# SUCCESSFUL THIRD TRANSPLANT FOR RECURRENT LEUKEMIA AFTER TLI-ATG CONDITIONING AND DESPITE EBV-ASSOCIATED MIXED DONOR CHIMERISM

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**Background:** Patients who relapse after hematopoietic cell transplantation (HCT) and undergo subsequent HCT have poor outcomes due to leukemia relapse and high non-relapse mortality. Myeloablative (MAC) and reduced intensity conditioning (RIC) regimens may be too toxic for these patients, necessitating alternatives. We report a 14-year-old male with acute myeloid leukemia (AML) who first received MAC and matched unrelated donor (MUD) HCT but relapsed one-year post-transplant. The patient had re-induction chemotherapy and received RIC and 2nd MUD HCT but relapsed after 18 months. He received re-induction chemotherapy and conditioning consisting of total lymphoid irradiation (TLI) and antithymocyte globulin (ATG), followed by HCT from a 3rd MUD. HLA typing for recipient and donors is shown in Figure 1.

**Figure 1. HLA typing for recipient and matched unrelated donors (MUDs).** HLA typing was performed by next generation sequencing (NGS, AlloSeq, CareDx). Mismatches with the patient are shown in gray. Direction of mismatch is indicated as bidirectional (BiD), graft versus host (GvH), or host versus graft (HvG). DP permissiveness (P) and direction of non-permissive (NP) mismatch was assessed with the DPB1 T-Cell Epitope Algorithm v2.0: https://www.ebi.ac.uk/ipd/imgt/hla/matching/dpb_v2/

| HLA | A* | C* | B* | DRB1* | DRB3/4/5* | DQB1* | DPB1* | DPB1 P/NP |
|---|---|---|---|---|---|---|---|---|
| Recipient | 02:01 | 05:01 | 44:02 | 04:01 | 4*01:03 | 03:01 | 04:01 | |
| | 01:01 | 12:02 | 52:01 | 15:02 | 5*01:02 | 06:02 | 04:02 | |
| MUD #1 | 02:01 | 05:01 | 44:02 | 04:01 | 4*01:03 | 03:01 | 04:01 | NP, HvG |
| | 01:01 | 12:02 | 52:01 | 15:02 | 5*01:02 | 06:02 | 14:01 (BiD) | |
| MUD #2 | 02:01 | 05:01 | 44:02 | 04:01 | 4*01:03 | 03:01 | 04:01 | P |
| | 01:01 | 12:02 | 52:01 | 15:02 | 5*01:02 | 06:02 | 02:01 (BiD) | |
| MUD #3 | 02:01 | 05:01 | 44:02 | 04:01 | 4*01:03 | 03:01 | 04:01 | P |
| | 01:01 | 12:02 | 52:01 | 15:02 | 5*01:02 | 06:02 | 04:01 (GvH) | |

**Transplant Course:** The patient's post-HCT inpatient course after 3rd HCT was uneventful and by day +32 post-HCT, his peripheral blood (PB) chimerism reached 91% donor 3 and 9% donor 2 (Figure 2); bone marrow (BM) chimerism reached 97% Donor 3 and 3% Donor 2 (not shown), with undetectable recipient contribution in either compartment. On day +25, the patient developed Epstein Barr Virus (EBV) reactivation and received a dose of anti-CD20 antibody, rituximab. Shortly following peak EBV DNA levels, his PB chimerism fell to 58% Donor 3 and 42% Donor 2, with no evidence of leukemia relapse. The drop in donor 3 chimerism coincided with an increase in $CD8^+$ T cell numbers (Figure 3), 99% Donor 2 $CD3^+$ T cell chimerism (data not shown), and resolution of EBV viremia. Other sources of graft rejection such as HLA mismatch in the Donor 2 to Donor 3 direction and donor-specific HLA antibodies were ruled out. The patient received 5 donor lymphocyte infusions (DLI) from Donor 3 starting on day +80 and achieved 100% Donor 3 chimerism. We theorized that Donor 2 was seropositive for EBV and expansion of EBV-specific Donor 2 T cells caused a transient decrease in Donor 3 chimerism and contributed to resolution of EBV. However, EBV serostatus for either donor was unavailable. The patient remains well and without evidence of relapse 21 months post-3rd HCT.

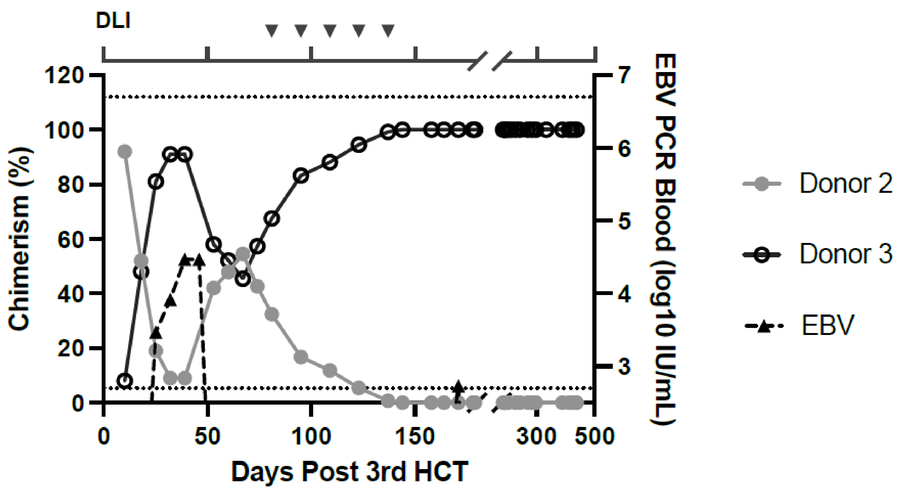
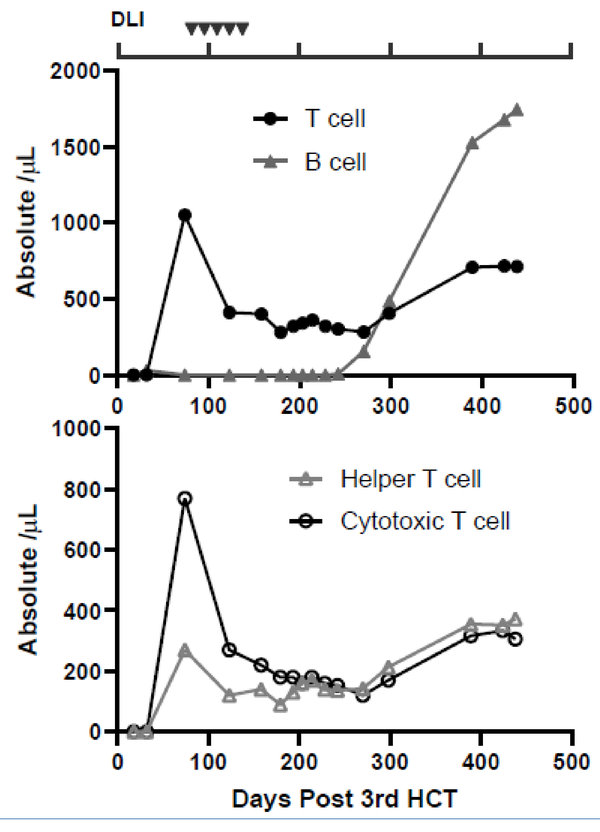



**Figure 2. Unsorted peripheral blood chimerism and EBV viral load post-transplant.**
Donor lymphocyte infusions (DLI) were given on days +80, 95, 109, 123 and 136 at doses of 1, 5, 10(3) x $10^6$ $CD3^+$ cells/kg respectively (noted by arrows). Chimerism was performed by NGS (AlloSeq HCT, CareDx). In this case, 65 informative single nucleotide markers were used to analyze recipient, donor 2 and donor 3 contributions. Values outside the reportable range of 0.4% -99.6% are reported as 0% and 100% respectively. Host chimerism was not detected in blood throughout the assessment period and is not plotted.

**Figure 3. Peripheral blood immune cell reconstitution post-transplant.**
Flow cytometric-based assay was used to enumerate absolute numbers of $CD3^+$ T cells and $CD19^+$ B cells (top panel) and the subsets of $CD3^+$, $CD4^+$ T helper cells and $CD3^+CD8^+$ cytotoxic T cells (bottom panel).




**Conclusions:**
TLI-ATG is a less toxic conditioning option for subsequent HCT in pediatric patients with refractory leukemia, although post-HCT chimerism profiles may differ from MAC or RIC regimens. Ideally, knowledge of donor viral seropositivity and close engraftment monitoring are crucial to management of mixed chimerism and prevention of relapse in this high-risk patient population.

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