Cluster of differentiation 33 single nucleotide polymorphism rs12459419 is a predictive factor in patients with *nucleophosmin1*-mutated acute myeloid leukemia receiving gemtuzumab ozogamicin

Gemtuzumab ozogamicin (GO) is a humanized anti-cluster of differentiation (CD) 33 monoclonal antibody linked to the cytotoxic agent calicheamicin.[1-4] GO binds the CD33 antigen and is internalized; calicheamicin is released inside the blasts leading to DNA damage and death of leukemic cells.[2,5,6]

GO was approved for the treatment of patients with acute myeloid leukemia (AML) with expression of CD33 on blasts by the European Medicines Agency (here only for newly diagnosed patients in combination with intensive therapy) and the Food and Drug Administration based on positive results from the ALFA-0701 trial.[1,7] However, a number of clinical trials with GO yielded partially conflicting results emphasizing the importance of a better understanding of factors influencing the clinical response to GO.[6,7]

Several factors can influence the response to GO. A meta-analysis by Hills *et al.* demonstrated a benefit of GO only in patients with Medical Research Council (MRC) favorable and intermediate cytogenetic risk, while GO did not improve outcome of patients with adverse cytogenetic risk.[7] Another factor that might have an impact on the response to GO is the CD33-coding single nucleotide polymorphism (SNP) rs12459419 (NM_001772.4:c.41 C>T; Ala14Val in exon 2) as GO needs to bind to CD33.

CD33 consists of an amino-terminal V-set immunoglobulin (Ig)-like domain, coded by exon 2, and a C2-set Ig-like domain in its extracellular component. The presence of rs12459419 in exon 2 affects alternative splicing of CD33 resulting in the loss of exon 2 for the T allele which leads to a shorter isoform lacking the GO binding site. The C allele leads to the CD33 full-length isoform including the GO binding site.[3,6] The genotype frequencies in the European population for the SNP are c.41C/C: 46.9%, c.41C/T: 44.1%, and c.41T/T: 8.9%.

Given the strong clinical need to clarify who could benefit from GO and the mechanisms responsible for a differential response to GO we studied the effect of rs12459419 in the AMLSG 09-09 phase III study (NCT00893399). This trial is a large, randomized study in adult AML patients who were eligible for intensive therapy and had a mutation in *nucleophosmin1 (NPM1)*. According to MRC definitions,[8] 99.5% of the patients in both treatment arms had intermediate-risk cytogenetics (*Online Supplementary Table S1*). Since the benefit of GO was only observed in favorable- and intermediate-risk groups,[7] our cohort is well suited for analyzing the effect of the CD33 SNP. Of 588 patients included in this study, 545 patients had samples available for SNP analysis. Patients were randomly assigned (1:1) to receive 3 mg/m^2 of GO (n=273, GO arm) *versus* no GO (n=272, standard arm) in combination with two cycles of induction chemotherapy.[9]

Samples were obtained from peripheral blood (n=459) and bone marrow (n=86); 507 samples were obtained at the time of diagnosis and 38 during follow-up. The SNP genotype was determined using the TaqMan SNP Genotyping Assay rs12459419 (Thermo Fisher Scientific, Waltham, MA, USA) on a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The allelic discrimination protocol was modified from that described by Easton *et al.*[10] The Genotyping Assay revealed robust results for 540 out of 545 samples (call rate >99%). DNA from the remaining five patients was genotyped by Sanger sequencing as previously reported.[11] In order to confirm the genotype we additionally analyzed three samples with a genotype known from the Genotyping Assay. Results from the Genotyping Assay and Sanger sequencing were concordant.

Genotype proportions were 45.2% c.41 C/C, 44.1% c.41 C/T and 10.7% c.41 T/T in the standard arm and 46.1% c.41 C/C, 45.3% c.41 C/T and 8.6% c.41 T/T in the GO arm.

We then analyzed whether rs12459419 genotypes influenced hematologic recovery times, response to therapy and clinical outcome in patients treated with or without GO.
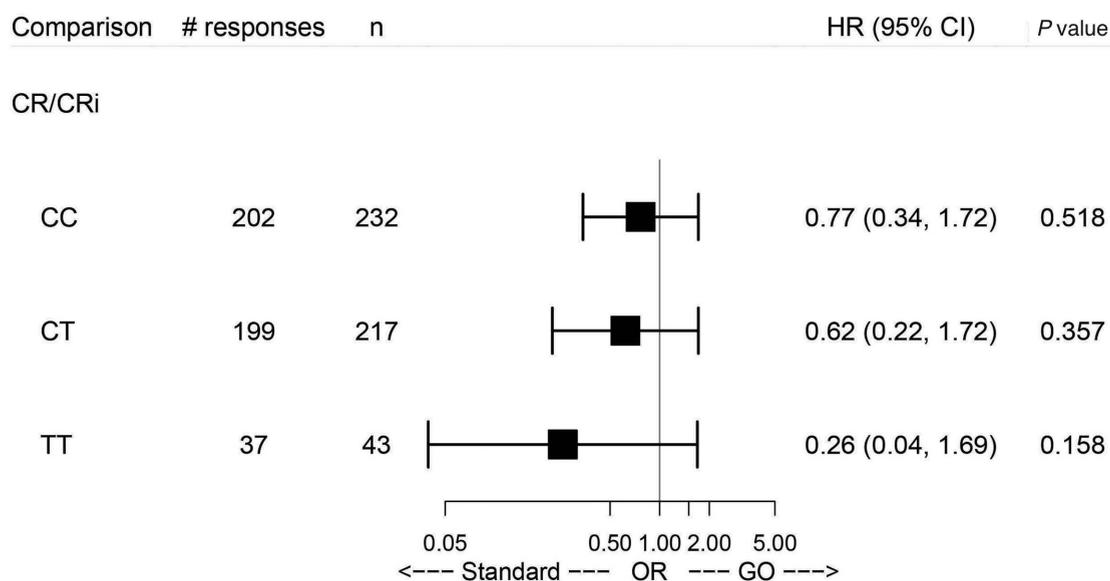

Figure 1. Analysis comparing gemtuzumab ozogamicin *versus* standard treatment in the three subgroups defined by the genotypes with regard to complete remission/complete remission with incomplete hematologic recovery. HR: hazard ratio; 95% CI: 95% confidence interval; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; OR: odds ratio; GO: gemtuzumab ozogamicin.
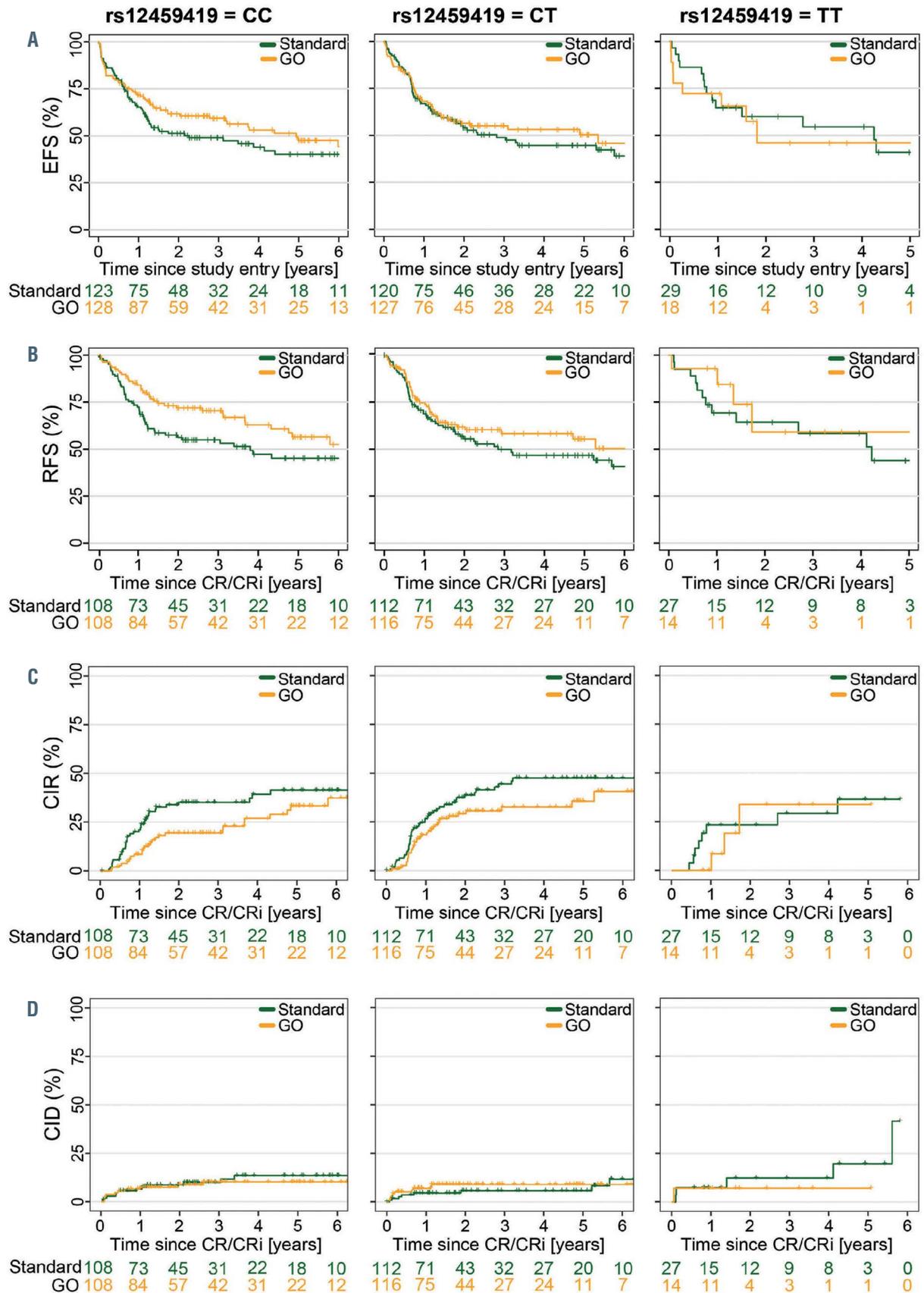
Figure 2. Association of rs12459419 genotypes with clinical outcome by treatment arm. (A) Event-free survival. (B) Relapse-free survival. (C) Cumulative incidence of relapse. (D) Cumulative incidence of death in remission. EFS: event-free survival; GO: gemtuzumab ozogamicin; RFS: relapse-free survival; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; CIR: cumulative incidence of relapse; CID: cumulative incidence of death in remission.

Comparison	# events	n	HR (95% CI)	*P* value
EFS				
CC	110	232	0.75 (0.52, 1.1)	0.141
CT	98	217	1.07 (0.72, 1.6)	0.73
TT	20	43	0.82 (0.32, 2.08)	0.673
RFS				
CC	80	201	0.61 (0.39, 0.95)	0.03
CT	80	198	0.96 (0.62, 1.49)	0.854
TT	14	37	0.38 (0.11, 1.4)	0.148
CIR				
CC	59	201	0.54 (0.32, 0.92)	0.023
CT	66	198	0.82 (0.5, 1.35)	0.439
TT	9	37	0.38 (0.08, 1.87)	0.236
CID				
CC	21	201	0.83 (0.35, 1.98)	0.676
CT	14	198	1.94 (0.65, 5.84)	0.238
TT	5	37	0.43 (0.05, 4.05)	0.464

0.05   0.50 1.00 2.00 5.00
<------- GO ------- HR --- Standard --->
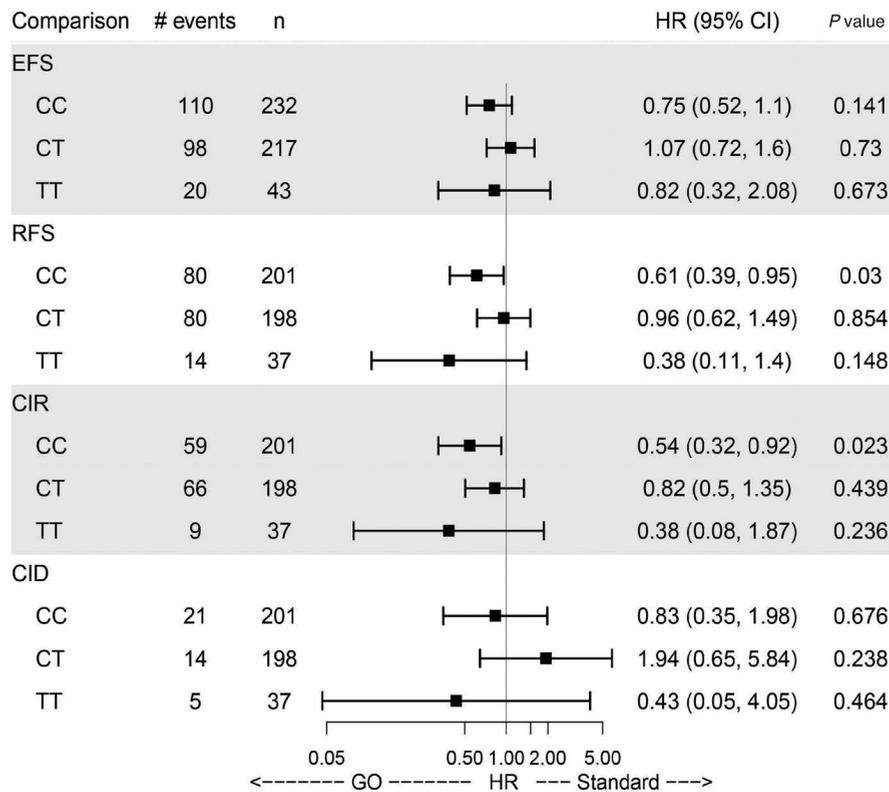
Figure 3. Analysis comparing gemtuzumab ozogamicin *versus* standard treatment in the three subgroups defined by the genotypes with regard to clinical endpoints. Effect estimates are taken from multivariate Cox models and are therefore adjusted for age, gender, European LeukemiaNet 2010 risk class and white blood cell count. HR: hazard ratio; 95% CI: 95% confidence interval; EFS: event-free survival; RFS: relapse-free survival; CR: complete remission; CIR: cumulative incidence of relapse; CID: cumulative incidence of death in remission: GO: gemtuzumab ozogamicin.

Time to hematologic recovery from the start of the first and second induction cycles was not influenced by the SNP genotype in the GO arm (*Online Supplementary Figure S1*).

With respect to the clinical endpoints complete remission (CR) or CR with incomplete hematologic recovery (CRi) as well as event-free survival, relapse-free survival, cumulative incidence of relapse and cumulative incidence of death in remission (defined according to European LeukemiaNet 2017 criteria[12]), multivariate regression models (logistic regression and Cox proportional hazards models) were used to evaluate whether the effect of GO varies with the SNP genotype. More explicitly, the likelihood ratio test was used to test for the presence of an interaction between treatment with GO and SNP genotype while other prognostic factors (age, gender, European LeukemiaNet 2010 risk class[13] and white blood cell count) were accounted for by including them as additional covariates. Of 485 patients who achieved CR/CRi, 157 patients experienced a relapse and 46 patients died in CR. No significant differences for CR/CRi could be observed between the genotypes in the treatment arms (Figure 1).

For event-free survival, no significant difference in the treatment effect of GO compared to the standard arm was observed across the SNP genotypes (Figures 2A and 3). Specifically, patients in the GO arm with the c.41 C/C genotype did not have a superior event-free survival compared to those with other genotypes. However, relapse-free survival was significantly improved for patients with the c.41 C/C genotype in the GO arm (*P*=0.03) compared to those in the standard arm (Figures 2B and 3). It is important to state that the early death rate during induction was significantly higher (10.3%) in the GO arm than in the standard arm (5.7%) (*P*=0.05) but not influenced by the different SNP genotypes (*data not shown*). However, the higher early death rate in the GO arm may explain why we see an effect of the CD33 SNP on relapse-free survival but not on event-free survival.

Furthermore, patients with c.41 C/C genotype had a significantly reduced cumulative incidence of relapse when treated with GO (*P*=0.023) (Figures 2C and 3). Patients with c.41 C/T or c.41 T/T genotype did not benefit from GO with regard to relapse-free survival and cumulative incidence of relapse (Figures 2B, C and 3). There were no significant differences in cumulative incidence of death in remission in relation to genotypes between the treatment arms or between the genotypes within a treatment arm (Figures 2D and 3).

Because subgroup analysis of the AMLSG 09-09 trial revealed a significant prognostic advantage in female patients, patients <70 years and *FLT3*-ITD-negative patients when receiving GO, we analyzed whether these observations could be explained by different distributions of the SNP genotypes. We found no significant association of the SNP with mutations in *FLT3*-ITD, *FLT3*-TKD or *DNMT3A* (*Online Supplementary Table S1*). For separate analysis of female and male patients see *Online Supplementary Figure S2A, B*.

Additionally, we separately analyzed the influence of rs12459419 on the effect of GO in younger patients (≤40 years) *versus* older ones (>40 years). Due to low event numbers (especially in the group of younger patients) additional covariate adjustment was omitted for these subgroup analyses. Here, younger patients with the c.41 C/C genotype had no extra benefit from GO compared to younger patients with other SNP genotypes (*data not shown*).

Published data about whether CD33 SNP rs12459419 can influence the response to GO have been conflicting. The first study to analyze the effect of the rs12459419 on the response to GO was the AAML0531 trial, a randomized phase III study in pediatric and adolescent AML patients (aged 0-29 years).[3] In that study, Lamba *et al.* showed that the T allele was significantly associated with

higher levels of the CD33 isoform lacking exon 2. Patients harboring the c.41 C/C genotype treated with GO had a significantly reduced cumulative incidence of relapse and higher relapse-free survival than patients in the non-GO arm. Importantly, this positive effect was restricted to patients with favorable and intermediate cytogenetic risk. The clinical benefit of GO was not visible in patients with the c.41 C/T or c.41 T/T genotype of SNP rs12459419.[3]

In contrast, in the UK MRC/NCRI AML 15 (ISRCTN17161961) and AML 17 (ISRCTN55675535) trials, GO was given to adult AML patients aged 13 to 69 years. Here, the SNP genotype had no impact on outcome, even if only patients with favorable-risk cytogenetics were considered.[14] Of note, the trial included multiple randomization steps with different chemotherapy regimens.[14]

Moreover, Short *et al.* could not show a significant impact of the SNP on overall survival or relapse-free survival in patients (n=113) treated with decitabine plus GO.[15] However, this study was performed in a population of patients unlikely to benefit from GO as it included patients with high-risk myelodysplastic syndrome and AML patients with unfavorable risk features.[15]

Our results show a similar signal as the data from Lamba *et al.* with regard to an improved relapse-free survival and reduced cumulative incidence of relapse for patients with the c.41 C/C genotype treated with GO. It is unlikely that the discrepancies between the results of the studies by Gale *et al.* and Lamba *et al.* are only related to the difference in age of the patients as we showed an impact of the SNP in adult patients.

In summary, our study suggests that CD33 SNP rs12459419 is one of the predictive factors that affects the rate of relapse in patients with *NPM1*-mutated AML receiving GO. As testing for the SNP is technically simple, it can be easily included in routine diagnostics. In order to support our findings a prospective study in a larger cohort is desirable.

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