original reports

Gemtuzumab Ozogamicin Improves Event-Free Survival and Reduces Relapse in Pediatric *KMT2A*-Rearranged AML: Results From the Phase III Children's Oncology Group Trial AAML0531

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abstract

PURPOSE We investigated the impact of the CD33-targeted agent gemtuzumab ozogamicin (GO) on survival in pediatric patients with *KMT2A*-rearranged (*KMT2A*-r) acute myeloid leukemia (AML) enrolled in the Children's Oncology Group trial AAML0531 (NCT01407757).

METHODS Patients with *KMT2A*-r AML were identified and clinical characteristics described. Five-year overall survival (OS), event-free survival (EFS), disease-free survival (DFS), and relapse risk (RR) were determined overall and for higher-risk versus not high-risk translocation partners. GO's impact on response was determined and outcomes based on consolidation approach (hematopoietic stem cell transplant [HSCT] *v* chemotherapy) described.

RESULTS Two hundred fifteen (21%) of 1,022 patients enrolled had *KMT2A*-r AML. Five-year EFS and OS from study entry were 38% and 58%, respectively. EFS was superior with GO treatment (EFS 48% with GO *v* 29% without, $P = .003$), although OS was comparable (63% *v* 53%, $P = .054$). For patients with *KMT2A*-r AML who achieved complete remission, GO was associated with lower RR (40% GO *v* 66% patients who did not receive GO [No-GO], $P = .001$) and improved 5-year DFS (GO 57% *v* No-GO 33%, $P = .002$). GO benefit was observed in both higher-risk and not high-risk *KMT2A*-r subsets. For patients who underwent HSCT, prior GO exposure was associated with decreased relapse (5-year RR: 28% GO and HSCT *v* 73% No-GO and HSCT, $P = .006$). In multivariable analysis, GO was independently associated with improved EFS, improved DFS, and reduced RR.

CONCLUSION GO added to conventional chemotherapy improved outcomes for *KMT2A*-r AML; consolidation with HSCT may further enhance outcomes. Future clinical trials should study CD33-targeted agents in combination with HSCT for pediatric *KMT2A*-r AML.

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INTRODUCTION

Chromosomal rearrangements involving *KMT2A* on chromosome band 11q23 (hereafter *KMT2A*-rearranged [*KMT2A*-r]) occur in approximately 20% of pediatric acute myeloid leukemia (AML) cases and represent the most common recurrent cytogenetic abnormality.[1–3] More than 80 fusion partners of *KMT2A* have been characterized,[4] and clinical outcome varies depending upon the translocation partner. Specifically, event-free survival (EFS) rates of 34%-61% and overall survival (OS) of 44%-64% have been reported, although outcomes are markedly inferior for higher-risk (HR) translocations.[1,2,4–7] A recent analysis of 1,257 heterogeneously treated children with *KMT2A*-r AML demonstrate 5-year EFS of 46% and OS of 62%.[8] Given these suboptimal outcomes, novel treatment approaches are needed.

CD33 is 67-kDA transmembrane glycoprotein present on the majority of AML blasts. Higher CD33 expression correlates with negative prognostic features and significantly lower OS and disease-free survival (DFS) from complete remission (CR).[9,10] CD33 is the target of gemtuzumab ozogamicin (GO; Mylotarg, Pfizer, New York, NY), a toxin-conjugated humanized IgG4 anti-CD33 monoclonal antibody. GO is US Food and Drug Administration–approved for treatment of adult and pediatric de novo AML based on previous studies demonstrating safety and efficacy when used as monotherapy or in combination with conventional chemotherapy.[11–29]

The Children's Oncology Group (COG) Trial AAML0531 (NCT01407757) was a phase III study in which 1,070 de novo pediatric AML patients received a conventional chemotherapy backbone and were randomly assigned to GO. Patients with high-risk disease underwent

ASSOCIATED CONTENT

Appendix

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Pediatric *KMT2A*-rearranged (*KMT2A*-r) acute myeloid leukemia (AML) is a heterogenous disease with suboptimal outcome and thus, novel therapeutic approaches. Within the context of Children's Oncology Group protocol AAML0531, a phase III randomized trial of the CD33-targeted agent gemtuzumab ozogamicin (GO) in combination with conventional chemotherapy, we studied whether GO provided therapeutic benefit in *KMT2A*-r AML, both overall and within higher- and lower-risk translocation partners.

Knowledge Generated

GO significantly improved event-free survival and reduced relapse risk in *KMT2A*-r AML, both overall and in higher- and lower-risk *KMT2A*-subsets. Although intensity of CD33 expression affected GO response, even patients with lower CD33 expression benefited from GO. GO in combination with hematopoietic stem cell transplant may provide additive clinical benefit; however, this needs to be studied further prospectively.

Relevance

Treatment of *KMT2A*-r AML should include the CD33-targeting agent GO; future trials should study second-generation CD33-targeting agents and further define the role of hematopoietic stem cell transplant in this disease subset.

hematopoietic stem cell transplant (HSCT) with an optimal donor source; intermediate-risk (IR) patients went to HSCT if a matched family donor (MFD) was available. For the 1,022 evaluable patients, GO significantly improved 3-year EFS (GO 53% *v* 47%, $P = .04$) but not OS (69% *v* 65%, $P = .39$). The lack of OS benefit may have reflected the increased toxic mortality observed in patients who received post-remission GO (7% *v* 4%, $P = .09$). Notably, relapse risk (RR) was significantly reduced among GO recipients (33% *v* 41%, $P = .006$), which translated into improved DFS (61% *v* 55%, $P = .07$).[27] In a multivariable model, high CD33 expression was a negative predictor of outcome[9] but imparted a more favorable response to GO.[10] Specifically, patients with higher CD33 expression who received GO had significantly reduced RR (GO: 32% *v* patients who did not receive GO [No-GO]: 49%, $P < .001$) and improved EFS (GO: 53% *v* No-GO 41%, $P = .005$). This differential effect was observed in all cytogenetic or molecular risk groups.[10]

As pediatric *KMT2A-r AML* is characterized by higher CD33 expression compared with *KMT2A* wild-type (WT) AML,[9,10] we wanted to determine if the addition of GO conferred survival benefit for patients with *KMT2A*-r AML enrolled on AAML0531 and, if so, whether GO benefit was seen in both HR and lower risk *KMT2A*-r subsets and/or was influenced by the degree of CD33 expression present. Moreover, as AAML0531 prospectively prescribed use of HSCT for patients with *KMT2A*-r AML with an MFD or co-occurring HR features, we explored whether GO followed by HSCT had additive clinical impact.

METHODS

Patients and Treatment

Pediatric patients with de novo AML enrolled in the COG trial AAML0531 (August 2006-June 2010) were eligible for this analysis. Details of the treatment regimen used in AAML0531 have been described previously.[27] In brief, patients were treated with five cycles of anthracycline and cytarabine–based chemotherapy, with the randomized addition of GO in the experimental arm. GO 3 mg/m^2 (0.1 mg/kg if body surface area < 0.6 m^2) was given by intravenous injection on day 6 of induction 1 and day 7 of intensification 2. Patients with high-risk features, defined by presence of monosomy 7, monosomy 5/5q deletion, or persistent morphologic disease at end of induction 1 (EOI1), received allogeneic HSCT following the third course of chemotherapy and thus did not receive a second GO dose. Patients with *KMT2A*-r AML without other high-risk features were allocated to the IR group and received HSCT if an MFD was available. All *KMT2A-r* samples from patients enrolled in AAML0531 were eligible for correlative study (eg, CD33 expression determination) if consent was obtained. The institutional review boards of all participating institutions approved the clinical protocol and the COG Myeloid Disease Biology Committee approved this research.

Cytogenetic Classification

Local laboratories performed conventional (G-banded) analyses of bone marrow or peripheral blood as well as fluorescence in situ hybridization (FISH) using a series of probes that included *KMT2A*. For normal conventional karyotype but abnormal interphase FISH showing a *KMT2A*-r, metaphase FISH was performed to characterize the fusion pattern and enable detection of cryptic signal deletion. All reports were reviewed centrally by COG cytogeneticists (University of Minnesota and St Jude Children's Research Hospital). The International System for Human Cytogenetic Nomenclature-2013 was used to interpret and report results.

TABLE 1. Disease Characteristics and Clinical Response for *KMT2A*-r Acute Myeloid Leukemia by Treatment Arm (GO *v* No-GO) and by Risk Designation (HR *v* NHR)

		***KMT2A*-r: No-GO *v* GO**					**HR *KMT2A*-r: No-GO *v* GO (n = 70)**					**NHR *KMT2A*-r: No-GO *v* GO (n = 107)**				
		***KMT2A*-r No-GO**		***KMT2A*-r GO**			**HR *KMT2A*-r No-GO**		**HR *KMT2A*-r GO**			**NHR *KMT2A*-r No-GO**		**NHR *KMT2A*-r GO**		
Characteristic	**Group**	**n = 107**		**n = 108**		***P***	**n = 33**		**n = 37**		***P***	**n = 56**		**n = 51**		***P***
Age, years	Median (range)	2.03	0.003-18.7	3.3	0.02-18.3	.287	1.3	0.003-18.7	3.7	0.09-1.82	.711	2.3	0.18-18.1	4.3	0.14-18.3	.097
Sex	Male	59	55%	48	44%	.117	19	58%	15	41%	.155	33	59%	22	43%	.103
WBC × $10^3/\mu L$	Median (range)	24.2	0.5-526	29.8	0.4-610	.79	25.6	0.5-519	43.7	0.8-263.1	.455	21.95	0.9-526	12.7	0.4-610	.566
CNS-positive	Yes	8	7%	6	6%	.568	2	6%	2	5%	1.000	5	9%	3	6%	.718
Non-CNS extramedullary disease	Yes	25	23%	28	26%	.663	10	30%	17	46%	.180	13	23%	8	16%	.328
FLT3/ITD	Positive	4	4%	1	1%	.192	1	3%	1	3%	1.000	2	4%	0	0%	.495
CEPBA	Mutant	0	0%	0	0%	—	0	0%	0	0%	—	0	0%	0	0%	—
NPM1	Mutant	0	0%	0	1%	—	0	0%	0	0%	—	0	0%	0	0%	—
Cytogenetic complexity	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1-2	82	77%	77	73%	.498	28	85%	27	73%	.227	41	73%	38	75%	.879
	3+	24	23%	28	27%	.498	5	15%	10	27%	.227	15	27%	13	25%	.879
Induction 1 response	CR	68	64%	82	77%	**.035**	20	61%	23	64%	.779	35	63%	42	82%	**.022**
Induction 1 MRD	Present	18	22%	17	20%	.755	6	22%	7	25%	.809	7	18%	7	18%	.958
HSCT received	Yes	11	10%	19	18%	.122	6	18%	3	8%	.290	4	7%	11	22%	**.032**

	***KMT2A*-r: No-GO *v* GO**					**HR *KMT2A*-r: No-GO *v* GO (n = 70)**					**NHR *KMT2A*-r: No-GO *v* GO (n = 107)**				
	***KMT2A*-r No-GO**		***KMT2A*-r GO**			**HR *KMT2A*-r No-GO**		**HR *KMT2A*-r GO**			**NHR *KMT2A*-r No-GO**		**NHR *KMT2A*-r GO**		
Clinical Outcome	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***
5-Year EFS from study entry	107	29 (20 to 38)	108	48 (38 to 57)	**.003**	33	6 (1 to 18)	37	27 (14 to 41)	**.013**	56	42 (29 to 55)	51	66 (51 to 77)	**.017**
5-Year OS from study entry	107	53 (43 to 62)	108	63 (53 to 72)	.054	33	36 (21 to 52)	37	49 (32 to 65)	.139	56	67 (53 to 78)	51	76 (61 to 85)	.244
5-Year DFS from end induction I (patients in CR)	68	33 (22 to 44)	82	57 (46 to 67)	**.002**	20	10 (2 to 27)	23	29 (12 to 49)	**.053**	35	50 (32 to 65)	42	75 (59 to 86)	**.025**
5-Year RR from end induction I (patients in CR)	68	66 (53 to 76)	82	40 (29 to 51)	**.001**	20	90 (60 to 98)	23	66 (42 to 83)	**.027**	35	47 (29 to 63)	42	22 (11 to 36)	**.026**
5-Year TRM from end induction I (patients in CR)	68	2 (0.1 to 7)	82	2 (0.5 to 8)	.609	20	0 (0 to 0)	23	4 (0.3 to 19)	.355	35	3 (0.2 to 13)	42	2 (0.2 to 11)	.884

NOTE. Bold indicates statistical significance.

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; HR, higher risk; HSCT, hematopoietic stem cell transplant; ITD, internal tandem duplication; *KMT2A*-r, *KMT2A*-rearranged; MRD, minimal measurable residual disease; NHR, not high-risk; No-GO, did not receive GO; OS, overall survival; RR, relapse risk; TRM, treatment-related mortality.

KMT2A-r AML: Risk Classification of Recurrent Translocation Partners

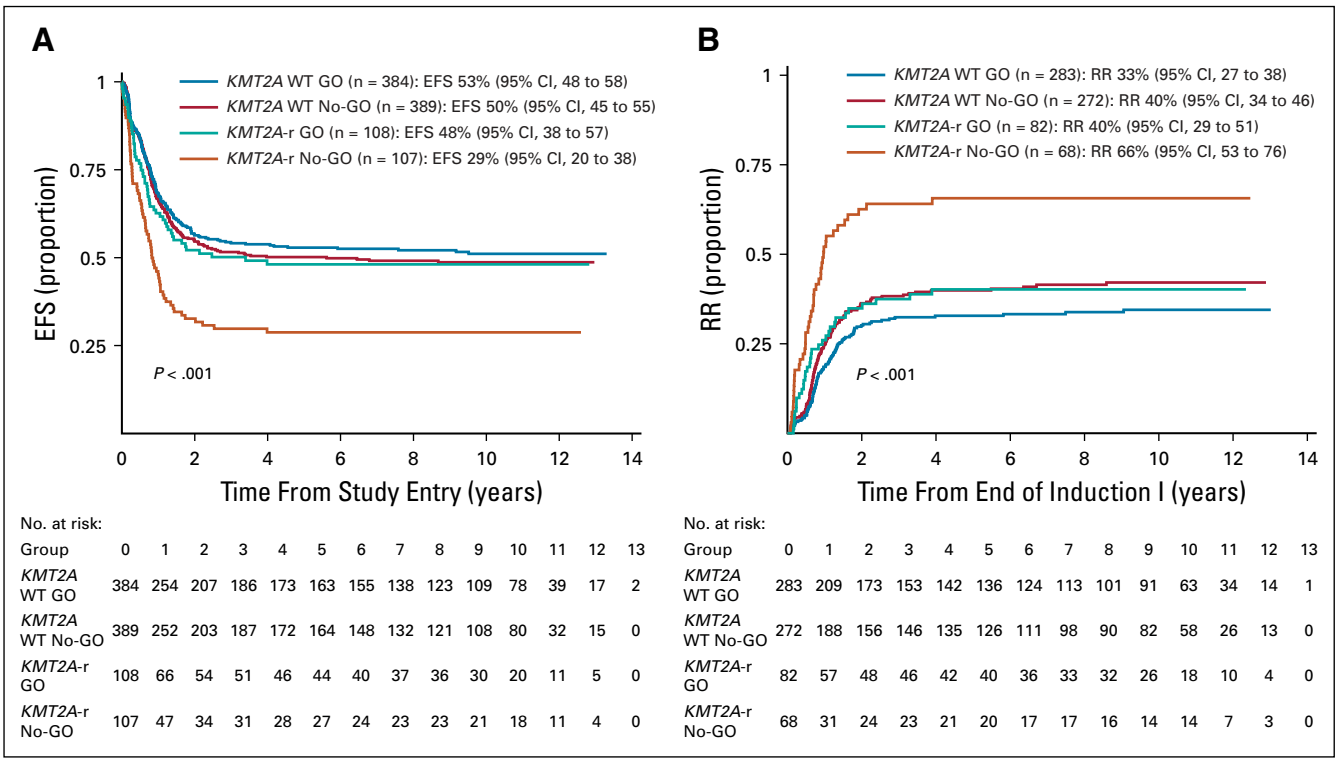
HR *KMT2A* translocation partners were defined as 6q27, 10p11.2, 10p12, 4q21.3, and 19p13.3 based on previously published data.[1,5,7,8] The non-HR (NHR) cohort included the remaining *KMT2A*-r cases but excluded other partners (defined as a NHR translocation with fewer than five cases) as their rarity precluded analysis of the impact of the fusion partner on prognosis, and the unknown partners, given the unclear origin of the fusion partner.

Assessment of CD33 Expression

Using difference from normal flow cytometry, CD33 expression was defined by mean fluorescence intensity (MFI) of leukemic blasts, as described previously.[9,10,30–32] CD33 expression data were then compared both overall and by *KMT2A*-r risk group. For univariable and multivariable analyses, the quartile of CD33 expression assigned for a given patient in the overall AAML0531 CD33 analysis[10] was used to determine whether GO response was affected by CD33 expression.

Statistical Analyses

Data on clinical outcomes for patients in AAML0531 were analyzed as of March 31, 2020. The median (range) follow-up time for patients alive at last contact was 9.3 (0.02-13.3) years. Significance of the observed difference in proportions was tested by the Pearson's χ^2 test or Fisher's exact test when data were sparse. The Kruskal-Wallis test was used to test differences in medians across multiple groups; the Mann-Whitney test was used when comparing two groups. CR was defined as $< 5\%$ blasts by morphology and absence of extramedullary disease. Minimal measurable residual disease (MRD) was determined by detecting flow cytometry-based disease and was typically defined as $> 0.02\%$ disease detected in the bone marrow by central difference from normal (ΔN) flow cytometry analysis.[33,34] The Kaplan-Meier method was used to estimate 5-year EFS, OS, and DFS.[35] Estimates are reported with corresponding log-log 95% CIs. EFS was defined as the time from study entry until death, induction failure, or relapse of any type; OS was defined as the time from study entry to death; and DFS as time from EOI1 for patients in CR until death or relapse. RR was defined as the time from EOI1 for patients in CR to relapse, where deaths without a relapse were considered competing events.[36] Treatment-related mortality (TRM) was defined as the time from EOI1 for those who continued therapy until death, where relapses were considered competing events.[36] To compare the consolidation approach (HSCT *v* chemotherapy), 5-year DFS and RR were also compared from end of intensification 1 in subset analyses. Differences between groups of patients were tested by the logrank test for OS, EFS, and DFS. Gray's test was used to test the significance of RR and TRM. Cox proportional hazard models were used



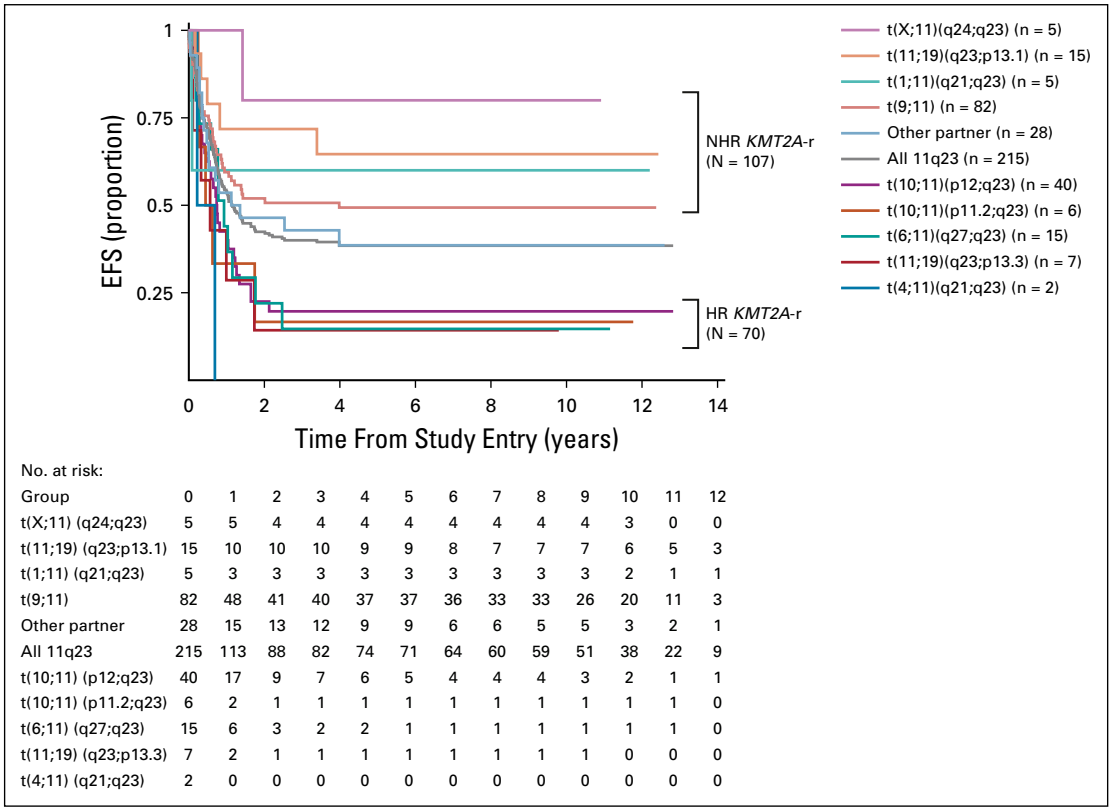
No. at risk:

Group	0	1	2	3	4	5	6	7	8	9	10	11	12	13
KMT2A WT GO	384	254	207	186	173	163	155	138	123	109	78	39	17	2
KMT2A WT No-GO	389	252	203	187	172	164	148	132	121	108	80	32	15	0
KMT2A-r GO	108	66	54	51	46	44	40	37	36	30	20	11	5	0
KMT2A-r No-GO	107	47	34	31	28	27	24	23	23	21	18	11	4	0

No. at risk:

Group	0	1	2	3	4	5	6	7	8	9	10	11	12	13
KMT2A WT GO	283	209	173	153	142	136	124	113	101	91	63	34	14	1
KMT2A WT No-GO	272	188	156	146	135	126	111	98	90	82	58	26	13	0
KMT2A-r GO	82	57	48	46	42	40	36	33	32	26	18	10	4	0
KMT2A-r No-GO	68	31	24	23	21	20	17	17	16	14	14	7	3	0

FIG 1. Outcomes for patients with *KMT2A-r* versus KMT2A WT outcome by GO exposure. (A) Five-year EFS from study entry and (B) 5-year RR from CR. CR, complete remission; EFS, event-free survival; GO, gemtuzumab ozogamicin; *KMT2A*-r, *KMT2A*-rearranged; No-GO, not receiving GO; RR, relapse risk; WT, wild-type.



No. at risk:

Group	0	1	2	3	4	5	6	7	8	9	10	11	12
t(X;11) (q24;q23)	5	5	4	4	4	4	4	4	4	4	3	0	0
t(11;19) (q23;p13.1)	15	10	10	10	9	9	8	7	7	7	6	5	3
t(1;11) (q21;q23)	5	3	3	3	3	3	3	3	3	3	2	1	1
t(9;11)	82	48	41	40	37	37	36	33	33	26	20	11	3
Other partner	28	15	13	12	9	9	6	6	5	5	3	2	1
All 11q23	215	113	88	82	74	71	64	60	59	51	38	22	9
t(10;11) (p12;q23)	40	17	9	7	6	5	4	4	4	3	2	1	1
t(10;11) (p11.2;q23)	6	2	1	1	1	1	1	1	1	1	1	1	0
t(6;11) (q27;q23)	15	6	3	2	2	1	1	1	1	1	1	1	0
t(11;19) (q23;p13.3)	7	2	1	1	1	1	1	1	1	1	0	0	0
t(4;11) (q21;q23)	2	0	0	0	0	0	0	0	0	0	0	0	0

FIG 2. EFS for patients with *KMT2A*-r acute myeloid leukemia. EFS from study entry for the entire study cohort (n = 215) and by specific translocation partners associated with higher-risk *KMT2A*-r AML (n = 70), non–high-risk *KMT2A*-r AML (n = 107), and other *KMT2A*-r subsets (n = 28). AML, acute myeloid leukemia; EFS, event-free survival; *KMT2A*-r, *KMT2A*-rearranged.

for OS, EFS, and DFS, whereas competing risk regression models were used for RR to estimate hazard ratios with 95% CIs for univariate and multivariable analyses.[37] Patients lost to follow-up were censored at the date of last known contact. An alpha level of 0.05 was used for *P* value significance.

RESULTS

Clinical Characteristics and Responses by KMT2A Cytogenetic Classification

Of 1,022 evaluable patients enrolled in AAML0531, 988 had evaluable cytogenetic data for central review and 215 (21%) had *KMT2A*-r AML (Appendix Fig A1, online only). Appendix Table A1 (online only) describes the differences in clinical characteristics and outcome for *KMT2A*-r versus *KMT2A* WT disease. Patients with *KMT2A*-r disease were younger and less likely to have clinically relevant co-occurring mutations than *KMT2A* WT patients and more likely to have cytogenetic complexity and non–central nervous system extramedullary disease, such as soft tissue chloromas or skin involvement (Appendix Table A1). Multivariable Cox regression models containing KMT2A WT versus *KMT2A*-r, treatment arm (GO *v* No-GO), and the corresponding interaction term yielded a significant interaction term for EFS ($P = .022$) and DFS ($P = .020$), suggesting a different GO treatment effect for *KMT2A*-WT and *KMT2A-r* AML for EFS and DFS but not for OS ($P = .119$) and RR ($P = .066$).

Comparison of disease characteristics across 11 *KMT2A*-r subgroups, including nine specific partner groups, other, and unknown *KMT2A*-r partners, revealed significant differences by age at presentation, non-CNS extramedullary AML, and presence of the *FLT3*/ITD mutation. GO exposure was equally distributed across the *KMT2A*-r subsets (Appendix Table A2, online only). Given the rarity of published data regarding the 28 patients in the other *KMT2A*-r subset, their clinical characteristics are further described in Appendix Table A3 (online only).

Impact of GO on CR and Outcome in *KMT2A*-r AML

Table 1 compares disease characteristics and induction response of patients with *KMT2A*-r AML treated with and without GO. Clinical characteristics were similar for the two treatment arms and for HR versus NHR *KMT2A*-r AML treated with and without GO (Table 1). Patients with *KMT2A*-r AML treated with GO had higher rates of EOI1 morphologic CR (77%) versus those treated without GO (64%, $P = .035$, Table 1) but comparable rates of EOI1 MRD (Table 1). GO use was associated with significant

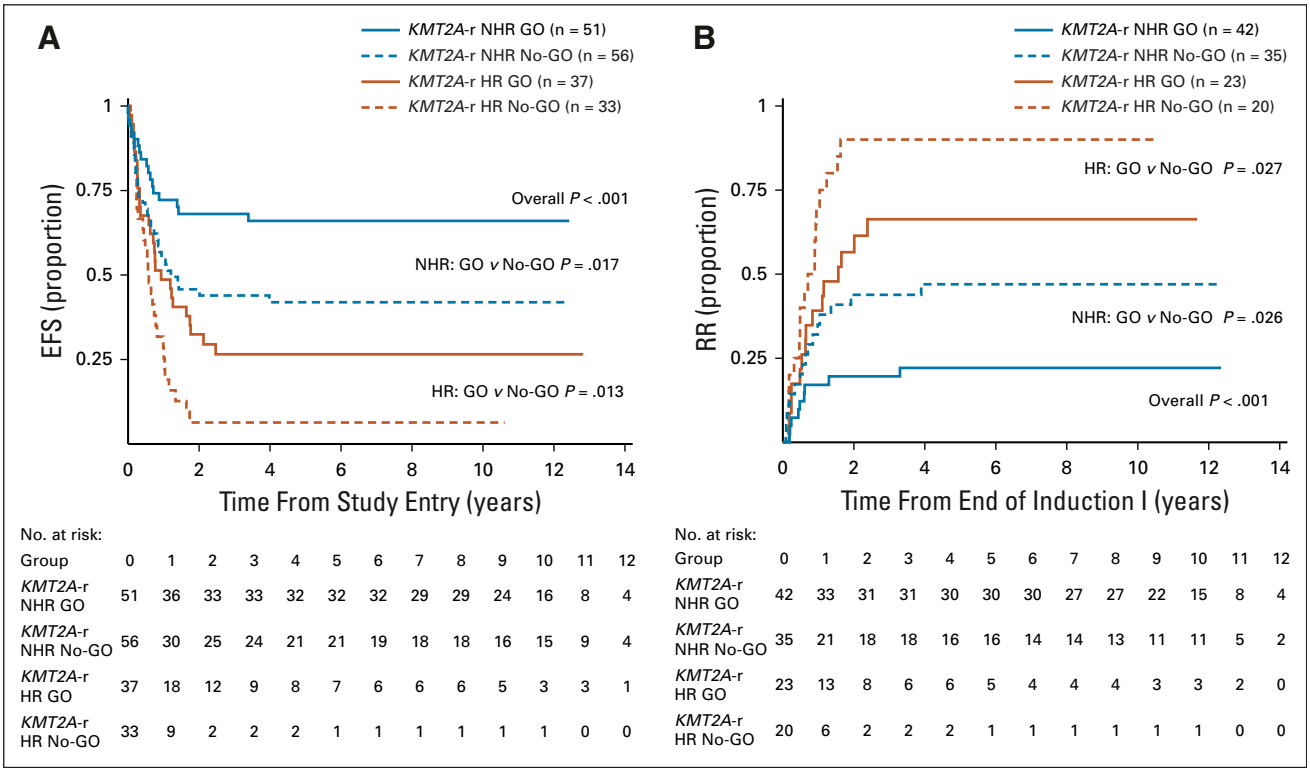


FIG 3. Outcomes for patients with HR versus NHR patients with *KMT2A*-r AML by GO exposure. (A) EFS from study entry and (B) RR from CR. CR, complete remission; EFS, event-free survival; GO, gemtuzumab ozogamicin; HR, higher risk; *KMT2A*-r, *KMT2A*-rearranged; NHR, not high-risk; No-GO, did not receive GO; RR, relapse risk.

improvements in long-term clinical outcomes for patients with *KMT2A*-r AML. Specifically, patients with *KMT2A*-r AML who received GO had 5-year EFS of 48% (95% CI, 38 to 57) versus 29% (95% CI, 20 to 38) for the No-GO cohort ($P = .003$, Table 1, Fig 1A) and RR of 40% (95% CI, 29 to 51) versus 66% (95% CI, 53 to 76, $P = .001$, Table 1 and Fig 1B). Although OS was not statistically different between the two arms, DFS was superior for patients treated with GO and rates of TRM were comparable (Table 1). Notably, patients with *KMT2A*-r AML treated with GO had, in general, comparable outcomes to *KMT2A* WT patients regardless of GO exposure (Appendix Table A1, Figs 1A and 1B).

Comparison of outcomes for historically defined HR versus NHR *KMT2A*-r AML revealed inferior EFS, OS, DFS, and RR for HR subsets (Appendix Table A4, online only, Fig 2). Specifically, EFS for patients with HR translocations was significantly better for those treated with GO (27%; 95% CI, 14 to 41) versus No-GO (6%; 95% CI, 1 to 18, $P = .013$, Table 1, Fig 3A). In addition, DFS trended toward superiority with GO (Table 1) and RR was significantly reduced (GO: 66%; 95% CI, 42 to 83% *v* no-GO: 90%; 95% CI, 60 to 98; $P = .027$; Table 1, Fig 3B). For the NHR subset (n = 107), GO improved EFS (GO: 66%; 95% CI, 51 to 77 *v* no-GO: 42%; 95% CI, 29 to 55; $P = .017$; Fig 3A), DFS (GO: 75%; 95% CI, 59 to 86 *v* no-GO: 50%; 95% CI, 32 to 65%; $P = .025$), and RR (GO: 22%; 95% CI, 11 to 36 *v* no-GO: 47%; 95% CI, 29 to 63; $P = .026$; Table 1, Fig 3B). Although GO improved outcomes for patients within both HR and NHR subsets (Table 1), outcomes remained significantly worse for GO-exposed HR versus NHR patients (Appendix Table A4).

Significance of GO and HSCT in *KMT2A*-r AML

Given the observed therapeutic benefit of GO and known benefit of HSCT in some AML subsets, we explored whether pre-HSCT GO affected post-HSCT outcomes. Of 215 patients with *KMT2A*-r AML, 30 (14%) received HSCT in first CR; 19/30 (63%) of these patients also received GO during induction 1. For HSCT recipients with prior GO exposure, DFS from end of intensification 1 was 72% (95% CI, 45 to 87) versus 27% (95% CI, 7 to 54) for patients in the no-GO cohort ($P = .004$, Fig 4A). RR was also reduced with GO/HSCT (28% CI, 10 to 50 *v* 73% CI, 32 to 91 for no-GO and HSCT, $P = .006$). For patients with *KMT2A*-r AML receiving chemotherapy without HSCT, there remained a trend toward improved outcome with GO (Fig 4B). The lowest rates of relapse were ultimately seen in patients with *KMT2A*-r AML who received GO and HSCT (Fig 4C).

CD33 Expression in *KMT2A-r* AML

Given the known association between CD33 expression and GO response and previous evidence that patients with *KMT2A*-r AML have a characteristic phenotype with higher CD33 expression in prospective analysis,[9,10,30] we analyzed

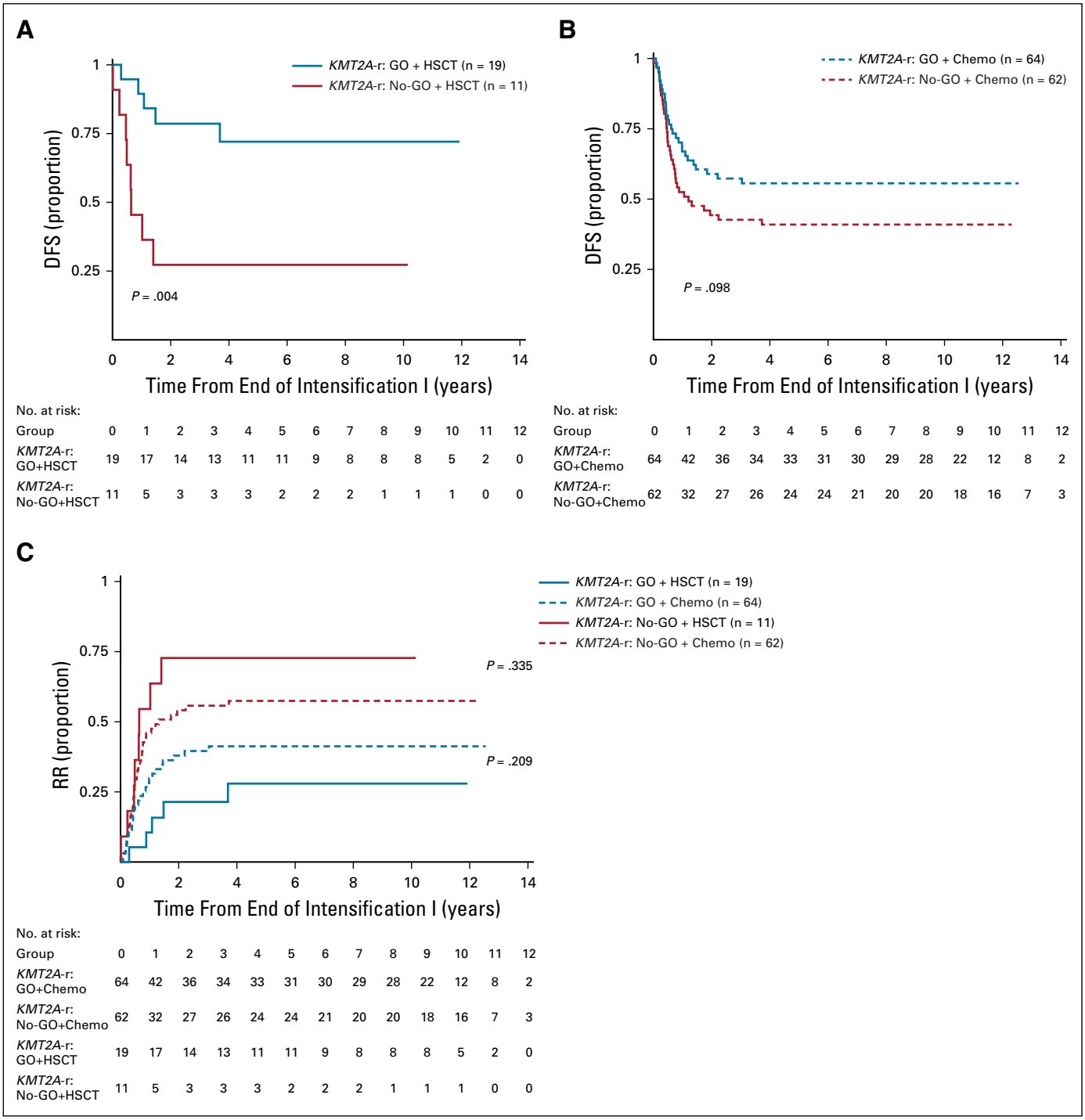


FIG 4. Outcome for *KMT2A*-r acute myeloid leukemia by GO exposure and consolidation approach. (A) DFS by GO exposure for patients treated with GO and HSCT, (B) DFS by GO exposure for patients treated with chemotherapy only, and (C) RR by GO exposure and consolidation approach. DFS, disease-free survival; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplant; *KMT2A*-r, *KMT2A*-rearranged; No-GO, did not receive GO; RR, relapse risk.

CD33 expression data for 168 of 215 (78%) patients with *KMT2A*-r AML with evaluable CD33 data. CD33 MFI was heterogenous in the patients with *KMT2A*-r AML but tended to cluster in higher AAML0531 CD33 expression quartiles [10] (Appendix Fig A2A, online only). Specifically, median CD33 MFI of leukemic cells isolated from *KMT2A*-r AML was 229.13 (range 6-1,351) versus 129 (range 2.68-1,225.87) for *KMT2A*-WT disease ($P \leq .001$, Appendix Fig A2B). Interestingly, HR *KMT2A-r* translocations had a comparable median CD33 MFI (median 267.32; range 22-1,119.5) to that of NHR translocations (median 226.5; range 7.6-1,351; $P = .480$, Appendix Fig A2B).

TABLE 2. Univariate and Multivariable Analyses

		5-Year OS From Study Entry			5-Year EFS From Study Entry				5-Year DFS From EOI1			5-Year RR From EOI1		
Univariable	**No.**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
KMT2A-r risk														
NHR	107	1.00			1.00			77	1.00			1.00		
HR	70	2.16	1.34 to 3.46	**.002**	2.38	1.61 to 3.50	**< .001**	43	3.04	1.82 to 5.07	**< .001**	3.20	1.90 to 5.40	**< .001**
Age, years														
1-15	141	1.00			1.00			102	1.00			1.00		
< 1	57	0.97	0.59 to 1.61	.915	1.19	0.80 to 1.77	.406	33	1.08	0.62 to 1.89	.785	1.11	0.61 to 2.03	.729
16+	17	2.07	1.11 to 3.86	**.023**	1.39	0.77 to 2.49	.275	15	1.85	0.97 to 3.56	.064	1.37	0.67 to 2.78	.385
WBC														
< 100,000	166	1.00			1.00			119	1.00			1.00		
≥ 100,000	49	1.11	0.68 to 1.81	.684	1.00	0.66 to 1.52	.989	31	0.68	0.38 to 1.24	.209	0.73	0.40 to 1.33	.306
Treatment arm														
No-GO	107	1.00			1.00			68	1.00			1.00		
GO	108	0.66	0.44 to 1.01	.056	0.59	0.42 to 0.84	**.003**	82	0.50	0.32 to 0.78	**.002**	0.48	0.30 to 0.75	**.001**
CD33 (original quartile assignment)[a]														
Q1-Q2	51	1.00			1.00			37	1.00			1.00		
Q3-Q4	117	1.26	0.73 to 2.15	.410	1.37	0.88 to 2.15	.164	84	1.60	0.91 to 2.81	.104	1.68	0.96 to 2.95	.071
HSCT as a time-dependent variable	30	0.58	0.28 to 1.21	.149	0.65	0.35 to 1.23	.187	24	0.78	0.38 to 1.58	.484	0.85	0.43 to 1.68	.637
Karyotype complexity														
1-2	159	1.00			1.00			115	1.00			1.00		
3+	52	2.05	1.32 to 3.20	**.002**	1.53	1.04 to 2.25	**.031**	31	1.40	0.82 to 2.37	.215	1.50	0.85 to 2.67	.166

(continued on following page)

TABLE 2. Univariate and Multivariable Analyses (continued)

		5-Year OS From Study Entry			5-Year EFS From Study Entry				5-Year DFS From EOI1			5-Year RR From EOI1		
Multivariable	**No.**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
KMT2A-r risk														
NHR	82	1.00			1.00			60	1.00			1.00		
HR	56	2.21	1.27 to 3.83	**.005**	2.62	1.68 to 4.09	**< .001**	37	2.85	1.62 to 5.00	**< .001**	3.28	1.84 to 5.86	**< .001**
Treatment arm														
No-GO	69	1.00			1.00			45	1.00			1.00		
GO	69	0.64	0.37 to 1.12	.117	0.52	0.33 to 0.82	**.005**	52	0.47	0.27 to 0.83	**.010**	0.45	0.25 to 0.82	**.009**
CD33 (original quartile assignment)[a]														
Q1-Q2	51	1.00			1.00			37	1.00			1.00		
Q3-Q4	117	1.42	0.74 to 2.71	.292	1.46	0.87 to 2.45	.150	84	1.77	0.90 to 3.49	.096	1.90	1.02 to 3.52	**.043**
Karyotype complexity														
1-2	104	1.00			1.00			77	1.00			1.00		
3+	34	1.86	1.04 to 3.32	**.038**	1.43	0.86 to 2.37	.166	20	1.22	0.60 to 2.46	.586	1.33	0.60 to 2.93	.485

NOTE. Bold indicates statistical significance.

Abbreviations: DFS, disease-free survival; EFS, event-free survival; EOI1, end of induction 1; GO, gemtuzumab ozogamicin; HR, higher risk; HSCT, hematopoietic stem cell transplant; *KMT2A*-r, *KMT2A*-rearranged; NHR, not higher risk; No-GO, did not receive GO; OS, overall survival; RR, relapse risk.

[a]Only 18 of 168 are in Q1 of original CD33 analysis.[10]

Importantly, patients with *KMT2A*-r AML who were in quartile (Q)1 or Q2 (Q1-Q2 median CD33: 84; range 6-146.94) in the composite AAML0531 CD33 analysis[10] retained clinical benefit from GO (Appendix Table A5, online only), demonstrating superior EFS and OS from study entry and DFS from CR. RR was also reduced in Q1-Q2 patients who received GO therapy (Appendix Table A5).

Univariate and Multivariable Analyses

Given the significant association between higher CD33 expression and *KMT2A*-r AML as well as impact of GO exposure on the *KMT2A*-r AML response, we performed Cox regression analyses to evaluate whether GO or CD33 expression had an independent impact on clinical outcomes in the context of established prognostic features. Age, presenting WBC count, risk designation of the *KMT2A* partner (HR *v* NHR), complex karyotype (≥ 3 cytogenetic abnormalities), GO exposure, HSCT exposure as a time-dependent variable, and CD33 expression, as defined by CD33 quartile classification from the original AAML0531 analysis[10] were assessed in a univariate analysis. HR *KMT2A*-r fusions were associated with inferior EFS and OS as well as DFS and RR. GO treatment was associated with superior EFS, DFS, and lower RR. CD33 expression, as defined by CD33 quartile designation (Q1-2 *v* Q3-4) was not independently associated with outcome. Older age was associated with inferior OS, and presence of complex karyotype affected OS and EFS (Table 2). In a multivariable model that included *KMT2A*-r risk group (HR *v* NHR), treatment arm (GO *v* no-GO), CD33 quartile assignment, and complex karyotype, GO exposure was independently associated with improved EFS and DFS and reduced RR. Higher CD33 expression (Q3-Q4) retained prognostic significance for RR. In addition, HR *KMT2A*-r disease was independently associated with reduced EFS, OS, and DFS, as well as higher RR. Complex karyotype was also an independent predictor of inferior OS (Table 2).

DISCUSSION

GO significantly improved EFS and DFS in children with *KMT2A*-r AML enrolled on AAML0531 by reducing rates of relapse without increasing TRM. This effect was observed in both HR and NHR *KMT2A*-r translocation cohorts. Importantly, the addition of GO abrogated the negative prognostic impact of a *KMT2A*-r, independent of CD33 expression, and resulted in comparable outcomes to that of *KMT2A* WT patients treated with or without GO. These findings support use of GO in all patients with *KMT2A*-r AML treated with a COG backbone of therapy. Moreover, the observation that treatment of *KMT2A*-r AML with GO followed by HSCT further improved outcomes suggests that GO exposure pre-HSCT may affect post-HSCT prognosis.

Children and adolescents with *KMT2A*-r AML have generally been treated as IR patients in cooperative group trials,[23,27,38–41] although it is clear that outcomes vary for specific translocation partners. The large retrospective analyses by Balgobind et al[1] of *KMT2A*-r pediatric AML demonstrated that patients with translocation partner 1q21 had favorable outcomes, whereas those with partners 10p11.2, 10p12, or 6q27 had markedly poor survival. Subsequent analyses, including our present study, confirmed the unfavorable effect of partners 10p11.2, 10p12, and 6q27, and added 19p13.3 and 4q21.3 as two additional unfavorable partner genes.[7,8]

Previous studies have also demonstrated that certain AML subsets like *FLT3*/ITD+ AML have high CD33 expression and that this confers poor outcome.[9] Importantly, however, higher CD33 expression is also associated with improved GO response, both overall and in the high-risk *FLT3*/ITD+ disease subset.[9,10,42] Both HR and NHR *KMT2A*-r subsets had high CD33 expression levels, although notably, our analysis demonstrates that even patients with lower CD33 expression appeared to have clinical benefit from GO. Together, this suggests that additional biologic factors in *KMT2A*-r AML might contribute to the favorable GO response seen. Surprisingly, despite the poor EFS and high RR in patients with *KMT2A*-r AML, EOI1 MRD was reported in < 20% of patients at EOI1. Although GO did not appear to decrease rates of MRD detection in our series, its use during induction ultimately affected DFS and RR particularly in patients receiving HSCT, suggesting GO may affect leukemic stem cells of more mature CD33+ origin resulting in additive benefit when used in combination with HSCT.

This study is limited as it is a retrospective analysis of a heterogeneous molecular subset within a larger prospective clinical trial that was not specifically designed to address the impact of GO or HSCT in *KMT2A*-r AML. Moreover, given that the other and unknown variants could not contribute to *KMT2A*-risk stratification, this missing data further limit the significance of our analyses. Nevertheless, this study includes a relatively large number of pediatric patients with *KMT2A*-r AML treated on a standard chemotherapy backbone in a randomized controlled trial. Although our analysis suggests that the combination of GO and HSCT may improve outcomes for pediatric *KMT2A*-r AML further, we concede that the number of patients who received both therapies was small and therefore further prospective studies are needed to explore the additive benefit of GO and HSCT. Importantly, our analysis has influenced *KMT2A*-r AML risk stratification for the current COG phase III pediatric AML trial, AAML1831, and provides additional rationale for including GO in the backbone of chemotherapy for all patients with *KMT2A*-r AML enrolled. However, given the higher TRM seen with GO therapy in COG AAML0531 and evidence in the NOPHO AML-2004 study that GO lacked clear benefit when given in consolidation, AAML1831 restricts GO use to the first cycle of treatment.[43] Investigation of second-generation CD33-targeting agents is prudent in this disease subset and may further aid identification of *KMT2A*-r disease features that predict for favorable response in this heterogenous group of patients.

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DISCLAIMER

L.E.B. is employed by Hematologics, and M.R.L. is equity owner and employed by Hematologics. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

EQUAL CONTRIBUTION

J.P. and E.G. contributed equally to this work.

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CLINICAL TRIAL INFORMATION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Gemtuzumab Ozogamicin Improves Event-Free Survival and Reduces Relapse in Pediatric KMT2A-Rearranged AML: Results From the Phase III Children's Oncology Group Trial AAML0531

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TABLE A1. Disease Characteristics and Clinical Response for KMT2A-r Versus WT Acute Myeloid Leukemia: Overall and Treated With or Without Gemtuzumab Ozogamicin

		***KMT2A*-WT *v* *KMT2A*-r**					**No-GO: *KMT2A*-WT *v* *KMT2A*-r**					**GO: *KMT2A*-WT *v* *KMT2A*-r**				
		***KMT2A* WT**		***KMT2A*-r**			***KMT2A* WT**		***KMT2A*-r**			***KMT2A* WT**		***KMT2A*-r**		
Characteristic	**Group**	**n = 773**		**n = 215**		***P***	**n = 389**		**n = 107**		***P***	**n = 384**		**n = 108**		***P***
Age, years	Median (range)	11.1	0.01-29.8	2.5	0.003-18.7	**< .001**	10.8	0.01-29.8	2.03	0.003-18.7	**< .001**	11.1	0.06-29.4	3.3	0.02-18.3	**< .001**
Sex	Male	388	50%	107	50%	.912	199	51%	59	55%	.465	189	49%	48	44%	.380
WBC × $10^3/\mu L$	Median (range)	24	0.2-827.2	25.6	0.4-610	.560	25.5	0.2-470	24.2	0.5-526	.964	22.7	0.6-827.2	29.8	0.4-610	.403
CNS-positive	Yes	53	7%	14	7%	.859	25	6%	8	7%	.700	28	7%	6	6%	.530
Non-CNS extramedullary disease	Yes	87	11%	53	25%	**< .001**	49	13%	25	23%	**.006**	38	10%	28	26%	**< .001**
FLT3/ITD	Positive	132	19%	5	3%	**< .001**	60	17%	4	4%	**.002**	72	21%	1	1%	**< .001**
CEPBA	Mutant	48	7%	0	0%	**< .001**	23	7%	0	0%	**.007**	25	7%	0	0%	**.005**
NPM1	Mutant	71	10%	0	1%	**< .001**	42	12%	0	0%	**< .001**	29	8%	0	1%	**.002**
Cytogenetic complexity	0	230	30%	0	0%	**< .001**	106	27%	0	0%	**< .001**	124	32%	0	0%	**< .001**
	1-2	401	52%	159	75%	**< .001**	215	55%	82	77%	**< .001**	186	48%	77	73%	**< .001**
	3+	142	18%	52	25%	**.042**	68	17%	24	23%	.226	74	19%	28	27%	.098
Treatment arm	GO treatment	384	50%	108	50%	.885	0	0%	0	0%	—	384	100%	108	100%	—
Induction 1 response	CR	555	73%	150	71%	.494	272	71%	68	64%	.174	283	75%	82	77%	.657
Induction 1 MRD	Present	199	33%	35	21%	**.003**	113	36%	18	22%	**.017**	86	30%	17	20%	.080
HSCT received	Yes	125	16%	30	14%	.429	64	16%	11	10%	.115	61	16%	19	18%	.671

	***KMT2A*-WT *v* *KMT2A*-r**					**No-GO: *KMT2A*-WT *v* *KMT2A*-r**					**GO: *KMT2A*-WT *v* *KMT2A*-r**				
	***KMT2A* WT**		***KMT2A*-r**			***KMT2A* WT**		***KMT2A*-r**			***KMT2A* WT**		***KMT2A*-r**		
Clinical Response	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***	**No.**	**% ± 2 SE%**	**No.**	**% ± 2 SE%**	***P***
5-Year EFS from study entry	773	51 (48 to 55)	215	38 (32 to 45)	**< .001**	389	50 (45 to 55)	107	29 (20 to 38)	**< .001**	384	53 (48 to 58)	108	48 (38 to 57)	.325
5-Year OS from study entry	773	66 (62 to 69)	215	58 (51 to 65)	**.020**	389	66 (61 to 70)	107	53 (43 to 62)	**.004**	384	66 (61 to 71)	108	63 (53 to 72)	.643
5-Year DFS from end induction I (patients in CR)	555	58 (54 to 62)	150	46 (38 to 54)	**.004**	272	56 (50 to 62)	68	33 (22 to 44)	**< .001**	283	60 (54 to 65)	82	57 (46 to 67)	.673
5-Year RR from end induction I (patients in CR)	555	36 (32 to 40)	150	52 (43 to 60)	**< .001**	272	40 (34 to 46)	68	66 (53 to 76)	**< .001**	283	33 (27 to 38)	82	40 (29 to 51)	.196
5-Year TRM from end induction I (patients in CR)	555	6 (4 to 8)	150	2 (0.6 to 5)	.068	272	4 (2 to 6)	68	2 (0.1 to 7)	.363	283	8 (5 to 11)	82	2 (0.5 to 8)	.100

NOTE. Bold indicates statistical significance.

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplantation; ITD, internal tandem duplication; *KMT2A*-r, *KMT2A*-rearranged; MRD, minimal measurable residual disease; OS, overall survival; RR, relapse risk; TRM, treatment-related mortality; WT, wild-type.

TABLE A2. Disease Characteristics and Induction Response by *KMT2A* Translocation Partner

	Other Partner		t(10;11) (p11.2;q23)		t(10;11) (p12;q23)		t(11;19) (q23;p13.1)		t(11;19) (q23;p13.3)		t(1;11) (q21;q23)		t(X;11) (q24;q23)		t(4;11) (q21;q23)		t(6;11) (q27;q23)		t(9;11)		Unknown Partner		
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	***P***
Total	28	13	6	3	40	19	15	7	7	3	5	2	5	2	2	1	15	7	82	38	10	5	
Sex																							
Male	14	50	4	67	20	50	6	40	4	57	2	40	3	60	0	0	6	40	44	54	4	40	.874
Female	14	50	2	33	20	50	9	60	3	43	3	60	2	40	2	100	9	60	38	46	6	60	
Treatment arm																							
Arm A (No-GO)	11	39	2	33	21	53	8	53	4	57	2	40	2	40	1	50	5	33	44	54	7	70	.784
Arm B (GO)	17	61	4	67	19	48	7	47	3	43	3	60	3	60	1	50	10	67	38	46	3	30	
Age, years																							
Median (range)	1.2	0.02-18.2	3.8	0.52-13.3	1.3	0.003-18.7	8.5	0.41-16.8	11.1	0.24-16.7	1.2	0.71-5.2	2.4	0.77-8.11	0.3	0.21-0.37	12.7	0.17-17.2	3.1	0.14-18.3	12.7	0.30-16.0	**.004**
WBC $\times$ 10^3 μL																							
Median (range)	25.4	1.5-334	48.2	39.9-95.7	19.3	0.5-299.4	21.1	1.7-122.5	43	19.7-216.3	9	2.7-43.5	52.2	2.5-169	316.7	114.3-519	72.2	6.7-263.1	18.2	0.4-610	51.4	1.3-332	.173
CNS disease																							
No	26	93	6	100	37	93	14	93	7	100	5	100	5	100	2	100	14	93	75	91	10	100	
Yes	2	7	0	0	3	8	1	7	0	0	0	0	0	0	0	0	1	7	7	9	0	0	.980
Non-CNS extramedullary chloroma																							
No	24	86	1	17	24	60	12	80	5	71	5	100	5	100	2	100	11	73	64	78	9	90	
Yes	4	14	5	83	16	40	3	20	2	29	0	0	0	0	0	0	4	27	18	22	1	10	**.009**
Unknown	0		0		0		0		0		0		0		0		0		0		0		
FLT3/ITD status																							
ITD+	0	0	0	0	0	0	0	0	2	33	0	0	0	0	0	0	0	0	2	3	1	11	**.002**
ITD–	26	100	6	100	36	100	13	100	4	67	4	100	5	100	2	100	14	100	73	97	8	89	
Unknown	2		0		4		2		1		1		0		0		1		7		1		
CEBPA status																							
CEBPA mutant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—
WT	26	100	6	100	35	100	13	100	6	100	4	100	5	100	2	100	14	100	73	100	8	100	
Unknown	2		0		5		2		1		1		0		0		1		9		2		
NPM status																							
NPM mutant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—
WT	26	100	6	100	35	100	13	100	6	100	4	100	5	100	2	100	14	100	73	100	8	100	
Unknown	2		0		5		2		1		1		0		0		1		9		2		
Cytogenetic complexity																							
1-2	19	68	6	100	31	78	11	73	6	86	4	80	3	60	1	50	11	73	61	74	6	100	.747
3+	9	32	0	0	9	23	4	27	1	14	1	20	2	40	1	50	4	27	21	26	0	0	

(continued on following page)

TABLE A2. Disease Characteristics and Induction Response by *KMT2A* Translocation Partner (continued)

Characteristic	Other Partner		t(10;11) (p11.2;q23)		t(10;11) (p12;q23)		t(11;19) (q23;p13.1)		t(11;19) (q23;p13.3)		t(1;11) (q21;q23)		t(X;11) (q24;q23)		t(4;11) (q21;q23)		t(6;11) (q27;q23)		t(9;11)		Unknown Partner		*P*
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
MRD at EOI1																							
Negative	19	79	4	100	29	88	10	77	3	60	2	40	4	100	1	50	5	45	49	86	4	57	
Positive	5	21	0	0	4	12	3	23	2	40	3	60	0	0	1	50	6	55	8	14	3	43	**.019**
Unknown	4		2		7		2		2		0		1		0		4		25		3		
Response by end of course 1																							
CR	21	81	5	83	23	58	12	80	4	67	3	60	2	40	1	50	10	67	60	73	9	90	.365
Not CR	5	19	1	17	17	43	3	20	2	33	2	40	3	60	1	50	5	33	22	27	1	10	
Not evaluable	2		0		0		0		1		0		0		0		0		0		0		
Protocol HSCT received?																							
Yes	6	21	0	0	5	13	2	13	1	14	2	40	1	20	0	0	3	20	10	12	0	0	.634
No	22	79	6	100	35	88	13	87	6	86	3	60	4	80	2	100	12	80	72	88	10	100	

Clinical Outcome	Other Partner		t(10;11) (p11.2;q23)		t(10;11) (p12;q23)		t(11;19) (q23;p13.1)		t(11;19) (q23;p13.3)		t(1;11) (q21;q23)		t(X;11) (q24;q23)		t(4;11) (q21;q23)		t(6;11) (q27;q23)		t(9;11)		Unknown Partner		*P*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
5-Year EFS from study entry	28	39 (21 to 56)	6	17 (0.8 to 52)	40	20 (9 to 33)	15	65 (35 to 84)	7	14 (0.7 to 46)	5	60 (13 to 88)	5	80 (20 to 97)	2	0 (0 to 0)	15	15 (2 to 37)	82	49 (38 to 60)	10	23 (3 to 52)	**.005**
5-Year OS from study entry	28	57 (37 to 73)	6	33 (5 to 68)	40	49 (33 to 64)	15	64 (34 to 83)	7	57 (17 to 84)	5	80 (20 to 97)	5	100 (100 to 100)	2	0 (0 to 0)	15	33 (12 to 56)	82	70 (59 to 79)	10	23 (3 to 52)	**.036**
5-Year DFS from EOI1 (course 1) (CR patients only)	21	42 (2 to 62)	5	20 (0.8 to 58)	23	25 (10 to 44)	12	64 (30 to 85)	4	0 (0 to 0)	3	100 (100 to 100)	2	100 (100 to 100)	1	0 (0 to 0)	10	20 (3 to 47)	60	61 (47 to 72)	9	25 (4 to 56)	**.007**
5-Year RR from EOI1 (course 1) (CR patients only)	21	58 (33 to 77)	5	80 (5 to 98)	23	70 (45 to 85)	12	27 (6 to 55)	4	100 (100 to 100)	3	0 (0 to 0)	2	0 (0 to 0)	1	100 (100 to 100)	10	80 (33 to 96)	60	37 (25 to 50)	9	75 (23 to 94)	**.003**
5-Year TRM from EOI1 (course 1) (CR patients only)	21	0 (0 to 0)	5	0 (0 to 0)	23	4 (0.3 to 19)	12	9 (0.4 to 35)	4	0 (0 to 0)	3	0 (0 to 0)	2	0 (0 to 0)	1	0 (0 to 0)	10	0 (0 to 0)	60	2 (0.1 to 8)	9	0 (0 to 0)	.921

Abbreviations: CR, complete remission; DFS, disease-free survival; EFS, event-free survival; EOI1, end of induction 1; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplant; MRD, minimal measurable residual disease; OS, overall survival; RR, relapse risk; TRM, treatment-related mortality; WT, wild-type.

TABLE A3. Clinical Characteristics and Descriptive Summary of Outcomes for the 28 Other Patients With *KMT2A*-r AML

Patient	Sex	KMT2A-r: Other Partner	Induction I Response	Induction II Response	HSCT Received	Days to Failure or Relapse From Study Entry	Days to OS From Study Entry	Status at Last Contact
1	Female	(10q11.2)	CR	CR	No	127	289	Dead
2	Male	(10q22)	CR	CR	No	173	219	Dead
3	Female	(10q26)	Unevaluable	CR	No		3,518	Alive
4	Male	(11p14)	CR	CR	No		4,062	Alive
5	Male	(11p15)	Unevaluable	CR	No	130	275	Dead
6	Female	(11q12)	CR	CR	No	105	379	Dead
7	Female	(11q13)	PD	CR	No	255	702	Dead
8	Female	(11q25)	Death	Death	No		10	Dead
9	Female	(12q24.3)	PR	CR	No	925	2,758	Alive
10	Female	(15q13)	CR	CR	No		2013	Alive
11	Female	(15q15)	CR	CR	No	103	198	Dead
12	Male	(17q12)	CR	CR	No	145	161	Dead
13	Male	(17q12)	CR	CR	No		2,793	Alive
14	Male	(17q21)	CR	CR	Yes		1,962	Alive
15	Female	(17q25)	CR	CR	Yes		2,097	Alive
16	Male	(17q25)	CR	CR	No	495	1,593	Alive
17	Female	(1p32)	Death	Death	No		15	Dead
18	Male	(1p32)	PR	CR	No		3,520	Alive
19	Female	(3p13)	CR	CR	No		3,797	Alive
20	Female	(4p11.2)	CR	Relapse	No	78	1,745	Alive
21	Male	(5p15)	CR	CR	No	411	974	Dead
22	Male	(5q31)	CR	Unevaluable	No	192	3,496	Alive
23	Male	(8q23)	CR	CR	No	191	215	Dead
24	Female	(Xq22)	CR	CR	Yes	1,455	4,284	Alive
25	Female	(Xq26.3)	CR	Unevaluable	Yes		1,343	Alive
26	Male	(Xq28)	CR	CR	No		4,601	Alive
27	Male	(Xq28)	CR	CR	Yes	290	434	Dead
28	Male	(Xq28)	CR	CR	Yes		1,155	Alive

Abbreviations: CR, complete remission; HSCT, hematopoietic stem cell transplant; *KMT2A*-r, *KMT2A*-rearranged; PD, persistent disease; PR, partial remission; OS, overall survival.

TABLE A4. Outcome Analysis for HR Versus NHR *KMT2A*-r Acute Myeloid Leukemia by GO Exposure

	HR KMT2A-r GO		NHR KMT2A-r GO			HR KMT2A-r No-GO		NHR KMT2A-r No-GO		
	n = 37		n = 51			n = 33		n = 56		
Clinical Outcome	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***
5-Year EFS from study entry	37	27 (14 to 41)	51	66 (51 to 77)	**.001**	33	6 (1 to 18)	56	42 (29 to 55)	**.001**
5-Year OS from study entry	37	49 (32 to 65)	51	76 (61 to 85)	**.023**	33	36 (21 to 52)	56	67 (53 to 78)	**.013**
5-Year DFS from end induction I (patients in CR)	23	29 (12 to 49)	42	75 (59 to 86)	**.001**	20	10 (2 to 27)	35	50 (32 to 65)	**.003**
5-Year RR from end induction I (patients in CR)	23	66 (42 to 83)	42	22 (11 to 36)	**.001**	20	90 (60 to 98)	35	47 (29 to 63)	**.002**
5-Year TRM from end induction I (patients in CR)	23	4 (0.3 to 19)	42	2 (0.2 to 11)	.686	20	0 (0 to 0)	35	3 (0.2 to 13)	.443

Abbreviations: DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; HR, higher risk; *KMT2A*-r, *KMT2A*-rearranged; NHR, not high-risk; OS, overall survival; RR, relapse risk; TRM, treatment-related mortality.

TABLE A5. Outcomes by Treatment Arm for Patients With *KMT2A*-r AML in CD33 Expression Q1-Q2[10]

	CD33 Q1-Q2: No GO		CD33 Q1-Q2: GO		
	n = 26		n = 25		
Additional Clinical Outcome	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	***P***
5-Year EFS from study entry	26	28 (13 to 46)	25	68 (46 to 83)	**.011**
5-Year OS from study entry	26	50 (30 to 67)	25	80 (58 to 91)	**.032**
5-Year DFS from end induction I (patients in CR)	19	32 (13 to 52)	18	83 (57 to 94)	**.002**
5-Year RR from end induction I (patients in CR)	19	68 (41 to 85)	18	11 (2 to 30)	**.001**
5-Year TRM from end induction I (patients in CR)	19	0 (0 to 0)	18	6 (0.3 to 23)	.305

NOTE. Q1 and Q2 are the original quartile assignments from the primary CD33 analysis,[10] not defined by just the patients with *KMT2A*-r AML.

Abbreviations: DFS, disease-free survival; EFS, event-free survival; GO, gemtuzumab ozogamicin; *KMT2A*-r, *KMT2A*-rearranged; OS, overall survival; RR, relapse risk; TRM, treatment-related mortality.

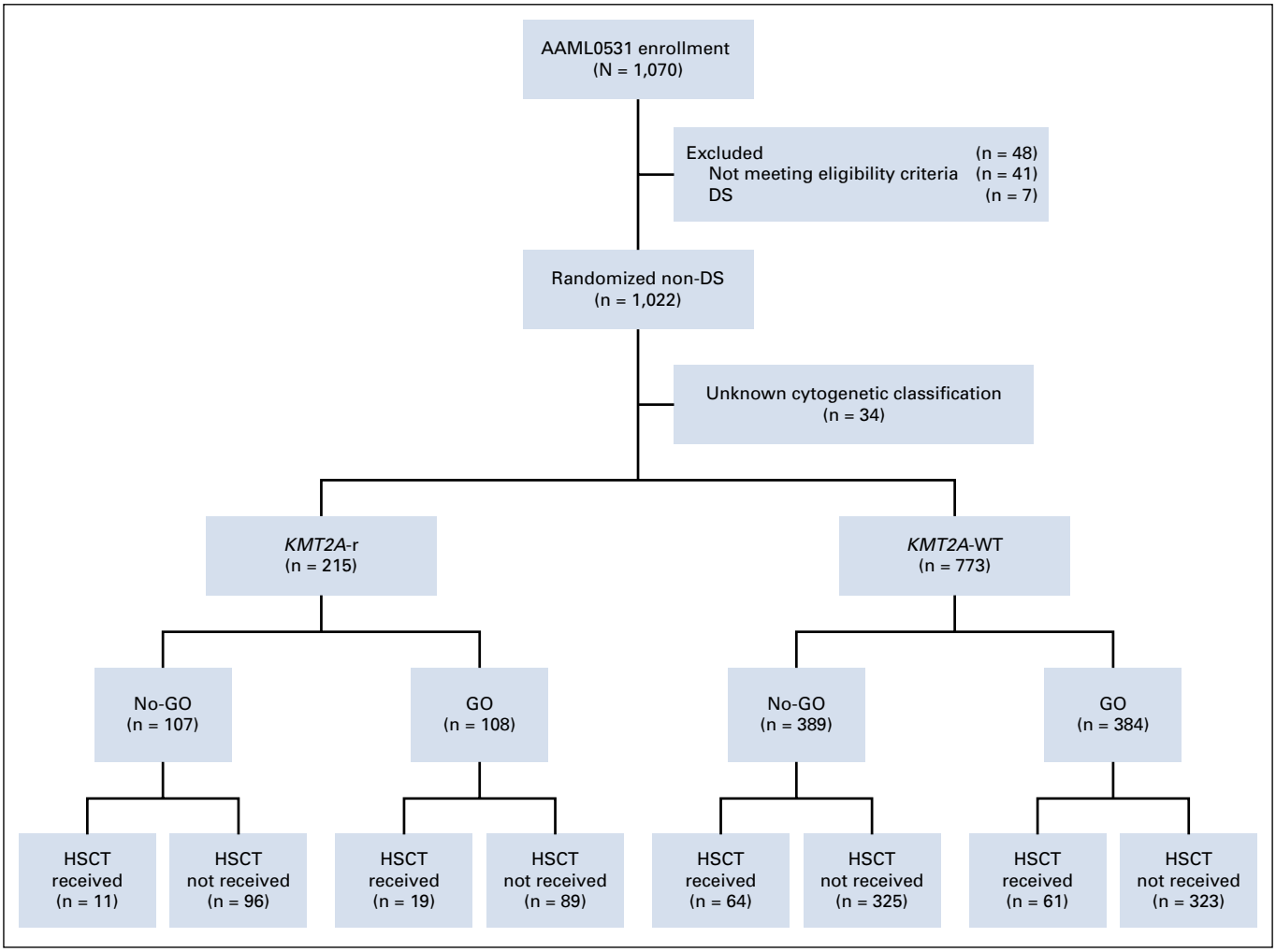

FIG A1. CONSORT diagram of the study population. DS, Down syndrome; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplant; *KMT2A*-r, *KMT2A*-rearranged; No-GO, did not receive GO; WT, wild type.

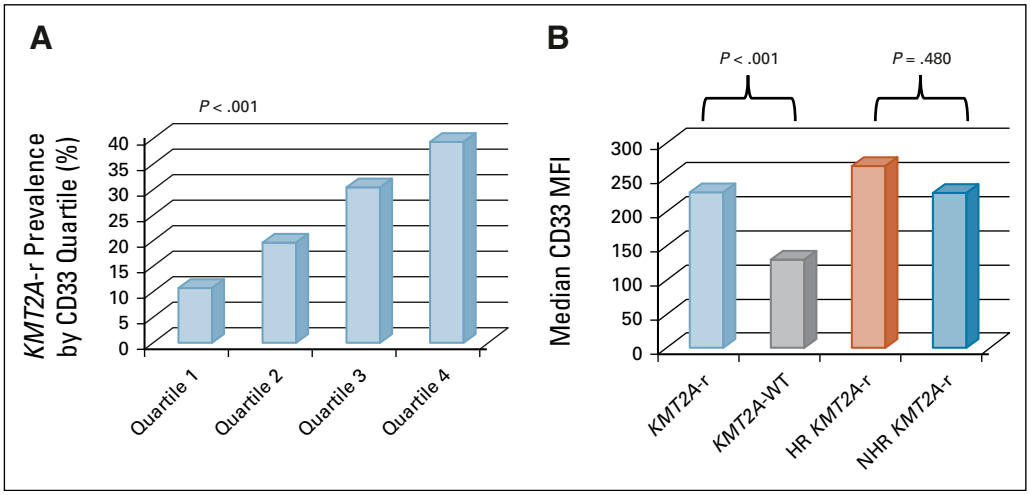

FIG A2. CD33 expression in *KMT2A*-r AML. (A) Distribution of patients with *KMT2A*-r AML in AAML0531-defined CD33 expression quartiles.[10] (B) Median CD33 MFI for *KMT2A*-r versus *KMT2A*-WT and for HR versus NHR *KMT2A*-r AML. AML, acute myeloid leukemia; HR, higher risk; *KMT2A*-r, *KMT2A*-rearranged; MFI, mean fluorescence intensity; NHR, non–high-risk; WT, wild-type.