Relapse of acute myeloid leukemia detected during routine cell subset purity assessment via flow cytometry as part of the myeloid cell lineage-specific chimerism analysis: A case report

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INTRODUCTION

Acute myeloid leukemia (AML) is a hematological malignancy characterized by the expansion of immature myeloid cells or myeloblasts resulting in failure of normal hematopoiesis and life-threating cytopenia. Allogeneic hematopoietic stem cell transplantation (allo-HCT) is an established therapy to control the disease. Recipient-donor chimerism is routinely analyzed after allo-HCT to monitor engraftment and graft rejection. For malignancies, chimerism can also be used to screen for disease relapse post-HCT which remains a major cause of treatment failure and is associated with high mortality. Sorted or cell lineage-specific chimerism analysis paired with next-generation sequencing (NGS) is one of the most sensitive strategies to perform this monitoring. Flow cytometry is the most common method for cell subset purity assessment allowing the proportion of each target cell type in the sample to be calculated. Moreover, flow cytometry technology also enables the phenotypic identification of a returning immature recipient cell population (suspected myeloblasts) based on low side scatter (SSC), dim to intermediate CD45 expression, and lack of maturation antigen markers typically present on mature CD33 expressing cells (CD14 and CD66b). Here, we report a clinically diagnosed case of AML relapse that was also detected during flow cytometric cell subset purity assessment and confirmed via myeloid cell lineage-specific NGS chimerism analysis.

METHODS

- EasySep™ HLA Chimerism Whole Blood CD3 and CD33 Positive Selection kits (STEMCELL Technologies) were used.
- Purity assessment via flow cytometry using staining reagents for specific cell lineages: CD5 marker for T CD3+ cells. And CD14 and CD66b markers for Myeloid CD33+ cells.
- Next Generation Sequencing (NGS) Chimerism testing was performed on DNA extracted samples using AlloSeq HCT kit (CareDx) and NGS data analysis was performed using AlloSeq HCT software version 1.1.0 (CareDx).

CONCLUSIONS

Purity assessment is critically important in chimerism analysis to ensure that cell subsets are not contaminated by non-target cells. This case exemplifies how informative routine cell subset purity assessments via flow cytometry can be in conjunction with lineage-specific chimerism testing for the detection of relapse and engraftment failure.

RESULTS

1st BMT with MUD#1 10/10 matched Txed on 10/27/2021

Mixed Chimerism: 97-99% Donor (from 01/28/2022 to 09/07/23)

1st Relapse and Engraftment Failure (09/26/2023)

Mixed Chimerism: 50% Donor (09/26/2023)

2nd BMT with MUD#2 9/10 matched Txed on 02/01/2024

Sorted Chimerism (04/29/2024):
-> T cells CD3+ 99% Donor
-> Myeloid Cells CD33+ 99% Donor

2nd Relapse and Engraftment Failure (02/10/2025)

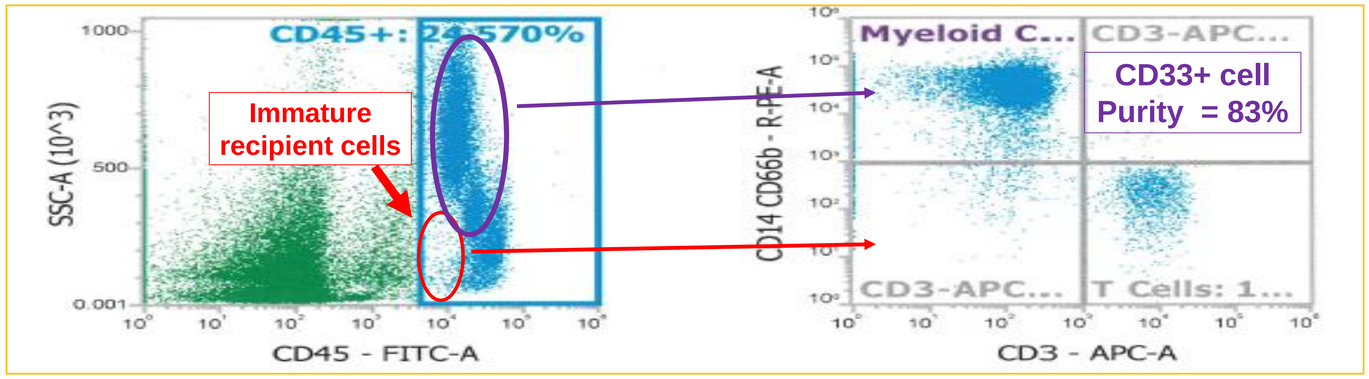
Sorted Chimerism (02/10/2025):
-> T cells CD3+ 95% Donor
-> Myeloid CD33+ < 1% Donor

(BMT Timeline)

In the context of this 2nd BMT

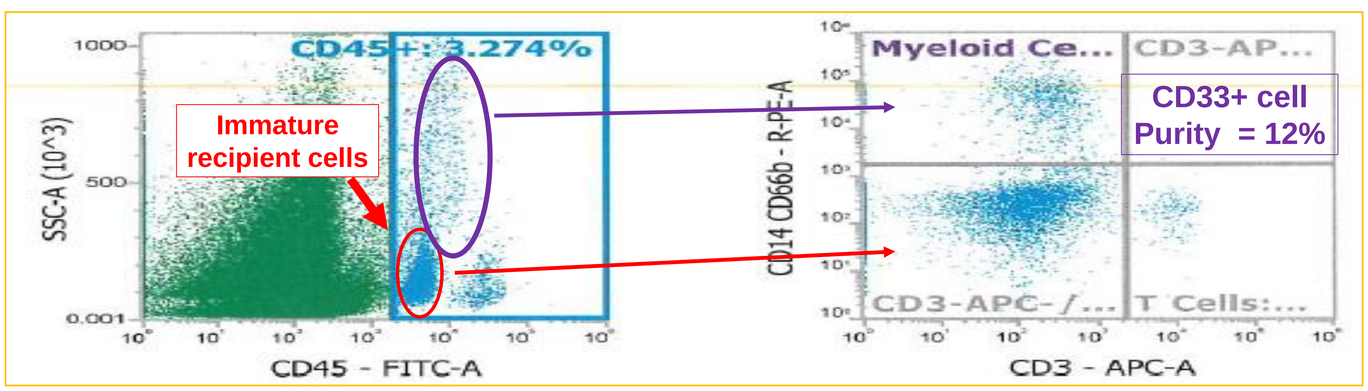
Myeloid CD33+ Positive-selected Cell Subset Purity Assessment via Flow Cytometry

as part of the Post-Transplant Engraftment Monitoring via NGS Chimerism Analysis after 2nd BMT



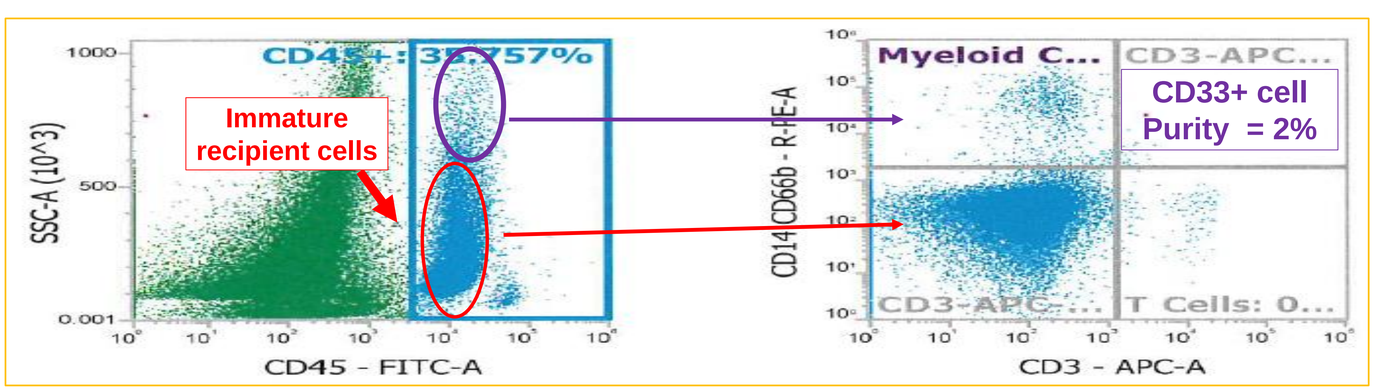
Post-Tx 04/29/24

Chim. Donor %
CD3+ 99%
CD33+ 99%



Post-Tx 09/05/24

Chim. Donor %
CD3+ 83%
CD33+ 14%



Post-Tx 02/10/25

Chim. Donor %
CD3+ 95%
CD33+ <1%

LOWER % of MATURE CD33+ expressing CD14 and CD66b antigen markers (dot-plots on the right side) WHILE the presence of suspected MYELOBLASTS population (IMMATURE RECIPIENT CELLS) INCREASES (dot-plots on the left side)

(2nd BMT Timeline)