# Undetected mixed chimerism in myeloid subset enrichments by an immunomagnetic bead-based method

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## BACKGROUND

Monitoring engraftment by accurate quantitative analysis of chimerism is critical in detecting relapse or graft failure and can guide therapeutic interventions in cases of mixed chimerism. Analysis of lineage-specific chimerism improves the sensitivity of the assay. For example, mixed chimerism of a low frequency cell population such as T cells following T cell depleted/reduced HCT could be below the limit of detection in unfractionated samples but would be detected in T cell-enriched populations. Timely detection of mixed chimerism could provide useful information to guide specific therapeutic interventions, such as tapering of immunosuppression, donor lymphocyte infusion or re-transplantation.

Enrichment of cell subsets is commonly performed using an immunomagnetic bead-based system, wherein antibodies directed against lineage-specific markers are coupled to magnetic beads for enrichment and recovery of cell populations expressing the target marker. This case illustrates limitations of immunomagnetic bead-based enrichment.

## CASE

A 71-year-old female with myeloproliferative disease had undergone allogeneic hematopoietic cell transplantation (alloHCT) in 2021. Post-transplant blood samples sent for subset chimerism (myeloid, T, B cell subsets) showed complete donor chimerism (≥95% donor) in all subsets. Subsequent bone marrow aspirate specimens showed mixed chimerism with a significant host fraction (77-80% donor). In December 2022 (day +545), whole blood (WB) chimerism was 80% donor, yet subsets were 100% donor, indicating a cell population was missed during subset enrichments. (**FIGURE 1**).

### Myeloid Subset Enrichment by CD33 and CD33/66b positive selection

Subsets are enriched using positive selection immunomagnetic beads (CD33+, CD3+, CD19+, respectively) (StemCell Technologies). Additional antibodies available for myeloid enrichment include a CD33 and CD66b cocktail. Parallel enrichment studies by CD33 vs CD33/66b were performed on samples from this patient. Chimerism assay was performed on the enrichments using a next-generation sequencing-based assay (Chimerism MD, Scisco-Genetics). CD33/66b enriched cells demonstrated mixed donor chimerism (73% donor), concordant with results from bone marrow (BM) and WB (**TABLE 1**). This indicates that recipient detected in WB originates from a myeloid population enriched by CD33/66b but not by CD33 antibodies. Flow cytometry identified two distinct CD33+ populations (dim and bright) (**FIGURE 2**). We considered a *CD33* polymorphism in the recipient leading to differential detection by the fluorescent antibody and less effective enrichment by anti-CD33 beads. This may account for the missing population.

**REFERENCES:**

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2. Lamba JK, Chauhan L, Shin M, Loken MR, Pollard JA, Wang YC, Ries RE, Aplenc R, Hirsch BA, Raimondi SC, et al. CD33 splicing polymorphism determines Gemtuzumab Ozogamicin response in De Novo Acute myeloid leukemia: Report from Randomized Phase III Children's Oncology Group Trial AAML0531. J Clin Oncol. 2017;35:2674–82. doi: 10.1200/JCO.2016.71.2513.
3. Chauhan L, Shin M, Wang YC, Loken M, Pollard J, Aplenc R, Hirsch BA, Raimondi S, Ries RE, Bernstein ID, Gamis AS, Alonzo TA, Meshinchi S, Lamba JK. CD33_PGx6_Score Predicts Gemtuzumab Ozogamicin Response in Childhood Acute Myeloid Leukemia: A Report From the Children's Oncology Group. JCO Precis Oncol. 2019 May 23;3:PO.18.00387. doi: 10.1200/PO.18.00387. PMID: 32914031; PMCID: PMC7446480.

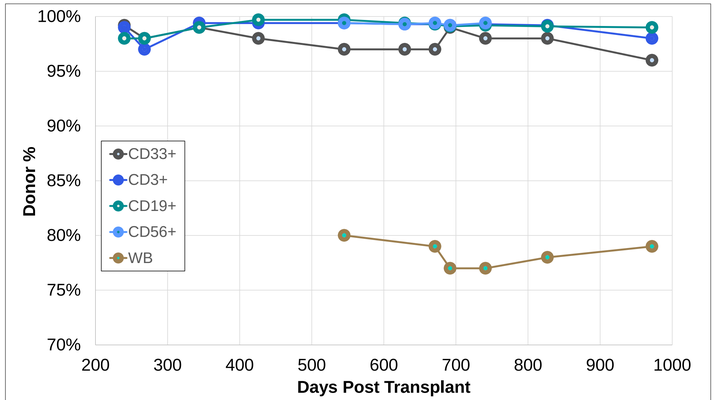


**FIGURE 1**: Donor chimerism (%) as measured in unfractionated peripheral blood (WB) and myeloid (CD33+), T cell (CD3+), B cell (CD19+) and NK cell (CD56+) subset enrichments from samples collected for post-transplant monitoring.

**TABLE 1:** Donor chimerism results from CD33/66b enrichment, compared to CD33+ enrichment, WB, and BM

| Days Post-Transplant | 671 | 692 | 741 | 827 |
|---|---|---|---|---|
| | | Donor Chimerism, % | | |
| BM* | | 77 | 80 | |
| WB | 79 | 77 | 77 | 78 |
| CD33+ | 97 | 99 | 98 | 98 |
| CD33/66b | 73 | 70 | 67 | 72 |

*Chimerism studies on BM were performed by STR-PCR. Chimerism studies on WB and CD33+ or CD33/66b+ enriched subsets were performed by NGS.

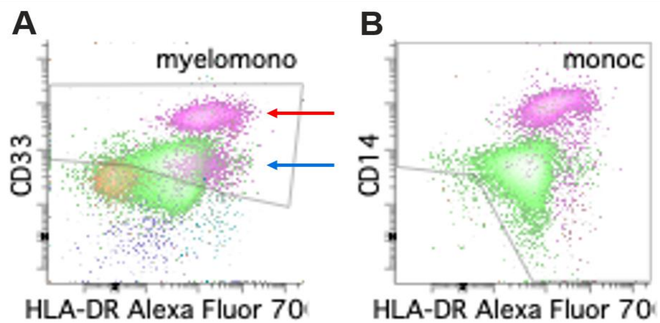


**FIGURE 2**: Flow cytometry of post-transplant peripheral blood. **A**) Two distinct populations of monocytes (*purple*): CD33dim (*blue arrow*), and CD33bright, (*red arrow*). Granulocytes (*green*) also show heterogeneity of CD33 expression (dim and bright). **B**) The monocytes are CD14+.

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### *CD33* Genotyping of Recipient and Donor

Whole Exome Sequencing (WES) was performed by Integrated Genomics Operation (IGO) at MSK on gDNA samples from donor and recipient (**TABLE 2**). Two variants (rs12459419 and rs35112940) were detected in both the donor and recipient. Both were heterozygous for these variants. The rs201074739 CCGG deletion (-/CCGG genotype) was detected in the recipient (heterozygous), but **NOT** in the donor (CCGG/CCGG).

**TABLE 2:** *CD33* germline variant analyses from WES of recipient and donor gDNA samples

| | Type | dbSNP_RS | Alteration | VAF | GPos | REF | ALT |
|---|---|---|---|---|---|---|---|
| RECIPIENT | **Frame_Shift_Del** | **rs201074739** | **p.G156Tfs*5** | **46.65%** | **19:51729104-51729107** | **CCGG** | **-** |
| | Missense_Mutation | rs12459419 | p.A14V | 44.13% | 19:51728477-51728477 | C | T |
| | Missense_Mutation | rs35112940 | p.G304R | 42.86% | 19:51738917-51738917 | G | A |
| DONOR | Missense_Mutation | rs12459419 | p.A14V | 47.85% | 19:51728477-51728477 | C | T |
| | Missense_Mutation | rs35112940 | p.G304R | 58.33% | 19:51738917-51738917 | G | A |

## CONCLUSIONS:

This case demonstrates limitations of anti-CD33 only enrichment of myeloid cells for patients with *CD33* polymorphisms. The variant rs201074739 CCGG deletion in exon 3 alters the reading frame, creating a premature termination codon, and thus a nonfunctional truncated CD33 protein[1] which affects cell surface expression. The heterozygous genotype in this recipient may result in relatively lower cell surface expression of CD33. Therefore, the failure of enrichment by CD33 antibody on recipient's cells maybe due to the variant rs201074739.

Several *CD33* polymorphisms have been described, some at high frequencies[2] **(FIGURE 3).** The CCGG deletion rs201074739 occurs at a relatively low frequency (minor allele frequency, MAF=0.02). Other *CD33* polymorphisms are more common, including rs12459419 C>T (MAF=0.32, white; MAF=0.13, African-American). The rs12459419 variant, located in the splice enhancer region, leads to a CD33 isoform lacking exon 2, which encodes the IgV domain. Commonly used CD33 antibodies target the IgV domain. Changes in the target domain may affect the efficiency of antibody binding to CD33. In this case, both the donor and recipient are heterozygous for the rs12459419 SNP, which may explain overall low recovery (*i.e.* lower DNA yield) of myeloid cells than typically expected, based on absolute neutrophil counts (*data not shown*).

Lower efficiency enrichment of recipient cells by CD33 alone can lead to undetected mixed chimerism, which may delay relapse detection. Correlation with WB/BM chimerism results can indicate cell populations missed by immunomagnetic cell separation. Limitations of CD33+ based enrichments can be addressed by using a cocktail of CD33/66b antibodies.

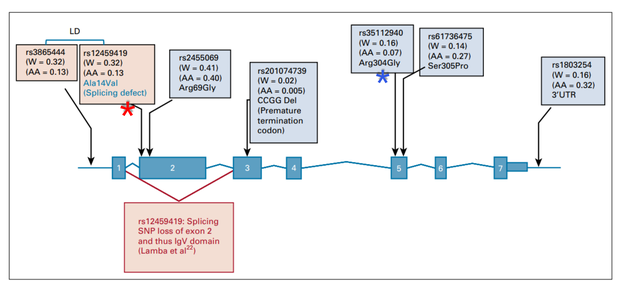


**FIGURE 3**: *CD33* SNP map. The variant rs12459419 (***red asterisk***) is located in exon 2, results in a splicing defect and loss of exon 2. The variant rs35112940 (***blue asterisk***) is located in exon 5, results in a frameshift and truncated protein with loss of cell surface expression. Figure from Chauhan *et al. JCO Precis Oncol*. 2019[3]