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Time to reconsider CD33 single nucleotide polymorphism in the response to gemtuzumab ozogamicin

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CD33 is a highly sought-after target in acute myeloid leukemia (AML), with gemtuzumab ozogamicin (GO), a CD33-antibody conjugated to a DNA-damaging cytotoxin currently approved for the treatment of CD33+ adult and pediatric AML.[1,2] The levels of expression of CD33 on the cell surface vary significantly between patients (up to 2 log-fold) and have been shown to be associated with disease characteristics as well as response to GO.[3-5] However, as a biological threshold of CD33 expression that correlates with response to GO is lacking for incorporation into prospective trials to guide CD33-directed therapeutics, there is an urgent and unmet need to better define genomic variants that might predict response to GO.

It has recently been reported that there is a splicing single nucleotide polymorphism (SNP) in CD33, rs12459419 (C>T, Ala14Val), which results in skipping of exon 2 and thus loss of the most immunogenic domain of CD33 – IgV. Given that the IgV domain is recognized by GO, this SNP holds great potential for predicting response to GO. Results from one of the largest studies to date (COG-AAML0531[6]) in children and young adults randomized to receive standard therapy with or without GO (GO arm, n=408; no-GO arm, n =408) indicated that there was a CD33 splicing SNP genotype-dependent clinical benefit from GO.[7] This study showed that the rs12459419C>T change was significantly associated with CD33 cell surface levels (P<0.001). With respect to clinical outcomes, in patients with the CC genotype (~50% of patients) who expressed high levels of full-length CD33, addition of GO resulted in a significant reduction in relapse risk (by ~50%, P<0.001), an improved disease-free survival and a trend to a better event-free survival in the whole cohort (disease-free survival, P=0.004; event-free survival, P=0.055). In contrast, patients with the CT/TT genotype had no benefit from the addition of GO to the standard no-GO therapy.[7] GO has been shown to improve outcomes in patients with favorable cytogenetics.[8] Among low-risk patients in the COG-AAML0531 trial, a significant improvement in outcome with GO was observed in those with the CC genotype (relapse risk, P<0.001; disease-free survival, P=0.001; event-free survival, P=0.001; and overall survival, P=0.014),[7] but not in patients with the CT/TT genotype. These results were consistent with the first report on the rs12459419 SNP which showed, albeit in a very small group of patients given GO after failing induction 1, an increase in minimal residual disease after GO treatment in patients with the TT genotype.[9]

These results raised hope for potentially personalizing GO treatment guided by germline SNP. However, initial attempts to validate these results in adult AML patients were apparently not successful, in two studies.[10,11] Some of the factors that might explain the inconsistencies from these studies are summarized in Table 1 along with key points from all the studies discussed in this article. There are a few points worth mentioning from the first study in 536 adult AML patients enrolled on MRC 15 and MRC 17.[10] First, in contrast to the COG-AAML0531 study, in which there was a single randomization, these studies included multiple randomizations with patients receiving varying numbers of courses (0, 1, or 2) and doses of GO (3 mg/m^2 or 6 mg/m^2) and different induction and consolidation therapies of varying intensity, with the outcome analysis based only on GO exposure at initial induction. This randomization complexity also led to a lack of

Table 1. Summary of major studies focused on the CD33 single nucleotide polymorphism and gemtuzumab response.

Study	Study cohort	Major findings	Other points/limitations
Lamba *et al.*, 2017	Pediatric *de novo* AML COG-AAML0531 trial N= 916, (GO arm=408, No-GO arm =408) -Single randomization ADE *vs.* ADE+GO (3 mg/m^2 in induction 1 and consolidation 2)	CD33 Splicing SNP rs12459419: - SNP genotype has strong association with CD33 expression. - CC genotype-benefits from GO addition: lower risk of relapse and higher DFS and EFS in GO *vs.* No-GO arm - CT/TT genotype-No benefit from GO addition: no difference in RR or DFS between GO *vs.* No-GO arms - Patients within low-risk group, CC genotype had significantly better outcome (RR, DFS, EFS and OS) with GO but no benefit of GO observed in CT/TT	- Study was limited to only pediatric AML patients.
Gale *et al.*, 2018	Adult AML patients: N=536, from different GO trials (MRC-AML15 and MRC-AML17) with varying GO and chemotherapy randomizations (induction and/or consolidation) and doses (3 or 6 mg/m^2)	-No difference in outcome (RFS, OS) by rs12459419 genotypes in whole cohort -Within favorable risk group no impact of CD33 splicing SNP on RFS or OS. - CD33 expression not associated with GO response - CD33 SNP demonstrated association with its expression in a subset (n=249) of patients.	- Patients received different GO randomizations (induction and/or consolidation) and doses (3 mg/m^2 or 6 mg/m^2) - Patients from GO in consolidation were combined with the No-GO group; - RFS and OS not different in randomized cohort although favorable cytogenetics showed a trend for better outcome by GO
Short *et al.*, 2020	Adult patients: N=104 (frontline =36; refractory/ relapsed =55) -20 mg/m^2 decitabine + 3 mg/m^2 GO on day 5 as induction; 5 consolidation cycles of decitabine + GO	-Trend to higher CR in patients with high 6 CD33-SNPscore ≥0 which includes the splicing SNP rs12459419. -3'UTR CD33-SNP rs1803254 associated with worse CIR and RFS	-Study population very poor risk disease with heterogeneous patients (AML/MDS/ CMML/PMF; frontline/ relapse). -Decitabine+ GO showed low global response rate, limiting the number of patients for evaluation
Teich *et al.*, 2021	Adult AML patients: AMLSG-0909 trial: NPM1 mutation-positive, intermediate risk N= 545 (GO arm=273; No-GO arm = 272)	-CD33 rs12459419 CC genotype improved RFS and CIR in GO *vs.* No-GO arm -CT/TT genotype no difference in RFS or CIR by arm	- Study only limited to NPM1 mutation positive AML within intermediate cytogenetic risk adult AML patients

DFS: disease free survival; RR: risk of relapse; RFS: relapse free survival; OS: overall survival; CR: complete remission; CIR: cumulative incidence of relapse.

demonstrated benefit of GO based on CD33 expression levels; Secondly, the fact that, in contrast to other studies, MRC studies did not show an association of CD33 expression and response to GO, lack of CD33 SNP-associated efficacy would not be surprising given that CD33 SNP are highly associated with CD33 expression. Thirdly, a trend to a difference in overall survival between patients included or not included in the study (P=0.06) implies a possible selection bias. Finally, relapse-free survival and overall survival were not significantly different in the randomized cohort although trends were observed for overall survival within patients with favorable cytogenetics (relapse-free survival, P=0.1; overall survival, P=0.05).

The study by Short *et al.* included a very heterogeneous group of patients with very poor risk spanning from frontline to relapsed/refractory AML, myelodysplastic syndromes, chronic myelomonocytic leukemia, and primary myelofibrosis.[11] Meta-analysis of results from MRC trials demonstrated a significant benefit of GO within favorable- and intermediate-risk groups but not in adverse cytogenetic risk groups,[8] thus only including poor-risk AML might have had an impact on the results of Short *et al.* Additionally, the treatment regimen included a combination of decitabine for 5 days followed by the addition of GO (3 mg/m^2) for induction and five consolidation cycles. However, it is worth mentioning that within this cohort, patients with a higher CD33 SNP score (≥0, which represents the CD33 rs12459419 CC genotype) tended to have a higher possibility of achieving complete remission.[11]

Thus, although the CD33 splicing SNP was observed to have an impact in AML in children and young adults, studies in adult AML were not able to demonstrate similar results, in part due to the complexities of the trial designs. In the study reported by Teich *et al.*, in this issue of *Haematologica*,[12] the impact of the CD33 splicing SNP in a homogeneous group of adult patients with *NPM* mutation-positive AML and primarily intermediate-risk cytogenetics randomized to receive standard therapy with or without the addition of GO (3 mg/m^2) in two cycles of induction was investigated. Of note, this regimen was closer to that used in the COG-AAML0531 trial. Consistent with the results from COG AAML0531, in Teich's study, the CC genotype of the CD33-rs12459419 SNP was associated with improved relapse-free survival and lower cumulative incidence of relapse in the GO arm than in the no-GO arm. Such a benefit of GO was not

observed in the rs12459419 CT/TT genotype group.[12] This study with a single randomization and uniformly treated patients confirms the COG AAML0531 findings, providing additional evidence that *CD33* genotype may constitute a valuable tool for predicting GO-responsive patients in cases of adult and pediatric AML. It also provides a caveat regarding the interpretation of studies with multiple GO randomizations that may obscure the clinical relevance of CD33 SNP in the context of GO treatment. The results published by Teich *et al.* show that the impact of CD33 SNP on GO response is not age-dependent and are of potential clinical utility in adult AML, especially given the abundance of the allele frequency (0.3) in patients with Caucasian ancestry. These exciting results warrant prospective and in-depth investigation and validation of the CD33 splicing SNP for its impact on response to GO in both pediatric and adult AML. Given that germline SNP genotyping is very quick and simple, and can be performed in a variety of specimens (blood sample, buccal swap, etc.), CD33 genotyping can be quickly translated to clinical testing.

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