

Brief report

CD33 expression and P-glycoprotein–mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy

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Gemtuzumab ozogamicin (GO) contains an anti-CD33 antibody to facilitate uptake of a toxic calicheamicin- γ_1 derivative. While recent in vitro data demonstrated a quantitative relationship between CD33 expression and GO cytotoxicity, previous correlative studies failed to identify a significant association between CD33 expression and clinical outcome. Studying patients undergoing GO monotherapy for

relapsed acute myeloid leukemia (AML), we now find that AML blasts of responders have a significantly higher mean CD33 level and lower P-glycoprotein (Pgp) activity compared with nonresponders. CD33 expression and Pgp activity are inversely correlated. While both variables are associated with outcome, Pgp remains significantly associated with outcome even after adjusting for CD33, whereas CD33

does not show such an association after adjusting for Pgp. The inverse relationship between CD33 and Pgp suggests a maturation-stage–dependent expression of both proteins, and offers the rationale for using cell differentiation–promoting agents to enhance GO-induced cytotoxicity. (Blood. 2007;109:4168-4170)

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Introduction

Due to its restricted, maturation stage–dependent expression on normal myeloid cells and its presence on tumor cells of 85% to 90% of adult and pediatric patients with acute myeloid leukemia (AML), CD33 has been exploited as a target for antibody-based AML therapies.^{1,4} The endocytic property of CD33 led to the development of gemtuzumab ozogamicin (GO; Mylotarg; Wyeth Pharmaceuticals, Collegeville, PA), an immunoconjugate consisting of a humanized IgG₄ anti-CD33 monoclonal antibody (hP67.6) joined to a toxic calicheamicin- γ_1 derivative.^{4,5} Encouraging results on 277 patients treated in phase 2 trials revealed that GO monotherapy induces a complete remission (CR) or CR with incomplete platelet recovery (CRp) in 26% of adults with relapsed CD33⁺ AML.^{6,7}

hP67.6 itself is largely nontoxic and primarily facilitates uptake of the calicheamicin- γ_1 derivative, which is then cleaved and eventually induces DNA damage and cell death,⁴ implying a critical role of the intracellular calicheamicin- γ_1 accumulation for GO-induced cytotoxicity. Indeed, drug efflux mediated by P-glycoprotein (Pgp) results in resistance to GO and predicts for adverse outcome after GO monotherapy; conversely, inhibition of Pgp function effectively increases GO-induced cytotoxicity in vitro.⁸⁻¹² Furthermore, recent in vitro studies revealed a quantitative relationship between CD33 expression and GO-induced cytotoxicity in human CD33⁺ AML cell lines,¹³ and higher CD33 expression levels on circulating AML blasts were associated with clearance of AML blasts from peripheral blood after one dose of GO.¹⁴ In contrast, an analysis of the first 142 patients treated in the phase 2

trials failed to show a significant impact of CD33 expression levels of bone marrow AML blasts in response to GO.⁶ Additional smaller studies similarly did not find a predictive role of CD33 expression.^{15,16} To resolve these apparent discrepancies, we re-examined the role of CD33 expression and Pgp function for clinical response to GO, using the complete phase 2 trial patient cohort for analysis.

Patients, materials, and methods

Patients and treatment

Details on the 3 multicenter, open-label, single-arm, phase 2 GO protocols have been reported previously.⁶⁻⁸ Briefly, adult patients with primary AML in untreated first relapse were eligible if they had CD33⁺ AML with CD33 at a fluorescence intensity more than or equal to 4 times the fluorescence intensity of an isotype-matched control antibody on at least 80% of the myeloid blasts. Patients were scheduled to receive 2 doses of GO 9 mg/m² as a 2-hour single-agent intravenous infusion with 14 to 28 days between doses. CR was defined as (1) absence of circulating leukemic blasts; (2) 5% or fewer leukemic blasts in the bone marrow by morphology; (3) peripheral blood counts with hemoglobin level more than or equal to 90 g/L, absolute neutrophil count more than or equal to $1.5 \times 10^9/L$, and platelets more than or equal to $100 \times 10^9/L$; and (4) RBC/platelet transfusion independence. CRp defined patients who fulfilled CR criteria except for failure to recover a platelet count of more than or equal to $100 \times 10^9/L$. All patients provided written informed consent before treatment, in accordance with the Declaration of Helsinki, and all studies were approved by the participating centers' institutional review boards.

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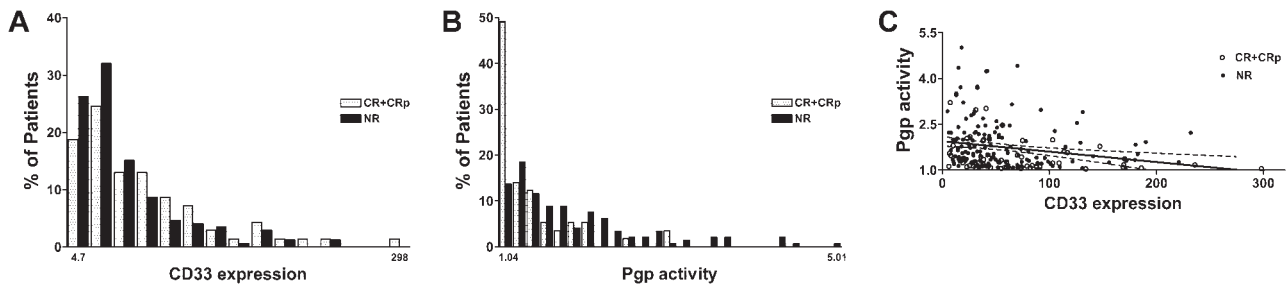


Figure 1. CD33 expression and Pgp activity in responders and nonresponders. (A) CD33 expression on AML blasts of responders (CR+CRp) and nonresponders (NR) who survived beyond day 28 of study initiation, determined by flow cytometry after staining with the anti-CD33 antibody hP67.6. (B) Pgp activity of AML blasts of responders and nonresponders who survived beyond day 28 of study initiation, determined by flow cytometry using the fluorescent Pgp substrate DiOC₂. (C) Correlation between CD33 expression and Pgp activity. Dot plot graph showing individual data on CD33 expression levels and Pgp activity in responders (CR+CRp; ○) as well as nonresponders (NR; ●) who survived beyond day 28 of study initiation; n = 208. The same magnitude of correlation was seen when the patients who died before day 28 were not excluded from the analysis (r = -0.23; n = 230; P < .001).

Assessment of CD33 expression and Pgp function

Pretreatment bone marrow samples were used to assess CD33 expression and Pgp function. Cell surface expression of CD33 on the myeloid blast population was determined by flow cytometry using hP67.6 as the primary antibody, and expressed as a ratio between the mean CD33 fluorescence of the CD33⁺ blast subfraction and the mean fluorescence of an isotype control antibody.¹⁴ Pgp function was cytofluorometrically assessed by efflux of the fluorescent Pgp substrate, DiOC₂, and expressed as mean DiOC₂ fluorescence intensity after dye loading divided by DiOC₂ intensity after dye efflux.⁸

Statistical analysis

Mean values between responders and nonresponders were compared using the 2-sample *t* test with nonequal variances. Additional analyses were conducted by dividing CD33 and Pgp into quartiles and using logistic regression to perform a trend test on the association between proportion of responders and quartile rank. The correlation between CD33 and Pgp was estimated using the Pearson correlation coefficient. The impact of Pgp on the probability of response after adjusting for CD33 (and vice versa) was assessed using logistic regression and the likelihood ratio test.

Results and discussion

There were 276 adult patients enrolled on the phase 2 trials (median age, 61 years; range, 20-87 years) who were available for analysis. Seventy-one (25.4%) patients achieved either a CR (n = 35) or a CRp (n = 36). Among patients who achieved a CR or CRp, the mean CD33 expression level and CD33 expression to blast percentage ratio were each statistically significantly higher, whereas the percentage of AML blasts in the bone marrow was statistically significantly lower than that among non-responders, that is, patients with a smaller amount of AML blasts but higher blast CD33 levels were more likely to respond to GO (Figure 1A and Table 1). Twenty-seven patients died within 28 days of therapy initiation,

and a separate analysis was done after exclusion of these patients. Qualitatively, the same results were seen as those among all patients (Table 1). Among patients in the first to fourth quartiles of mean CD33 expression, response rates were 23%, 23%, 27%, and 41%, respectively, after exclusion of patients who experienced early death (P < .03, trend test). Previous reports failed to identify a significant association between CD33 expression level and outcome.^{6,15,16} This discrepancy may be explained by differences in the method of quantifying CD33 expression, the larger sample size yielding increased statistical power compared with previous reports, and/or differences in the manner in which associations were assessed.

Previous analysis of a subset of the phase 2 trial patients has demonstrated that Pgp activity was associated with a poorer response.^{6,8} Consistent with these earlier findings, we found that patients who achieved a CR or CRp had statistically significantly lower mean Pgp efflux, even after excluding patients who died before day 28 (Figure 1B and Table 1). This association was also apparent when the relationship between Pgp activity and clinical outcome was analyzed by quartiles of Pgp, with response rates of 54%, 27%, 23%, and 12% among patients in the first to fourth quartiles, respectively, after exclusion of patients who experienced early death (P < .001, trend test). This observation for a GO-based therapy is similar to anthracycline-based conventional anti-AML chemotherapies.^{17,18}

Finally, since increasing evidence suggests that Pgp function is related to the maturation stage of the AML blast, with higher activity in more immature cells¹⁹⁻²¹ and CD33 being expressed in a maturation-stage-dependent manner in myeloid cells,¹ we estimated the correlation between these 2 parameters. We found a statistically significant inverse relationship (P < .001) between CD33 expression and Pgp efflux, although the magnitude of the correlation was only moderate (r = -0.23, Figure 1C). Given this correlation, it was of interest to examine the association of one parameter with outcome after adjusting for the other. The addition

Table 1. Association of parameters with response among all patients and among those who survived beyond day 28 of therapy initiation

	All nonresponders		Nonresponders surviving beyond day 28*		Responders†		P‡
	n	Mean (95% CIs)	n	Mean (95% CIs)	n	Mean (95% CIs)	
CD33 expression	197	54.8 (48.6-61.0)	172	53.9 (47.2-60.7)	69	71.2 (57.2-85.2)	.04; .03
Pgp efflux	170	1.84 (1.73-1.96)	148	1.86 (1.74-1.99)	60	1.44 (1.31-1.57)	<.001; <.001
CD33 expression-blast % ratio	197	1.52 (1.26-1.78)	172	1.56 (1.27-1.85)	69	3.31 (2.11-4.53)	.005; .006
% AML blasts	197	55.9 (51.8-60.0)	172	54.7 (50.3-59.2)	69	44.7 (37.7-51.8)	.008; .02

n indicates the number of patients with available data; 95% CIs, 95% confidence intervals.
 *This group excludes patients who died within 28 days of therapy initiation.
 †CR + CRp.
 ‡First P value among all patients; second value excludes patients who died within 28 days of therapy initiation.

of Pgp statistically significantly improved a logistic regression model containing only CD33 ($P < .001$), whereas the addition of CD33 did not lead to such an improvement relative to the model containing only Pgp ($P = .14$).

In conclusion, our data indicate that CD33 expression levels and Pgp efflux are each associated with clinical outcome of patients treated with GO monotherapy, and Pgp remains statistically significantly associated with outcome even after adjusting for CD33 whereas CD33 does not show such an association after adjusting for Pgp function. Importantly, these variables may not necessarily be independent of other prognostic factors, or in all situations. The inverse relationship between CD33 abundance and Pgp activity is consistent with the notion of maturation-stage-dependent expression of these proteins. This offers the rationale for using drug efflux inhibitors and/or cell differentiation-promoting agents (eg, cytokines or growth factors) to enhance GO-induced cytotoxicity by increasing CD33 expression/drug uptake and/or reducing drug efflux,^{16,22-24} thereby possibly improving clinical outcome of patients undergoing GO-containing AML therapy.

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Authorship

Contribution: R.B.W. designed research, analyzed data, and wrote the paper; T.A.G. analyzed data and wrote the paper; V.H.J.v.d.V. and J.J.M.v.D. collected data and wrote the paper; M.R.L. and D.A.F. collected data; and I.D.B. and F.R.A. designed research and wrote the paper.

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