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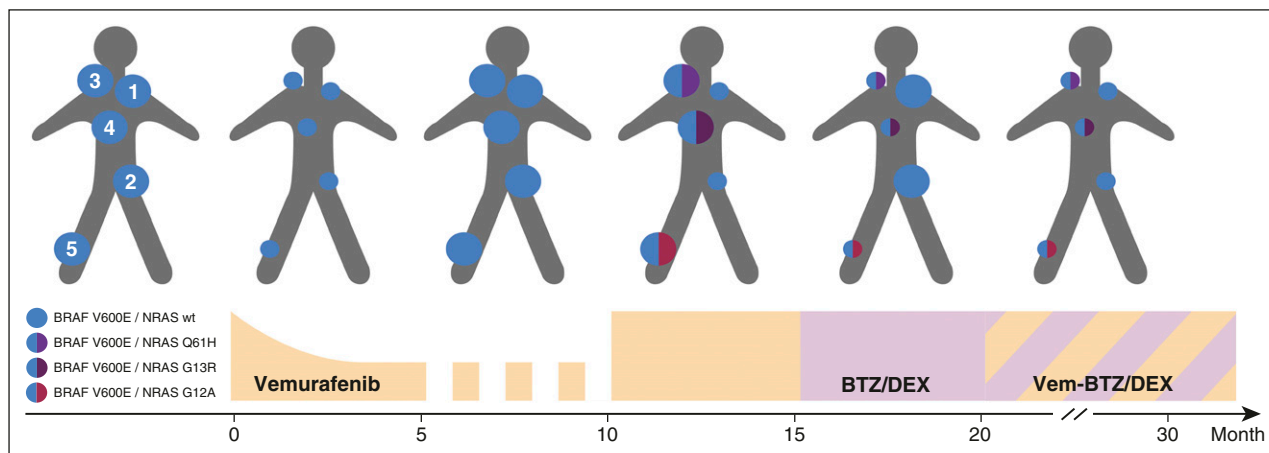


Figure 2. Schematic overview of the representative myeloma lesions in the course of treatment. The 5 representative lesions were located at the left shoulder above the acromial bone (lesion 1), the left iliac bone (2), the right clavicle (3), adjacent to the sternal bone (4), and the right proximal tibia (5). Lesions 1, 3, and 5 were extramedullary tumors; lesions 2 and 4 were intramedullary computed tomography-guided biopsies. Bullet sizes depict growth and regression, respectively.

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To the editor:

The level of blast CD33 expression positively impacts the effect of gemtuzumab ozogamicin in patients with acute myeloid leukemia

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Gemtuzumab ozogamicin (GO) is an immunoconjugate, combining an anti-CD33 monoclonal antibody to calicheamicin, a highly cytotoxic antibiotic. First developed as single agent in adults with relapsed acute myeloid leukemia (AML),¹ it was then evaluated in combination with chemotherapy in newly diagnosed patients.²⁻⁷ In the randomized Acute Leukemia French Association (ALFA)-0701 study, we reported that sequential administration of a lower dose of GO allowed the safe

delivery of a high cumulative dose associated with a substantial improvement in patient outcome.⁵ A recent meta-analysis has confirmed that adding GO to chemotherapy may provide a survival advantage in patients without adverse cytogenetic characteristics.⁸ However, these results were obtained regardless of the level of blast CD33 expression. In vitro, a clear relationship between CD33 expression and GO efficacy has nevertheless been shown.^{9,10} In vivo, contradictory results have

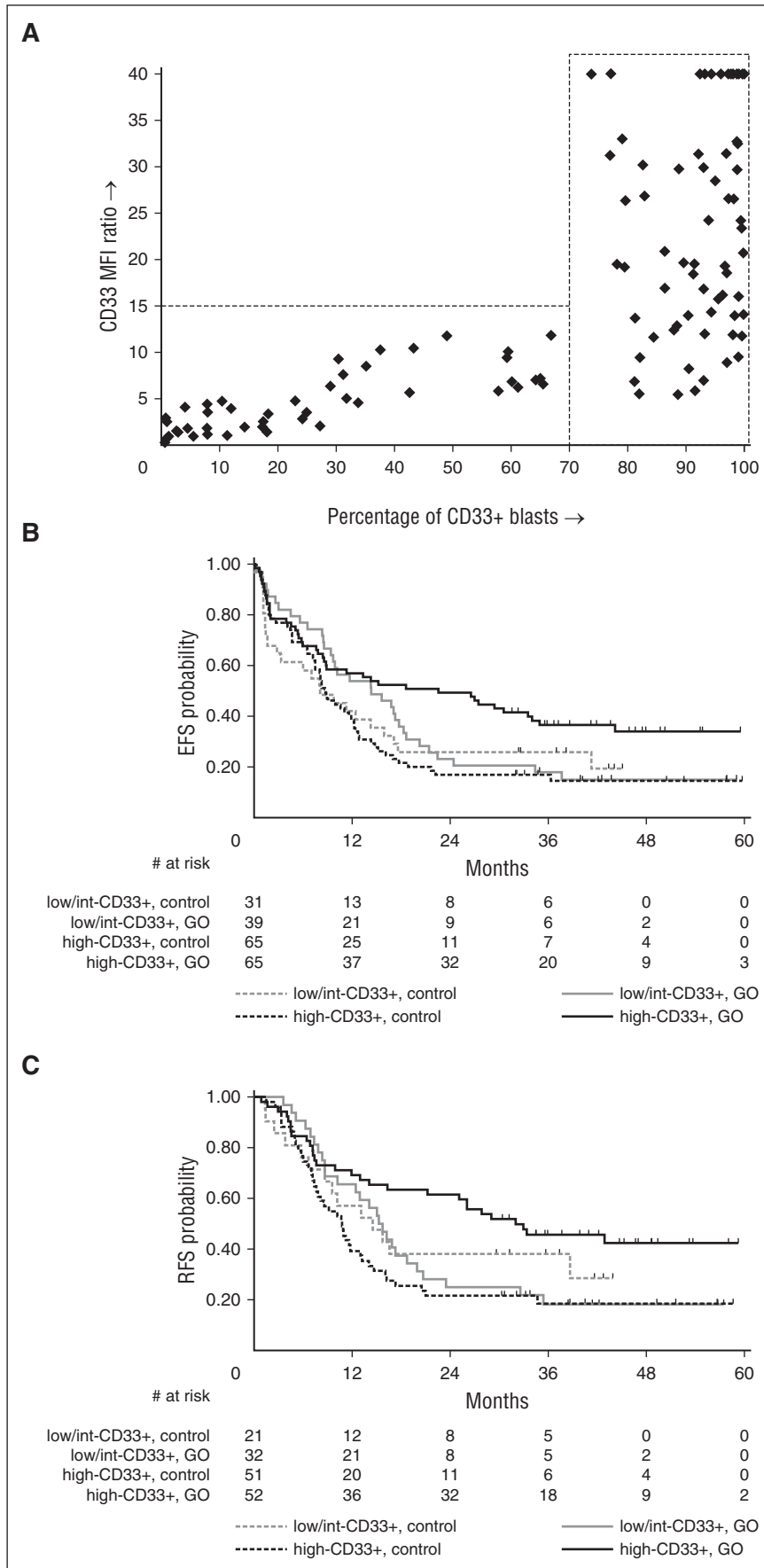


Figure 1. CD33 expression and impact on outcome according to treatment arm. (A) The concordance between the percentage of CD33⁺ blast cells and the CD33 MFI ratio. To properly calculate the CD33 MFI ratio, at least 500 lymphocytes and blast cells had to be acquired and fewer than 20% of acquired lymphocyte events had to fall in channel 0 at the electronic digitizing step of fluorescence light intensities for CD33 labeling. The 2 boxes separate low/int-CD33⁺ and high-CD33⁺ patients on the basis of a threshold of 70% for CD33⁺ blasts and of 15 for CD33 MFI ratio. (B) In low/int-CD33⁺ patients (<70%), EFS was estimated at 23% vs 26% at 2 years in the GO and control arms, respectively (HR, 0.89; 95% confidence interval [CI], 0.52-1.50; *P* = .66). Conversely, in high-CD33⁺ patients (≥70%), 2-year EFS was 49% vs 17% in the GO and control arms, respectively (HR, 0.56; 95% CI, 0.37-0.85; *P* = .0051). Tests for interaction did not reach statistical significance (*P* = .155). (C) In low/int-CD33⁺ patients (<70%), RFS was estimated at 25% vs 38% at 2 years in the GO and control arms, respectively (HR, 1.17; 95% CI, 0.61-2.24; *P* = .66). Conversely, in high-CD33⁺ patients (≥70%), 2-year RFS was 62% vs 22% in the GO and control arms, respectively (HR, 0.47; 95% CI, 0.29-0.76; *P* = .0016). Tests for interaction reached statistical significance (*P* = .022). (B-C) The 29 patients who received allogeneic SCT in first CR/CRp were not censored at the time of SCT.

Table 1. Patient characteristics according to CD33 expression

	Low/int-CD33 ⁺ (<70%)	High-CD33 ⁺ (≥70%)	P value
Patients, n	70	130	—
Treatment arm, GO/control	39/31	65/65	.46
Median age (range), years	62.3 (50-70)	61.6 (50-70)	.33
Gender, M/F	39/31	60/70	.24
ECOG-PS, 0/1/2/3/NA	25/34/9/1/1	46/72/12/0/0	.31
Median WBC (range), 10 ⁹ /L	3.0 (0.8-211)	11.8 (0.6-320)	<.001
Median BM blast (range), %	52 (20-99)	93 (20-100)	<.001
Cytogenetics, fav/int/unfav/NA	4/45/14/7	3/85/29/13	.65
<i>NPM1</i> status, mut/WT/NA	7/62/1	59/69/2	<.001
<i>FLT3</i> -ITD status, mut/WT/NA	2/67/1	32/97/1	<.001
Genotype, fav/other/NA*	6/56/8	28/87/15	.057
ELN classification, fav/int-1/int-2/unfav/NA	10/24/15/14/7	31/46/11/29/13	.10

ECOG, Eastern Cooperative Oncology Group performance status; ELN, European Leukemia Net; F, female; fav, favorable; int, intermediate; M, male; mut, mutation; NA, not available; unfav, unfavorable; WT, wild-type.

*A favorable genotype was defined as normal karyotype and *NPM1* mutation without *FLT3*-ITD or normal karyotype with *CEBPA* mutation, according to international ELN recommendations.

been reported so far. No impact was found when CD33 expression was evaluated as a continuous covariable^{1,5,11} or using a 20% cutoff.³ Higher response rates were nevertheless reported for patients with CD33⁺ expression ≥98% in 1 phase 2 study¹¹ or when showing CD33 expression as mean fluorescence intensity (MFI) using an isotype antibody as control.¹² To further evaluate the impact of CD33 expression on GO treatment effect, we retrospectively analyzed the results of the ALFA-0701 study.

The first results of the ALFA-0701 study (European Union Drug Regulating Authorities Clinical Trial 2007-002933-36; clinicaltrials.gov #NCT00927498), which included 278 patients whose CD33 expression levels made them ineligible for study inclusion, were published in 2012.⁵ A final updated analysis was performed in 2014 with a longer median follow-up of 43 months.¹³ In this study, CD33 expression was evaluated locally in the 24 participating centers, as described previously.¹⁴ For the current study, which was not planned for in the initial protocol, flow cytometry data from 200 patients (104 from the GO group; 96 from the control group) were centrally reassessed. Patient characteristics are shown in supplemental Table 1, available on the *Blood* Web site. Apart from a higher white blood cell count (WBC), there were no significant differences between these 200 patients and the 78 remaining patients. Blast CD33 expression was expressed as a percentage of CD33⁺ blasts using lymphocytes as an internal negative control and as a ratio of CD33 MFI between AML blasts and lymphocytes. Because of technical issues in this retrospective setting, the MFI ratio was accurately calculated in 132 patients only. Details are provided in Figure 1A. The primary clinical end point was event-free survival (EFS), defined as the time from randomization to the date of response assessment if complete remission/incomplete platelet recovery (CR/CRp) had not been achieved or there was a relapse or death. Secondary end points were relapse-free survival (RFS), defined as the time from CR/CRp achievement to the date of relapse or death and overall survival. Analysis was performed on the 2014 updated study database using Stata/IC 12.1 software (Stata, College Station, TX).

Because GO efficacy was likely influenced by the drug uptake and thus the amount of CD33 molecules on a blast surface as reflected by MFI, we first correlated CD33⁺ percentage and MFI ratio to define the optimal CD33⁺ percentage cutoff to be used for the prognostic analysis. As shown in Figure 1A, this correlation was not linear. MFI ratio remained relatively low in patients until there were 70% CD33⁺ blasts and started to more markedly increase when there were more than 70% CD33⁺ blasts, even if some patients with high CD33 expression still had a relatively low MFI ratio. We thus graphically defined 70% as

a cutoff of interest and classified the whole patient population in 2 subgroups using this cutoff: 70 patients had low or intermediate CD33 expression (low/int-CD33⁺ <70%; 39 GO patients and 31 controls) and 130 patients had high CD33 expression (high-CD33⁺ ≥70%; 65 GO patients and 65 controls). Characteristics of these 2 subgroups are shown in Table 1. Consistent with the literature,¹⁵⁻¹⁸ patients from the high-CD33⁺ subgroup had higher a WBC count and bone marrow (BM) blast percentage, and the large majority of patients with *NPM1* gene mutation or internal tandem duplication of the *FLT3* gene (*FLT3*-ITD) belonged to the high-CD33⁺ subgroup (Table 1).

Among the 200 study patients, 155 (77%) achieved CR/CRp. There was no difference in CR/CRp rate between low/int-CD33⁺ and high-CD33⁺ patients (74% vs 79%, *P* = .48). Twenty-nine (14.5%) patients received allogeneic stem cell transplantation (SCT) in the first CR/CRp (7/70 low/int-CD33⁺ and 22/130 high-CD33⁺; 11 GO patients and 18 controls). Overall, 96 patients relapsed (48%; 35/70 low/int-CD33⁺ and 61/130 high-CD33⁺; 50 GO patients and 46 controls) and 128 patients died (58%; 49/70 low/int-CD33⁺ and 79/130 high-CD33⁺; 64/104 GO patients and 64/96 controls), including only 13 (7.5%) deaths in first CR (7 after SCT).

High CD33⁺ expression did not significantly affect patient outcome (not shown), but the beneficial effect of adding GO was observed only in high-CD33⁺ patients. The CR/CRp rate was similar in the GO and control arms even in high-CD33⁺ patients (80% vs 78%). However, prolonged EFS and RFS associated with GO administration were observed in high-CD33⁺ patients only (hazard ratio [HR], 0.56 and 0.47; *P* = .0051 and 0.0016, respectively), but not in low/int-CD33⁺ patients (Figure 1B-C). This interaction reached statistical significance for RFS but not for EFS. In this updated analysis,¹³ overall survival was not significantly affected by GO treatment, even in the high-CD33⁺ subgroup. Notably, the benefit associated with GO treatment in high-CD33⁺ patients was still observed after adjustment for cytogenetics and genotype (HR, 0.64 and 0.51 and *P* = .032 and 0.007 for EFS and RFS, respectively). Supplemental Figure 1 illustrates the effect of GO in these high-CD33⁺ patients, according to various patient subsets. Significant gains were still observed in patients with intermediate-risk cytogenetics (HR, 0.52 and 0.51 and *P* = .011 and 0.016 for EFS and RFS, respectively). Furthermore, GO effects were consistently observed in these patients whatever their *NPM1* and *FLT3*-ITD status, meaning that high-CD33⁺ patients without the *NPM1* mutation or *FLT3*-ITD may also benefit from GO. This is illustrated in supplemental Figure 2. Finally, even if limited by the relatively low number of patients, the

MFI ratio level did not seem to significantly influence the effect of GO in high-CD33⁺ patients (supplemental Figure 1).

In this study, we confirmed the heterogeneity of CD33 expression on AML blasts.^{19,20} We confirmed that high CD33 expression was associated with higher WBC and BM blast infiltration as well as with higher incidences of *FLT3*-ITD and *NPM1* mutations.¹⁵⁻¹⁸ We also found that a CD33⁺ expression level as high as 70% or more (observed here in 65% of the patients and in 64% of those with nonadverse cytogenetics) appeared to be necessary to allow a beneficial response to GO, independent of the *NPM1* and *FLT3*-ITD mutational status. This study supplies the first convincing data suggesting individualized use of GO with respect to individual AML blast CD33 expression. It will be important to confirm these results in future retrospective or prospective studies, including quantification of both CD33⁺ percentage and CD33 MFI, to validate this 70% expression cutoff as a practical biomarker for GO efficacy and to further analyze the potential role of CD33 MFI in these patients.

The online version of this article contains a data supplement.

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Contribution: G.O., E.G., and J.F. performed the centralized flow cytometry analysis; S. Castaigne, H.D., and J.F. designed the study; J.G., J.-Y.P., F.D., A.d.L., O.L., A.P., O.W.-B., E.C., V.S., and C. Preudhomme provided patient flow cytometry data and participated in the analysis; J.-N.B., E.R., X.T., C. Pautas, S. Chantepie, P.T., S. Castaigne, and H.D. entered patients in the study and participated in the analysis. All authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: S. Castaigne received consultancy fees from Pfizer; H.D. received honoraria for advisory boards from Pfizer; and J.F. received research funding from Pfizer. All other authors declare no competing financial interests. In this study, gemtuzumab ozogamicin was used off-label during front-line acute myeloid leukemia therapy.

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