patient in whom AML relapsed after uBMT and who received GO as pretherapy prior to CBT is duplicated.

sion of GO tended to be lower than in NR patients (OR, 14.0/ $\mu$ L; NR, 790/ $\mu$ L;  $P = 0.15$ ). In a multivariate analysis, the following factors were significantly associated with OS: ECOG performance status ( $P = 0.001$ ), duration of CR1 ( $P = 0.007$ ), time between diagnosis of leukemia and first GO infusion ( $P = 0.01$ ), monocyte count in the BM ( $P = 0.005$ ), and the number of karyotype abnormalities before GO infusion ( $P = 0.002$ ).

#### *Survival according to efficacy measures*

The median OS was 77 days (391.5 days and 55.5 days for patients with OR and NR, respectively). The OS was significantly longer in patients with OR than those with NR ( $P = 0.03$ ; Figs. 1 and 2). All the OR patients experienced relapses and the median event-free survival of OR patients was 140 days (range, 79-176 days; Fig. 3). The causes of death were leukemia, sepsis, and multiple organ failure.

Table 4. Pretreatment factors according to the gemtuzumab ozogamicin efficacy measures; mean (range)

	OR	NR	<i>P</i> value in Univariate analysis	<i>P</i> value in Multivariate analysis
Status at the time of diagnosis of leukemia				
WBC counts ( $\times 10^3/\mu\text{L}$ )	32.9 (2.19-67.0)	110 (1.50-381)	0.57	
NCC in BM ( $\times 10^4/\mu\text{L}$ )	21.2 (1.4-38)	46.3 (7-108)	0.28	
Status at 1 <sup>st</sup> GO infusion				
Age	73.0 (64-85)	51.6 (27-85)	0.11	
Times of relapse	2.3 (1-3)	1.7 (1-2)	0.31	
ECOG Performance status	1.3 (1-2)	2.8 (1-4)	0.03*	0.001*
Duration of CR1 (day)	1093.0 (397-1474)	296.7 (96-525)	0.01*	0.007*
Day from diagnosis of disease	1747.5 (1113-2232)	501 (201-803)	0.004*	0.01*
No. of treated chemotherapy regimens	2.75 (2-4)	4.63 (2-8)	0.15	
Status of PB at 1 <sup>st</sup> GO infusion				
WBC counts ( $\times 10^3/\mu\text{L}$ )	6.33 (2.80-9.50)	11.1 (0.500-24.0)	0.68	
Leukemic blasts (%)	51.5 (1.0-78.0)	46.8 (1.0-99.0)	0.93	
Leukemic blast counts ( $\times 10^3/\mu\text{L}$ )	3.99 (0.028-741)	711 (0.044-170)	0.57	
Monocytes (%)	0.5 (0.0-2.0)	10.3 (0.0-60.0)	0.15	
Monocyte counts ( $\times 10^3/\mu\text{L}$ )	0.014 (0.00-0.056)	0.790 (0.00-5.22)	0.15	
Status of BM before GO infusion				
Leukemic blasts (%)	47.7 (4.2-89.0)	54.2 (3.7-78.6)	> 0.99	
Leukemic blast counts ( $\times 10^3/\mu\text{L}$ )	100.0 (0.504-352.4)	84.1 (2.22-151.5)	0.46	
Monocytes (%)	0.65 (0.0-2.2)	0.85 (0.0-2.1)	0.57	
Monocyte counts ( $\times 10^3/\mu\text{L}$ )	0.100 (0.00-0.264)	1.08 (0.00-3.20)	0.048*	0.005*
CD14 expression of leukemic blasts (%)	0.35 (0.2-0.5)	2.6 (1.2-5.1)	0.004*	0.66
CD33 expression of leukemic blasts (%)	58.4 (17.2-95.0)	69.8 (12.3-98.5)	0.46	
No. of abnormalities of karyotypes	0.5 (0-2)	9.5 (0-30)	0.03*	0.002*
Karyotypes grouped by SWOG cytogenetic risk category				
Favorable	0	0	] 0.01*	
Intermediate	3	1		
Unfavorable	0	7		
Unknown	1	0		
Karyotypes grouped by MRC cytogenetic risk category				
Favorable	0	1	] 0.049*	
Intermediate	4	2		
Adverse	0	5		

OR, overall response; NR, no remission; AML, acute myeloid leukemia; FAB, French-American-British classification; WBC, white blood cells; NCC, nucleated cell counts; GO, gemtuzumab ozogamicin; PB, peripheral blood; BM, bone marrow; ECOG, Eastern Cooperative Oncology Group; CR1, initial complete remission; SWOG, Southwest Oncology Group; MRC, Medical Research Council; \*,  $p < 0.05$ .

### Outcome in patients who received allogeneic stem cell transplantation

Overall, 6 patients received allogeneic stem cell transplantation (allo-SCT) - 5 before GO therapy and 2 after GO therapy; 1 patient, whose AML relapsed after unrelated BM transplantation and who had received GO as pretherapy prior to cord blood transplantation, underwent allo-SCT both before and after GO therapy. All patients who underwent allo-SCT before or after GO treatment showed NR and died within 91 days of the initial GO infusion. Venous-occlusive disease (VOD) was not observed in any of these patients.



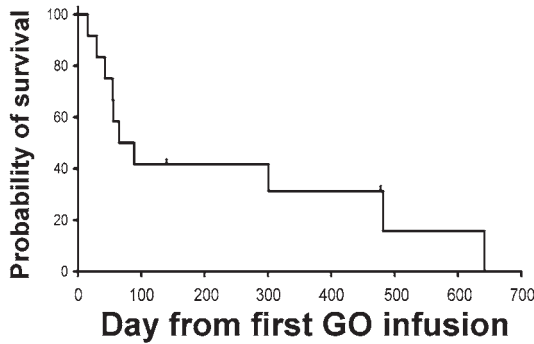


Fig. 1. Overall survival among all patients who received gemtuzumab ozogamicin (n = 12).

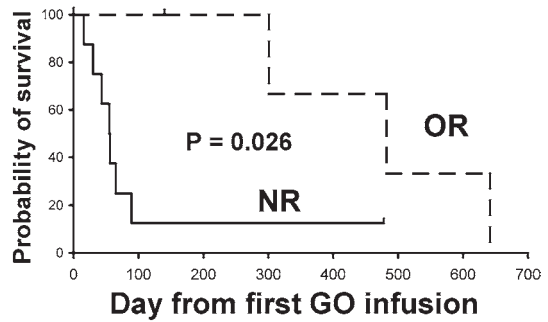


Fig. 2. Overall survival (OR vs. NR).

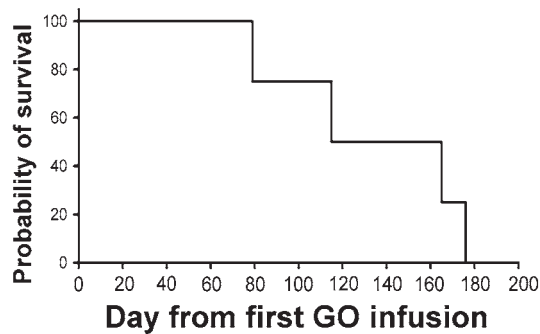


Fig. 3. Event-free survival among patients who attained OR (n = 4).

### Adverse events

Elevation of transaminase and total bilirubin (grade 3) was observed in 1 case. In another case, an infusion reaction was observed with a transient decrease in percutaneous oxygen saturation and fever (grade 3). The infusion reaction was resolved with glucocorticoid therapy. BM suppression resulting in neutropenia ( $\leq 500/\mu\text{L}$ , grade 4) and thrombocytopenia ( $\leq 50,000/\mu\text{L}$ , grade 3) was observed in all patients. Febrile neutropenia occurred in 11 patients (91.7%), while grade 2 nausea and vomiting was observed in 1 patient.

WBC counts and leukemic blasts in PB decreased in all patients after GO infusion. The kinetics of WBC decline after 14 days following the initial GO infusion did not differ significantly between OR and NR patients (data not shown).

### Discussion

In this study we retrospectively evaluated the use of GO in 12 patients and analyzed the factors that affected the outcome of GO therapy. The OR rate for GO monotherapy has been previously reported as 17%–33%<sup>2)</sup>, which corresponds with the rate observed in our study (33%). In this study, a significant difference was observed between OR and NR

patients for factors including OS, ECOG performance status, duration of CR1, time between diagnosis of leukemia and initial GO infusion, number of karyotype abnormalities before GO infusion, cytogenetic risk as defined by the SWOG and MRC classification systems, CD14 expression rate of leukemic blasts in BM, and monocyte count in the BM.

The duration of CR1 was significantly longer in OR patients, which was similar to the results of a previous study<sup>7)</sup>. Additionally, the time from diagnosis of leukemia to GO infusion was significantly longer in OR patients. These findings suggest that GO therapy is effective in patients who have been previously successfully treated with conventional chemotherapy. Previous studies have reported that P-glycoprotein and multi-drug resistance protein expressions are associated with GO therapy outcomes<sup>1, 4)</sup> and conventional chemotherapy outcomes<sup>11)</sup>. Thus, we consider sensitivity to conventional chemotherapy an important prognostic factor for GO therapy.

Similar to previous reports, no relationship was observed between the CD33 expression rate on leukemic blasts and the outcome of GO therapy<sup>1, 5, 12)</sup>. In our study, a patient (patient no. 3) with a CD33 expression rate of < 20% achieved CR, while CR has also been reported in a CD33-negative patient<sup>5)</sup>. Furthermore, internalization of GO without intermediation by CD33 via endocytosis has been previously demonstrated<sup>13)</sup>. These results suggest that it is not possible to predict the effects of GO therapy based on CD33 expression rates alone, and that GO should not be excluded from the therapeutic options even for patients deficient in CD33-positive leukemic cells.

Interestingly, CD33 is known to be expressed on monocytes, and GO-induced apoptosis of monocytes has been previously reported<sup>14, 15)</sup>. Van der Velden *et al* demonstrated that a high CD33-antigen load in PB is an independent adverse prognostic factor, likely due to peripheral consumption of GO<sup>6)</sup>. In our study, the CD14 expression rate and monocyte count in the BM were significantly lower in the OR group as compared to the NR group. Additionally, the monocyte count in the BM was significantly associated with OS in a multivariate analysis. However, there are no reports regarding an association between monocyte count or CD14 expression rate and GO therapy outcomes. The reason why GO therapy failed in patients with high monocyte counts is not clear. Further studies are needed to clarify the association between monocyte counts and GO therapy outcomes.

In our study, both the number of karyotype abnormalities and the SWOG/MRC cytogenetic risk were significantly different between NR and OR patients before GO infusion, which support a previous study reporting an association between MRC cytogenetic risk and clinical outcomes of GO therapy<sup>7)</sup>. These findings suggest that the number of karyotype abnormalities and cytogenetic risk are predictive factors for GO therapy outcomes.

In classical AML treatment, older patients are known to have poorer outcomes than younger patients<sup>16)</sup>. However, a previous study has demonstrated that GO is well-tolerated by elderly patients aged  $\geq 60$  years<sup>16)</sup>; moreover, no significant difference has been observed in the OR rate of GO therapy between patients aged < 60 and  $\geq 60$  years<sup>7)</sup>. For patients

aged > 75 years, Amadori *et al* concluded that a dose of 9 mg/m<sup>2</sup> of GO was too toxic<sup>17</sup>. Since the OR patients in our study comprised only those aged ≥ 64 years (age range, 64–85 years), the tolerability of GO at a dosage of 9 mg/m<sup>2</sup> could be verified for those aged at least ≤ 75 years.

An adverse event of grade 3 or higher neutropenia was encountered in all patients. This finding was similar to the 98% incidence of high-grade neutropenia reported by Larson *et al* in 2005<sup>7</sup>. The presentation rate for febrile neutropenia in our study was 92%, which was higher than that in previous reports (6%<sup>7</sup>, 31%<sup>18</sup>, and 52%<sup>17</sup>). A grade 3 or higher infusion reaction was noted in 1 patient (8%); this incidence was lower than that described in previous reports (24%<sup>18</sup>, and 30%<sup>7</sup>).

Previous analyses concluded that patients undergoing SCT within a short interval following GO administration are at an increased risk of developing VOD<sup>19</sup>. In our study, no cases of VOD or sinusoidal obstruction syndrome were observed in the 6 patients who underwent allo-SCT following GO therapy. The absence of VOD/sinusoidal obstruction syndrome in our study may be due to the small number of patients evaluated.

Although our study was limited by the small number of patients included, we consider the following inferences important. Our results suggest that patients showing good sensitivity to conventional chemotherapy with mild disease progression would benefit from GO therapy as well, and that GO is well tolerated even in elderly patients with relapsed leukemia. Our study also suggests that a low monocyte count in the BM at the time of GO infusion might indicate an improved efficacy of GO. However, GO monotherapy is limited in its efficacy. The combination of GO with other antileukemic agents is known to improve the response to GO therapy<sup>2</sup>. Additionally, the administration of GO a few days after conventional chemotherapy appears to provide improved response and survival as compared to GO monotherapy<sup>20</sup>. Further clinical trials with large sample groups are necessary to establish the efficacy of GO and to determine the group of patients most likely to benefit from GO therapy.

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