Sustained Donor Chimerism and Rapid Immune Cell Reconstitution With a Low Probability of GVHD Following Familial Haploidentical (FHI) CD34 Enriched Stem Cell Transplantation with PBMNC AddBack in Patients with High Risk Sickle Cell Disease (SCD) (IND 14359)

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Background

Allogeneic stem cell transplantation (AlloSCT) from an HLA-matched sibling donor is the only known curative therapy in patients with high-risk SCD (Talano/Cairo, *EJH*, 2015). Unfortunately only about 15% of high risk patients with SCD have an HLA-matched unaffected sibling donor. T cell depletion has been employed to reduce AGVHD e.g., CD3/CD19 cell depletion (Barfiled RC, et al, *Cytotherapy,* 2004), αβ T-cell/CD19 cell depletion (Locatelli F, et al, *Blood,* 2017), CD34+ positive selection (Aversa F, et al, *NEJM*, 1998). MUD transplantation in high-risk SCD recipients has shown unexpectedly high rates of CGVHD (Shenoy et al, *Blood*, 2016). We reported a very low incidence of acute and chronic GVHD in pediatric recipients receiving CD34 enriched HPC products with PBMNC addback with 2 x 10^5 CD3/kg from MUD donors (Geyer/Cairo et al, *BJH,* 2012). Furthermore, rapid NK cell reconstitution after AlloSCT is associated with a significant improvement in 1yr OS (Pical-Izard, *BBMT*, 2015; Dunbar et al, *Hematologica*, 2008). Recently, we reported promising results for high-risk SCD patients at 1 year follow-up after FHI CD34 enriched/PBMNC with addback AlloSCT with the probability of 1-year overall survival (OS) n=17; 88.2% (CI_{95}: 60.6-96.9) (Cairo, *ASH*, 2018), expanding the donor pool and hopefully improving outcomes for high-risk patients with SCD. We further investigated the incidence of GVHD in patients, donor chimerism, immune cell reconstitution and NK cell function in high-risk patients with SCD following AlloSCT using FHI CD34 enrichment/PBMNC (2 x 10^5 CD3/kg) addback.

Objective

To investigate donor chimerism, immune cell reconstitution and NK cell function in high-risk patients with SCD following FHI CD34 enrichment/PBMNC (2 x 10^5 CD3/kg) addback.

Methods

Twenty-one eligible SCD patients (2-<21 yrs) were enrolled. Nineteen patients received hydroxyurea, azathioprine, fludarabine, busulfan, thiotepa, cyclophosphamide, R-ATG, and TLI followed by FHI AlloSCT to date (Cairo, *ASH*, 2018). CD34 cells were enriched using the CliniMACS® system, kindly provided by Miltenyi Biotec, with a target dose of 10 x 10^6 CD34+ cells/kg with a PBMNC addback dose of 2x10*5 CD3/kg in the final product. Whole blood and RBC chimerism (estimated using CD71 to isolate an erythroid lineage-enriched fraction) were determined by STR. Immune cell and subset reconstitution was assessed by flow cytometry as previously described (Geyer/Cairo et al. *BJH*, 2012). NK function was determined by cytotoxic activity against K562 tumor targets at 10:1 E:T ratio by europium release assay and intracellular LAMP-1 (CD107a) and granzyme B expression by flow cytometry as previously described (Chu/Cairo et al, *Can Imm Res*, 2015).

Disclosure

Dr. Mitchell Cairo has research funding from Otsuka and MTA with Miltenyi Biotech. Dr. Johnson has equity ownership in Cell Vault and research funding from Miltenyi Biotech.
Other co-authors have no relevant conflicts of interest to disclose.

Results

Figure 1. The cumulative incidence of grade II-IV AGVHD and late AGVHD was 6·2 % (A) and moderate and/or severe CGVHD was 6·7% (B), respectively

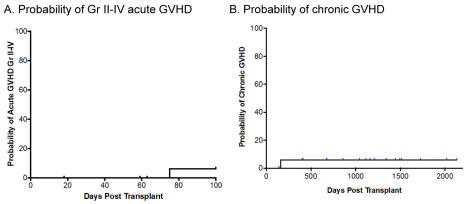


Figure 2. 100% engraftment of neutrophils (A) and 92.1% platelets

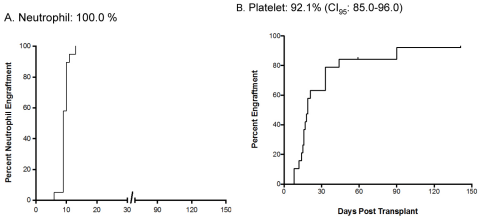
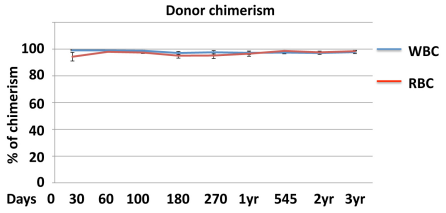


Figure 3. Whole blood donor chimerism (mean±SEM) at 1-year, 2-year, and 3-year post-HISCT was 97±1%, 97±1%, 97±1%, respectively. Donor chimerism for CD71+ RBCs (mean ±SEM) at 1-year, 2-year, 3-year post-HISCT was 97±2%, 98±1%, 98±1%, respectively.



Results

Figure 4. Probability of Cell Immune Reconstitution in High Risk Patients with SCD Following FHI AlloSCT Utilizing CD34 Enrichment and PB MNC Addback

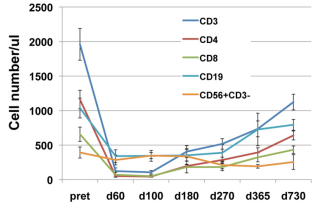


Figure 5. Probability of EFS/OS in 19 enrolled patients following familial haploidentical stem cell transplantation utilizing donor CD34+ enrichment and mononuclear (MNC) add-back determined by the product limit method of Kaplan and Meier.

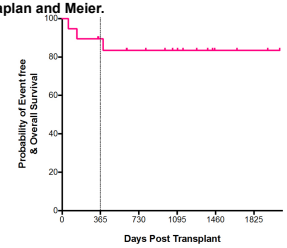
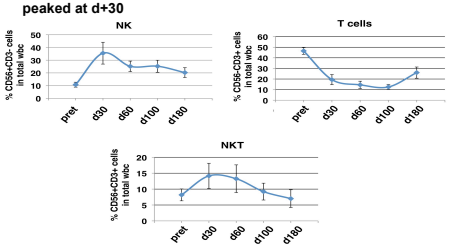


Figure 6. NK cells (CD56+/CD3-) reconstitution following FHI AlloSCT with CD34 enrichment/T cells add back was rapid and peaked at d+30



Results

Figure 7. NK cytotoxicity against K562 rapidly peaked at d+30 with enhanced CD107a and granzyme B expressions

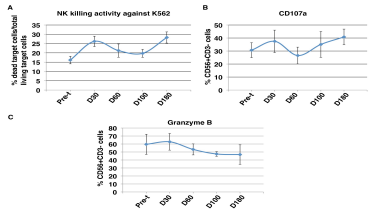


Figure 8. reconstituted NK cells expressed high level of activating receptors NKp46, NKG2D and KIR2DS and inhibitory receptors NKG2A, CD94 and KIR2DL2/3 at d+30.

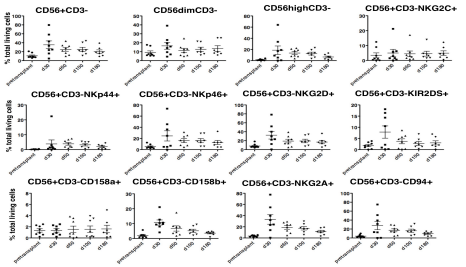
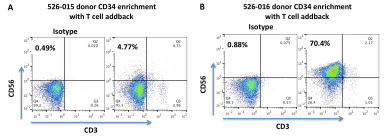


Figure 9.% CD3-/CD56+ NK cells by flow cytometry in the final products of FHI CD34 enriched/PB MNC addback



Conclusions

Despite a 5 log depletion of T cells, the PBMNC addback (fixed at 2 x 10^5 CD3/kg) facilitated rapid donor chimerism and immune reconstitution with a low probability of Grade II-IV AGVHD. The rapid NK reconstitution may have in part contributed to the excellent 1yr OS in the FHI study.
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