explored further in our dataset. Detailed flow-based analysis of T cell subset recovery after alloHCT can potentially provide additional biological insights to post HCT adverse events and guide strategies to decrease post alloHCT complications and non-relapse mortality.

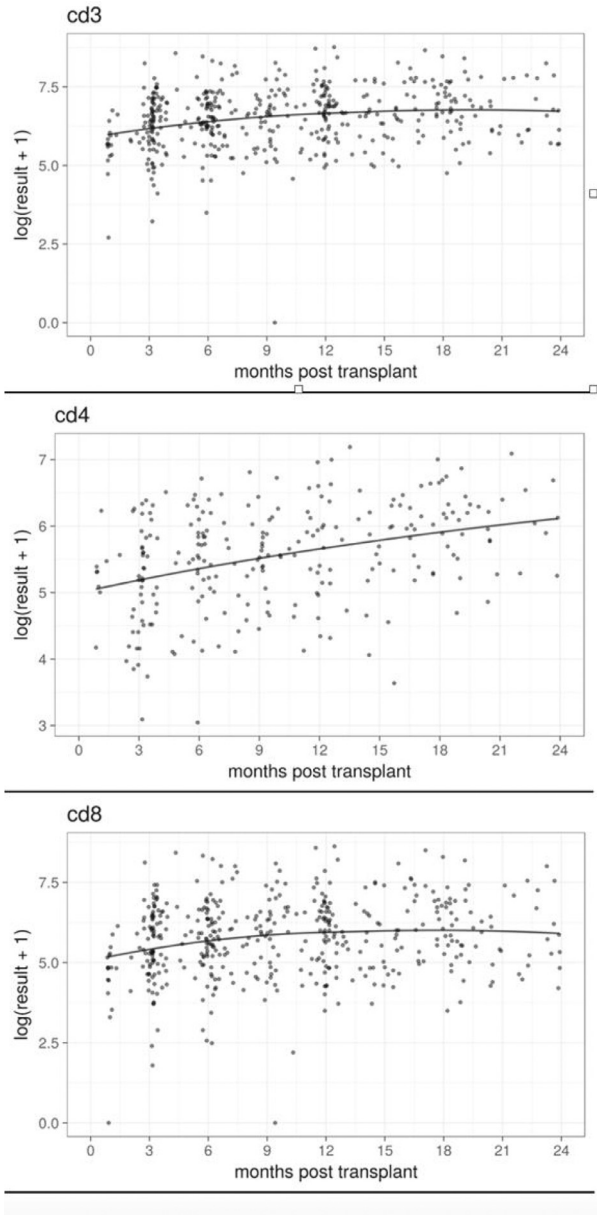


**Figure 2.** T cell subset recovery after allogeneic stem cell transplantation.

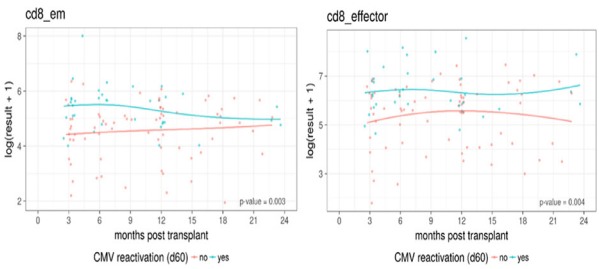


**Figure 3.** CE8+ T cell recovery by CMV reactivation by day +60.

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### Dynamics of Immune Cell Reconstitution in Allogeneic Hematopoietic Cell Transplant Patients Receiving Post-Transplant Cyclophosphamide (PTCy)

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In the setting of haploidentical hematopoietic cell transplantation (haplo-HCT), post-transplant cyclophosphamide (PTCy) selectively eliminates alloreactive T cells *in-vivo*, resulting in favorable graft versus host disease (GVHD), non-relapse mortality (NRM) and relapse outcomes. However, few studies have examined the impact of PTCy on immune reconstitution (IR).

We quantified IR in 63 patients after haplo-HCT with PTCy, mofetil mycophenolate and tacrolimus (TAC) and compared results to 93 patients with 8/8 HLA matched related or unrelated donors (MD) receiving TAC, methotrexate and sirolimus for GVHD prophylaxis. Both groups received reduced intensity conditioning for hematologic malignancies. The median age of the Haplo-PTCy and MD cohorts was 55 and 57 years. Patient samples were analyzed using multi-color flow cytometry panels to characterize distinct lymphocyte populations. All IR values are expressed as median absolute cell count per $\mu$L.

One month after HCT, recovery of all T cell subsets (CD3, CD4Tcon, Treg, CD8) was significantly reduced in the PTCy cohort compared to MD (Figure A, B, C). Recovery of CD4Tcon was also reduced at 2 and 3 months after PTCy ($p<=0.01$) but recovery of Treg was significantly suppressed for one year after HCT (Figure B). By 2 months post-HCT, CD8 T cell recovery was similar in both cohorts. Despite delayed Treg recovery, the Treg:Tcon ratio was significantly higher after PTCy at 1 and 3 months after HCT (0.18 vs 0.09, $p<0.0001$, 0.12 vs 0.08, $p=0.0006$). As CD4Tcon recovery improved, the Treg:Tcon ratio became significantly lower in the Haplo-PTCy cohort at 9 (0.09 vs 0.12, $p=0.03$) and 12 months (0.07 vs 0.1, $p=0.01$) post HCT.

NK cells were lower 1 month after PTCy (52.7 vs 91.1, $p=0.08$), but were significantly higher at 2, 3 and 6 months (153.4 vs 94.8, $p=0.001$, 153.7 vs 87.5, $p=0.008$, 180 vs 102, $p=0.01$, respectively, Figure D) compared to the MD cohort. Delayed NK cell recovery at 1 month after PTCy was due entirely to reduced numbers of CD56dim NK cells (Figure E). Subsequently recovery of CD56dim NK cells was similar in both cohorts. Recovery of CD56bright NK cells was significantly increased in the PTCy cohort ($p<0.0001$. Figure F).

Consistent with prior reports, 1 year cumulative incidence of extensive cGvHD was lower in the PTCy cohort compared to the MD cohort, 13% (5-26%, 95% CI) and 40% (30-50%, 95% CI) respectively, $p=0.003$, without increased NRM ($p=0.28$) or relapse ($p=0.17$).

In summary, the effect of PTCy on IR was most pronounced 1 month after transplant with significantly delayed recovery of CD3, CD4Tcon, Treg, CD8 and CD56dim NK cells. Slow recovery of CD4Tcon persisted for 3 months and delayed recovery of

Treg persisted for 1 year. Beginning 2 months after HCT, recovery of both CD56dim and CD56bright NK cells was more rapid in the PTCy cohort. Further studies will examine the effects of these differences in IR on clinical outcomes such as relapse, infections and GVHD.

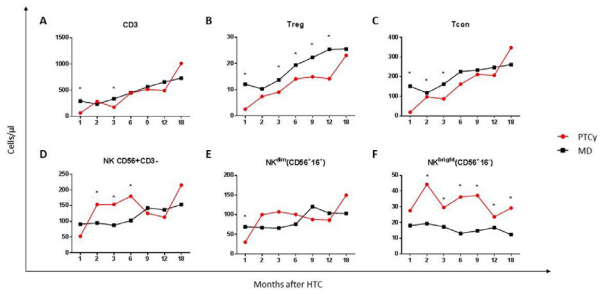


**Figure 1. Reconstitution of major T and NK cell populations after haplo-HCT using post-transplant cyclophosphamide (PTCy, red line) compare to matched donor (MD, black line) treated with standard GVHD prophylaxis.** (A) Median absolute $CD3^+$ t-T-cell counts. (B) Median absolute regulatory T-cell (Treg) counts. (C) Median absolute conventional T-cell (Tcon) counts. (D) Median absolute $CD56^+CD3^-$ NK-cell counts. (E) Median absolute $CD56^{dim}CD16^+$ $CD3^-$ ($NK^{dim}$)NK-cell counts. (F) Median absolute $CD56^{bright}CD16^-CD3^-$ ($NK^{bright}$) NK-cell counts.

*:$p<0.05$

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### Early Expansion of $CD56^{dim}$ $NKG2A^{low}$ with Late Surge and Persistence of $CD56^{dim}NKG2A^{neg}NKG2C^{bright}$ NK Cells Attenuate Cytomegalovirus (CMV) Replication and Recurrence As Well As Leukemia Relapse Following Haploidentical HSCT with T Cell Co-Stimulation Blockade and Ptcy

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CMV related morbidity and mortality remains a major limiting factor in Haploidentical HSCT. We have developed a novel approach to Haploidentical PBSCT combining T cell Costimulation Blockade (T-COSB) with PTCy and Cyclosporine or Sirolimus (BBMT 2018). We studied the pattern of CMV reactivation (CMV-R) and its progression in 60 patients undergoing Haploidentical HSCT in this protocol in relation to the kinetics of Immune Reconstitution (IR) and pattern of leukemia relapse. PCR based CMV monitoring was carried out until 180 days with preemptive anti-CMV therapy.

All patients and donors were CMV seropositive. CMV-R was 54.2% with median onset at 32 days (14-75) in the study group [compared to 52.5% in 42 who had received PTCy alone (p=0.8)]. The median peak viral load was $5.7 \times 10^3$/ml (1.8-25.8). The median duration of anti-CMV therapy was 16 days (7-60 days). There was no recurrence noted before100 days and only 2/32 (6.2%) beyond 100 days with no CMV disease. CMV-R was higher in those transplanted for malignant diseases compared to non-malignant diseases (62.5% vs 8.2%, p=0.01).

Analysis of IR in relation to CMV-R showed no difference in CD3 or CD4+T cell reconstitution. However, $CD56^{dim}CD16^+$ NK cells at D+60 were significantly higher in patients without CMV-R (median 95 vs 45 cells/µl, p=0.02) and the same population of cells were associated with lower peak of viral load (p=0.04). In those with a lower viral peak and lower duration of viral replication, $CD56^{dim}CD16^+$ NK cells had a higher KIR repertoire with lower NKG2A expression. In those with CMV-R, a subsequent surge in absolute NK cells was observed (median 188 vs 112 cells/µl, p=0.01). This was primarily contributed by massive expansion of $CD56^{dim}NKG2A^{neg}NKG2C^{bright}$ NK cells (median 7.8% [5-14.6%] at day +30 to 35% [28-65%] at day +90, p=0.01, Fig 1). This phenomenon was not witnessed in patients treated with PTCy alone. Peak viral load inversely correlated with NKG2C+ NK cells and CD45RO+CD8+ T cells at day +90 (p=0.03). The incidence of aGVHD (10.2%), cGVHD (18%), NRM (9.5%), and overall survival (75.8%) were not influenced by CMV-R. Incidence of relapse was 23.5 % at 28-144 days (median 85 days). However, none of the patients who relapsed before 100 days had proliferation of NKG2C+ NK cells and only 1/38 patients with proliferation and persistence of this subpopulation beyond 100 days experienced leukemia relapse at a median follow-up of 18 months (p=0.01).

The kinetics of NK cell reconstitution in relation to the pattern of viral replication suggests that an early surge of $CD56^{dim}NKG2A^{low}$ NK cells with cytotoxic potential is responsible for the initial containment of viral replication followed by late surge and persistence of memory type NKG2C+NK cells preventing late recurrence as well as leukemia relapse. This unique synaptic response was possibly responsible for improved outcome with this novel approach of T-COSB based haploidentical HSCT.

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### Effect of Heterozygosity of Human Leukocyte Antigen on Outcomes Following Allogeneic Hematopoietic Cell Transplant for Myeloid and Lymphoid Malignancies

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**Background:** A recent study demonstrated that heterozygosity at HLA class I loci is associated with improved survival in patients with advanced solid tumors treated with immune checkpoint inhibitors when compared to patients homozygous in at least one HLA class I locus (Chowell et al. *Science* 2018). HLA heterozygosity allows for greater diversity of peptide presentation to T-cells and activation of a diversified immune response. In HCT, we hypothesize that HLA heterozygosity impacts disease control and survival in patients with myeloid and lymphoid malignancies.

**Design:** Patients who underwent 8/8 HLA-matched first allogeneic HCT for AML, MDS, ALL, or NHL between 2000 – 2015 were identified from CIBMTR database. Patients who received non-myeloablative and reduced intensity conditioning were excluded from analysis, except for those with NHL. HLA zygosity was characterized to the allele level as either heterozygous at all HLA class I loci or homozygous in at least one HLA class I locus. Primary outcomes of overall survival (OS) and relapse