CD34 selection in peripheral blood to support diagnosis of relapsed leukemia

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Background

- Chimerism assessment by short tandem repeat (STR) testing permits post-hematopoietic stem cell transplantation (HSCT) engraftment monitoring.
- Chimerism testing is generally tested at regular intervals post transplant, with many programs testing on Days 30, 90, 180, and at 1-year post-transplant (1,2).
- Chimerism testing is also performed for cause including:
  - Suspected poor engraftment, graft failure or disease relapse.
  - Evidence of minimal residual disease (MRD)
  - Declining donor chimerism
  - Persistent or new cytopenias
  - After adjustment of immunosuppressive therapy or other interventions (e.g., donor lymphocyte infusion)
- In addition to bone marrow and whole blood, subset analysis can be performed of different cell lineages:
  - Myeloid cells (e.g., CD33+cells) are commonly monitored for residual or recurrent myelogenous leukemia (3).
  - T-cells (e.g., CD3+ cells) are routinely monitored to assess the risk of graft versus host disease (4).
- Examining chimerism by lineage has been shown to increase the sensitivity of detecting small percentages for recipient- or donor-derived cells (37), permitting earlier interventions if needed.

CASE PRESENTATION

Herein we present a case in which the use of CD33 selection alone of peripheral blood (PB) would have missed the detection of disease relapse.

Methods

- DNA Extraction: DNA was extracted from PB using the Qiagen EZ1 DNA Blood 350 µL kit and Robotic workstation.
- Cell Selection: Cell subset isolations were performed utilizing RoboSep™ instrument platform (STEMCELL Technologies) and the CD3, CD33, and CD34 selection kits.
- Chimerism: STR was performed via using the PowerPlex the GlobalFiler PCR Amplification kit (Thermo Fisher Scientific):
  - STR loci were amplified using genomic DNA by PCR employing fluorescently labeled primers.
  - Amplicons were run on capillary electrophoresis (Applied Biosystems 3500xL Genetic Analyzer; Thermo Fisher Scientific).
  - Donor and recipient DNA percentages were quantified by ChimeRMarker Automated Chimerism Analysis Software (SoftGenetics).
  - Results are reported as percent donor in the post-HSCT sample.
- Flow cytometry:
  - Performed in the flow cytometry laboratory using in-house leukemia panel of antibody combinations and fluorochromes.
  - Also performed as part of a minimal residual disease assessment performed by an outside reference laboratory (University of Washington Medical Centers, Seattle, WA).

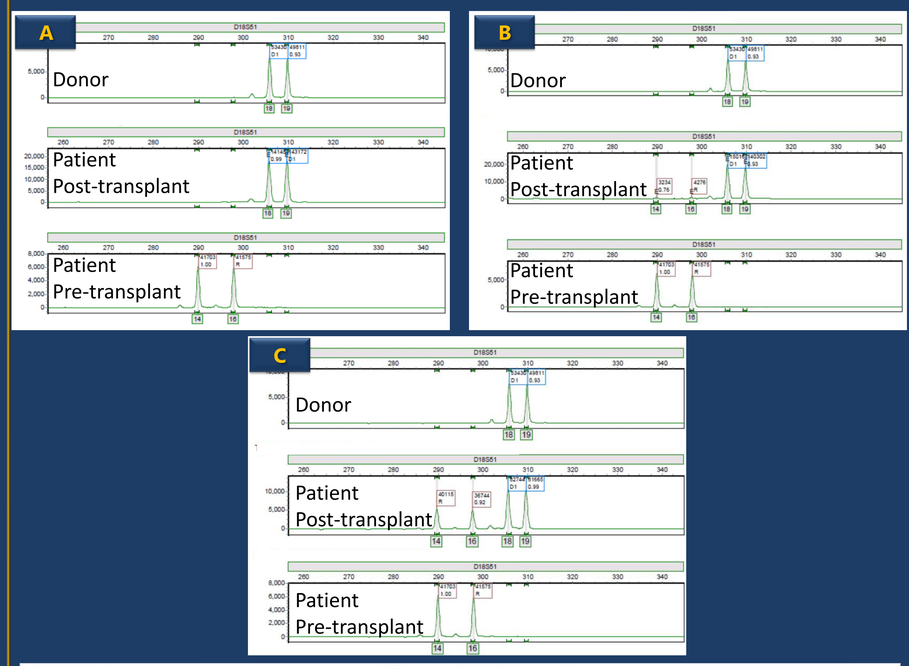


FIGURE 1. Electropherogram of a representative STR locus (D18S51) from 6-months post-transplant.

A) CD3 cell subset from peripheral blood demonstrating 100% donor chimerism

B) CD33cell subset from peripheral blood demonstrating ~98% donor chimerism

C) Bone marrow aspirate demonstrating 68% donor chimerism

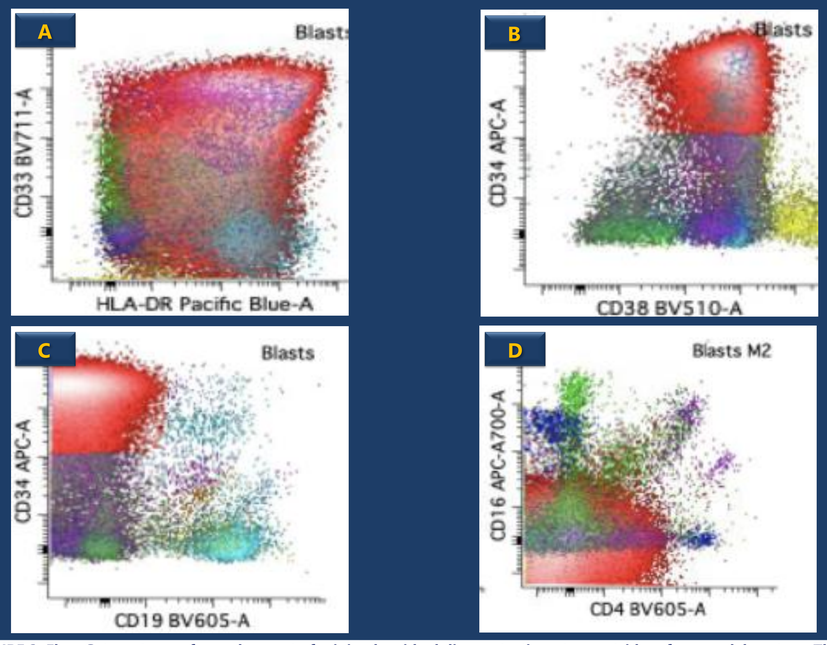


FIGURE 2. Flow Cytometry performed as part of minimal residual disease testing at an outside references laboratory. The red-colored population represents the myeloblasts.

A) CD33 staining demonstrates variable expression in the myeloblast population, ranging from minimal-to-absent to bright expression.

B) Myeloblasts demonstrate increased CD34 expression and are also CD38-positive.

C) Myeloblasts are CD34-positive and CD19-negative, demonstrating absent CD19 expression for comparison with variable CD33 expression.

D) CD4 staining demonstrates low (dim) expression, highlighting the distinction between dim expression and variable expression

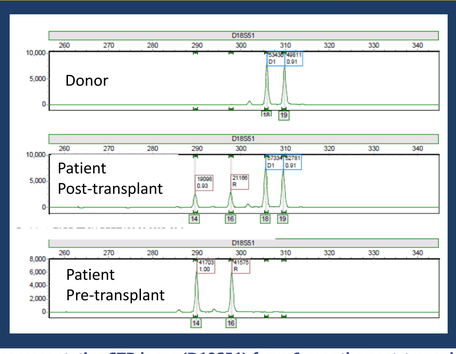


FIGURE 3. Electropherogram of a representative STR locus (D18S51) from 6-months post-transplant. CD34 cell subset from peripheral blood demonstrating ~70% donor chimerism.

CASE PRESENTATION

- The patient is a 64-year-old male with acute myeloid leukemia who received a 6/8 HLA-matched unrelated HSCT.
- STR testing performed on days 30 and 90 post HSCT demonstrated 100% donor chimerism in the CD3 and CD33 PB compartments.
- An unfractionated bone marrow (BM) sample from day 90 also indicated 100% donor chimerism.
- Six months post HSCT, the CD3 PB compartment remained 100% donor while the CD33 PB compartment showed a slight decrease at 98% donor chimerism (Figure 1A and 1B).
- In contrast, the unfractionated BM demonstrated a stark decrease in donor chimerism at 68% (Figure 1C).
- To investigate discrepancy between the PB and BM, the medical record was reviewed for clinical context and correlation with other laboratory findings.
- Flow cytometry performed on the BM aspirate sample demonstrated a population of blasts, constituting approximately 30% of the total cells.
- Minimal residual disease testing by flow cytometry also showed a population of myeloblasts. Interestingly, even though the blasts expressed CD33, expression was variable (dim to bright); but all blasts expressed CD34 (Figure 2).
- Together, the findings were consistent with relapse of AML.
- Given that CD33 expression was variable on the blasts and that CD33 is also present on mature myeloid cells, chimerism was repeated after CD34 selection of the PB sample.
- Focusing on the CD34 cells, donor chimerism resulted at approx. 70%, which was more consistent with the finding on the BM aspirate (68%) (Figure 3).

Conclusions

- This case highlights the relevance of chimerism testing in both PB and BM samples when relapse is suspected.
- In this case, had the BM sample not been examined, STR testing of the PB sample may have missed the relapse. Changes in PB chimerism often lag behind BM chimerism.
- Additionally, since CD33 expression was variable on blasts, CD33 selection may have excluded some recipient derived leukemic cells.
- Using CD34 selection, a marker expressed on immature myeloid cells (eg, blasts), detection of leukemic cells was facilitated.

References

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