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ORIGINAL REPORT

CD33 Expression and Its Association With Gemtuzumab Ozogamicin Response: Results From the Randomized Phase III Children's Oncology Group Trial AAML0531

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ABSTRACT

Purpose

CD33 is variably expressed on acute myeloid leukemia (AML) blasts and is targeted by gemtuzumab ozogamicin (GO). GO has shown benefit in both adult and pediatric AML trials, yet limited data exist about whether GO response correlates with CD33 expression level.

Patients and Methods

CD33 expression levels were prospectively quantified by multidimensional flow cytometry in 825 patients enrolled in Children's Oncology Group AAML0531 and correlated with response to GO.

Results

Patients with low CD33 expression (lowest quartile of expression [Q1]) had no benefit with the addition of GO to conventional chemotherapy (relapse risk [RR]: GO 36% *v* No-GO 34%, $P = .731$; event-free survival [EFS]: GO 53% *v* No-GO 58%, $P = .456$). However, patients with higher CD33 expression (Q2 to Q4) had significantly reduced RR (GO 32% *v* No-GO 49%, $P < .001$) and improved EFS (GO 53% *v* No-GO 41%, $P = .005$). This differential effect was observed in all risk groups. Specifically, low-risk (LR), intermediate-risk (IR), and high-risk (HR) patients with low CD33 expression had similar outcomes regardless of GO exposure, whereas the addition of GO to conventional chemotherapy resulted in a significant decrease in RR and disease-free survival (DFS) for patients with higher CD33 expression (LR RR, GO 13% *v* No-GO 35%, $P = .001$; LR DFS, GO 79% *v* No-GO 59%, $P = .007$; IR RR, GO 44% *v* No-GO 57%, $P = .044$; IR DFS, GO 51% *v* No-GO 40%, $P = .078$; HR RR, GO 40% *v* No-GO 73%, $P = .016$; HR DFS, GO 47% *v* No-GO 28%, $P = .135$).

Conclusion

We demonstrate that GO lacks clinical benefit in patients with low CD33 expression but significantly reduces RR and improves EFS in patients with high CD33 expression, which suggests a role for CD33-targeted therapeutics in subsets of pediatric AML.

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INTRODUCTION

The majority of patients with acute myeloid leukemia (AML) expresses the myeloid antigen CD33 on leukemic blasts.[1] CD33, a 67-kDa transmembrane glycoprotein, is a member of the sialic acid–binding immunoglobulin-like lectins (Siglecs) and is targeted by gemtuzumab ozogamicin (GO), a toxin-conjugated humanized immunoglobulin G4 anti-CD33 monoclonal antibody that has efficacy within subsets of adult patients with de novo AML, particularly those with favorable or normal cytogenetics.[1-10] GO has also been studied in a number of pediatric oncology trials,[11-16] which include three conducted within the Children's Oncology Group (COG).[11,13,16] The first, AAML00P2, determined the maximum tolerated dose of GO when used in combination with conventional chemotherapy for patients whose condition had relapsed.[11] The subsequent study, COG AAML03P1, was a pilot in which patients with de novo AML received GO in combination with Medical Research Council–based conventional chemotherapy.[13] The successor study, COG AAML0531, used the same chemotherapy regimen as that for AAML03P1, but patients were randomly assigned to receive standard chemotherapy alone or in combination with GO. This study found that GO recipients had significantly improved event-free survival (EFS) as well as relapse risk (RR) and disease-free survival (DFS) compared with patients treated only with conventional chemotherapy.[16]

We previously demonstrated within the context of AAML03P1, in which all patients received GO, that high CD33 expression was correlated with negative prognostic features and significantly lower overall survival (OS) and DFS from complete remission (CR). In a multivariable model, high CD33 expression remained a negative predictor of outcome.[17] The aim of the current study was to determine, within the context of the GO randomization trial COG AAML0531, whether GO had efficacy in patients with the lowest CD33 expression and, conversely, whether it significantly improved outcomes in patients with higher CD33 expression compared with the control arm. Toward that end, we prospectively quantified CD33 expression on the surface of leukemic blasts and correlated these findings with disease characteristics and clinical outcome by treatment arm.

PATIENTS AND METHODS

Patients and Treatment

Pediatric patients with de novo AML enrolled in COG AAML0531 were eligible for this study. Details of the treatment regimen have been described previously.[16] In brief, patients were randomly assigned to one of two study arms: a backbone of standard chemotherapy alone (No-GO arm) or in combination with 3 mg/m^2 GO administered on day 6 of induction I and day 7 of intensification II (GO arm). Patients designated as high risk (HR) received the best allogeneic hematopoietic stem cell transplantation (HSCT) after intensification I chemotherapy. Selection of an alternative donor was by the discretion of the treating transplantation center. Intermediate-risk (IR) patients, defined as those without low-risk (LR) or HR features, underwent HSCT if a matched family donor was available. IR patients without a matched family donor and LR patients did not undergo HSCT; their therapy was limited to five chemotherapy courses. There was no threshold of CD33 expression for enrollment in this clinical protocol. All samples from patients enrolled in AAML0531 were eligible for our correlative study if consent for biology studies was obtained. The institutional review boards of all participating institutions approved the clinical protocol, and the COG Myeloid Disease Biology Committee approved this research.

Risk Stratification

For the purposes of this correlative biology study, cytogenetic and molecular abnormalities were used to stratify the study population into risk groups. The LR group included patients with core-binding factor AML [t(8;21) or inv(16)/t(16;16)] and/or nucleophosmin 1 (*NPM1*) or *CEBPA* mutations without *FLT3/ITD* mutations. The HR group included patients with high allelic ratio (> 0.4) *FLT3/ITD*+ disease and/or monosomy 5, del (5q), or monosomy 7. The remaining patients with known cytogenetics were designated as IR.

Assessment of CD33 Expression

By using flow cytometry, CD33 mean fluorescent intensity (MFI) of myeloid progenitor cells, as defined by CD45 low and side scatter, was determined with a previously described protocol.[17-19]

Statistical Analyses

Clinical outcome data for patients enrolled in COG AAML051 were analyzed as of September 30, 2014. The median follow-up for eligible patients who were alive at last contact and included in our analysis was 1,856 days (range, 4 to 2,829 days). Patients were considered in CR if they had less than 5% blasts and an absence of extramedullary disease after one course of induction chemotherapy. Minimal residual disease (MRD) was defined by using flow cytometry and considered positive if 0.1% or more disease was detected at the end of induction I.[20] OS was defined as the time from study entry or from the end of course I for patients in CR to death. EFS was defined as the time from study entry until death, induction failure, or relapse of any type. DFS was defined as the time from the end of course I for patients in CR until relapse or death of any cause. RR was defined as the time from the end of course I for patients in CR to relapse, where deaths without a relapse were considered competing events.[21] The significance of predictor variables was tested with the log-rank statistic for OS, EFS, and DFS and with the Gray statistic for RR. Patients lost to follow-up were censored at their date of last known contact. The significance of observed difference in proportions was tested by the χ^2 test between patient groups. Alternatively, the exact test was used if data were sparse. The Kruskal-Wallis test was used to determine the significance between differences in medians of the groups.

RESULTS

CD33 Expression Levels and Correlation With Disease Characteristics

We prospectively evaluated CD33 expression levels in samples from 825 pediatric patients with de novo AML enrolled in COG AAML0531. CD33 MFI of the blast population varied more than two-log-fold, with a median MFI of 146.00 (range, 2.68 to 1,351.00). The study population was divided into four quartiles on the basis of CD33 expression, and expression levels were correlated with clinical characteristics and outcomes. Median MFI for quartile (Q) 1 to 4 was as follows: Q1 (n = 208), 34.61 (range, 2.68 to 67.00); Q2 (n = 205), 100.7 (range, 67.13 to 146.94); Q3 (n = 206), 207.01 (range, 147.00 to 296.38); and Q4 (n = 206), 435.9 (range, 296.98 to 1,351.00). Correlation of CD33 expression with somatic mutations revealed that *FLT3/ITD*, *NPM1*, and *CEBPA* mutations were detected in 16%, 8%, and 6% of evaluable samples, respectively. Similar to our previous analysis,[17] there was a statistically significant increase in the proportion of *FLT3/ITD* and *NPM1* mutations with increasing CD33 expression and a trend toward decreased numbers of patients with *CEBPA* mutations with high levels of CD33 expression (Fig 1A; Table 1). This translated into higher median CD33 expression for patients with *FLT3/ITD*+ disease (median MFI, 215.31; range, 4.27 to 1,225.87) compared with patients with the wild-type *FLT3* (median MFI, 135; range, 2.68 to 1,351; $P < .001$). Similar findings were observed for patients with *NPM1* + disease (median MFI, 275.6; range, 6.84 to 1,160) compared with those with the wild-type *NPM1* (median MFI, 139; range, 2.68 to 1,351; $P < .001$).

Cytogenetic data were available for 799 of 825 patient samples (97%). Prevalence of core-binding factor AML was inversely associated with CD33 expression, with a prevalence of 47% in patients with the lowest (Q1) and 5% in patients with the highest (Q4) CD33 expression ($P < .001$; Fig 1A). The prevalence of *KMT2A* gene alterations (11q23) also increased with increase in CD33 expression ($P < .001$; Table 1). There was no association between CD33 expression and HR cytogenetics; however, analysis was limited by the small number of patients (27 of 825 [3%]) with such alterations.

For risk group classification, complete cytogenetic and molecular data were available for 811 of 825 patients (98%); 307 (38%) were classified as LR, 390 (48%) as IR, and 114 (14%) as HR.

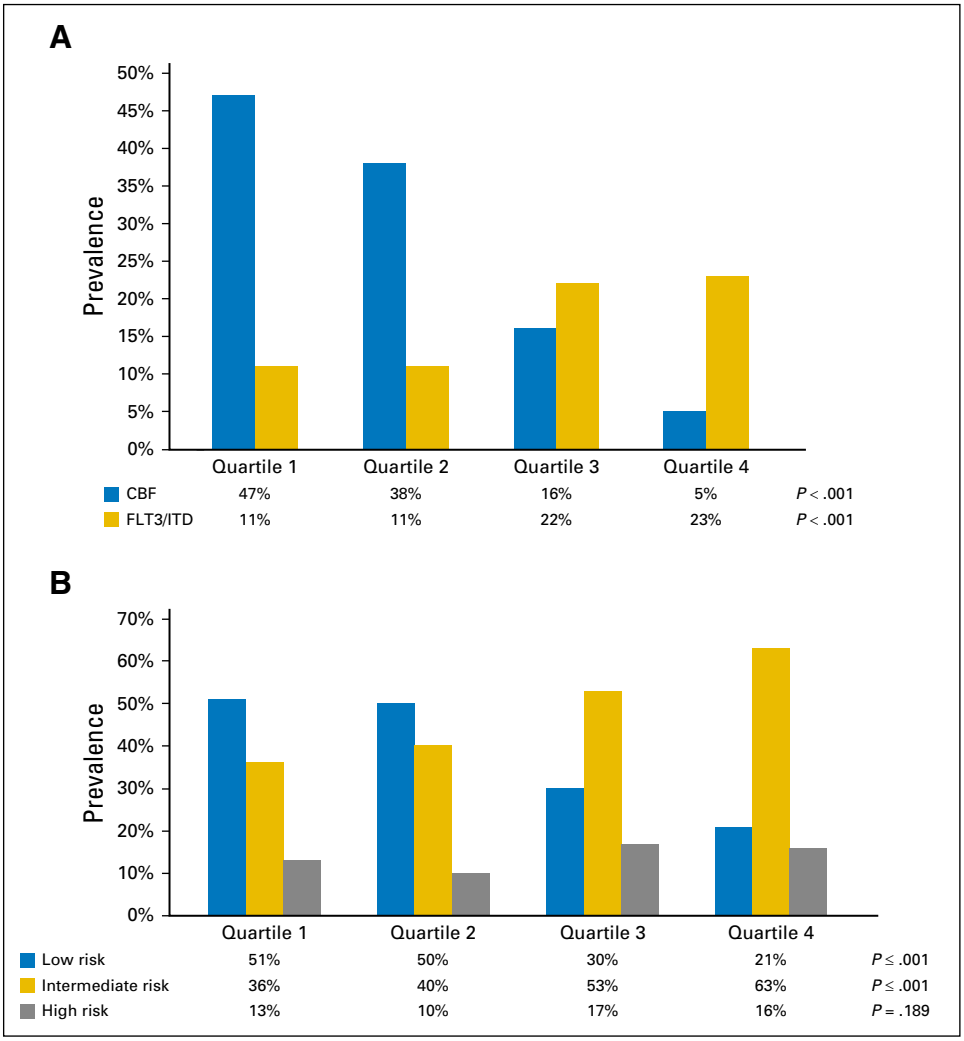

Fig 1. Distribution of CD33 expression in patients. (A) Correlation of CD33 expression data with specific cytogenetic/molecular disease characteristics by quartile. (B) CD33 expression and disease risk group classification by quartile. CBF, core-binding factor; FLT3/ITD, FLT3 internal tandem duplication.

LR disease was associated with low CD33 expression ($P < .001$; Fig 1B; Table 1), whereas the prevalence of IR disease increased significantly with increasing quartile ($P \leq .001$, Fig 1B; Table 1). There was no clear trend in prevalence by quartile for HR disease ($P = .189$; Fig 1B; Table 1), although there was a significantly higher median CD33 MFI with HR (median MFI, 191.05; range, 4.27 to 1,225.87) versus LR disease (median MFI, 98; range, 5 to 876; $P < .001$).

Association of CD33 Expression and GO Response

Induction I and induction II remission rates were determined for all patients independent of risk group classification and were similar across all quartiles (Table 1). CR rates across quartiles also lacked statistical significance when analysis was conducted by treatment arm (Table 1). Given the mechanism of action of GO, we were particularly interested in determining whether GO had differential efficacy in patients who expressed low (Q1) versus higher (Q2 to Q4) levels of CD33. For patients with low CD33 expression, CR rates were not significantly different for those receiving and not receiving GO (75% *v* 73%; $P = .750$). However, for patients with higher CD33 expression, end–induction I CR rates were significantly higher for those who received GO than those who did not (77% *v* 68%; $P = .012$). Similarly, patients with low CD33 expression did not have significantly different rates of end–induction I MRD when compared by treatment arm (GO 27% *v* No-GO 33%; $P = .426$). However, patients with higher (Q2 to Q4) CD33 expression treated with GO had lower rates of MRD than those treated with conventional chemotherapy only (GO 28% *v* No-GO 36%; $P = .052$).

Patients with the lowest CD33 expression had similar survival outcomes regardless of GO exposure (Table 2; Figs 2A and 2C). In contrast, patients with higher CD33 expression (Q2 to Q4) who received GO had a significant improvement in clinical outcome compared with those who did not receive GO, with an RR of 32 ± 6% versus 49% ± 7% ($P < .001$) and a corresponding EFS of 53 ± 6% versus 41 ± 6% ($P = .005$) observed for those treated with and without GO therapy (Table 2; Figs 2B and 2D). The relative impact of consolidation with HSCT versus chemotherapy was also determined. Analysis of DFS from time of consolidation therapy supported our observation that GO provided clinical benefit for patients with high CD33 expression. However, consolidation with HSCT versus chemotherapy resulted in similar DFS from CR (Appendix Fig A1, online only).

Table 1. Disease Characteristics and Induction Response by Quartile of CD33 Expression

Characteristic	CD33 Q1 (n = 208)		CD33 Q2 (n = 205)		CD33 Q3 (n = 206)		CD33 Q4 (n = 206)		*P*
	No.	%	No.	%	No.	%	No.	%	
Sex									
Male	110	53	105	51	96	47	108	52	.563
Female	98	47	100	49	110	53	98	48	
Treatment arm									
No-GO	115	55	99	48	96	47	101	49	.310
GO	93	45	106	52	110	53	105	51	
Cytogenetics									
Normal	28	14	34	17	60	30	60	30	< .001
t(8;21)	64	32	40	20	13	6	0	0	< .001
inv(16)	31	15	34	17	20	10	11	5	.001
t(9;11)/11q23	16	8	32	16	50	25	66	33	< .001
t(6;9)(p23;q34)	2	1	0	0	5	2	5	2	.112
Monosomy 7	6	3	5	3	2	1	3	1	.462
del(7q)	4	2	3	2	3	1	2	1	.880
-5/5q-	1	0	6	3	4	2	0	0	.035
+8	12	6	7	4	16	8	17	8	.183
Other	38	19	36	18	28	14	38	19	.505
Unknown	6		8		5		4		
FLT3/ITD status									
ITD+	23	11	22	11	45	22	46	23	< .001
ITD−	183	89	179	89	157	78	156	77	
Unknown	2		4		4		4		
CEBPA status									
CEBPA mutant	10	5	17	8	16	8	6	3	.065
CEBPA WT	197	95	185	92	186	92	197	97	
Unknown	1		3		4		3		
NPM1 status									
NPM1 mutant	5	2	11	5	17	8	31	15	< .001
NPM1 WT	202	98	190	95	185	92	172	85	
Unknown	1		4		4		3		
Risk group (cyto/molecular)									
Intermediate	73	36	80	40	108	53	129	63	< .001
Low	105	51	99	50	60	30	43	21	< .001
High	27	13	20	10	34	17	33	16	.189
Unknown	3		8		4		1		
Induction I response									
CR	153	74	151	75	143	70	145	73	.701
Not in CR	54	26	50	25	61	30	54	27	
Not evaluable	1		4		2		7		
Induction II response									
CR	183	90	169	87	171	86	169	86	.684
Not in CR	21	10	26	13	27	14	27	14	
Not evaluable	4		10		8		10		
No-GO patients only									
Induction I response									
CR	84	73	73	74	59	62	65	67	.208
Not in CR	31	27	25	26	36	38	32	33	
Not evaluable	0		1		1		4		
Induction II response									
CR	101	90	82	84	73	81	84	88	.228
Not in CR	11	10	16	16	17	19	11	12	
Not evaluable	3		1		6		6		
GO patients only									
Induction I response									
CR	69	75	78	76	84	77	80	78	.945
Not in CR	23	25	25	24	25	23	22	22	
Not evaluable	1		3		1		3		
Induction II response									
CR	82	89	87	90	98	91	85	84	.065
Not in CR	10	11	10	10	10	9	16	16	
Not evaluable	1		9		2		4		

Abbreviations: CR, complete remission; cyto, cytogenetic; GO, gemtuzumab ozogamicin; ITD, internal tandem duplication; Q, quartile; WT, wild type.

Table 2. Clinical Outcome Analysis by Treatment Arm (No-GO *v* GO) and Quartile as Well as Aggregate Analysis for Q2 to Q4 for All Patients

	All Patients (%)														
	CD33 Q1 (n = 208)		*P*	CD33 Q2 (n = 205)		*P*	CD33 Q3 (n = 206)		*P*	CD33 Q4 (n = 206)		*P*	CD33 Q2-Q4 (n = 617)		*P*
	No-GO (n = 115)	GO (n = 93)		No-GO (n = 99)	GO (n = 106)		No-GO (n = 96)	GO (n = 110)		No-GO (n = 101)	GO (n = 105)		No-GO (n = 296)	GO (n = 321)	
5-year OS from study entry, mean ± SE	75 ± 8	72 ± 9	.495	59 ± 10	71 ± 9	.174	62 ± 10	64 ± 9	.758	54 ± 10	60 ± 10	.341	58 ± 6	65 ± 6	.132
5-year EFS from study entry, mean ± SE	58 ± 9	53 ± 10	.456	43 ± 10	61 ± 10	.010	43 ± 10	53 ± 10	.151	36 ± 10	44 ± 10	.359	41 ± 6	53 ± 6	.005
	No-GO (n = 84)	GO (n = 69)		No-GO (n = 73)	GO (n = 78)		No-GO (n = 59)	GO (n = 84)		No-GO (n = 65)	GO (n = 80)		No-GO (n = 197)	GO (n = 242)	
5-year DFS from end of induction I, mean ± SE	65 ± 11	59 ± 12	.437	50 ± 12	70 ± 11	.016	51 ± 13	57 ± 11	.377	39 ± 12	55 ± 11	.046	47 ± 7	61 ± 6	.003
5-year RR from end of induction I, mean ± SE	34 ± 11	36 ± 12	.731	47 ± 12	21 ± 10	< .001	45 ± 13	39 ± 11	.361	55 ± 13	34 ± 11	.016	49 ± 7	32 ± 6	< .001

NOTE. SE is two standard errors of the mean (± 2SE %).
Abbreviations: DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; OS, overall survival; Q, quartile; RR, relapse risk.

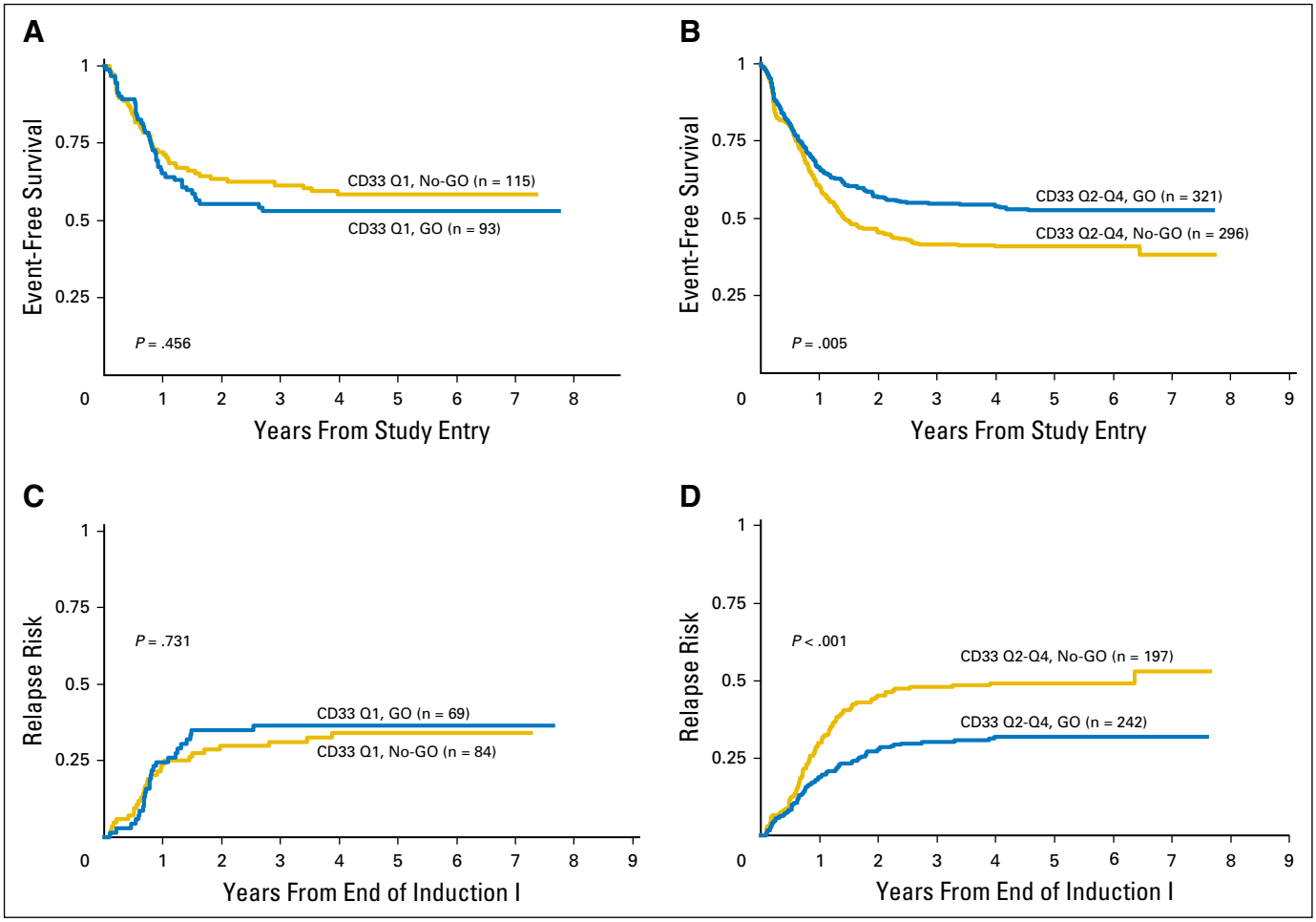

Fig 2. Association of CD33 levels and GO response. (A) Event-free survival from study entry for patients with low CD33 expression levels (Q1) when analyzed by treatment arm. (B) Event-free survival from study entry for patients with higher CD33 expression levels (Q2 to Q4) when analyzed by treatment arm. (C) Relapse risk from complete remission for patients with low CD33 expression levels (Q1) when analyzed by treatment arm. (D) Relapse risk from complete remission for patients with higher CD33 expression levels (Q2 to Q4) when analyzed by treatment arm. GO, gemtuzumab ozogamicin; Q, quartile.

Association of CD33 Expression and GO Response By Disease Risk Group

We also assessed the clinical impact of GO within the context of CD33 expression and risk group. LR patients with the lowest CD33 expression (Q1) had comparable outcomes regardless of GO (Table 3; Fig 3A), whereas LR patients with higher CD33 expression (Q2 to Q4) had improved EFS from study entry as well as DFS from CR when treated with GO (EFS from study entry: GO 77 ± 8% versus No-GO 60 ± 10%, $P = .020$; DFS from CR: GO 79 ± 9% versus No-GO 59 ± 11%, $P = 0.007$; Table 3). This translated into a lower RR for patients with higher CD33 expression treated with GO therapy (RR from CR: GO 13 ± 7% *v* No-GO 35 ± 11%, $P = .001$; Table 3; Fig 3B). Outcomes for patients with IR disease also demonstrated a differential GO effect. Specifically, IR patients with the lowest CD33 expression had similar outcomes regardless of GO therapy (Table 3; Fig 3C), whereas IR patients with higher CD33 expression seemed to have a significant improvement in EFS from study entry, a trend toward improved DFS from CR, and a reduction in RR when treated with GO versus conventional chemotherapy alone (EFS from study entry: GO 44 ± 8% *v* No-GO 33 ± 8%, $P = .021$; DFS from CR: GO 51 ± 9% *v* No-GO 40 ± 10%, $P = .078$; RR from CR: GO 44 ± 9% *v* No-GO 57 ± 10%, $P = .044$; Table 3; Fig 3D). HR patients with low CD33 expression had no benefit from GO (Table 3; Fig 3E). However, HR patients with high CD33 expression treated with GO had an RR from CR of 40 ± 18% compared with 73 ± 22% for patients treated without GO ($P = .016$) with a corresponding DFS from CR of 47% ± 18% *v* 28 ± 21% ($P = 0.135$; Table 3; Fig 3F).

DISCUSSION

In this prospective clinical trial in which pediatric patients with de novo AML were randomly assigned to receive GO in combination with conventional chemotherapy, we demonstrate that GO has limited benefit in patients with low CD33 expression but significantly decreases relapse and improves EFS and DFS in patients with high CD33 expression. This differential effect in which GO benefits only patients with higher CD33 expression was observed in all risk groups.

Previous in vitro analysis found a direct quantitative relationship between CD33 expression and GO-induced cytoxicity in

Table 3. Clinical Outcome Analysis by Treatment Arm (No-GO *v* GO) and Risk Group for Patients With Low (Q1) Versus Higher (Q2 to Q4) CD33 Expression

	All Patients (%)					
	CD33 Q1 (n = 105)		*P*	CD33 Q2-Q4 (n = 202)		*P*
Low-risk patients	No-GO (n = 57)	GO (n = 48)		No-GO (n = 96)	GO (n = 106)	
5-year OS from study entry, mean ± SE	83 ± 11	85 ± 10	.959	78 ± 9	83 ± 8	.425
5-year EFS from study entry, mean ± SE	69 ± 12	67 ± 14	.750	60 ± 10	77 ± 8	.020
	No-GO (n = 46)	GO (n = 39)		No-GO (n = 79)	GO (n = 89)	
5-year DFS from end of induction I, mean ± SE	71 ± 14	69 ± 15	.725	59 ± 11	79 ± 9	.007
5-year RR from end of induction I, mean ± SE	29 ± 14	26 ± 14	.837	35 ± 11	13 ± 7	.001
	CD33 Q1 (n = 73)			CD33 Q2-Q4 (n = 317)		
Intermediate-risk patients	No-GO (n = 40)	GO (n = 33)		No-GO (n = 157)	GO (n = 160)	
5-year OS from study entry, mean ± SE	78 ± 13	59 ± 18	.116	50 ± 8	59 ± 8	.100
5-year EFS from study entry, mean ± SE	52 ± 16	41 ± 17	.290	33 ± 8	44 ± 8	.021
	No-GO (n = 28)	GO (n = 25)		No-GO (n = 95)	GO (n = 119)	
5-year DFS from end of induction I, mean ± SE	57 ± 19	43 ± 20	.428	40 ± 10	51 ± 9	.078
5-year RR from end of induction I, mean ± SE	43 ± 19	53 ± 21	.647	57 ± 10	44 ± 9	.044
	CD33 Q1 (n = 27)			CD33 Q2-Q4 (n = 87)		
High-risk patients	No-GO (n = 16)	GO (n = 11)		No-GO (n = 39)	GO (n = 48)	
5-year OS from study entry, mean ± SE	41 ± 26	61 ± 31	.251	44 ± 16	48 ± 15	.805
5-year EFS from study entry, mean ± SE	38 ± 24	36 ± 29	.977	24 ± 14	31 ± 14	.608
	No-GO (n = 8)	GO (n = 4)		No-GO (n = 20)	GO (n = 30)	
5-year DFS from end of induction I, mean ± SE	63 ± 34	75 ± 43	.653	28 ± 21	47 ± 18	.135
5-year RR from end of induction I, mean ± SE	25 ± 33	25 ± 50	.997	73 ± 22	40 ± 18	.016

NOTE. SE is two standard errors of the mean (± 2SE %).
Abbreviations: DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; OS, overall survival; Q, quartile; RR, relapse risk.

an AML cell line forced to express different levels of CD33 by way of lentivirus-mediated gene transfer.[22] Furthermore, Jager et al[23] demonstrated in an in vitro model that high CD33 production predicted high intracellular GO exposure, a surrogate marker for GO response. Culture of $CD34^+/CD38^-/CD123^+$ leukemia stem/progenitor cells with GO was also affected by CD33 expression; those samples with high CD33 expression grew less in the presence of GO.[24] Despite these findings, initial correlative analyses of single-agent phase II AML trials of GO in adults suggested that GO response is independent of CD33 expression levels.[25,26] However, reanalysis of these data with the use of a larger sample size and different methodology for CD33 analysis refuted the results and demonstrated a direct relation between CD33 expression and GO response.[27]

To date, five large adult de novo AML trials have included GO randomization. Four of the five suggested that GO treatment during induction, particularly for LR and IR patients, significantly improves outcome.[1-5,7] The fifth trial, Southwest Oncology Group S0106, did not show a clinical benefit of GO. However, this study used lower anthracycline doses for patients on the GO arm compared with the control arm, which may have affected the ability to detect GO benefit.[28] Despite this study design limitation, patients with favorable cytogenetics demonstrated a trend toward improved survival with GO treatment. A meta-analysis of these five randomized trials suggested that GO significantly reduced RR and improved relapse-free survival (RFS) and OS despite slightly higher rates of early mortality.[7,9] LR patients, and to some degree IR patients, experienced the most pronounced GO effect.[9] A second meta-analysis of 11 randomized clinical trials of GO suggested that GO improved RFS without clear benefit on OS as a result of increased induction deaths. Analysis by risk classification suggested that GO improved OS in patients with favorable cytogenetics but not IR or HR cytogenetics.[8] The current data further support the benefit of GO for LR patients but as demonstrated in Table 3, suggest that the benefit of GO may be restricted to LR patients with higher CD33 expression.

Initial single-agent GO trials mandated a threshold level of CD33 expression for patients to be eligible for study enrollment. Subsequent adult combination chemotherapy trials did not exclude CD33-negative disease, which raises the question of whether GO is efficacious in all patients or only in those with a threshold level of CD33 expression. To date, only the Medical Research Council has published data on this issue. Analysis of the study cohort by treatment arm and CD33 positivity versus negativity found that CR, OS, and cumulative incidence of death in CR were comparable for patients with CD33-negative versus -positive disease and was independent of GO exposure. However, RFS was better and RR lower for patients with CD33-positive disease receiving GO treatment.[2]

Pediatric studies of GO plus combination chemotherapy also suggested that GO may provide benefit. In the COG pilot study AAML03P1, 350 patients with de novo AML received GO and had 3-year OS and EFS rates of 66% and 53%, respectively, which compared favorably to previously published cooperative pediatric AML trials.[13] High CD33 expression was correlated with negative prognostic features, and patients with the highest CD33 expression had significantly lower OS and DFS from CR.[17] COG AAML0531, the GO randomization trial, found that GO significantly improved 3-year EFS (GO 53% *v* No-GO 47%, $P = .04$) but not OS (69% *v* 65%, $P = .39$) possibly due to increased postremission toxic mortality for those patients who received GO (7% *v* 4%, $P = .09$). Notably, RR was significantly reduced among GO recipients (33% *v* 41%, $P = .006$), which translated into improved DFS for those receiving GO (61% *v* 55%, $P = .07$).[16] Two additional pediatric studies explored the value of GO as postremission therapy in AML. Hasle et al[14] failed to show a clear benefit for using GO during consolidation therapy, although time to relapse was prolonged, but not significantly, with GO treatment. A second study demonstrated that GO consolidation was

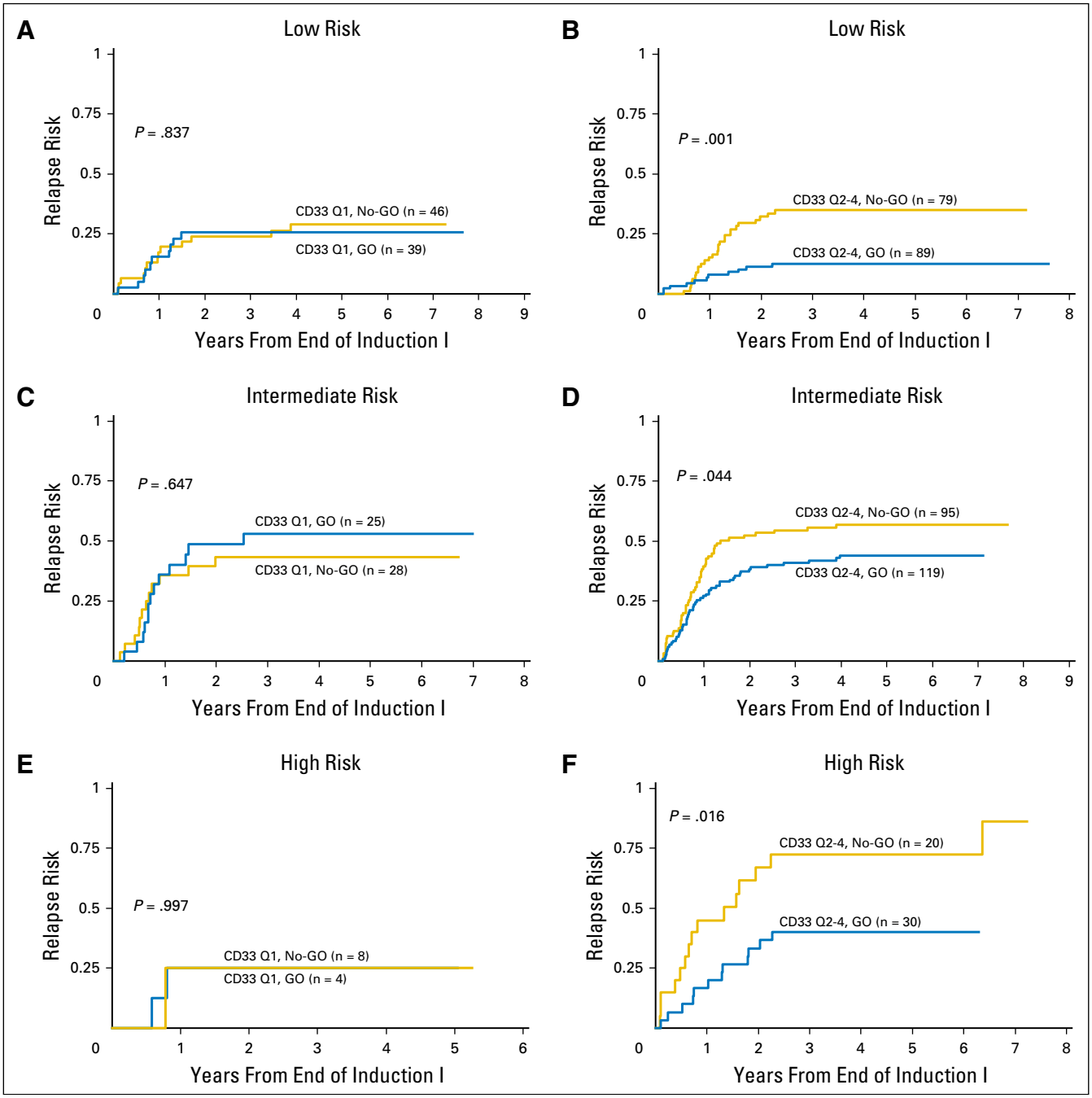

Fig 3. Association of CD33 levels and GO response by treatment arm and risk group. Relapse risk from complete remission by treatment arm. (A) Low-risk patients with low CD33 expression (Q1). (B) Low-risk patients with high CD33 expression (Q2 to Q4). (C) Intermediate-risk patients with low CD33 expression (Q1). (D) Intermediate-risk patients with high CD33 expression (Q2 to Q4). (E) High-risk patients with low CD33 expression (Q1). (F) High-risk patients with high CD33 expression (Q2 to Q4). GO, gemtuzumab ozogamicin; Q, quartile.

effective at reducing MRD levels pre-HSCT without increasing transplant-related toxicity.[12,15] Taken together, CD33-targeted agents may show promise in pediatric AML within the context of induction treatment and possibly during consolidation cycles.

We acknowledge that the current data did not demonstrate a discrete biologically defined threshold of CD33 expression in which CD33-targeted therapeutics would be clearly justified. However, analysis of outcome by individual quartile (Table 2; Appendix Fig A2, online only) suggests that patients with the lowest CD33 expression do not benefit from GO. In contrast, Q2 patients had significantly improved EFS, DFS, and RR when treated with GO. Patients in Q3 and Q4 had either a statistically significant improvement or a trend toward better outcome with GO therapy (Table 2; Appendix Fig A2), which suggests that GO can abrogate inferior clinical outcomes observed in patients with higher (Q2 to Q4) CD33 expression. Analysis with alternative cut points (Q1 to Q2 *v* Q3 to Q4; Appendix Table A1, online only) demonstrates a clear benefit of GO for patients in Q3 to Q4 as well a relative contribution of GO toward reduction of

RR within Q1 to Q2, which likely reflects the positive impact GO has on Q2 treatment response (Table 2).

The current study is significant as the first to our knowledge to demonstrate, within the context of a large randomized pediatric AML trial, that GO therapy is unlikely to have clinical benefit in patients with low CD33 expression. Conversely, in patients with high CD33 expression, GO seems to significantly improve disease-free response in all risk groups. Given limited commercial access to GO, additional studies of novel CD33-targeted agents, like SGN-CD33A, are needed in pediatric AML. However, the efficacy of SGN-CD33A needs to be critically evaluated in patients with intrinsically low CD33 expression in light of preclinical data that suggest it may have limited efficacy when CD33 expression is low or absent.[29] If second-generation CD33-targeted therapeutics further demonstrate a selective benefit in patients with high CD33 expression, future clinical trials may warrant conventional therapy de-escalation for this subset of patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

CD33 Expression and Its Association With Gemtuzumab Ozogamicin Response: Results From the Randomized Phase III Children's Oncology Group Trial AAML0531

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Appendix

Table A1. Clinical Outcome Analysis by Treatment Arm (No-GO *v* GO) for Lower (Q1 to Q2) Versus Higher (Q2 to Q4) CD33 Expression

	All Patients (%)					
	CD33 Q1-Q2 (n = 413)		*P*	CD33 Q3-Q4 (n = 412)		*P*
	No-GO (n = 214)	GO (n = 199)		No-GO (n = 197)	GO (n = 215)	
5-year OS from study entry, mean ± SD	68 ± 7	71 ± 7	.706	58 ± 7	62 ± 7	.350
5-year EFS from study entry, mean ± SD	51 ± 7	57 ± 7	.230	40 ± 7	49 ± 7	.086
	No-GO (n = 157)	GO (n = 147)		No-GO (n = 124)	GO (n = 164)	
5-year DFS from end of induction I, mean ± SD	58 ± 8	65 ± 8	.281	45 ± 9	56 ± 8	.035
5-year RR from end of induction I, mean ± SD	40 ± 8	28 ± 8	.036	50 ± 9	37 ± 8	.016

Abbreviations: DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; OS, overall survival; Q, quartile; RR, relapse risk.

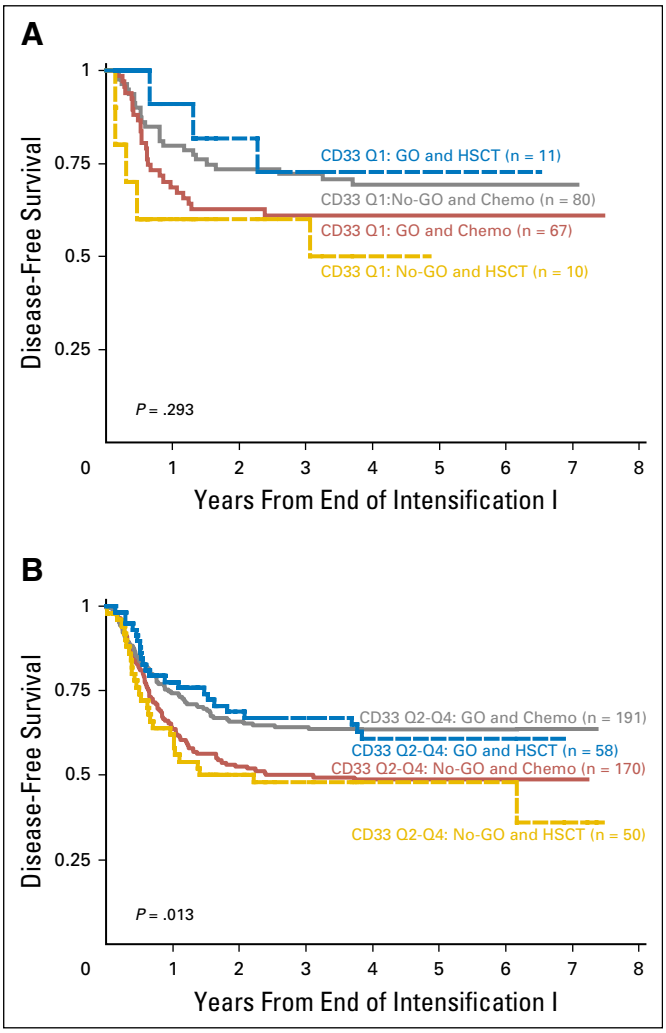

Fig A1. Association of CD33 levels and GO response by treatment arm (GO *v* No-GO) and consolidation therapy (HSCT *v* chemotherapy) for patients with (A) low (Q1) CD33 expression and (B) higher (Q2to Q4) CD33 expression. Chemo, chemotherapy; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplantation; Q, quartile.

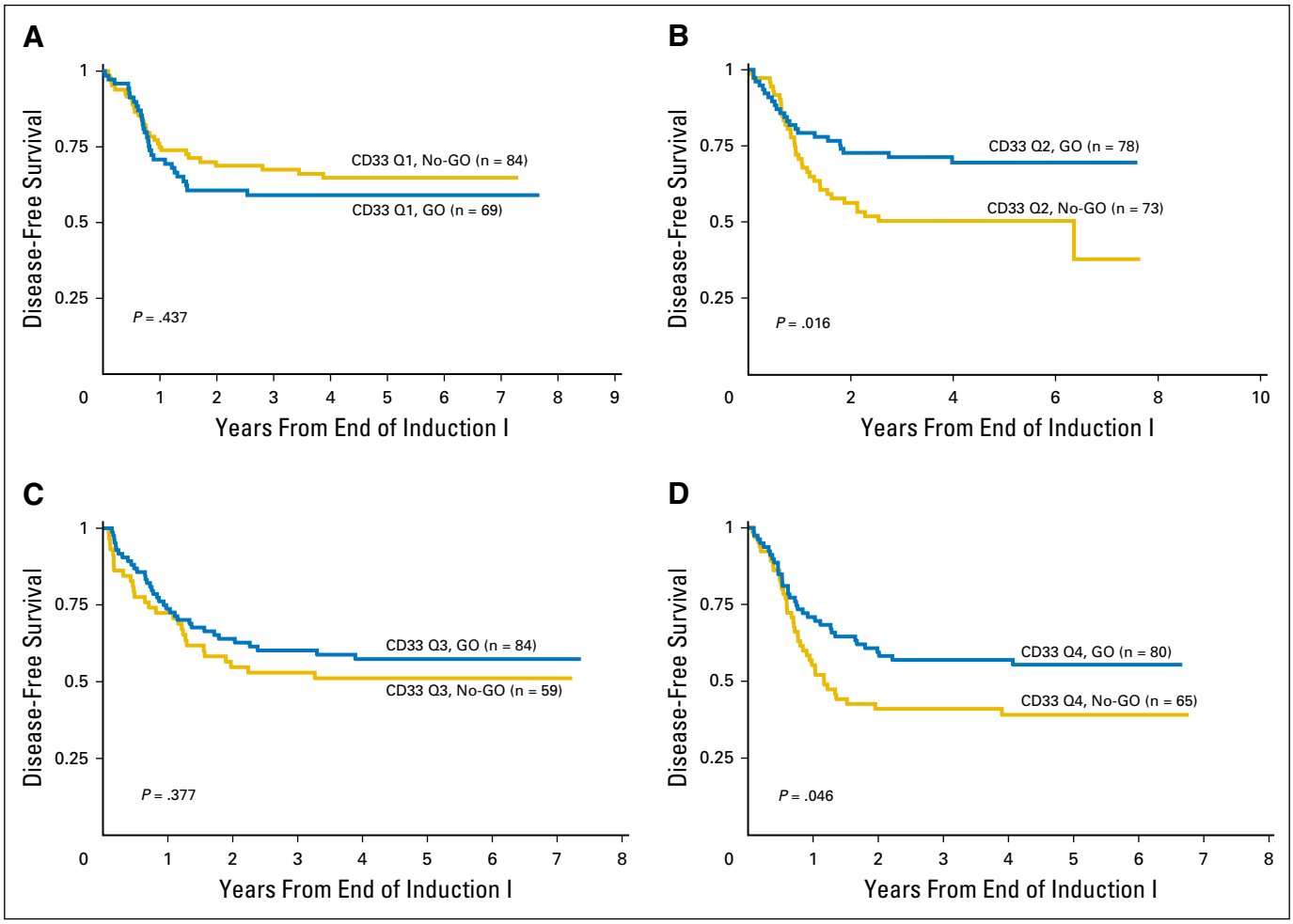

Fig A2. Disease-free survival by quartile and treatment arm (GO *v* No-GO) for (A) Q1, (B) Q2, (C) Q3, and (D) Q4. Chemo, chemotherapy; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplantation; Q, quartile.