To the editor:

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

Guillaume Olombel,1 Estelle Guerin,1 Julien Guy,2 Jean-Yves Perrot,3 Florent Dumezy,4 Adrienne de Labarthe,5 Jean-Noël Bastie,6 Olivier Legrand,7 Emmanuel Raffoux,5 Adriana Plesa,8 Orianne Wagner-Ballon,9 Edouard Cornet,10 Véronique Salaun,10 Claude Preudhomme,4 Xavier Thomas,11 Cécile Pautas,12 Sylvain Chantepie,13 Pascal Turlure,14 Sylvie Castaigne,15 Hervé Dombret,5 and Jean Feuillard1

1Laboratoire d’Hématologie et Unité Mixte de Recherche 7276 Centre National de la Recherche Scientifique, Centre Hospitalier Universitaire (CHU) de Limoges, Faculté de Médecine, Université de Limoges et Centre National de la Recherche Scientifique, Limoges, France; 2Service d’Hématologie Biologique, CHU, Dijon, France; 3Service d’Hématologie Biologique, Hôpital Saint Antoine, Assistance Publique–Hôpitaux de Paris (AP-HP), Paris, France; 4Laboratoire d’Hématologie, CHU, Lille, France; 5Service d’Hématologie Adulte, Hôpital Saint-Louis, AP-HP, Université Paris Diderot, Paris, France; 6Service d’Hématologie, CHU, Dijon, France; 7Service d’Hématologie, Hôpital Dieu, Hôpital Saint Antoine, AP-HP, Paris, France; 8Centre de Biologie et d’Anatomie Pathologique, Hématologie Cellulaire et Immunophénotypage des hémopathies, CHU de Lyon–Hospices Civils de Lyon, Pierre Bénite, France; 9Département d’Hématologie et Immunologie Biologiques, Hôpitaux universitaire Henri Mondor, AP-HP, University Paris-Est Créteil, Créteil, France; 10Laboratoire d’Hématologie, CHU, Caen, France; 11Service d’Hématologie, Centre Hospitalier Lyon Sud, Pierre-Bénite, France; 12Service d’Hématologie Clinique, Hôpital Henri Mondor, AP-HP, Créteil, France; 13Service d’Hématologie, CHU, Caen, France; 14Service d’Hématologie, CHU, Limoges, France; and 15Service d’Hématologie-Oncologie, Centre Hospitalier, Versailles, France

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.2-4 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.6 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 already been shown.7,8 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.
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.12 Higher response rates were nevertheless reported for patients with CD33 expression $\geq 98\%$ 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; clinicaltrial.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$^+$ $\geq$70%; 65 GO patients and 65 controls). Characteristics of these 2 subgroups are shown in Table 1. Consistent with the literature,15-18 patients from the high-CD33$^+$ subgroup had a higher 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,17 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

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**Table 1. Patient characteristics according to CD33 expression**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low/int-CD33$^+$ (&lt;70%)</th>
<th>High-CD33$^+$ (≥70%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>70</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Treatment arm, GO/control</td>
<td>39/31</td>
<td>65/65</td>
<td>.46</td>
</tr>
<tr>
<td>Median age (range), years</td>
<td>62.3 (50-70)</td>
<td>61.6 (50-70)</td>
<td>.33</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>39/31</td>
<td>60/70</td>
<td>.24</td>
</tr>
<tr>
<td>ECOG-PS, 0/1/2/3/NA</td>
<td>25/54/9/1/1</td>
<td>46/72/12/0/0</td>
<td>.31</td>
</tr>
<tr>
<td>Median WBC (range), $10^9$/L</td>
<td>3.0 (0.8-211)</td>
<td>11.8 (6.0-320)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Median BM blast (range), %</td>
<td>52 (20-99)</td>
<td>93 (20-100)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cytogenetics, fav/int/ unfav/NA</td>
<td>4/45/14/7</td>
<td>3/85/29/13</td>
<td>.65</td>
</tr>
<tr>
<td>NPM1 status, mut/WT/NA</td>
<td>7/62/1</td>
<td>59/69/2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FLT3-ITD status, mut/WT/NA</td>
<td>2/67/1</td>
<td>32/97/1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Genotype, fav/other/NA*</td>
<td>6/56/8</td>
<td>28/87/15</td>
<td>.057</td>
</tr>
<tr>
<td>ELN classification, fav/int-1/int-2/ unfav/NA</td>
<td>10/24/15/14/7</td>
<td>31/46/11/29/13</td>
<td>.10</td>
</tr>
</tbody>
</table>

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 CEBPA mutation, according to international ELN recommendations.

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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.

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Correspondence: Jean Feuillard, Laboratoire d’Hématologie, Centre Hospitalier Universitaire de Limoges, 2 Av Martin Luther King, 87042 Limoges, France; e-mail: jean.feuillard@unilim.fr; and Hervé Dombret, Hématologie Adulète, Hôpital Saint-Louis, 1 Av Claude Vellefaux, 75010 Paris, France; e-mail: herve.dombret@lslsaph.fr.

References


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