

Gemtuzumab Ozogamicin Reduces Relapse Risk in *FLT3*/ITD Acute Myeloid Leukemia: A Report from the Children's Oncology Group

Katherine Tarlock¹, Todd A. Alonzo², Robert B. Gerbing³, Susana C. Raimondi⁴, Betsy A. Hirsch⁵, Lillian Sung⁶, Jessica A. Pollard⁷, Richard Aplenc⁸, Michael R. Loken⁹, Alan S. Gamis¹⁰, and Soheil Meshinchi¹

Abstract

Purpose: Gemtuzumab ozogamicin (GO), a calicheamicin-conjugated mAb against CD33, has been used in the treatment of acute myeloid leukemia (AML). We evaluated the impact of the addition of GO to standard chemotherapy and hematopoietic stem cell transplant (HCT) in patients with *FLT3*/ITD.

Experimental Design: We analyzed children with *FLT3*/ITD-positive AML ($n = 183$) treated on two consecutive Children's Oncology Group AML trials (NCT00070174 and NCT00372593). Outcomes were assessed for *FLT3*/ITD patients receiving standard chemotherapy with or without GO (GO vs. No-GO, respectively), and the impact of consolidation HCT for high-risk *FLT3*/ITD patients [high *FLT3*/ITD allelic ratio (ITD-AR)].

Results: For all *FLT3*/ITD patients, complete remission (CR) rates for the GO versus No-GO cohorts were identical (64% vs.

64%; $P = 0.98$). Relapse rate (RR) after initial CR was 37% for GO recipients versus 59% for No-GO recipients ($P = 0.02$), disease-free survival (DFS) was similar (47% vs. 41%; $P = 0.45$), with higher treatment-related mortality (TRM) in GO recipients (16% vs. 0%; $P = 0.008$). Among high-risk *FLT3*/ITD patients with high ITD-AR, those who received HCT in first CR with prior exposure to GO had a significant reduction in RR (15% vs. 53%; $P = 0.007$), with a corresponding DFS of 65% versus 40% ($P = 0.08$), and higher TRM (19% vs. 7%; $P = 0.08$).

Conclusions: CD33 targeting with HCT consolidation may be an important therapeutic strategy in high-risk *FLT3*/ITD AML and its efficacy and associated toxicity warrant further investigation. *Clin Cancer Res*; 22(8); 1951–7. ©2015 AACR.

Introduction

CD33 is a cell surface myeloid antigen that is variably expressed on the majority of blasts in patients with acute myeloid leukemia (AML), but is absent from early hematopoietic progenitor cells (1, 2). CD33 is the target of the calicheamicin toxin-conjugated humanized mAb gemtuzumab ozogamicin (GO). High expression of CD33 is associated with high-risk disease features, such as internal tandem duplications of *FLT3* (*FLT3*/ITD), and poor

prognosis in pediatric AML (3, 4). *In vitro* studies have shown a correlative relationship between CD33 expression and GO-mediated cytotoxicity (5). The safety of combining GO with conventional chemotherapy in adult trials for relapsed AML ultimately resulted in the accelerated FDA approval of GO in 2000 by the FDA (6–8). However, the initial phase III study of GO combined with conventional chemotherapy in adults failed to demonstrate improved outcomes (9), and the drug was withdrawn from the U.S. market. Subsequent randomized controlled trials have shown that GO improves outcomes in certain subsets of patients with AML, particularly those with low- or intermediate-risk cytogenetics (8, 10, 11). The Children's Oncology Group (COG) phase III pilot trial AAML03P1 and the subsequent phase III trial AAML0531 confirmed the safety and benefit of adding GO to intensive chemotherapy in pediatric AML (6, 12).

Therapy intensification and advancements in supportive care have led to improvement in overall outcomes in pediatric AML. However, patients with *FLT3*/ITD have a poor prognosis with chemotherapy alone, especially those with a high allelic ratio (ITD-AR; >0.4) of mutant to wild-type *FLT3* (13–18). Although allogeneic hematopoietic stem cell transplant (HCT) increases the survival to approximately 65% for this cohort, alternative therapeutic approaches are needed to improve long-term survival in a significant number of children with this lesion (13, 19). Because the limits to which conventional treatment can be intensified have been reached, the use of alternative approaches such as immunotherapy are necessary to further improve outcomes. In this study, we investigated the impact of CD33 targeting with GO in

¹Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, Washington. ²Keck School of Medicine, University of Southern California, Los Angeles, California. ³Department of Statistics, Children's Oncology Group, Monrovia, California. ⁴Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee. ⁵Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota. ⁶Division of Haematology, The Hospital for Sick Children, Toronto, Ontario, Canada. ⁷Maine Children's Cancer Program, Maine Medical Center, Scarborough, Maine. ⁸Pediatric Oncology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania. ⁹Hematologics Incorporated, Seattle, Washington. ¹⁰Hematology/Oncology, Children's Mercy Hospitals and Clinics, Kansas City, Missouri.

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Corresponding Author: Katherine Tarlock, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109. Phone: 206-667-7121; Fax: 206-667-7084; E-mail: ktarlock@fredhutch.org

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Translational Relevance

In this study, we assess the impact of combining gemtuzumab ozogamicin (GO) with conventional chemotherapy in pediatric patients with *FLT3/ITD*-positive acute myeloid leukemia (AML) and show that the addition of GO in induction results in reduction of relapse. This antileukemic effect was even more pronounced in high allelic ratio (AR) *FLT3/ITD* patients. Ours is the first study to examine the impact of CD33 targeting and GO in pediatric *FLT3/ITD* AML and assess its impact by AR, which is recognized as a marker of poor prognosis. This high-risk group of patients is in need of new therapeutic strategies as a significant number of patients experience relapse despite intensive chemotherapy and hematopoietic stem cell transplant (HCT). Our study finds that *FLT3/ITD* patients experience significant benefit from CD33 targeting, and these results may be further improved when combined with additional therapies including HCT.

FLT3/ITD patients as a method to improve outcomes for this high-risk group of patients.

Methods

Patients and treatment

Pediatric patients (ages 1 month to 30 years) with *de novo* AML enrolled on the COG phase III pilot study AAML03P1 and the subsequent phase III trial AAML0531 were eligible for this study. Between December 2003 and November 2005, 30 of the 339 eligible patients treated on AAML03P1 were positive for the *FLT3/ITD* mutation. Between August 2006 and June 2010, 153 of 1,022 eligible patients treated on AAML0531 were positive for the *FLT3/ITD* mutation. The trials were conducted in accordance with the Declaration of Helsinki. Complete eligibility criteria, risk stratification based on cytogenetic and molecular features as well as randomization and complete treatment details have been previously reported (6, 12). In brief, patients treated on AAML0531 were randomly assigned to one of two study arms—Arm A which included standard therapy alone (No-GO) or Arm B which included GO (dose 3 mg/m²) administered once during induction course I on day 6 and again during intensification course II on day 7. All patients on AAML03P1 received GO on the same schedule as patients on Arm B of AAML0531. Initially, all patients with *FLT3/ITD* were treated under a biologic randomization; wherein patients with a matched sibling donor (MSD) underwent HCT and those without a MSD continued along in the assigned chemotherapy arm, with patients on Arm B receiving a second dose of GO during intensification. Following definitive recognition of HAR *FLT3/ITD* as a high-risk disease feature, AAML0531 was amended and all patients with ITD-AR>0.4 enrolled after April 14, 2008, were allocated to receive consolidation HCT from a suitable donor.

Statistical analysis

The significance of observed difference in proportions was tested using Pearson χ^2 test and Fisher exact test when data were sparse. Complete remission (CR) was defined as <5% blasts on morphologic examination and no evidence of extramedullary disease

following the first course of induction therapy. The Kaplan–Meier method was used to estimate overall survival (OS) and disease-free survival (DFS). Data were current on AAML03P1 and AAML0531 as of March 31, 2014. Estimates of OS and DFS at 5 years were reported along with corresponding two times Greenwood standard errors. OS was defined as the time from study entry until death, DFS as the time from end of induction (EOI) I for patients in CR until relapse or death. Relapse risk (RR) was defined as the time from either EOI I for patients in CR or from end of intensification I to relapse where deaths without a relapse were considered competing events. Treatment-related mortality (TRM) was defined as the time from EOI I for patients in CR to death without relapse where relapses were considered competing events. Estimates of RR and TRM at 5 years were obtained by methods that account for competing events. For results that compare patients who receive HCT or consolidation chemotherapy alone, analyses are defined as the time from completion of one course of intensification for patients who continue on chemotherapy. Patients lost to follow-up were censored at their date of last known contact. The significance of predictor variables was tested with the log-rank statistic for OS and DFS and with Gray statistic for RR and TRM (20). Cox proportional hazard models were used to estimate hazard ratios (HR) for univariate and multivariate analyses of OS, event-free survival (EFS), and DFS (21). Competing risk regression models were used to estimate HRs for analyses of RR and TRM (22).

Results

GO and disease response

A total 1,214 patients with *de novo* AML treated on COG AAML03P1 and AAML0531 were evaluated for inclusion and we identified 183 patients positive for *FLT3/ITD* and their clinical outcome data were included in further analysis. The median follow-up time (and range) for patients alive at last contact 7.45 (3.96–8.94) years for 30 patients on AAML03P1 and 4.59 (0.3–6.44) years for 153 patients on AAML0531. Of these 183 patients with *FLT3/ITD*, 112 patients received GO in addition to standard chemotherapy (GO arm), treated on AAML0531 Arm B ($n = 82$) and AAML03P1 ($n = 30$). The remaining 71 patients all treated on AAML0531 Arm A received chemotherapy only on the No-GO arm. There were no significant differences by age, gender, race, ethnicity, extramedullary involvement, cytogenetic features, presenting white blood cell count, and bone marrow blast percentages between the GO and No-GO cohorts (Table 1). There were significantly more patients with *NPM1* mutations in the No-GO versus GO cohort ($P = 0.01$). Although *NPM1* mutations have subsequently been demonstrated to be a low-risk mutation and confer a favorable prognosis (23, 24), patients treated on AAML03P1 and AAML0531 were not stratified by *NPM1* status and some received HCT in first CR per protocol stratification. There were no significant differences between *FLT3/ITD* high versus low ARs between the two treatment cohorts (Table 1).

Patients in the GO cohort received the initial dose of GO during induction I, we therefore analyzed the impact of GO on CR rates at the EOI I. After the initial induction course, patients with *FLT3/ITD* had a CR rate of 64% versus 77% for the *FLT3/ITD*-negative patients ($P < 0.001$). Among *FLT3/ITD*-positive cohort, patients with or without GO exposure had an identical CR rate of 64% ($P = 0.98$). Minimal residual disease (MRD) as detected by flow cytometry was also analyzed and 56% of GO recipients achieved MRD-negative status at EOI I versus 49% in the No-GO cohort

Table 1. Clinical and biologic characteristics of FLT3/ITD patients in the GO and No-GO cohorts

	No-GO (n = 71) n (%)	GO (n = 112) n (%)	P
Gender			
Male	41 (58%)	61 (54%)	0.66
Age, median (range)	13.2 (0.7–20.4)	12.1 (1.6–20.9)	0.49
0–2 y	2 (3%)	4 (4%)	1
3–10 y	20 (48%)	41 (37%)	0.24
11–21 y	49 (69%)	67 (60%)	0.21
Race			
Asian	3 (5%)	12 (12%)	0.13
Black or African	7 (11%)	13 (13%)	0.77
White	53 (84%)	78 (76%)	0.20
CNS disease			
Yes	5 (7%)	7 (6%)	1
Chloroma			
Yes	9 (13%)	9 (8%)	0.30
WBC × 10 ³ /μL, median (range)	66.5 (0.2–470)	53.4 (1.2–827.2)	0.94
Bone marrow blasts %	80 (3–98)	79 (23–100)	0.90
Cytogenetics			
Normal	29 (58%)	57 (54%)	0.61
t(8;21)	2 (3%)	3 (3%)	1
inv(16)	3 (4%)	2 (2%)	0.38
Abnormal 11	4 (6%)	2 (2%)	0.21
t(6;9)(p23;q34)	5 (7%)	10 (10%)	0.64
Del 7q	0 (0%)	0 (0%)	1
Trisomy 8	9 (13%)	21 (20%)	0.27
Other	4 (6%)	9 (9%)	0.53
NPM1 mutant	17 (27%)	12 (11%)	0.01
CEBPA mutant	4 (6%)	7 (7%)	1
FLT3/ITD AR			
≤0.4 (Low)	30 (42%)	38 (34%)	0.26
>0.4 (High)	41 (58%)	74 (66%)	

Abbreviation: CNS, central nervous system.

($P = 0.44$), with 63% of both cohorts achieving MRD-negative status at EOI II ($P = 0.96$).

Postinduction clinical outcome was evaluated on the basis of induction exposure to GO. FLT3/ITD patients treated with GO had a reduction in RR of 37% ± 12% versus 59% ± 16% for the No-GO cohort ($P = 0.02$; Fig. 1A). However, this GO-associated improvement in relapse did not translate into an improvement in survival, as the GO versus No-GO cohorts had a DFS of 47% ± 12% versus 41% ± 15% ($P = 0.5$; Fig. 1B) and an OS of 50% ± 10% versus 49% ± 13%, respectively ($P = 0.74$). Evaluation of

TRM demonstrated that patients who received GO had a significantly higher rate of TRM compared with the No-GO recipients (16% ± 9% vs. 0%; $P = 0.008$; Fig. 1C).

In univariable and multivariable Cox regression analyses that included treatment arm, NPM1 mutation status, diagnostic white blood cell count, and race, treatment with GO was associated with lower RR compared with No-GO (HR = 0.4, $P = 0.01$; Supplementary Table S1). There were no differences among the GO versus No-GO cohorts in OS (HR = 0.99, $P = 0.99$), EFS (HR = 0.91, $P = 0.64$), or DFS (HR = 0.77, $P = 0.36$; Supplementary Table S1). Given that all TRM events occurred in the GO arm, there was no convergence for further analysis, again demonstrating higher TRM for patients treated with GO.

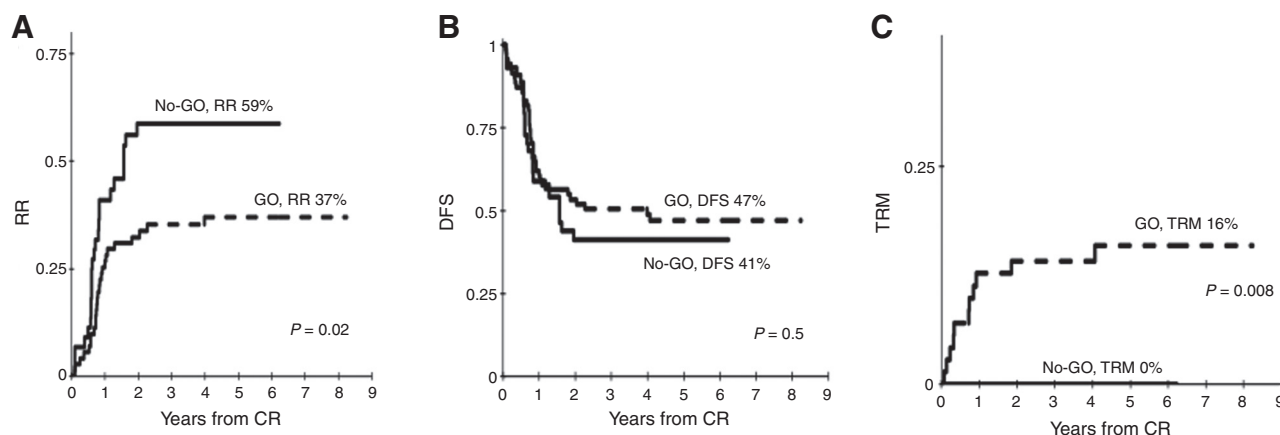
GO and HCT

Our group and others have demonstrated the poor prognostic impact of high ITD-AR on outcome, and that these patients may benefit from intensification of consolidation therapy with allogeneic HCT (13, 15, 25). As a result, an amendment was introduced into AAML0531 that allocated FLT3/ITD patients with high ITD-AR to the high-risk arm of therapy and to receive allogeneic HCT in first CR. Of the 58 patients with FLT3/ITD who received HCT in first CR, 33 had received induction GO and 25 were treated on the No-GO arm. Cumulative incidence of relapse for the GO recipients was 22% ± 15% versus 56% ± 21% for the No-GO cohort ($P = 0.003$; Fig. 2A) with a corresponding DFS of 56% ± 18% versus 40% ± 20% ($P = 0.09$; Fig. 2B). TRM for GO recipients compared with No-GO recipients was 22% ± 15% versus 4% ± 8% ($P = 0.08$; Fig. 2C). The OS for patients in the GO and No-GO cohorts was 65% ± 17% versus 49% ± 22% ($P = 0.21$).

Among FLT3/ITD patients who received consolidation chemotherapy alone ($n = 57$), patients who received GO ($n = 25$) versus No-GO ($n = 22$) had similar RR (43% ± 17% vs. 58% ± 23%, $P = 0.28$), OS (59% ± 17% vs. 60% ± 22%, $P = 0.90$), DFS (45% ± 17% vs. 42% ± 22%, $P = 0.80$), and TRM (12% ± 11% vs. 0%; $P = 0.11$; Supplementary Fig. S1).

Impact of GO and high AR FLT3/ITD

We evaluated the role of GO in this high-risk cohort with high ITD-AR who underwent HCT in first CR ($n = 41$) according to treatment with induction GO ($n = 26$) versus No-GO ($n = 15$). Posttransplant RR in GO recipients was 15% ± 15% versus

**Figure 1.**

Clinical outcomes for FLT3/ITD patients in the No-GO and GO cohorts. RR (A), DFS (B), and TRM (C).

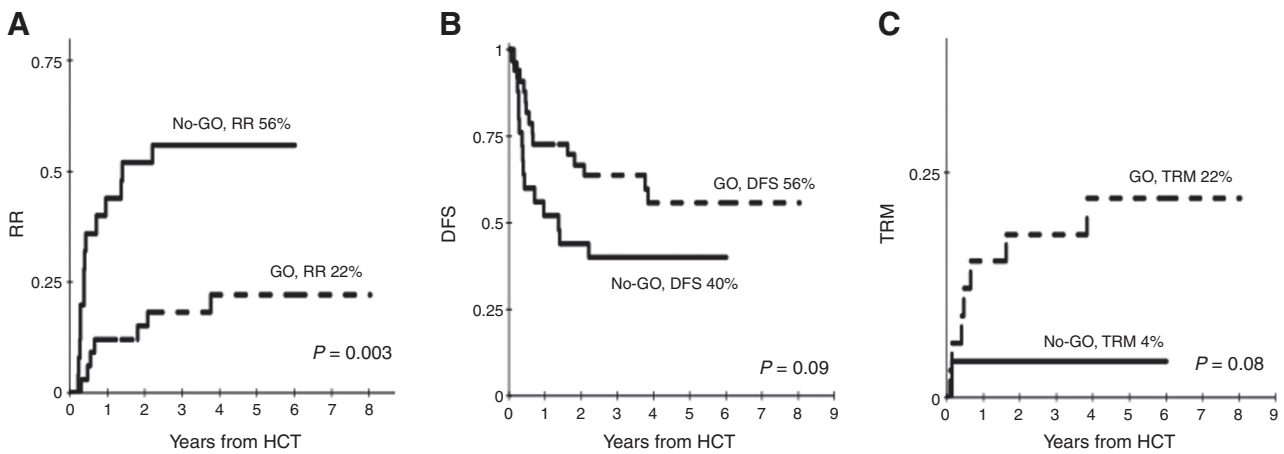


Figure 2. Outcomes for *FLT3/ITD* patients in the No-GO and GO cohorts who received consolidation HCT in first CR. RR (A), DFS (B), and TRM (C).

53% ± 27% for the No-GO cohort ($P = 0.007$; Fig. 3A) with a corresponding DFS of 65% ± 19% versus 40% ± 25% ($P = 0.08$; Fig. 3B) and OS of 68% ± 19% versus 51% ± 27% ($P = 0.33$). The TRM among GO versus No-GO recipients treated with HCT was 19% ± 16% versus 7% ± 13% ($P = 0.30$; Fig. 3C). Evaluation of the effect of GO on high ITD-AR patients who did not receive HCT demonstrated that GO recipients ($n = 17$) had a RR 47% ± 25% versus 64% ± 36% for the No-GO cohort ($n = 10$; $P = 0.42$), with a corresponding DFS of 47% ± 24% versus 36% ± 32%, ($P = 0.66$), OS of 64% ± 24% versus 56% ± 33% ($P = 0.67$), and a TRM of 6% ± 12% versus 0%, respectively ($P = 0.44$).

We analyzed outcomes of low AR (≤ 0.4) patients and found GO recipients ($n = 25$) had an OS of 53% ± 22% versus 58% ± 22% for the No-GO cohort ($n = 22$; $P = 0.99$), with a corresponding DFS of 34% ± 21% versus 44% ± 22% ($P = 0.87$) and a RR of 42% ± 22% versus 56% ± 22% ($P = 0.19$; Supplementary Table S2). High TRM was again noted in GO recipients compared with No-GO (24% ± 20% vs. 0%; $P = 0.04$). Most patients with low AR

received consolidation chemotherapy alone so these patients were not stratified further according to consolidation therapy.

GO and TRM

The increase in TRM that was seen in the GO arm for *FLT3/ITD* patients was further analyzed, especially with regard to initial concerns that GO was associated with increased incidence of post-HCT sinusoidal obstructive syndrome (SOS). Among all patients treated on AAML03P1 and AAML0531, 31 had SOS of any grade that occurred after HCT. Among *FLT3/ITD* patients, 8 had SOS of any grade (GO, $n = 6$; No-GO, $n = 2$), although the overall numbers were too low to draw any significant conclusions. In almost all cases, SOS was not fatal. However, in 3 patients treated on the two studies, SOS was listed as the cause or significant contributing cause of death and occurred in the first 30 days post-HCT. Of the 3 patients, 2 were *FLT3/ITD* patients who received GO and 1 was a *FLT3* wild-type patient who did not receive GO.

In an analysis of the causes of TRM among *FLT3/ITD* patients, we identified 14 cases of TRM in the GO cohort, equally divided

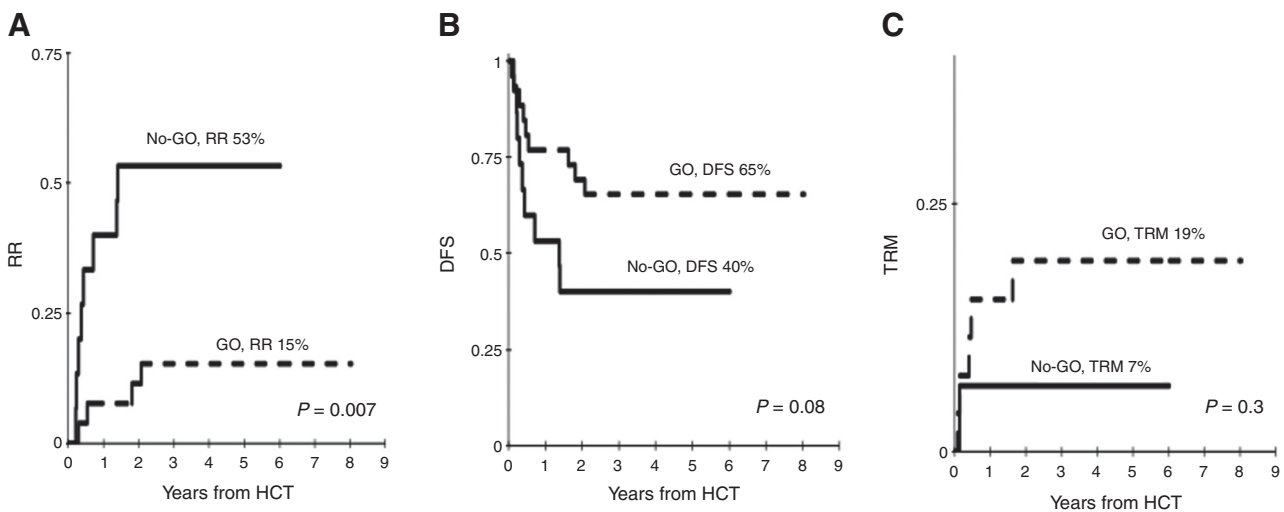


Figure 3. Outcomes for HAR *FLT3/ITD* patients treated in the No-GO and GO cohorts who received HCT. RR (A), DFS (B), TRM (C).

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between no-HCT ($n = 7$) and HCT ($n = 7$) recipients. Infections were the major cause of TRM among GO recipients ($n = 9$, 64%). Among the no-HCT recipients, 6 of 7 deaths were attributed to infection, with 1 of these deaths occurring more than 1,000 days after the end of the intensification course II, which included the last dose of GO. Among the HCT recipients in the GO cohort, infections were the cause of death in 4 patients. TRM was attributed to SOS in 1 patient and was the primary contributing cause where no primary cause was listed in 1 other patient. Among the GO cohort who received HCT, 5 of 7 deaths occurred in more than 100 days from HCT and more than 250 days from the EOII course which contained the only GO dose these patients received. Two of the deaths were very late and occurred 1.6 and 3.8 years post-HCT. There was only case of TRM in the No-GO cohort and was attributed to infection.

Because of the higher rate of TRM observed among patients in the GO cohort compared with the No-GO cohort, we evaluated the toxicity on both treatment arms. We measured the time to neutrophil and platelet recovery only for patients treated on AAML0531 ($n = 153$), because the complete data were not available for AAML03P1. After induction I, patients in the GO cohort showed a trend toward an increase in median time to neutrophil recovery [absolute neutrophil count (ANC) $> 500/\mu\text{L}$] compared with those in the No-GO cohort (33 vs. 30 days; $P = 0.06$) and an increase in median time to platelet recovery (platelet count $> 50 \times 10^9/\text{L}$; 29.5 vs. 26.5 days, respectively; $P = 0.003$). Following HCT, there was no difference in median time to neutrophil recovery among the GO versus No-GO cohort (30 vs. 30 days; $P = 0.66$). However, the median time to post-HCT platelet recovery was higher for patients in the GO cohort compared with No-GO (44 vs. 39 days; $P = 0.05$). Among FLT3/ITD patients who did not receive HCT, there were no differences in median time to recovery for ANC or platelets in intensification II or intensification course III among the GO and No-GO cohorts, with the GO cohort receiving an additional dose of GO in intensification II (Supplementary Table S3).

There were no differences between the cohorts for all grade 3 or higher toxicities (CTC v3.0 Toxicity) for any of the treatment cycles, including among patients who received HCT (Supplementary Table S4). Importantly, there were no differences among grade 3 or higher infection rates, incidence of hemorrhage, or liver dysfunction between both cohorts (Supplementary Table S4).

Discussion

In this retrospective study of *de novo* pediatric FLT3/ITD AML patients, we found that although the addition of GO to standard chemotherapy did not improve the initial CR rate, exposure to GO during induction led to significant reduction in relapse in this high-risk population. Furthermore, the impact of GO persisted into consolidation HCT, where those who had received induction GO had a significantly lower rate of relapse post-HCT. The disease benefit was most pronounced in high ITD-AR patients, the cohort with the highest risk disease. Although the improvement in RR was very clear in both univariate and multivariate analyses, the findings suggest that an increase in TRM may have attenuated the direct translation of the reduction in RR into corresponding improvements in OS. Historically, patients with high ITD-AR have had the poorest outcomes with standard chemotherapy regimens. Data from COG as well as a number of adult trials have shown the benefit of HCT in first CR for this group of patients

(13, 15, 19, 26–28). For patients in the No-GO cohort, there were no differences in outcome measures according to HCT versus no-HCT status, suggesting that GO may enhance the beneficial impact of HCT in reducing relapse risk. Our findings suggest that indeed the most optimal outcome for this high-risk cohort may be achieved with the use of induction GO followed by HCT in first CR.

The data we present are consistent with the increasing evidence from adult and pediatric clinical trials that targeting CD33 is a promising therapeutic strategy in AML and can enhance the effects of conventional chemotherapy and allogeneic HCT (8, 10, 12). A recent meta-analysis on the use of GO in AML found that GO was associated with reduction in RR and improved OS, with the most significant survival benefit in patients with good and intermediate cytogenetic risk characteristics, but not in patients with FLT3/ITD (11). Here, we show that patients with the high-risk molecular lesion FLT3/ITD do experience significant disease benefit from GO, especially when combined with HCT. In addition to our study, Castaigne and colleagues have reported results of the French ALFA-0701 study which combined fractionated dosing of GO with conventional chemotherapy, and found that patients with FLT3/ITD derived a distinct benefit from the addition of GO to induction chemotherapy (10, 29). The strategy of GO fractionated dosing is supported by data that the binding and internalization of GO results in renewed CD33 expression on myeloid blasts, which can be subsequently exploited to further saturate the blasts with additional GO and increase cell death (30). Thus, fractionated dosing may provide further improvements in clinical outcomes for FLT3/ITD patients, especially when combined with consolidation HCT.

The single-dose GO regimen utilized in the COG trials was in part selected due to initial concerns for GO-induced hepatotoxicity, particularly SOS when GO was given in close proximity to HCT (31, 32). Although we did observe a few cases of SOS in the GO cohort, there was no apparent increase and the overall numbers are too low to draw any definitive conclusions. Patients in the GO cohort had significantly higher TRM overall, with the majority of deaths attributed to infection. It is important to note that the TRM in the No-GO cohort was very low, with only 1 death, which occurred post-HCT. This very low TRM rate is atypical for the intensity of standard AML therapy and lower than what was seen in AAML03P1 and AAML0531 overall, where there were no observed differences in overall TRM for patients in the GO versus No-GO cohorts (6, 12). Importantly, half of the TRM events in our study occurred over 100 days from the last dose of GO, and do not immediately seem to be directly attributable to GO. Even among patients who received GO prior to HCT, the majority of TRM events also occurred at over 100 days following HCT.

The GO recipients did have longer duration to platelet and neutrophil recovery in the courses prior to HCT, including post-induction I courses that did not include GO, as well as delayed platelet recovery following HCT. The delayed platelet recovery we observed correlates with that previously reported in adult AML patients receiving GO (10). Our findings suggest that there may be significant GO-induced effects on normal CD33-positive myeloid progenitors, leading to potential marrow exhaustion, which may be exacerbated by exposure to subsequent cytotoxic therapy and lead to significantly prolonged myelosuppression. This delayed hematopoietic recovery and prolonged neutropenia could be a potential contributor to the higher TRM seen in the GO cohort, which was largely due to infectious causes. We observed no

differences in infection rates between the two cohorts overall; however, it is possible that prolonged myelosuppression could contribute to increased morbidity and mortality in the setting of infection. Further understanding of the lasting effects of CD33-targeting on myeloid progenitors could inform the safety of combining these agents with additional myelosuppressive therapies, including HCT.

We have previously shown that high-risk disease features, including *FLT3/ITD*, have high CD33 expression (4). Studies by van der Velden and colleagues demonstrated that the level of bone marrow blast CD33 expression correlates with peripheral blood blast clearance by GO (30). This suggests that high CD33 expression may mediate the antileukemic efficacy of GO in *FLT3/ITD*-positive AML. Adequate targeting of leukemia stem cells (LSC) is essential to therapeutic efficacy and similar to bulk blasts, the sensitivity of LSCs to GO has been correlated with the level of CD33 expression (33). Importantly, LSCs that harbor *FLT3/ITD* mutations are more sensitive to GO-mediated cytotoxicity when compared with non-*ITD* LSCs (33), suggesting that additional biologic features may be involved. High levels of CD33 expression, which is often present on *FLT3/ITD* blasts, may enhance the efficacy of GO. However, it is likely that multiple biologic features, including overall disease burden and Pgp status, contribute to moderate the sensitivity to GO and other CD33-targeted agents.

FLT3/ITD is one of the first genomic lesions in AML to be targeted for therapeutic benefit with tyrosine kinase inhibitors (TKI), as the blasts are uniquely susceptible to apoptotic effects of FLT3 inhibition (34–37). To date, FLT3 inhibitors have demonstrated the ability to favorably affect initial disease response, but have thus far failed to achieve sustained remissions and improvements in overall outcomes (38–42). For many patients, FLT3 inhibition might be an inadequate therapeutic strategy, even when combined with conventional chemotherapy, as *FLT3/ITD* is often not the only driver mutation and can be a later event absent in the founding clone (43, 44). Thus additional therapeutic strategies, including those that target the LSC population, are necessary. The strategy of bulk reduction, a setting wherein TKIs can be very effective, might be combined with CD33-directed therapeutics, which can effectively target bone marrow blasts, to create more effective therapeutic regimens. TKIs can also be used for prolonged periods of time, whereas GO or anti-CD33 agents may have myelosuppressive effects limiting the timing and duration that they can be safely used.

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Our findings confirm that CD33 targeting is an effective therapeutic strategy in AML and *FLT3/ITD* patients may derive a distinct disease benefit from this therapy, especially when combined with HCT for high *ITD-AR* patients. The increased TRM in this population warrants further investigation regarding optimal dosing and timing, especially in conjunction with HCT, to improve overall outcomes. Newly developed CD33-targeted agents, which may have distinct therapeutic profiles, are currently under investigation in clinical trials (45, 46). *FLT3/ITD* patients might experience significant benefit from this treatment strategy, especially when combined with additional therapies such as HCT and FLT3 inhibition.

Disclosure of Potential Conflicts of Interest

A. Gamis is a consultant/advisory board member for Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: K. Tarlock, T.A. Alonzo, A.S. Gamis, S. Meshinchi
Development of methodology: K. Tarlock, M.R. Loken, A.S. Gamis, S. Meshinchi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Tarlock, S.C. Raimondi, B.A. Hirsch, L. Sung, J.A. Pollard, R. Aplenc, M.R. Loken, A.S. Gamis, S. Meshinchi

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Tarlock, T.A. Alonzo, R.B. Gerbing, R. Aplenc, M.R. Loken, S. Meshinchi

Writing, review, and/or revision of the manuscript: K. Tarlock, R.B. Gerbing, S.C. Raimondi, B.A. Hirsch, L. Sung, J.A. Pollard, R. Aplenc, M.R. Loken, A.S. Gamis, S. Meshinchi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.B. Gerbing, R. Aplenc, S. Meshinchi

Study supervision: A.S. Gamis, S. Meshinchi

Other (review of cytogenetics data): B.A. Hirsch, S.C. Raimondi

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