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CLINICAL SIGNIFICANCE OF CD33 NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN PEDIATRIC PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH GEMTUZUMAB-OZOGAMICIN-CONTAINING CHEMOTHERAPY

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R.B.W. received research funding from and has advisory and consultant role at Seattle Genetics Inc., J.F is employed at leadership position, has stocks and obtained research funding at Amgen Inc. M.R.L is funded and employed with leadership position at Hematologics Inc. The remaining authors declare no competing financial interests.

Abstract

Purpose—Purpose of this study was to evaluate clinical implications of CD33 SNPs in pediatric acute myeloid leukemia (AML) patients treated with gemtuzumab ozogamicin (GO) based therapy.

Experimental Design—We genotyped four CD33 SNPs: rs35112940 (G>A; Arg304Gly), rs12459419 (C>T; Ala14Val), rs2455069 (A>G; Arg69Gly) and rs1803254 (G>C; 3' UTR) in pediatric patients undergoing induction chemotherapy containing GO (COG-AAML03P1 trial; n=242) or not containing GO (St. Jude AML02 trial; n=172).

Results—CD33 SNPs were correlated significantly with clinical characteristics and treatment outcome. The coding SNPs, rs35112940 and rs12459419, were significantly associated with clinical endpoints in COG-AAML03P1 but not in the St. Jude AML02 trial. Specifically, among white patients in COG-AAML03P1, the 3-year overall survival (OS) from remission was 84±8% for those homozygous (GG) for rs35112940 vs. 68±15% for the other genotypes (p=0.018); these patients also had a lower relapse risk and superior disease-free survival. Likewise, patients homozygous for variant allele (TT) for rs12459419 were more likely have favorable-risk disease as compared CC and CT genotypes (52% vs. 31%, p=0.034) and significantly lower diagnostic blast CD33 expression as compared to other genotypes (p<0.001).

Conclusion—Our data suggest that genetic variations in CD33 could impact clinical outcome of GO-based therapy in pediatric AML.

Introduction

Acute myeloid leukemia (AML) remains a difficult-to-treat disease, and novel, efficacious therapies are needed. A promising approach includes monoclonal antibodies as specific means to deliver anti-AML therapy. The cell surface antigen currently most exploited is CD33, given its expression on AML blasts of 85–90% of all patients and preclinical studies indicating that ablation of CD33⁺ cells could eliminate leukemia *in vitro* and allow re-establishment of normal hematopoiesis in some cases (1).

The recognition that antibodies were internalized after binding to CD33 led to the development of gemtuzumab ozogamicin (GO, MylotargTM), an immunoconjugate between an anti-CD33 antibody and a toxic calicheamicin- γ derivative (1–3). As the first cancer immunoconjugate ever to receive FDA approval by the U.S. Food and Drug Administration (FDA), GO was indicated for the treatment of CD33⁺ AML in first relapse for patients >60 years of age who were not considered candidates for conventional chemotherapy (4). This approval was based on results from interim analyses of phase 2 monotherapy trials showing achievement of complete remission (CR) or CR with incomplete platelet recovery (CRp) in ~30% of such patients (4). Recent studies from several large, well controlled trials now suggest a benefit of GO when added to conventional chemotherapy for significant subsets of patients with untreated AML(5, 6).

Nevertheless, while efficacious in some patients, clinical response to GO is heterogeneous, emphasizing the need for a better understanding of the subsets of patients that will/will not benefit from GO. Although response to therapy is generally multi-factorial in origin, there is often a significant contribution of host genetic factors. A recent small pilot study from our laboratory suggested the possibility that single nucleotide polymorphisms (SNPs) in CD33, the drug target could constitute such a genetic factor (7). Herein, we sought to investigate the clinical impact of CD33 SNPs in a larger cohort of patients enrolled on the Children's Oncology Group (COG) AAML03P1 trial, a pilot phase 3 study that explored the feasibility and safety of adding a single dose of GO to intensive induction chemotherapy for newly diagnosed AML (8). As a control group, we evaluated CD33 SNPs in patients enrolled on St Jude AML02 clinical trial who did not receive GO.

PATIENTS AND METHODS

Patient Samples

Cohort I—Details of COG-AAML03P1 (NCT00070174) were reported previously (8). Briefly, from 2003–2005, this trial enrolled 340 children (aged 1 month to 21 years) with newly diagnosed *de novo* AML, excluding those with acute promyelocytic leukemia and Down syndrome. Cycle regimens were cytarabine/daunorubicin/etoposide (ADE) plus GO (induction I), ADE (induction II), high-dose cytarabine and etoposide (intensification I), mitoxantrone/cytarabine plus GO (intensification II), and sequential high-dose cytarabine and asparaginase (intensification III). Patients with matched family donor could receive SCT on study after intensification I and were not excluded or censored from the analyses. Patients who were taken off protocol at the end of induction 1 due to marrow blasts exceeding 20% were considered an event (induction failure) for EFS, but were not included for analyses from the end of course 1 (DFS, RR, TRM).

CD33 SNPs were determined in genomic DNA isolated from bone marrow aspirate specimens from patients who consented to biological studies and had such specimens available at the end of Induction I (n=242). Informed consent was obtained in accordance with the Declaration of Helsinki. The institutional review boards of all participating institutions approved the clinical protocol, and the COG Myeloid Disease Biology Committee approved this study.

Cohort II—Details of the St Jude AML02 study trial (NCT00136084) were described elsewhere (9). Briefly, from 2002–2008, 232 children with *de novo* AML (n=206), therapy-related or myelodysplastic syndromes (MDS)-related AML (n=12), or mixed-lineage leukemia (n=14) were randomized to receive high-dose cytarabine (n=113) or low-dose cytarabine (n=117) plus daunorubicin and etoposide. Subsequent therapy was adapted based on minimal residual disease (MRD) as assessed by flow cytometry and diagnostic risk features. Out of 232 patients, 176 patients who did not receive GO and had specimens available were included in the present study. The Institutional review board approved the study.

Identification and Genotyping of SNPs in CD33

To characterize the spectrum of frequently occurring CD33 SNPs, we sequenced the coding region of *CD33* in 100 randomly selected samples from the COG-AAML03P1 cohort, using methods described previously (7). Sequencing studies identified 4 CD33 SNPs (rs12459419, rs35112940, rs2455069, and rs1803254) that occurred with the minor allele frequency (MAF) of >10%. These 4 SNPs were genotyped in the AAML03P1 (n=242) and the St. Jude AML02 (n=176) cohort using the Sequenom platform at Biomedical Medical Genomics Center (BMGC) at University of Minnesota.

Risk Stratification

AAML03P1—A combination of cytogenetic and molecular abnormalities was used for the retrospective risk stratification for this analysis. A patient was considered *favorable-risk* if a chromosomal abnormality/mutation was present in core-binding factors (CBF, t(8;21) or inv(16)/t(16;16)), nucleophosmin-1 (*NPM1*), or *CEBPA*. Patients were classified as *high-risk* if they had -5/5q-, monosomy 7, or *FLT3*/ITD with high allelic ratio. All other patients with data sufficient for classification were considered *standard-risk*. Patients of unknown risk were those for whom cytogenetic/molecular data were insufficient for classification.

St Jude AML02—Patients were provisionally classified as having *low-risk* AML if their leukemic cells had t(8;21)/*AML1-ETO*, inv(16)/*CBFβ-MYH11*, or t(9;11)/*MLL-AF9*. *High-risk* cases included those with -7, *FLT3*-ITD, t(6;9), megakaryoblastic leukemia, treatment-

related AML, or AML arising from myelodysplastic syndrome. All other patients were provisionally classified as having *standard-risk* AML.

Assessment of CD33 Expression

CD33 expression on viable leukemic cells, as identified by CD45/side scatter gating properties, was prospectively quantified by multiparameter flow cytometry after staining of diagnostic bone marrow specimens (AAML03P1) with CD14, CD33, CD45, and CD34 (BD Biosciences, San Jose, CA) in a centralized laboratory. Linear mean fluorescence intensity (MFI)(10) of CD33 was determined using WinList (Verity Software House, Topsham, ME) for each patient without knowledge of other clinical characteristics.

Statistical Analysis

The significance of observed differences in proportions was tested using the Chi-squared test and Fisher's exact test when data were sparse. The Kruskal-Wallis test was used to determine the significance between differences in medians. The Kaplan-Meier method was used to estimate overall survival (OS), event-free survival (EFS), and disease-free survival (DFS). OS was defined as time from study entry or from end of course 1 for patients in complete remission (CR) to death. EFS was defined as time from study entry until death, failure to achieve remission during induction therapy, or relapse. DFS was defined as time from end of course 1 for patients in CR until relapse or death. Relapse-free survival (RFS) was defined as the time from end of course 1 for patients in CR until relapse or death due to progressive disease where deaths from non-progressive disease were censored. Estimates of relapse risk (RR) and treatment related mortality (TRM) were obtained using methods that account for competing events. RR was defined as time from end of course 1 for CR patients to relapse where deaths from non-progressive disease were considered competing events. Within the AAML03P1 cohort 5 patients died by the end of course 1, however none of these were included in this analysis hence TRM during induction 1 was not included. Rather TRM was defined as time from end of course 1 for CR patients to death from non-progressive disease where relapses were considered competing events. The significance of predictor variables was tested with the log-rank statistic for OS, EFS, DFS, and with Gray's statistic for RR and TRM. Clinical outcome data were analyzed through November 18, 2010. Children lost to follow-up were censored at their date of last known contact or at a cut off 6 months prior to November 18, 2010. Cox proportional hazard models were also used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for univariate and multivariate analyses.

RESULTS

CD33 SNPs in COG-AAML03P1 and St. Jude AML02

Among 340 participants of COG-AAML03P1, 242 (71%) had DNA available from bone marrow aspirates done at end of Induction I. In order to initially identify frequently occurring CD33 SNPs, sequencing was performed in 100 samples that were randomly selected from these 242 specimens. A total of 8 SNPs (1 synonymous and 7 non-synonymous) were identified in the coding region, one each in a splice site (intron 6) and in 3'UTR (Table 1). All but one of these SNPs has been reported earlier³. Of these 10 SNPs, 4 SNPs had a minor allele frequency (MAF) of >10% (rs12459419, rs35112940, rs2455069, and rs1803254) and were selected for further analysis. Three of these were missense SNPs, resulting in a change in amino acid, and one (rs1803254) was located in the 3' untranslated region (UTR) (Table 1).

Supplementary Table 1 summarizes the comparisons of demographics as well as laboratory and clinical characteristics of the patients across the genotype groups for these four SNPs in COG-AAML03P1 cohort. CD33 SNPs rs12459419, rs35112940, and rs1803254 occurred with significantly different frequency in African American vs. white patients ($P<0.05$). Hence, we

performed subsequent analyses in all the patients as well as African Americans and Caucasians separately. Within the COG-AAML03P1 cohort, patients with the TT genotype of rs12459419 were significantly more likely to have *favorable-risk* disease than those with at least one C allele (CC or CT genotype; 52% vs. 31%; $P=0.034$). Similar observation was not seen in St. Jude cohort, as the risk group definition was slightly different than COG. Supplementary Table 2 summarizes the comparisons of demographics as well as other patient characteristics for CD33 SNPs (rs1803254, rs35112940 and rs12459419) in the St. Jude AML02 clinical trial.

Association between CD33 SNPs and CD33 Expression in COG-AAML03P1

Quantitative data on CD33 expression were available from 222 (91.7%) of the 242 patients from COG-AAML03P1. As expression of CD33 has recently been linked to clinical outcome (11), we wondered about a relationship between CD33 SNPs and CD33 expression. Indeed, we found a significant association between 3 CD33 SNPs and CD33 expression on AML blasts. As shown in Figure 1, patients homozygous for the reference allele (AA) of the SNP, rs2455069 (A>G; Arg69Gly), had a significantly lower median CD33 expression compared to those with either AG or AA genotype (AA vs. AG vs. GG: 90.2 vs. 144.4 vs. 166.6 respectively; $p=0.022$). rs2455069 also occurs in LD with a promoter SNP rs386544. Furthermore, SNP rs12459419 (C>T; Ala14Val) and the 3'UTR SNP rs1803254 (G>C) were both associated with lower median CD33 expression (rs12459419: CC vs. CT vs. TT; 152.2 vs. 97.4 vs. 44.8; $p<0.001$ and rs1803254: GG vs. CG vs. CC; 146.1 vs. 94.7 vs. 46.4; $p=0.029$). rs35112940 SNP that results in coding change was not associated CD33 expression levels in the diagnostic leukemic blasts (AA vs. AG vs. GG; 39.6 vs. 99.2 vs. 139.1; $p=0.39$) (Figure 1).

Association of CD33 SNPs with Clinical Outcome

Finally, we evaluated clinical outcome data for the various genotypes of each SNP (Table 2); due to the varying prevalence of the SNPs across different races, clinical outcomes were also compared separately for African Americans and Caucasians. For SNPs rs35112940, rs12459419, and rs1803254 but not rs2455069, our analyses indicated significant associations with clinical outcome as follows:

SNP rs35112940: relative to patients with the GG genotype, those with variant alleles (AA and AG genotypes) had a significantly higher RR ($48\pm14\%$ vs. $29\pm7\%$; $p=0.019$). As rs35112940 was predominantly present in Caucasians (MAF = 0.16) and was quite rare in African Americans (MAF= 0.01), we restricted further analyses to Caucasian patients. As shown in Figure 2A, OS from end of course 1 for patients with variant allele (genotype AA and AG, $n=40$) was significantly worse than for those with the GG genotype ($n=101$; $68\pm15\%$ vs. $84\pm8\%$; $p=0.036$). OS from study entry followed a similar trend but did not reach statistical significance ($67\pm14\%$ vs. $78\pm8\%$ for AA+AG vs. GG; $p=0.09$). Consistently, patients carrying variant allele (AA+ AG) for rs35112940 had the higher RR than those with the GG genotype ($45\pm16\%$ vs. $24\pm8\%$; AA+AG vs. GG $p=0.018$; Figure 2B and Table 2). Furthermore, DFS from the end of course 1 was marginally lower in patients homozygous for variant allele as compared to patients with at least one G allele ($66\pm8\%$ vs. $33\pm39\%$; $p=0.05$; Table 2).

SNP rs12459419: A trend towards better 3-year OS from study entry was observed for patients homozygous with TT genotype as compared to patients having at least one C allele (CC+CT) genotype ($88\pm13\%$ vs. $69\pm6\%$; $p=0.056$).

SNP rs1803254: Patients with at least one variant allele (CC+CG) had a 3-year TRM of $13\pm12\%$ compared to $3\pm3\%$ for patients wild-type allele (GG) ($p=0.027$, Figure 3).

Multivariate analyses using Cox regression models were performed for OS, EFS from study entry as well as OS, DFS and RFS from end of course 1 for patients with rs12459419,

rs35112940, and rs1803254 SNPs separately. After adjusting for race (white vs. not white), and risk group (standard, favorable, high), in multivariate analyses rs35112940 SNP retained significance for RFS (HR=1.91, 95% CI: 1.10 – 3.32, $p=0.022$). Additional multivariate analyses including adjustments for WBC (<50k vs. $\geq 50K$) yielded similar results. We also evaluated association of CD33 SNPs (rs35112940, rs12459419, and rs1803254) in 176 patients enrolled on St. Jude AML02 study who did not receive GO as part of their chemotherapy. Compared to COG-AAML03P1, participants on this trial were relatively similar with regard to gender, age, race, cytogenetics, and FLT3 status (all $p>0.1$). Equally comparable were allele frequency of these CD33 SNPs (15% vs. 19% for rs1803254; 15% vs. 13% for rs35112940, and 28.2% vs. 28.7% for rs12459419 in St. Jude AML02 and COG-AAML03P1, respectively). None of the CD33 SNPs demonstrated significant association with clinical outcome (MRD day 22, OS, DFS, RFS or RR) within this cohort (results for rs35112940 are shown in Figure 4A and 4B).

Discussion

In this study, we evaluated 3 non-synonymous coding SNPs and one 3'UTR SNP in *CD33* for their association with disease features and outcomes of intensive chemotherapy in pediatric AML. We found that several SNPs were significantly associated with outcome in patients who received GO-containing therapy but not in those who did not receive GO, suggesting that CD33 SNPs impact clinical response to GO.

The long-term goal of pharmacogenetic studies such as ours is to use genotype data to predict beneficial or adverse response to specific drug and then utilize this information to optimize the drug's therapeutic efficacy and minimize its toxicity by helping in the selection of appropriate patient subsets.

Emerging clinical data from several studies now indicate that GO might be efficacious for subgroups of AMLs (5, 6, 12, 13). Pharmacogenetic information may thus be particularly useful for a drug like GO, which has been shown to provide important benefit for some patients with AML but is ineffective in others, in which it may only lead to increased TRM. Given this significant inter-patient variation in response, the drug's benefit may be missed if used in unselected patients. In fact, the lack of benefit of GO across the entire study population of the Southwest Oncology Group S0106 trial and the slightly higher TRM observed in this study led the manufacturer of GO to withdraw commercial drug availability in the U.S. in 2010 (1, 14). We analyzed association of CD33 SNPs with clinical outcome measures both from entry and end of course 1 and found most significant association from end of induction 1. By identifying CD33 SNPs that are associated with response to GO-containing therapy, in particular SNP rs35112940 – which we found associated with lower OS, DFS, and higher RR from the end of course 1 – our data suggest that pharmacogenetic information might be useful to individualize the use of GO in the clinic.

The potential molecular mechanisms underlying the observed associations between CD33 SNPs and CD33 expression and/or clinical outcome are currently unclear and under active investigation. However, as the rs35112940 SNP results in an amino acid substitution close to the ITIM motif in CD33, it is tempting to speculate that this SNP could impact the formation of a CD33-GO complex or the rate of its internalization. On the other hand, rs12459419 SNP that was significantly associated with CD33 expression, results in an amino acid change in the signal peptide in CD33, which may interfere with protein trafficking and cell surface expression of CD33 molecules. In pediatric AML patients receiving GO as part of a multi-agent therapy, higher CD33 expression has been shown to be associated with high-risk disease (FLT3/ITD + disease, high risk cytogenetics e.g. -7, -5, -5q)(11). This SNP was also more frequent in patients within favorable risk group in AAML03P1 cohort but not in AML02 cohort, the reason

for this discrepancy is not clear. While the risk classifications of the two protocols were similar, they were not identical and it is not possible to fully reconcile the risk classifications of the two protocols because they each measure some molecular characteristics that were not captured on the other protocol. Future work to define the interplay between CD33 SNP, CD33 expression and risk group is needed to understand the molecular mechanism responsible for this observation.

A major limitation of our study is that the conclusion that clinical impact of CD33 SNPs is limited to GO is based on a comparison of data from 2 independent clinical trials (COGAAML03P1 and St. Jude AML02). It is theoretically possible that CD33 and CD33 SNPs can impact response to non-CD33-targeted chemotherapy. In fact, although the biological role of CD33 remains obscure, CD33 has been implicated in cellular proliferation and survival of AML cells (15); thus, more global effects of CD33 SNPs need to be considered. Another major limitation is that we performed a large number of comparisons which can increase the likelihood of a false positive result. Therefore, further validation of our findings is critical. To this end, our future studies are targeted towards evaluation of COG-AAML0531, a phase 3 trial of >1,000 patients in which the value of GO addition to intensive chemotherapy was studied in a randomized fashion. This will allow a direct assessment as to whether the association between outcome and CD33 SNPs is limited to GO, as our data indicate.

In conclusion, our findings suggest that CD33 SNPs identified in the genomic DNA are associated with clinical features of AML and outcome after GO-containing combination chemotherapy in AML patients. If these data are confirmed, CD33 SNPs could serve as prognostic markers and to help in the selection of patient subsets most suitable for therapies containing GO and possibly other CD33-targeted immunotoxins in future prospective trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Walter RB, Appelbaum FR, Estey EH, Bernstein ID. Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. *Blood*. 2012; 119(26):6198–208. Epub 2012/01/31. [PubMed: 22286199]
2. Bernstein ID, Singer JW, Andrews RG, Keating A, Powell JS, Bjornson BH, et al. Treatment of acute myeloid leukemia cells in vitro with a monoclonal antibody recognizing a myeloid differentiation antigen allows normal progenitor cells to be expressed. *The Journal of clinical investigation*. 1987; 79(4):1153–9. Epub 1987/04/01. [PubMed: 3470307]
3. Bernstein ID. Monoclonal antibodies to the myeloid stem cells: therapeutic implications of CMA-676, a humanized anti-CD33 antibody calicheamicin conjugate. *Leukemia: official journal of the Leukemia Society of America, Leukemia Research Fund, UK*. 2000; 14(3):474–5. Epub 2000/03/17.
4. Bross PF, Beitz J, Chen G, Chen XH, Duffy E, Kieffer L, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clinical cancer research: an official journal of the*

- American Association for Cancer Research. 2001; 7(6):1490–6. Epub 2001/06/19. [PubMed: 11410481]
5. Castaigne S, Pautas C, Terre C, Raffoux E, Bordessoule D, Bastie JN, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet*. 2012; 379(9825):1508–16. Epub 2012/04/10. [PubMed: 22482940]
 6. Burnett AK, Hills RK, Milligan D, Kjeldsen L, Kell J, Russell NH, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2011; 29(4):369–77. Epub 2010/12/22. [PubMed: 21172891]
 7. Lamba JK, Pounds S, Cao X, Downing JR, Campana D, Ribeiro RC, et al. Coding polymorphisms in CD33 and response to gemtuzumab ozogamicin in pediatric patients with AML: a pilot study. *Leukemia: official journal of the Leukemia Society of America, Leukemia Research Fund, UK*. 2009; 23(2):402–4. Epub 2008/07/11.
 8. Cooper TM, Franklin J, Gerbing RB, Alonzo TA, Hurwitz C, Raimondi SC, et al. AAML03P1, a pilot study of the safety of gemtuzumab ozogamicin in combination with chemotherapy for newly diagnosed childhood acute myeloid leukemia: a report from the Children's Oncology Group. *Cancer*. 2012; 118(3):761–9. Epub 2011/07/19. [PubMed: 21766293]
 9. Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *The lancet oncology*. 2010; 11(6):543–52. Epub 2010/05/11. [PubMed: 20451454]
 10. Wells DAL, MR. Flow cytometric mean fluorescence intensity: The biophysics behind the number. *Leukemia research*. 2008; 32(6)
 11. Pollard JA, Alonzo TA, Loken M, Gerbing RB, Ho PA, Bernstein ID, et al. Correlation of CD33 expression level with disease characteristics and response to gemtuzumab ozogamicin containing chemotherapy in childhood AML. *Blood*. 2012 Epub 2012/03/02.
 12. Estey E. Treatment of AML: resurrection for gemtuzumab ozogamicin? *Lancet*. 2012; 379(9825):1468–9. Epub 2012/04/10. [PubMed: 22482941]
 13. Burnett AK, Russell NH, Hills RK, Kell J, Freeman S, Kjeldsen L, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2012; 30(32):3924–31. Epub 2012/08/02. [PubMed: 22851554]
 14. Petersdorf SKK, Stuart RK, et al. Preliminary results of Southwest Oncology Group Study S0106: an international intergroup phase 3 randomized trial comparing the addition of gemtuzumab ozogamicin to standard induction therapy versus standard induction therapy followed by a second randomization to post-consolidation gemtuzumab ozogamicin versus no additional therapy for previously untreated acute myeloid leukemia. *Blood*. 2009; 114:Abstract 790.
 15. Mingari MC, Vitale C, Romagnani C, Falco M, Moretta L. p75/AIRM1 and CD33, two sialoadhesin receptors that regulate the proliferation or the survival of normal and leukemic myeloid cells. *Immunological reviews*. 2001; 181:260–8. Epub 2001/08/22. [PubMed: 11513147]

Translational Relevance

Acute myeloid leukemia (AML) remains a difficult-to-treat disease, and novel, efficacious therapies are needed. Gemtuzumab-Ozogamicin (GO), a CD33 antibody conjugated with the toxin calicheamicin, is a promising agent for AML therapy. However, there is substantial inter-patient variability in clinical response to GO. In the present study we evaluated CD33 genetic variation and our results suggest that coding polymorphisms within CD33 are significantly associated with clinical outcome in AML patients receiving GO containing chemotherapy. Integration of this information as independent prognostic marker into current cytogenetic/molecular-based risk classification models would present an opportunity to increase our accuracy in forecasting therapeutic outcome in AML. This refinement would help our efforts to inform patients properly about expected treatment results and provide better-quality guidance in their decision making process. An improvement in disease-risk classification would also allow more tailored, risk-stratified treatment approaches, which will be a major advancement over current strategies.

Figure 1.

Association of CD33 SNPs with its expression determined as mean fluorescence intensity (MFI) in diagnostic leukemic blasts from AML patients enrolled in AAML03P1. CD33 levels were determined in the diagnostic leukemic blast by multiparameter flow cytometry.

Figure 2. Prognostic significance of CD33 SNP rs35112940 (Arg304Gly) in white AML patients treated with GO+ADE on AAML03P1 study. Kaplan-Meier estimates of (A) Relapse risk and (B) Overall survival in white AML patients for different genotypes of rs35112940 SNP.

Figure 3.

Prognostic significance of CD33 3'UTR SNP rs1803254 in white AML patients treated with GO+ADE on AAML03P1 study. Kaplan-Meier estimates of treatment related mortality in white AML patients with or without at least one C allele for rs180254.

Figure 4. Prognostic significance of CD33 SNP rs35112940 (Arg304Gly) in white AML patients treated with regimen excluding GO on St Jude AML02 study. Kaplan-Meier estimates of (A) Relapse risk and (B) Overall survival in white AML patients for different genotypes of rs35112940 SNP.

Table 1

CD33 SNPs identified by sequencing and genotyped in the present study.

CD33 SNP	rs#	Location	Allele Frequency	Flanking sequence
Leu10Leu	rs117255828	Exon 1	T=0.02	GCTGCCCTG-C-TGTGGGCAGG
Ala14Val	rs12459419	Exon 2	T=0.31	CCCACAGGGG-C-CCTGGCTATG
Arg62Glu		Exon 2	A=0.005	TACTGGTTCC-G-GGAAGGAGCC
Ala65Pro		Exon 2	C=0.005	CCGGGAAGGA-G-CCATTATATC
Arg69Gly	rs2455069	Exon 2	G=0.4	CCATTATATCC-A-GGGACTCTCC
Phe243Leu	rs11882250	Exon 4	C=0.01	AACTGGTATC-T-TTCCAGGAGA
Val267Ile	rs58981829	Exon 5	A=0.02	AGGAGCTGGT-G-TTACAGCCCT
Arg304Gly	rs35112940	Exon 6	A=0.13	CCCTACCACA-G-GGTCAGCCTC
Ser305Pro	rs61736475	Exon 6	C=0.04	TACCACAGGG-T-CAGCCTCCCC
Ala331Thr	rs35632246	Exon 7	G=0.004	TGCCGCCCT-A-CTGTGGAGAT
intron 6	rs273621	Intron 6 (Splice site)	C=0.01	GCCTCCCCGG-T-GAGTGATGGG
3'UTR	rs1803254	3'UTR	C=0.22	CATTGCTAA-G-TGTATGATGT

SNPs in bold occur with minimum allele frequency (MAF) of more than 0.1 were selected for genotyping.

Table 2

Association of CD33 SNPs with clinical endpoints in white AML patients treated on COG AAML30P1 and St Jude AML02 trials.

CD33 SNP	COG-AAML03P1(with GO+ADE)		St Jude AML02 (without GO treatment)	
rs 35112940 (Arg304Gly)	3 yr OS from end of course 1		3 yr OS from end of course 1	
	AA (n=6)	50 ± 41	AA (n=5)	100 ± 0
	AG (n=34)	71 ± 16	AG (n=34)	64.8 ± 8.6
	GG (n=101)	84 ± 8	GG (n=81)	77.7 ± 4.9
	AA vs. AG vs. GG; p=0.036 AA+AG vs. GG; p=0.018		AA vs. AG vs. GG; p=0.23 AA+AG vs. GG; p=0.61	
rs 35112940 (Arg304Gly)	3 yr DFS from end of course 1		3 yr DFS from end of course 1	
	AA (n=6)	33 ± 39	AA (n=5)	80 ± 16
	AG (n=34)	56 ± 17	AG (n=29)	67.6 ± 9.3
	GG (n=101)	70 ± 9	GG (n=59)	79.3 ± 5.8
	AA vs. AG vs. GG; p=0.064 AA vs. AG+GG; p=0.05		AA vs. AG vs. GG; p=0.50 AA vs. AG+GG; p=0.76	
rs 35112940 (Arg304Gly)	3 yr RR from end of course 1		3 yr RR from end of course 1	
	AA (n=6)	67 ± 38	AA (n=5)	20 ± 20
	AG (n=34)	41 ± 17	AG (n=29)	25.3 ± 8.5
	GG (n=101)	24 ± 9	GG (n=59)	15.7 ± 4.9
	AA vs. AG vs. GG; p=0.018 AA vs. AG+GG; p=0.021 AA+AG vs. GG; p=0.025		AA vs. AG vs. GG; p=0.55 AA vs. AG+GG; p = 0.93 AA+AG vs. GG; p = 0.29	
rs180234 (3'UTR)	3 yr TRM from end of course 1		3 yr TRM from end of course 1	
	CC (n=3)	0 ± 0	CC (n=4)	0 ± 0
	CG (n=29)	14 ± 13	CG (n=12)	8.3 ± 8.4
	GG (n=109)	3 ± 3	GG (n=73)	9.9 ± 3.6
	CC vs. CG vs. GG; p=0.051 CC+CG vs. GG; p=0.029		CC vs. CG vs. GG; p=0.81 CC+CG vs. GG; P=0.67	