

CD33 Splicing Polymorphism Determines Gemtuzumab Ozogamicin Response in De Novo Acute Myeloid Leukemia: Report From Randomized Phase III Children's Oncology Group Trial AAML0531

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A B S T R A C T

Purpose

Gemtuzumab ozogamicin (GO), a CD33-targeted immunoconjugate, is a re-emerging therapy for acute myeloid leukemia (AML). CD33 single nucleotide polymorphism rs12459419 C>T in the splice enhancer region regulates the expression of an alternatively spliced CD33 isoform lacking exon2 (D2-CD33), thus eliminating the CD33 IgV domain, which is the antibody-binding site for GO, as well as diagnostic immunophenotypic panels. We aimed to determine the impact of the genotype of this splicing polymorphism in patients with AML treated with GO-containing chemotherapy.

Patients and Methods

CD33 splicing single nucleotide polymorphism was evaluated in newly diagnosed patients with AML randomly assigned to receive standard five-course chemotherapy alone (No-GO arm, n = 408) or chemotherapy with the addition of two doses of GO once during induction and once during intensification (GO arm, n = 408) as per the Children's Oncology Group AAML0531 trial.

Results

The rs12459419 genotype was CC in 415 patients (51%), CT in 316 patients (39%), and TT in 85 patients (10%), with a minor allele frequency of 30%. The T allele was significantly associated with higher levels of D2-CD33 transcript ($P < 1.0E^{-6}$) and with lower diagnostic leukemic cell surface CD33 intensity ($P < 1.0E^{-6}$). Patients with the CC genotype had significantly lower relapse risk in the GO arm than in the No-GO arm (26% v 49%; $P < .001$). However, in patients with the CT or TT genotype, exposure to GO did not influence relapse risk (39% v 40%; $P = .85$). Disease-free survival was higher in patients with the CC genotype in the GO arm than in the No-GO arm (65% v 46%, respectively; $P = .004$), but this benefit of GO addition was not seen in patients with the CT or TT genotype.

Conclusion

Our results suggest that patients with the CC genotype for rs12459419 have a substantial response to GO, making this a potential biomarker for the selection of patients with a likelihood of significant response to GO.

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease that is associated with poor outcome. Although intensive chemotherapy and hematopoietic stem-cell transplantation (SCT) remain the mainstay of current AML therapy, targeted therapies such as monoclonal antibodies and small-molecule inhibitors have emerged as

promising novel approaches. Gemtuzumab ozogamicin (GO), a humanized anti-CD33 antibody linked to the toxin calicheamicin, targets CD33 antigen present on the majority of AML blasts.^{1,2} GO received an accelerated approval in 2000 by the US Food and Drug Administration for the treatment of relapsed AML in patients older than 60 years of age,³ but it was withdrawn because of a lack of benefit and a high early mortality in the SWOG-S0106 study.⁴ However, four

ASSOCIATED CONTENT



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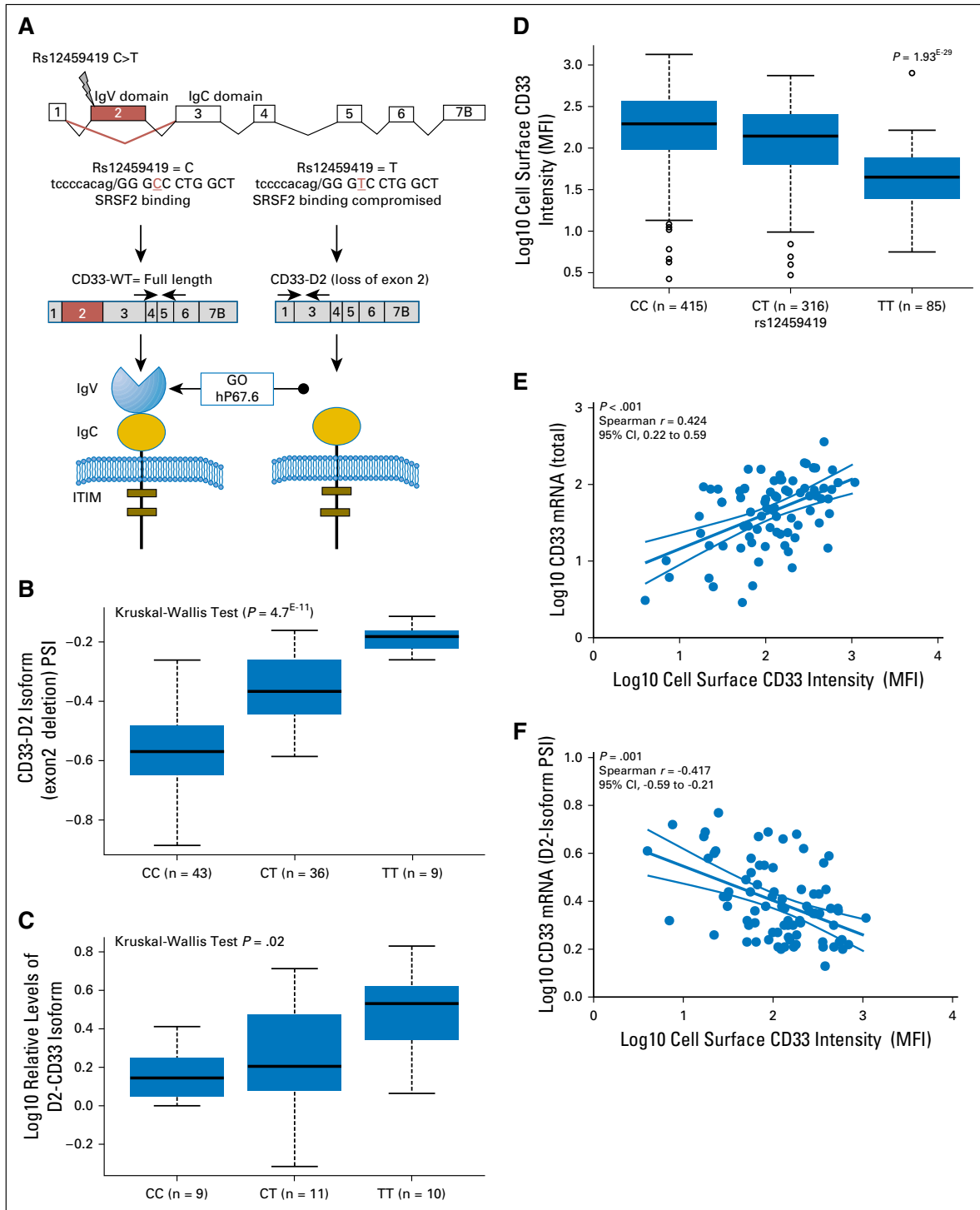


Fig 1. CD33 exon2 single nucleotide polymorphism (SNP) rs12459419 influences the alternative splicing of CD33. (A) CD33 gene and location and potential functional consequences of rs12459419 SNP. The presence of rs12459419 in exon2 affects the exonic enhancer binding site for SRSF2, thereby resulting in the loss of exon2 (shown in red) in the T allele. The loss of exon2 results in a shorter CD33 isoform lacking the IgV domain, which is recognized by gemtuzumab ozogamicin (GO) and currently, used antibodies. (B) Transcriptome-sequencing data from AAML0531 patients (n = 88) shown as percent spliced isoform (PSI) with loss of exon2 for different rs12459419 SNP genotypes. (C) Quantitative real-time polymerase chain reaction (Data Supplement) using isoform-specific primers shown in (A) in an independent set of patient specimens (n = 30) obtained at diagnosis. Log10 relative levels of the D2-CD33 isoform are shown for the rs12459419 genotypes (patient numbers in parentheses). Box plots show medians as a line between the boxes representing the first and third quartiles; the whiskers represent the range after excluding the outliers. The outliers are defined as data points that fall outside the first and third quartiles by > 1.5 times the interquartile range. Circles outside the whiskers represent outliers. Cell membrane was created using clipart from ctker.com. (D) Association of rs12459419 with CD33 expression determined as mean fluorescence intensity (MFI) in diagnostic leukemic blasts from patients treated in AAML0531 (n = 816). CD33 levels were determined in the diagnostic leukemic blasts by multiparameter flow cytometry. Log10 CD33 MFI is shown for different rs12459419 genotypes (patient numbers in parentheses). Correlation plots show the association of CD33 MFI with levels of (E) the CD33 total mRNA levels and (F) D2-CD33 isoform. Plots were created using the GraphPad Prism software. ITIM, immunoreceptor tyrosine inhibitory motif; WT, wild-type.

large randomized studies conducted in Europe demonstrated congruently that GO lowered the relapse risk (RR) and prolonged survival in favorable-risk and, to a lesser degree, intermediate-risk, AML.⁴⁻¹¹ In pediatric AML, GO was investigated in the Children's Oncology Group (COG) trials AAML03P1 and AAML0531.^{12,13} Results from these trials suggested that GO has the potential to improve outcomes in pediatric AML and may have been withdrawn prematurely from the market.¹³⁻¹⁵

We reported previously in a small pilot study in the St Jude AML02 trial that the CD33-coding single nucleotide polymorphism (SNP) rs12459419 was associated with response.¹⁶ A follow-up study showed significant association of this SNP with CD33 cell surface expression in leukemic blasts.¹⁷ rs12459419 (C > T; Ala14Val) in exon2 is present within 4 base pairs of the intron/exon junction and resides within the exonic splicing enhancer (ESE) binding site for SRSF2.¹⁸ It has been related to the skipping of exon2, resulting in the shorter CD33 isoform (D2-CD33), which lacks the IgV domain.¹⁹⁻²¹ The exon2-encoding IgV domain is an epitope for the hP67.6-CD33 antibody, which is used for diagnostic immunophenotyping, and for the antibody that is conjugated to calicheamicin in GO. As a result, loss of this domain can not only interfere with the detection of total CD33, but also affect the binding, internalization, and clinical efficacy of GO (Fig 1A).

In this study, we evaluated rs12459419 for an association with CD33 cell surface expression in leukemic blasts and clinical outcome in patients treated with or without the addition of GO to standard chemotherapy in the AAML0531 trial.

PATIENTS AND METHODS

Patients and Treatment

Details of the AAML0531 study design, treatment regimen, and clinical outcome have been reported previously.¹³ Overall, patients with de novo AML (0 to 29 years of age) were randomly assigned to either standard five-course chemotherapy (No-GO arm) or to the same chemotherapy with the addition of two doses of 3 mg/m² GO (GO arm). Risk groups were defined as follows: low risk (LR), which included t(8;21), inv(16), or t(16;16) presence of CEBP- α and NPM mutations; high risk, which included monosomy 7, monosomy 5/5q deletion, *FLT3-ITD*, or persistent disease at the end of induction 1; and intermediate or standard risk, which included all other patients.^{13,22} This study included 816 patients with specimens and CD33 cell surface expression data available. The institutional review boards of all participating institutions approved the clinical protocol, and the COG Myeloid Disease Biology Committee approved this research.

Genotyping CD33 SNPs

CD33-coding SNP rs12459419-Ala14Val and linked promoter SNP rs3865444 were genotyped using the Sequenom (San Diego, CA) platform at the Biomedical Genomics Center, University of Minnesota. Both SNPs had a call rate of > 0.98 and were in Hardy-Weinberg equilibrium ($P > .05$).

CD33 Expression Levels on Leukemic Blasts

CD33 expression levels were determined by measuring the mean fluorescence intensity (MFI) of myeloid blasts obtained at diagnosis by flow cytometry, as described previously.²²⁻²⁴

mRNA Levels of CD33 and Its Isoforms

CD33 wild-type and alternatively spliced transcript levels were extracted from RNA sequencing (RNA-seq) data obtained from diagnostic

leukemia blasts from 88 patients. In addition, CD33 wild-type and splice variants lacking exon2 were quantitated in 30 samples by quantitative real-time polymerase chain reaction (qRT-PCR), using isoform-specific primers (Data Supplement).

Statistical Analyses

The Kruskal-Wallis exact test was used to compare CD33 cell surface levels or CD33 transcript levels across genotype groups. Clinical outcome data for patients treated in AAML0531 were analyzed as of September 30, 2014. The Kaplan-Meier method was used to estimate overall survival (OS), event-free survival (EFS), and disease-free survival (DFS; Data Supplement).²⁵ For patients in complete remission (CR), RR was defined as the time from the end of induction 1 to relapse or death, where deaths without a relapse were considered competing events. All the time-to-event end points are reported at 5 years; for DFS, it was 5 years from the end of induction 1. The significance of predictor variables was tested with the log-rank statistic for OS, EFS, and DFS and with Gray's statistic for RR. Cox proportional hazards models were used to estimate the hazard ratio (HR) for OS and EFS.²⁶ The χ^2 test was used to test the significance of observed differences in proportions. The Mann-Whitney or Wilcoxon signed-rank test was used to compare differences in medians. A P value < .05 was considered statistically significant (Data Supplement).

RESULTS

Genotype Frequency of rs12459419

Of the 816 patients genotyped for rs12459419, 415 (51%) had the CC genotype, 316 (39%) had the CT genotype, and 85 (10%) had the TT genotype, with a corresponding minor allele frequency of 30%.

Comparison of the demographics and disease characteristics across the different genotype cohorts showed no difference in genotype frequency by sex, treatment arm, disease characteristics, or receipt of SCT during treatment (Data Supplement). However, frequency of the SNP was significantly higher in white patients (0.31) than in black patients (0.13; $P < .001$). There was no difference in the clinical outcome measures between patients included in the study and those who were part of the parent clinical trial but were not included in this study (Data Supplement).

rs12459419 C>T at the ESE Binding Site Is Associated With Expression of Exon2-Skipped Transcript Lacking GO Binding Site

Splicing SNP rs12459419 is located within 4 base pairs of the exonic junction of exon2, a known binding domain for splicing factor SRSF2. The C>T change results in an Ala14Val change and alters the splicing enhancer binding site, thereby altering CD33-exon2 splicing (Fig 1A). We used RNA-seq data from 88 patients treated in AAML0531 to determine whether the minor allele (T) is associated with alternative splicing of CD33 transcript in AML. The presence of rs12459419 variant T allele was significantly associated with increased levels of an alternatively spliced D2-CD33 isoform lacking exon2 ($P < 1.0E^{-6}$; Fig 1B). qRT-PCR of RNA from leukemic blasts obtained at diagnosis in a representative set of 30 patients in AAML0531 (CC = 9, CT = 11, and TT = 10) using isoform-specific primers (Fig 1A; Data Supplement) revealed a significant association of the T allele with increased levels of the D2-CD33 isoform (Fig 1C; $P = .02$). There was no association

between total CD33 transcript levels and rs12459419 in samples from this cohort ($P > .05$) in either the RNA-seq or the qRT-PCR analysis.

Association of rs12459419 With CD33 Cell Surface Intensity in Leukemic Blasts

Given that commonly used antibodies in diagnostic immunophenotypic panels target the IgV domain (coded by exon2), we reasoned that those with minor allele (D2 isoform) would not be amenable to detection by available antibodies. rs12459419 genotype data and CD33 cell surface expression of the blast population were available for 816 patients (No-GO arm, $n = 408$; GO arm, $n = 408$). The MFI was determined using the commonly used P67.6 antibody that targets the IgV domain of CD33. Our results demonstrated that the presence of rs12459419 T allele was highly associated with lower cell surface CD33 MFI ($P < 1.0E^{-6}$; Table 1; Fig 1D) and that genotype associations were balanced within each arm, risk group, and *FLT3-ITD* status (Table 1; Data Supplement).

Correlation of RNA-seq data with CD33 cell surface expression levels by MFI showed significant positive correlation with total CD33 mRNA expression (Spearman $r = 0.424$; $P < .001$; Fig 1E) and negative correlation of the D2-CD33 isoform with CD33 MFI (Spearman $r = -0.417$; $P = .001$; Fig 1F), further supporting our speculation that the lower cell surface expression of CD33 might be, in part, a result of the lack of detection of the expressed D2-CD33 isoform by current diagnostic immunophenotypic panels.

rs12459419 Genotype Defines the Clinical Response to GO

Given that the rs12459419 variant influences the ESE binding site and is associated with the expression of the variant CD33 lacking the GO binding domain, we hypothesized that the response to GO would be limited to the rs12459419 genotype encoding the full-length protein. We correlated the rs12459419 SNP genotype with clinical end points OS, EFS, DFS, RR, and treatment-related mortality (TRM) in 816 patients in the GO versus the No-GO arms (No-GO arm, $n = 408$; GO arm, $n = 408$). Comparisons between patients included in the analyses with eligible AAML0531 patients who consented to banking ($n = 816$) versus those who were not

included in the analyses ($n = 163$) showed no statistically significant differences in the outcome (Data Supplement). Patients with the rs12459419 homozygous CC had an RR of $26\% \pm 7\%$ in the GO arm, whereas those in the No-GO arm had an RR of $49\% \pm 9\%$ (HR, 0.468; $P < .001$). The corresponding DFS rates for patients with the CC genotype in the GO and No-GO arms were $65\% \pm 7\%$ and $46\% \pm 9\%$, respectively (HR, 0.597; $P = .004$; Table 2; Fig 2). In contrast, GO exposure provided no clinical benefit in RR in patients with the heterozygous CT or the homozygous TT genotype (CT: $38\% \pm 9\% \nu 37\% \pm 10\%$ [GO ν No-GO]; $P = .975$; TT: $46\% \pm 20\% \nu 46\% \pm 20\%$ [GO ν No-GO]; $P = .798$) or DFS (CT: $56\% \pm 9\% \nu 60\% \pm 10\%$ [GO ν No-GO]; $P = .821$; TT: $51\% \pm 20\% \nu 54\% \pm 18\%$ [GO ν No-GO]; $P = .972$; Fig 2). Given the similar impact of GO on the CT and TT genotypes, these two cohorts were combined in all additional analyses (Data Supplement). We also performed tests of interaction, and our results show a significant interaction between SNP genotype and treatment of DFS ($P = .008$) and RR (.031).

Clinical Outcome for Patients With rs12459419 Genotypes by Risk Group and CD33 Expression

Previous studies have reported that the clinical impact of GO is limited to those with favorable cytogenetics or to those with high CD33 expression.^{12,13} Thus, we evaluated the impact of the CD33 genotype in the efficacy of GO in different risk groups, as well as in high versus low CD33 expression cohorts. Patients in the LR group, as defined by the presence of t(8;21) or Inv(16) translocations or NPM1 or CEBPa mutations, with the CC genotype treated with GO had an RR from remission of $10\% \pm 8\%$ versus $37\% \pm 13\%$ for No-GO patients ($P < .001$). In contrast, those with LR disease with the CT or TT genotype did not benefit from GO (Table 2; Fig 3). Standard-risk patients with the CC genotype had an RR of $41\% \pm 12\%$ versus $59\% \pm 12\%$ ($P = .056$); high-risk patients had an RR of $36\% \pm 27\%$ versus $70\% \pm 32\%$ ($P = .073$) for the GO and No-GO arms, respectively. As with the LR patients, addition of GO in patients with the CT or TT genotype showed no benefit (Table 2; Fig 3). These data provide the rationale that GO is efficacious in patients with the CC genotype (expression of antibody-binding domain), with a significant effect in patients in the LR group and a moderate impact in those in the high- and standard-risk groups.

Table 1. Association of rs12459419 Genotype With Cell Surface CD33 Expression in Leukemic Cells From Patients With AML (AAML0531 Clinical Trial)

rs12459419	Total	CC (n = 415)			TT (n = 85)			CT (n = 316)			Genotype Comparison
	No.	No.	Median	Range	No.	Median	Range	No.	Median	Range	P
CD33 expression											
All	816	415	195.00	(2.65-1,351)	85	44.50	(6-813)	316	140.84	(3-748.47)	< .001
No-GO arm	408	204	205.15	(4.27-1,077.7)	46	33.40	(6-813)	158	126.47	(3-748.47)	< .001
GO arm	408	211	175.00	(2.68-1,351)	39	58.62	(6.84-134.47)	158	158.77	(4-713)	< .001
Standard-risk group	385	192	279.26	(2.68-1,351)	44	56.49	(6-813)	149	183.00	(3-713)	< .001
Low-risk group	306	159	124.00	(11.23-876)	28	25.34	(6.84-91)	119	87.31	(5-545.03)	< .001
High-risk group	111	55	225.74	(4.27-1,225.87)	11	50.55	(12-139.47)	45	178.73	(9.83-748.47)	< .001
FLT3+	133	66	254.26	(4.27-1,225.87)	15	55.90	(12-165.1)	52	213.28	(25-706)	< .001
FLT3-	670	341	173.00	(2.68-1,351)	68	35.48	(6-165.14)	261	133.85	(3-748.47)	< .001

Abbreviations: AML, acute myeloid leukemia; FLT3, FMS-like tyrosine kinase 3 receptor; GO, standard five-course chemotherapy with the addition of two doses of 3 mg/m² gemtuzumab ozogamicin; No-GO, standard five-course chemotherapy.

Table 2. Association of CD33 SNP rs12459419 With Clinical Outcome by Treatment Arm in Patients Treated in the COG AAML0531 Clinical Trial

Clinical Outcome	All Patients (N = 816)			CC (n = 415)			CT (n = 316)			TT (n = 85)		
	No-Go	GO	P	No-Go	GO	P	No-Go	GO	P	No-Go	GO	P
All patients												
No.	408	408		204	211		158	158		46	39	
5-year outcome from study entry												
OS	64 ± 5	66 ± 5	.506	58 ± 7	66 ± 7	.159	69 ± 7	64 ± 8	.658	70 ± 14	69 ± 15	.824
EFS	46 ± 5	53 ± 5	.055	41 ± 7	54 ± 7	.041	53 ± 8	51 ± 8	.749	44 ± 15	49 ± 17	.461
No.	408	408		145	154		101	125		34	30	
5-year outcome from end of course 1												
DFS	52 ± 6	60 ± 6	.050	46 ± 9	65 ± 7	.004	60 ± 10	56 ± 9	.821	54 ± 18	51 ± 20	.972
RR	44 ± 6	33 ± 5	.004	49 ± 9	26 ± 7	< .001	37 ± 10	38 ± 9	.975	46 ± 18	46 ± 20	.798
TRM	3 ± 2	7 ± 3	.071	4 ± 3	9 ± 5	.135	3 ± 3	5 ± 4	.496	—	3 ± 7	.290
Low risk												
All Patients (n = 306) CC (n = 159) CT (n = 119) TT (n = 28)												
No.	153	153		79	83		61	58		16	12	
5-year outcome from study entry												
OS	81 ± 7	83 ± 6	.688	73 ± 11	90 ± 7	.014	88 ± 8	74 ± 12	.058	88 ± 17	83 ± 22	.761
EFS	63 ± 8	73 ± 7	.096	55 ± 12	82 ± 9	.001	73 ± 11	64 ± 13	.276	60 ± 26	58 ± 28	.929
No.	125	128		65	69		46	48		14	1	
5-year outcome from end of course 1												
DFS	63 ± 9	76 ± 8	.045	57 ± 13	84 ± 9	.001	72 ± 13	70 ± 14	.794	61 ± 28	55 ± 30	.683
RR	33 ± 9	17 ± 7	.005	37 ± 13	10 ± 8	< .001	26 ± 13	19 ± 11	.483	39 ± 30	45 ± 32	.683
TRM	4 ± 4	8 ± 5	.284	6 ± 6	6 ± 6	.944	2 ± 4	11 ± 10	.107	0 ± 0	0 ± 0	1.000
Standard risk												
All patients (n = 385) CC (n = 192) CT (n = 149) TT (n = 44)												
No.	195	190		101	91		72	77		22	22	
5-year outcome from study entry												
OS	56 ± 7	59 ± 8	.488	49 ± 11	56 ± 11	.599	64 ± 12	60 ± 12	.936	59 ± 21	64 ± 21	.684
EFS	37 ± 7	44 ± 7	.139	33 ± 10	42 ± 10	.397	44 ± 12	44 ± 12	.532	31 ± 20	47 ± 23	.197
No.	122	142		68	67		40	60		14	15	
5-year outcome from end of course 1												
DFS	44 ± 9	49 ± 9	.276	38 ± 12	51 ± 12	.181	55 ± 16	46 ± 14	.721	43 ± 26	50 ± 28	.439
RR	54 ± 9	47 ± 9	.174	59 ± 12	41 ± 12	.056	43 ± 16	52 ± 14	.635	57 ± 28	50 ± 30	.441
TRM	2 ± 3	4 ± 3	.445	3 ± 4	8 ± 7	.248	3 ± 5	2 ± 3	.766	0 ± 0	0 ± 0	1.000
High risk												
All patients (n = 111) CC (n = 55) CT (n = 45) TT (n = 11)												
No.	54	57		24	31		24	21		6	5	
5-year outcome from study entry												
OS	43 ± 15	49 ± 14	.460	46 ± 23	40 ± 19	.767	32 ± 20	62 ± 21	.056	56 ± 50	—	.639
EFS	28 ± 13	33 ± 13	.636	29 ± 19	23 ± 15	.222	21 ± 18	48 ± 22	.037	—	—	.981
No.	28	34		10	14		14	16		4	4	
5-year outcome from end of course 1												
DFS	37 ± 19	50 ± 17	.233	30 ± 29	43 ± 26	.500	28 ± 26	56 ± 25	.112	—	—	.620
RR	60 ± 20	38 ± 17	.071	70 ± 32	36 ± 27	.073	65 ± 31	44 ± 26	.261	25 ± 50	25 ± 50	.919
TRM	4 ± 7	12 ± 11	.255	0 ± 0	21 ± 23	.129	7 ± 14	0 ± 0	.286	0 ± 0	25 ± 53	.330

NOTE. Data are presented as 5-year estimates ± 2 SE. P values < .05 are indicated in bold.

Abbreviations: COG, Children's Oncology Group; DFS, disease-free survival; EFS, event-free survival; GO, standard five-course chemotherapy with the addition of two doses of 3 mg/m² gemtuzumab ozogamicin; No-GO, standard five-course chemotherapy; OS, overall survival; RR, relapse risk; SNP, single nucleotide polymorphism; TRM, treatment-related mortality.

On the basis of our recently published study on the impact of CD33 expression and the efficacy of GO, we evaluated the association of rs12459419 genotypes in patients with low and high CD33 cell surface expression quartile 1 (n = 206) and quartiles 2 to 4 (n = 610), respectively, as defined by Pollard et al.²² As expected, patients with the CC genotype (n = 353 [58%]) were more common than those with the CT or TT genotype (n = 260 [42%]) in the higher CD33 expression cohort (quartiles 2 to 4). Among patients in quartile 1, the CT or TT genotype (n = 144 [70%]) was more common than the CC genotype (n = 62 [30%]). The Data Supplement provides the genotype distribution of patients in the whole patient cohort, as well as within each risk group for quartile

1 and quartiles 2 to 4. In patients with high CD33 expression, those with the CC genotype in the GO arm showed significant improvement in RR compared with those in the No-GO arm (P = .001), but this effect was not observed in patients with the CT or TT genotype (P = .125). As expected, there were fewer patients with the CC genotype (n = 44 [28%]) than with the CT or TT genotype (n = 110 [72%]) in the lower CD33 expression (quartile 1) cohort. However, even within this small subset, patients with the CC genotype in the GO arm showed a similar magnitude and a trend toward improvement in RR compared with those in the No-GO arm (P = .055), but this trend was not seen in patients with the CT or TT genotype (P = .125; Data Supplement), thus further

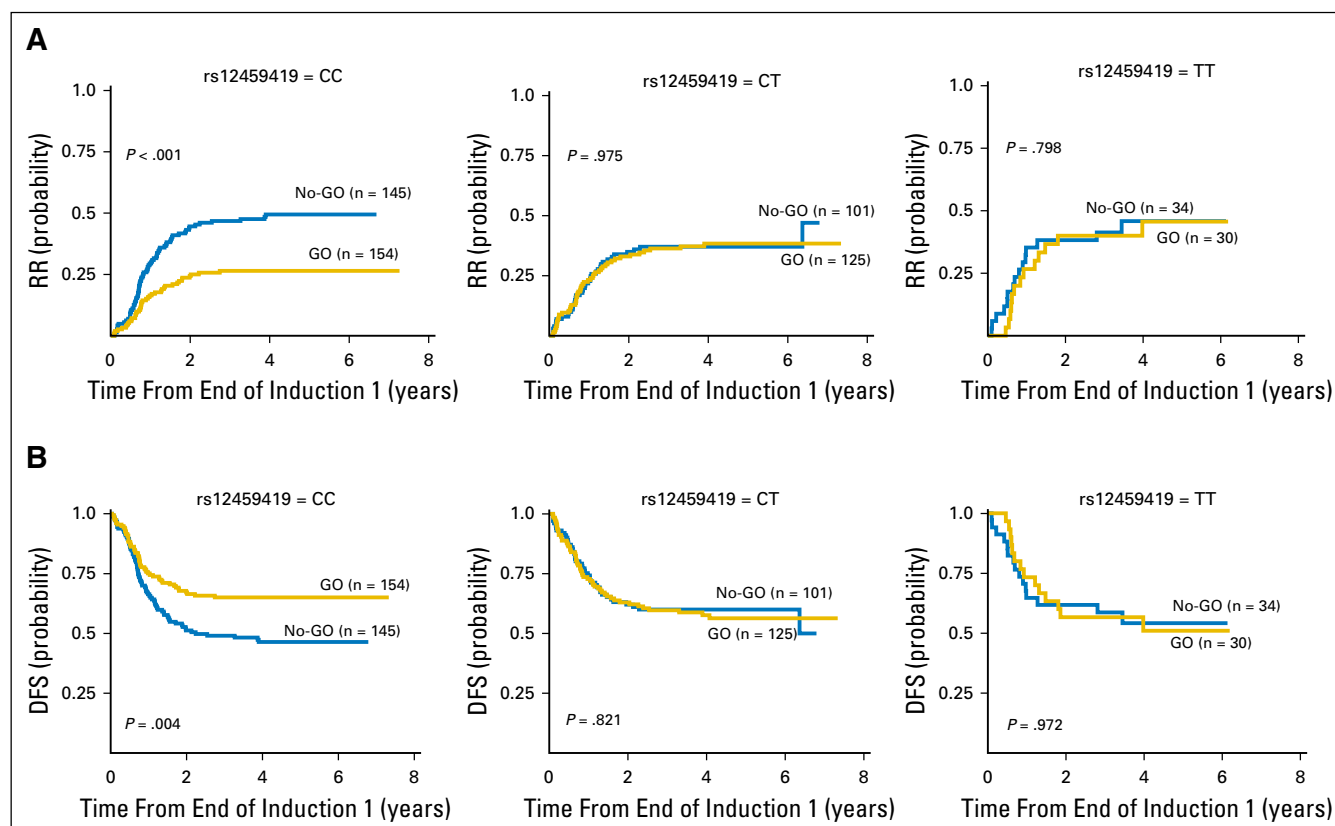


Fig 2. Association of rs12459419 genotypes with clinical response by treatment arm in patients treated in AAML0531. (A) Differences in relapse risk (RR) and (B) disease-free survival (DFS) from the end of course 1 in patients with the CC, CT, or TT genotype in the standard five-course chemotherapy with the addition of two doses of 3 mg/m² gemtuzumab ozogamicin (GO) versus the standard five-course chemotherapy alone (No-GO) arm.

emphasizing the CD33 genotype as a significant biomarker, irrespective of CD33 cell surface expression. We further evaluated the impact of this SNP in response to GO within the high CD33 expression cohort on the basis of risk status. Despite a limited sample size for LR and standard-risk patients with high CD33 expression, LR patients with the CC genotype who received GO had a significantly lower RR compared with those in the No-GO arm ($P = .003$). In standard-risk patients with the CC genotype, there was a trend in a consistent direction toward GO response ($P = .09$; Data Supplement).

A multivariate Cox regression analysis that included CD33 genotype, risk status, and CD33 expression demonstrated that the CD33 CC genotype was an independent predictor of response to GO (HR, 0.45 and $P < .001$ for RR; HR, 0.57 and $P = .003$ for DFS). In this model, LR status remained significantly associated with response to GO, whereas CD33 expression was no longer associated with response to GO (Data Supplement).

All the results were consistent for the rs3865444 promoter SNP that occurs in linkage disequilibrium (LD) with rs12459419 (Data Supplement).

DISCUSSION

The addition of GO to standard chemotherapy has shown promising results in AML, with variable outcomes in different

trials. Results from recent meta-analyses in adults with AML show that GO, when added to standard induction chemotherapy, improves survival in patients with favorable-risk and intermediate-risk cytogenetics.^{10,11} In pediatric AML, COG AAML0531 demonstrated that patient outcomes are modestly improved when GO is added to standard chemotherapy.¹³ This study further demonstrated wide interpatient variation (approximately two log-fold) in CD33 expression in leukemic cells and indicated that the impact of GO was limited to those with higher CD33 expression.²⁷⁻²⁹ These results were validated in the French ALFA trial³⁰ and are consistent with in vitro studies relating GO-induced cytotoxicity to an abundance of cell surface CD33.³¹

CD33 SNP rs12459419 has been associated with the expression of an alternatively spliced variant of CD33 lacking the GO binding site (IgV domain).¹⁹ Results from this study show that this SNP defines a genotypic-driven prediction of response to GO. In addition to defining a clinical response to GO, and given that all available diagnostic antibodies also target the CD33 IgV domain, this SNP defines the ability to reliably identify CD33 expression level.

We reported previously on the coding and regulatory polymorphisms in CD33^{16,17} and showed an association between these SNPs and cell surface CD33 expression as well as clinical outcome. In the context of the recent COG randomized trial, we were able to extend these findings and highlight the significance of SNP rs12459419 in defining response to GO. This SNP is unique by

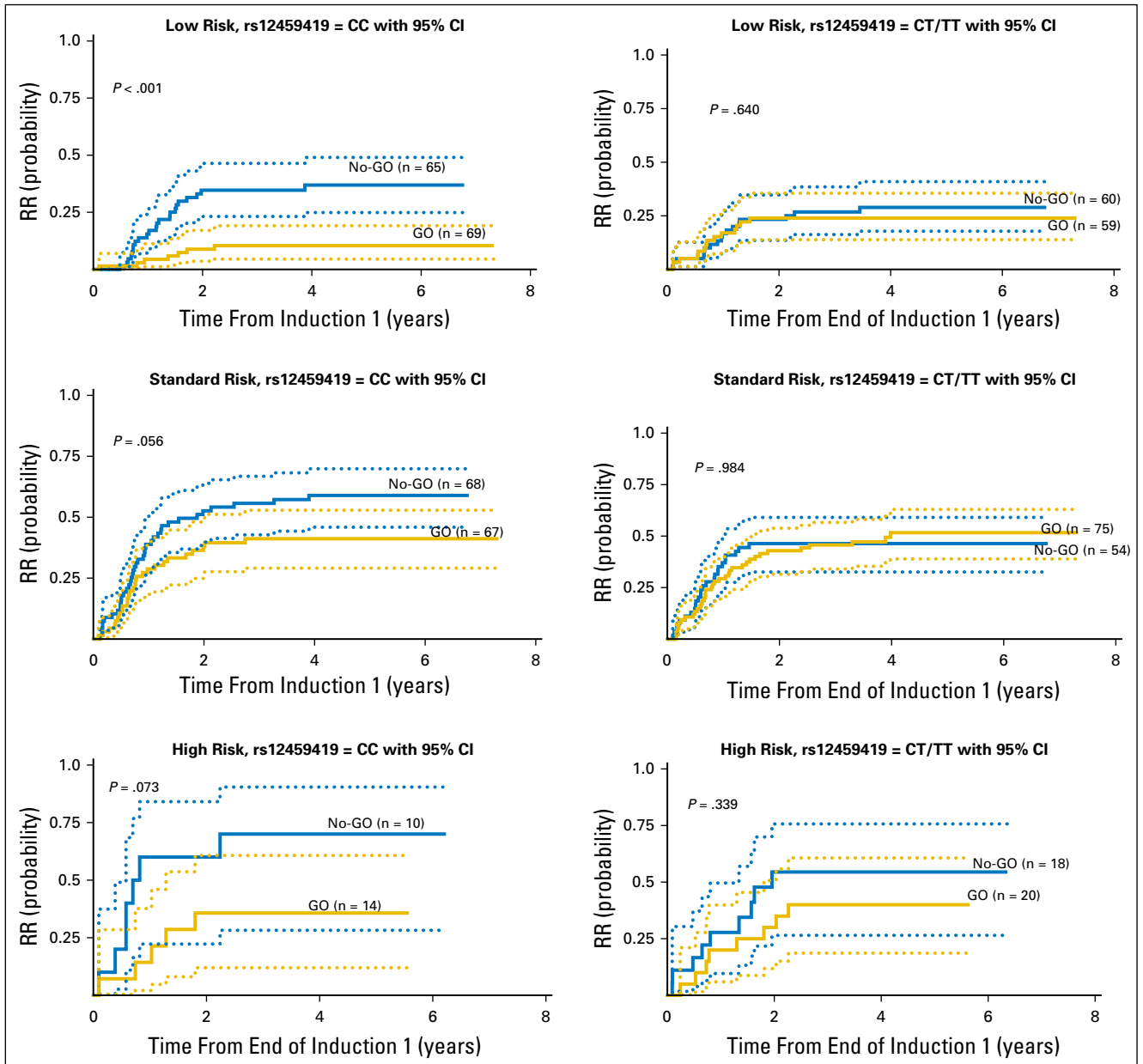


Fig 3. Association of rs12459419 genotypes with relapse risk (RR) for patients treated in AAML0531 in the low-, standard-, and high-risk groups in the standard five-course chemotherapy with the addition of two doses of 3 mg/m² gemtuzumab ozogamicin (GO) versus the standard five-course chemotherapy alone (No-GO) arm.

virtue of its location in the ESE region of exon2, and it regulates the splicing of CD33 exon2. Using transcriptome-sequencing data from AML diagnostic leukemic cells, we demonstrated that the presence of the T allele leads to a higher expression of a shorter, alternatively spliced CD33 transcript that lacks exon2 (the D2-CD33 isoform), thus lacking the GO-binding IgV domain of CD33. We further demonstrated a strong association between the rs12459419 genotype and CD33 cell surface expression (detection) on leukemic blasts.

Consistent with the fact that GO recognizes the IgV domain, our studies identified rs12459419 as a significant determinant of an eventual benefit from GO when added to conventional chemotherapy. Patients with the CC genotype had an almost 50%

reduction in RR and experienced superior DFS with the addition of GO, compared with those receiving standard chemotherapy alone. Conversely, patients with at least one T allele (which increases the production of the CD33 isoform lacking the IgV domain) did not experience any improvement in outcome when GO was administered. This was true for patients in all risk categories. As an example, the OS from study entry for patients with the CC genotype in the LR group was 90% in the GO arm versus 73% for patients with the CC genotype in the No-GO arm (Table 2).

This finding has multiple significant clinical as well as biologic implications. Because most available CD33 antibodies used for the detection or generation of CD33-targeted antibody-drug conjugates (ADCs) are directed against the IgV domain of the CD33

protein, the truncated CD33 variant lacking the IgV domain cannot be detected by hP67.7 (used in the generation of GO and the most common antibody in diagnostic panels). As a result, one would predict that patients with the CC genotype would respond to GO, whereas those with the CT genotype would have no response to GO. It is of interest that the heterozygous CT genotype, which would be expected to have both the full length as well as the short isoform, shows no response to GO. This is a presence of biologically important observation because it would indicate that the short CD33 isoform interferes with the cytotoxicity of GO. This might be a result of an interference with internalization of the ligated CD33 or of altered trafficking of the GO-CD33 conjugated complex.

CD33 rs12459419 and linked promoter SNP rs3865444 have been associated previously to late-onset Alzheimer's disease in genome-wide association analyses³² and to reduced CD33 expression levels in the hematopoietic-derived microglial cells.^{33,34} Although in-depth mechanistic studies of CD33 SNPs are warranted, our results show that the CD33 rs12459419 genotype allows for the identification of patients expressing the CD33 isoform lacking the antibody-binding site for GO. In a recent report, we characterized CD33 splice variants and demonstrated that this variant is unable to serve as a target of GO.³⁵ Perhaps more importantly, it holds promise as a marker for selecting patients who are likely to benefit from the addition of GO to chemotherapy (CC genotype) regardless of the clinical risk or the CD33 cell surface expression levels. The fact that patients heterozygous for rs12459419 (CT) do not experience any benefit from the addition of GO indicates that the prediction of outcome by SNP is more powerful than the prediction by CD33 cell surface expression and suggests that CD33 expression alone may not fully inform on the GO responsiveness of the leukemic cell subsets most relevant to disease progression or relapse.

Knowledge of the CD33 genotype and prediction of response to GO provides opportunities to use patient genotypes for selecting CD33-targeted therapies. Given that only one half of patients are expected to have a response to GO, we propose that all prior GO-containing studies be re-evaluated for response on the basis of the patients' CD33 genotype. Furthermore, the fact that patients with the full-length CD33 respond to GO argues for the potential efficacy of a CD33-ADC using an antibody targeting the IgC domain, which is shared by all CD33 isoforms, thus creating the next generation of CD33 ADCs with potential benefit for all patients with AML. Given the significance of this observation and its impact in therapeutic decision making regarding which patients should receive GO, external validation of these findings is required. These findings will likely be relevant to other CD33-targeted therapies such as SGN-CD33A and lintuzumab.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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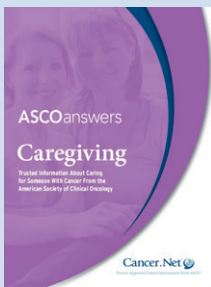
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

CD33 Splicing Polymorphism Determines Gemtuzumab Ozogamicin Response in De Novo Acute Myeloid Leukemia: Report From Randomized Phase III Children's Oncology Group Trial AAML0531

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